Improving The Sustainability Of Aquaculture: Investigating Novel Experimental Concepts And Techniques.

Daryl E. Gunning (BSc., MSc., MSc.)

Submitted for the qualification of PhD degree

University College Cork
School of Biological, Earth, and Environmental Sciences
University College Cork, Co. Cork, Ireland
Year of submission: 2017

Head of School: Prof. Andrew J. Wheeler

Supervisors:
Dr Timothy Sullivan
Prof. Gavin Burnell
Dr Julie Maguire (Industry Supervisor)

Declaration

"This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism."

Candidate: Daryl E. Gunning

Signature: Daryl Gunning

Date: 08/08/20

Table of Contents

Abstract

The overall aim of this thesis is to investigate novel concepts and techniques that have the potential to improve the sustainability of the marine aquaculture industry. The focus of the research described here is on novel ecosystem approaches to aquaculture management by integrating species from multiple trophic levels into one system. This has been termed Integrated Multi-Trophic Aquaculture (IMTA), a concept that combines (ideally, in the appropriate proportions) the cultivation of fed aquaculture species (e.g. finfish or shrimp), with organic and inorganic extractive species (e.g. bivalve molluscs, seaweed or halophytes). Emphasis throughout has been placed on improving techniques and novel concepts that have the potential to be of practical sustainable use to existing and future industrial aquaculture operations.

Chapter 1 specifically details the development of sustainable saltwater-based food production systems, with a focus on established and emerging concepts. In Chapter 2, the biofiltering capacity of the halophyte *Salicornia europaea* is assessed, with a focus on biofiltering capacity when irrigated with wastewater from an oyster hatchery and cultivated via the novel hydroponic techniques. In Chapter 3, the efficacy of different stratification methodologies on *S. europaea* seed germination and growth are assessed, while Chapter 4 deals with the effectiveness of three anaesthetics in reducing error when measuring the size of cotton-spinner sea cucumber *Holothuria forskali*. The efficacy of passive integrated transponder (PIT) tags for *H. forskali* are assessed in Chapter 5 and Chapter 6 describes the cultivation of six species of seaweed in small-scale zero exchange maraponic systems with blue mussels (*Mytilus edulis*), Japanese abalone (*Haliotis discus hannai*) and *Holothuria forskali*. Finally, Chapter 7 details practical considerations and theoretical aspects of set-up and operation of a pilot-scale IMTA system (seaweed longlines containing *Alaria esculenta* and *Saccharina latissima*) in conjunction with a commercial organic salmon farm in Southern Ireland.

Chapter 1

The development of sustainable saltwater-based food production systems: a review of established and novel concepts*

***Published review article**

Citation: Gunning, D., Maguire, J., and Burnell, G. (2016). The development of sustainable saltwater-based food production systems: a review of established and novel concepts. *Water*, 8(12), p. 598, DOI: 10.3390/w8120598

Abstract

The demand for seafood products on the global market is rising, particularly in Asia, as affluence and appreciation of the health benefits of seafood increase. This is coupled with a capture fishery that, at best, is set for stagnation and, at worst, significant collapse. Global aquaculture is the fastest growing sector of the food industry and currently accounts for approximately 45.6% of the world's fish consumption. However, the rapid development of extensive and semi-extensive systems, particularly intensive marine-fed aquaculture, has resulted in worldwide concern about the potential environmental, economic, and social impacts of such systems. In recent years, there has been a significant amount of research conducted on the development of sustainable saltwater-based food production systems through mechanical (e.g. recirculating aquaculture systems {RAS}) methods and ecosystem-based approaches (e.g. Integrated Multi-Trophic Aquaculture (IMTA)). This chapter reviews the potential negative impacts of monocultural saltwater aquaculture operations, established (RAS) and novel (IMTA; constructed wetlands; saltwater aquaponics) saltwater-based food production systems, and discusses their existing and potential contribution to the development of sustainable and environmentally friendly systems.

1.1 Introduction

The human population is rising at a dramatic rate, doubling from 3 billion in the early 1960s to 6.5 billion in 2008, and currently standing at approximately 7.34 billion. It is expected to reach 9.7 billion by 2050 (United States Census Bureau, 2017; FAO, 2016; Klinger and Naylor, 2012). Global demand for fish (i.e. finfish, crustaceans, molluscs, and other aquatic animals) has increased significantly in recent decades, per capita consumption increasing from 9.9 kg in 1960 to 19.7 kg in 2013 (FAO, 2016; Klinger and Naylor, 2012). Fish are considered an important source of essential micronutrients (i.e. vitamins and minerals), proteins, and polyunsaturated omega-3 fatty acids and have been shown to have positive effects in relation to the prevention of heart disease, stroke, high blood pressure, muscular degeneration, some cancers, and inflammatory disease, to name but a few (Granada *et al*. 2016; Lund, 2013). Over 3 billion people worldwide now obtain approximately 17% – 20% of their animal protein (6.5% of total protein) from fish (Troell *et al*. 2014).

Capture fisheries have grown from a production of c.20 million metric tons (Mt) in the early 1950s to c.90 million Mt (70 million Mt for food use) in the late 1980s, providing the vast majority of global fish supplies during this period (e.g. 91% in 1980). Capture fisheries' production levels have remained stable since the late 1980s. In contrast, aquaculture has seen an annual worldwide production growth rate of 6.3% - 7.8% between 1990 and 2010 and is now the fastest growing food production sector (FAO, 2016; Granada *et al*. 2016; Troell *et al*. 2014; Diana, 2009). This rapid expansion of the aquaculture industry resulted from: wild fisheries reaching or exceeding their sustainable limit; a high level of global investment; improvements in aquaculture technology and management; and innovative techniques/technologies (e.g. RAS) (Troell *et al*. 2014; Naylor and Burke, 2005; Eagle *et al*. 2004). It was estimated that, in 2011, 61.3 % of marine fish stocks were fully exploited, 28.8 % were overexploited, and only 9.9 % were underexploited. Also, 13 of the world's 15 major oceanic fishing areas are now fished at or beyond capacity (Granada *et al*. 2016; Naylor and Burke, 2005).

In 2014, a landmark was reached when, for the first time, the contribution to the global supply of fish for human consumption from aquaculture (c.74 million Mt) exceeded that from capture fisheries (c.70 million Mt). This is in stark contrast to 1950, when

only 1 million Mt of finfish, crustaceans, and molluscs were cultivated (FAO, 2016; Klinger and Naylor, 2012). The majority of aquaculture today (by tonnage) takes place in freshwater (c.60 %), with the remaining taking place in seawater (c.32.3%) and brackish water (c.7.75 %). Most aquaculture operations take place in the Asia-Pacific region (88%-89 % of volume), with the vast majority occurring in China (60 %-62 % by volume & 51 % by global value) (Granada *et al*. 2016; Troell *et al*. 2014; Klinger and Naylor, 2012). Aquaculture production is composed mainly of freshwater finfish (c.55 %) and marine/brackish molluscs (c.25 %), finfish (c.10%), and crustaceans (9.5 %) (Klinger and Naylor, 2012; FAO, 2010). However, saltwater aquaculture will most likely increase over the coming decades, as global supplies of freshwater continue to decrease (FAO, 2016). In this chapter, saltwater aquaculture refers to offshore and on-land (e.g. coastal) marine aquaculture and on-land aquaculture which utilises non-coastal saline water (e.g. groundwater and artificial saltwater).

As the human population continues to expand and the capture fisheries industry stagnates, the reliance on farmed fish as a fundamental source of protein will also increase. Aquaculture has a number of potential positive impacts such as: reducing the pressure on wild stocks; rebuilding depleted wild stocks through stock enhancement; bioremediation and wastewater treatment (e.g. in RAS); providing a vital source of affordable fish-based protein and employment (Klinger and Naylor, 2012; Kawarazuka and Béné, 2010; Smith *et al*. 2010; Diana, 2009; Subasinghe *et al*. 2009; Gifford *et al*. 2007; Bell *et al*. 2006; Bunting, 2004). However, there are also potential negative environmental, social, economic, and health impacts resulting from aquaculture (predominantly monoculture operations). Some of the main concerns include: environmentally damaging levels of effluent discharge; water consumption; farmed fish escapes; transmission of parasites and disease; presence of contaminants; reliance on wild fish for fishmeal & oil addition to aquaculture feed; and negative employment and income effects (Granada *et al*. 2016; Troell *et al*. 2014; Klinger and Naylor, 2012; Cole *et al*. 2009; Diana, 2009; Naylor *et al*. 2009; Naylor and Burke, 2005; Naylor *et al*. 2000). To ensure the sustainable development of the aquaculture industry it is of paramount importance to develop technologies and production systems that mitigate these impacts.

This chapter will examine the potential negative impacts of monocultural saltwater aquaculture operations and review established (i.e. recirculating aquaculture systems (RAS)) and novel (i.e. Integrated Multi-Trophic Aquaculture (IMTA); constructed

wetlands; saltwater aquaponics) saltwater-based food production systems and discuss their contribution (or potential contribution) to the development of sustainable and environmentally-friendly systems.

1.2 Potential Negative Aquacultural Impacts

Aquaculture has become one of the most promising avenues for increasing fish production against a backdrop of continued human pressure on marine fisheries and ocean resources. However, extensive research has identified a number of potential ecological, social, and health impacts resulting from the aquaculture industry. The main impacts identified in the literature are discussed in this section.

Effluent Discharge and Contaminants

The discharge of effluent from aquaculture to the aquatic environment falls under three main categories: (1) continuous from aquaculture production; (2) periodic from farm activities; and (3) periodic discharges of chemicals (Granada *et al*. 2016; Read and Fernandes, 2003). Discharged aquacultural effluent contains metabolic waste products such as faeces, pseudofaeces, excreta, and uneaten feed. These are major contributors to organic and nutrient loading in the vicinities of aquaculture farms (Granada *et al*. 2016; Grigorkis and Rigos, 2011; Naylor *et al*. 2003). The scale of uneaten feed is dependent upon: farm operator's personal experience and qualifications; feeding management (automated or manual); and feed ingredients (Granada *et al*. 2016; Grigorkis and Rigos, 2011). It is estimated that 52% - 95% of the nitrogen and 85% of the phosphorus input to marine aquaculture systems through feed may be lost to the environment through fish excretion, faeces production, and feed wastage. The resulting organic enrichment causes environmental damage to receiving water bodies and sediments (Marinho-Soriano *et al*. 2011; Zhou *et al*. 2006a).

Chemical inputs to aquaculture, such as prescribed compounds (e.g., pesticides and pharmaceuticals), antifoulants, anaesthetics, and disinfectants, are an environmental concern when released in effluent water. The use of antibiotics is of particular concern as it may affect non-target species resulting in antibiotic resistance and other toxic effects. The prophylactic use of therapeutants is also a great concern due to their

persistence in the environment (Burridge *et al*. 2010; Cole *et al*. 2009; Read and Fernandes, 2003).

It has been shown that environmental levels of copper and zinc are significantly elevated close to aquaculture sites, in particular, areas where intensive cage aquaculture takes place. Antifouling paints applied to cages and nets to prevent the unwanted attachment of biofouling organisms often contain copper. Copper has low solubility in water and accumulates in sediments (Granada *et al*. 2016; Burridge *et al*. 2010; Le Jeune *et al*. 2006; Winner and Owen, 1991; Brand *et al*. 1986). Zinc, like copper, binds to fine particles and sulphides in sediments. Zinc is used as an additive to aquafeed, sometimes in excess of the species' dietary requirement. Fortunately, a number of feeds utilise zinc methionine, a more nutritionally accessible source of zinc, resulting in a feed with extremely low levels of required zinc. Algae, crustaceans, and molluscs also require copper and zinc additives for successful growth (Granada *et al*. 2016; Russell *et al*. 2011; Burridge *et al*. 2010).

Several studies have shown that natural and man-made contaminants are found in higher concentrations in farmed than wild fish. Such contaminants include polyaromatic hydrocarbons (PAH), dioxin, organophosphates (OP), polybrominated diphenyl ethers (PBDE), and polychlorinated biphenyl (PCB). Antibiotic contamination was only found to occur in farmed fish. Exposure of consumers to these contaminants can have a number of associated risks such as antibiotic resistance, memory impairment, cancer, and neurocognitive, endocrine, hormonal, immune, and cardiovascular abnormalities (Cole *et al*. 2009; Pinto *et al*. 2008; Blanco *et al*. 2007; Carubelli *et al*. 2007; Dewailly *et al*. 2007; Hayward *et al*. 2007; Hastein *et al*. 2006; Minh *et al*. 2006; Montory *et al*. 2006; Foran *et al*. 2005; Hites *et al*. 2004a; Hites *et al*. 2004b). Mercury contamination of fish has been linked to neurocognitive abnormalities in populations with a high level of fish consumption and to the occurrence of Minamata disease (Axelad *et al*. 2007; Davidson *et al*. 2006; Hites *et al*. 2004a; Easton *et al*. 2002).

Such contaminants can be found in fish feed and in areas of high natural occurrence c (Cole *et al*. 2009; Pinto *et al*. 2008; Blanco *et al*. 2007; Hites *et al*. 2004a; Hites *et al*. 2004b). A number of approaches can be taken to reduce the potential for contamination of farmed fish meat. Firstly, locating farms in areas with low levels of naturally occurring contaminants (Cole *et al*. 2009). Secondly, dioxins and PCD-like contaminants should be removed from fish feed through partitioning and

decontamination processes (Oterhals and Nygard, 2008). Thirdly, advisory bodies can recommend fish consumption limits, especially for susceptible people (e.g. pregnant women) (Cole *et al*. 2009; Kiljunen *et al*. 2007).

The most commonly practiced waste management solution for cage aquaculture is "dilution is the solution" and untreated effluent is released to the surrounding waters. In locations that have little flushing by tides and currents, this type of philosophy is problematic, as cage aquaculture effluent can have an enormous impact on the ocean floor extending from 30.5 to 152.4 metres in diameter. However, for areas that are well flushed, water quality problems and benthic impacts should be minimal. In closed systems (e.g. onshore RAS), waste management technology is utilised to minimise harmful effluent discharge into surrounding waters (Naylor and Burke, 2005; Naylor *et al*. 2003; Bridger and Garber, 2002; Brown, 2002).

Water Consumption

One of the most common solutions for excess nitrogen removal from on-land aquaculture farms is the frequent exchange and replacement of water, however, this method has a number of restrictions. Many nations have governmental regulations that limit the release of nutrient-rich water to the environment and there is an enormous cost associated with the pumping of large volumes of water (Granada *et al*. 2016; Avnimelech, 1999). Depending upon local conditions, grow-out stage, and feeding cycle, the daily water exchange rate of pond aquaculture systems, for example, can range from 3% to 30% of the pond's volume (Páez-Osuna, 2001; Páez-Osuna *et al*. 1998). A reduction in effluent volume would considerably decrease the volume of water that would need to be exchanged or replaced while also limiting the potential polluting impacts of on-land aquaculture (Granada *et al*. 2016; Boyd and Gross, 2000).

Farmed Fish Escapes

Accidental release of farmed fish into natural waters can lead to a number of ecological risks, including: increased competition for space, prey, and/or mates; introduction of alien species; pathogen, disease, and parasite transmission; interbreeding between farmed and wild fish resulting in reduced fitness of wild cousins or wild stock enhancement resulting in genetically distinct fish from their wild cousins; habitat damage; and water quality alterations (Granada *et al*. 2016; Arthur *et al*. 2010; Diana, 2009; Naylor and Burke, 2005; Kolmes, 2004; McGinnity *et al*. 2003; Levin *et al*. 2001;

Fleming *et al*. 2000; Volpe *et al*. 2000; McGinnity *et al*. 1997). Many of the general features of successful invasive species (i.e. rapid growth, early sexual maturity, high genetic variability, broad environmental range, and a short generation time) are also common features of aquaculture species (Granada *et al*. 2016; Diana, 2009). The escape of farmed salmon through sporadic and mass events is well recorded (Foran *et al*. 2005; Naylor *et al*. 2005; Gross, 1998; McKinnell and Thomson, 1997; Hansen *et al*. 1993). In the early 1990s, a study conducted by Hansen *et al*. (1993) found that up to 40% of Atlantic salmon caught by fishermen in oceanic waters north of the Faroe Islands were of farmed origin (Hansen *et al*. 1993). Since the 1980s, over 255,000 farmed Atlantic salmon have escaped and been caught by fishermen from Washington to Alaska (McKinnell and Thomson, 1997). Various studies have provided evidence that farmed Atlantic salmon (*Salmo salar*) escapees may hybridise and alter the genetic composition of wild populations, potentially exacerbating the decline of local endangered populations of wild Atlantic salmon (Gross, 1998; McGinnity *et al*. 1997; Slaney *et al*. 1996). Naylor *et al*. (2005) showed that farmed Atlantic salmon introduced to their native range are more likely to hybridise with local populations than, for example, farmed Atlantic salmon escaping into non-native regions (e.g. the Pacific) (Naylor *et al*. 2005).

Parasite and Disease Transmission

There are a number of diseases and parasites that have the capability to spread from farmed to wild fish and their transmission can occur when infected farmed fish come in contact with wild host species (e.g. infected farmed escapees) or when wild fish migrate or move through plumes of an infected cage or disease outbreak (Naylor and Burke, 2005; Naylor *et al*. 2005). In a lot of cases, pathogens originate from wild populations, but reach epidemic proportions in intensive cage aquaculture operations, risking further, more intensified infection of wild stocks (Naylor and Burke, 2005). A number of studies have provided modelled and empirical evidence indicating that sea lice do transmit from farmed to wild salmon and this transmission causes massive mortalities or collapse of infected wild stock (Ford and Myers, 2008; Krkosek *et al*. 2007; Krkosek *et al*. 2006). The movement of aquaculture stock can increase the risk of spreading pathogens to wild species. For example, in Europe, serious epidemics of *Gyrodactylus salaris* in wild Atlantic salmon stocks have been linked to the movement of fish for aquaculture and re-stocking (Naylor *et al*. 2000; McVicar, 1997). Studies

have also indicated that the movement of aquafeed around the world can be a vector for disease transmission (Naylor and Burke, 2005; Dalton, 2004).

Fishmeal and Oil

Most carnivorous, diadromous fish and marine finfish farm operations require an input of wild fish (i.e. live pelagic fish or low value "trash fish") or feed containing components of wild fish origin (i.e. fishmeal or fish oil) (Naylor and Burke, 2005; Tacon, 1997). The proportion of farmed aquatic species raised on supplementary feed inputs continues to rise, reaching almost 70% of total aquaculture production in 2012. Mollusc species (e.g. mussels and oysters) account for approximately 23% of global farmed seafood production and take their nutrition from the surrounding environment (e.g. plankton & detritus), resources that are otherwise not directly exploitable by humans (FAO, 2014; Troell *et al*. 2014). The efficiency of feed utilisation by farmed fish (known as feed conversion ratio {FCR}), the quantities of fishmeal and fish oil contained in the feed, and the amount of wild fish used to produce the feed, are important factors determining the economic profitability and environmental impacts of aquaculture (Klinger and Naylor, 2012; Boissy *et al*. 2011; Tacon *et al*. 2011; Hardy, 2010; Naylor *et al*. 2009; Tacon *et al*. 2006; Naylor and Burke, 2005; Naylor *et al*. 2000). For example, fishmeal and fish oil generally constitute 50% - 75% by weight of carnivorous marine farmed finfish aquafeeds. For salmon, feeds typically contain 35% - 40% fishmeal and 25% fish oil, however, new diets containing less than 20% fish oil are becoming more common (Goldburg and Naylor, 2005; Naylor and Burke, 2005; Tacon, 1997).

Overall, the aquaculture industry has made significant strides in increasing feed efficiency. The ratio of wild fish input to farmed fish have fallen to 0.63 for aquaculture overall. However, it is important to note that this figure remains as high as 5.0 for Atlantic salmon. Improvements in FCR ratios and reductions in fishmeal and fish oil inclusion rates in aquaculture feeds have also been made (Klinger and Naylor, 2012; Hardy, 2010; Naylor *et al*. 2009; Tacon and Metian, 2008). Despite these improvements, the continued growth of feed-reliant aquaculture has resulted, within the last decade, in the doubling of aquaculture's share of global fishmeal and fish oil consumption to 68% and 88%, respectively (Naylor *et al*. 2009; Tacon and Metian, 2008). An estimated 20–30 million Mt of reduction fish are fished from the oceans each year to produce fishmeal and fish oil. These fish tend to be low on the marine food

chain and include small pelagic fish species such as Peruvian and Japanese anchovy, blue whiting, Atlantic herring, and chub and Chilean jack mackerel. Additionally, an estimated 5–9 million Mt of "trash fish" and other small pelagic fish are used in non-pelleted, farm-made feeds (Klinger and Naylor, 2012; Tacon *et al*. 2010). Most forage fish are either fully exploited, overexploited, or in the process of recovery from overexploitation. These forage fish play an essential role in converting plankton into food for higher trophic level species such as: humans, larger fish, marine mammals, and seabirds (Klinger and Naylor, 2012; Naylor *et al*. 2009; Alder *et al*. 2008). A number of alternatives to fishmeal and fish oil from forage fish are possible and are currently being researched, including: vegetable proteins and oils; terrestrial animal byproducts (e.g. rendered animal products); fish/seafood processing waste; oils produced by industrial fermentation technology; and the use of less-common feed inputs such as krill, polychaetes, insects, and macroalgae (i.e. lower trophic level organisms) Also, new genetic and metabolic engineering techniques to produce long-chain omega 3 fatty acids and the development of single-cell organisms, microbial (e.g. bacterial) and algal proteins are being researched (Troell *et al*. 2014; Klinger and Naylor, 2012; Bendiksen *et al*. 2011; Naylor *et al*. 2009; Naylor *et al*. 2000).

Social Welfare

Aquaculture can generate a large amount of employment for communities. In some coastal regions of Scotland and Norway, for example, the salmon farming industry is the largest private-sector employer. Also, in Maine, where communities once relied on now-collapsed wild fisheries, the benefits from employment in the salmon aquaculture industry have been significant (Naylor and Burke, 2005).

However, in a broader context, experiences from the growth of the salmon farming industry have shown us that the employment and income losses in the fish capture industry may be as large, or larger, than the employment and income generated for coastal communities through aquaculture (Naylor *et al*. 2005; Marshall, 2003; Naylor *et al*. 2003). There is also no guarantee that those fishermen who have lost their jobs due to overfishing and/or as a direct or indirect result of aquaculture growth will find employment in the aquaculture industry or that local communities will benefit from this growth. In Canada, most of the employment gains resulting from the aquaculture industry were limited to areas where hatcheries and processing facilities are located (Naylor *et al*. 2003). Grow-out operations can often lack community roots, depending

upon a supply of feed, larvae, supplies, equipment, and a skilled workforce from areas distant from the production site. This situation rarely has a noteworthy income multiplier effect for local communities (Costa-Pierce, 2002; Bailey *et al*. 1996). Finally, if large multinational companies control a vast majority of the aquaculture industry, as is the case for salmon aquaculture, a large share of the sector's income gains are secured by these companies and the benefits to local communities become limited (Naylor *et al*. 2003).

The aquacultural production of high trophic level fish species often relies on fishmeal and fish oil from pelagic fish for the production of aquafeeds. These high trophic level fish are mainly aimed towards the markets of developed countries.This situation has negative implications for developing countries that depend on pelagic fish, or wild fish that feed upon pelagic fish, as a direct source of protein for human nutrition. This demand for pelagic fish for direct/indirect consumption will most likely rise as the population grows in developing countries (Naylor and Burke, 2005; Naylor *et al*. 2000). Other potential social conflicts and impacts on other users of water-bodies that can arise from the development of the aquaculture industry, include: blocked access to water-body resources by pond or cage structures; navigational hazards; privatisation of public waterways and lands; and the conversion of agricultural (e.g. rice paddies, pastures), residential, and common waterways and land (Primavera, 2006; Primavera, 1997; Bailey, 1988).

1.3 Recirculating Aquaculture Systems (On-Land)

A lack of space for expansion, competition with other users for sites, concerns over pollution, and the high costs associated with pumping large volumes of water (e.g. with flow-through on-land aquaculture farms) are major obstacles to the environmentally and economically sustainable expansion of the saltwater aquaculture industry (Badiola *et al*. 2012; Boyd and Gross, 2000). One effective solution is the rearing of fish in recirculating aquaculture systems (RASs); defined in a paper by Zhang *et al*. (2011) as "land-based aquatic systems where the water is (partially) re-used after mechanical and biological treatment in an attempt to reduce the consumption of water and energy and the release of nutrients into the environment" (Martins *et al*. 2011; Zhang *et al*. 2011). In general, with RASs, large solid particles of uneaten feed, faeces, and bacteria are

concentrated and removed by settling or mechanical filtration. Fine particles (<100 microns) can be removed by foam fractionation. Ozone treatment can be utilised to reduce Total Suspended Solids (TSS) and Dissolved Organic Carbon (DOC), as well as controlling the level of Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) (Klinger and Naylor, 2012; Timmons and Ebeling, 2010). Some forms of dissolved nitrogenous wastes (i.e. ammonia and nitrite) are toxic to fish and are removed from the wastewater in biofilters containing nitrifying bacteria (e.g. biofilm filtration). In the biofilter, *Nitrosomonas* sp. and *Nitrosococcus* sp. oxidise ammonia into nitrite and then *Nitrospira* sp. oxidise nitrite into nitrate (Schreier *et al*. 2010; Helfman *et al*. 2009; Gutierrez-Wing and Malone, 2006). Although high levels of nitrate are tolerable to fish (Helfman *et al*. 2009), long-term exposure can be harmful to some species (e.g. Turbot {*Psetta maxima*}) (Van Bussel *et al*. 2012; Helfman *et al*. 2009). To combat this, many RASs will use anaerobic ammonium oxidation (anammox) to convert ammonia and nitrite directly into nitrogen gas (Van Bussel *et al*. 2012; Chavez-Crooker, 2010; Schreier *et al*. 2010; Van Rijn *et al*. 2006). Fish and bacterial metabolism strips water of dissolved oxygen while increasing concentrations of carbon dioxide (CO_2). Therefore, many operators will run air through the CO_2 rich wastewater to degas the CO_2 and increase the oxygen concentration (Timmons and Ebeling, 2010). Ozone gas and ultraviolet lamps are also often used to kill fungal, viral, bacterial, and protozoan pathogens in the water prior to its re-entry to the culture tanks or being discharged (Gonçalves and Gagnon, 2011; Schroeder *et al*. 2011).

RASs have a number of advantages over conventional aquaculture systems. They vastly reduce water consumption. RASs enable up to 90% - 99% of the water to be recycled and water use in saltwater RASs can be as low as 16 L/kg of fish. This is in stark contrast to conventional aquaculture systems that use 3000–45,000 L of water/kg of seafood produce (Badiola *et al*. 2012; Tal *et al*. 2009; Verdegem *et al*. 2006). Due to this low water requirement, RASs can be located on land unsuitable for other food production methods (e.g. deserts, post-mining lands, urban areas) and/or close to markets, which results in local employment and revenue opportunities and reduced shipping and transportation costs (Klinger and Naylor, 2012; Martins *et al*. 2010; Miller, 2008; Singer *et al*. 2008). RASs improve opportunities for waste management, nutrient recycling, and biological pollution control. The majority of excess nutrients and waste material (uneaten feed, faeces, dead bacteria) are removed before water is released to the environment. Thus, RASs reduce potential negative impacts on marine and saline

environments and ecosystems (Badiola *et al*. 2012; Klinger and Naylor, 2012; Zhang *et al*. 2011). RASs improve conditions for cultured fish by having greater control over environmental and water quality parameters and enhance feeding efficiency. Subsequently, RASs can allow for higher stocking densities than most aquacultural systems (Brown *et al*. 2011; Martins *et al*. 2010; Mirzoyan *et al*. 2010; Timmons and Ebeling, 2010; Tal *et al*. 2009; Marsh *et al*. 2005; Piedrahita, 2003; Cripps and Bergheim, 2000; Heinen *et al*. 1996). By sterilising the water prior to (re)entry to the fish tanks, pathogens and contaminants are removed, reducing the risk of disease outbreaks and contaminant uptake by the fish (Jeffery *et al*. 2010; Martins *et al*. 2010; Cole *et al*. 2009). Due to the on-land and recirculatory nature of RASs, the potential for fish escapes is greatly reduced (Klinger and Naylor, 2012; Martins *et al*. 2010). Ultimately, the wastes removed from RAS water must be dealt with. The solid wastes removed from a RAS can be utilised in methane production, polychaete culture, vermicomposting, and as an agricultural fertiliser. Therefore, the by-products of RASs can be sold to other industries. Also, the higher stocking densities, year-round production, and reduced water costs are an economic advantage (Klinger and Naylor, 2012; Cripps and Bergheim, 2000).

Despite having a number of advantages over conventional aquaculture systems, RASs also have a number of constraints, namely, high capital and operational costs, a requirement for extremely careful management, and difficulties in treating disease (Badiola *et al*. 2012; Klinger and Naylor, 2012). The cost of setting up a RAS is very high, therefore, future profitability is uncertain, discouraging many from investing (Matins *et al*. 2010; Timmons and Ebeling, 2010). A high amount of electricity is required to run recirculating systems that function on a continuous basis, subsequently, RASs consume far more energy than most other types of aquaculture (Pelletier *et al*. 2011; Ayer and Tyedmers, 2009). The total energy consumption (including feed) of carnivorous finfish RAS facilities is estimated to range from 16 to 98 kilowatt hours per kilogram (kWh/kg) of fish produced. In comparison, net pen aquaculture consumes approximately 7.4 kWh/kg and flow-through farms approximately 27.2 kWh/kg for similar species of fish (Ayer and Tyedmers, 2009; D'Orbcastel *et al*. 2009). Surveys of RAS operators conducted by Badiola *et al*. (2012) identified the following barriers to the successful operation of RASs: poor system design, poor management (mainly due to unskilled labourers taking responsibility of water quality and mechanical problems), a lack of communication between parties (e.g. between different operators or suppliers),

and a disincentive to share information and knowledge within the industry. Badiola *et al*. (2012) identified two key priorities necessary to improve RAS operations: (1) Improvement of equipment performance. This can be achieved through commercial-scale research to try and identify the best combination of devices on a site-specific basis; (2) The development of a specialised RAS platform for the sharing of knowledge amongst the relevant personnel (Badiola *et al*. 2012). If recirculated water is not properly sterilised, the reuse of water in RASs can lead to contaminants from feed and system components and diseases/pathogens accumulating in the system (Martins *et al*. 2010; Jeffery *et al*. 2010; Cole *et al*. 2009). However, two studies by Tal *et al*. (2009) and Martins *et al*. (2011) found that contaminants in RASs were either below harmful levels or undetectable (Martins *et al*. 2011; Tal *et al*. 2009). The use of denitrifying bacteria in RAS biofilm filtration systems has three possible constraints that may negatively impact survival, growth, and reproduction of the cultured organism. Firstly, nitrifying bacteria compete with the cultured organism for oxygen. Secondly, nitrate can be converted into the toxic nitrite under anaerobic conditions. Thirdly, RASs using biofilm filtration tend to acidify over time due to the respiration of the biofilm and the cultured organisms (Cahill *et al*. 2010; Watten and Sirbrell, 2006; Greiner and Timmons, 1998; Van Rijn, 1996). Although RASs improve feed efficiency, the high cost of setting up and running a RAS means that most operators will choose to cultivate high value carnivorous fish, which consume relatively high levels of fishmeal and fish oil (Martins *et al*. 2010; Timmons and Ebeling, 2010). To improve upon some of these constraints, feed inputs need to be altered, energy efficiency needs to be improved, and the conditions for bacterial growth need to be optimised (Martins *et al*. 2010). Novel solutions include: bio-floc technology, which greatly reduces the flow rate and suspended communities of microbes (i.e. flocs) convert toxic nutrients into biomass that can be consumed directly by fish or shrimp (De Schryver *et al*. 2008). Periphyton-based systems whereby artificial substrates (e.g. poles, bamboo) are added to the culture system to attract organisms which remove nutrients and provide (additional) food for the cultured animals (Azim *et al*. 2006).

1.4 Integrated Multi-Trophic Aquaculture (Offshore and On-Land)

Another approach to tackling the negative impacts of aquaculture is an ecosystem-based approach to aquaculture management. To be considered an ecologically sound system, these "ecological aquaculture" systems should be designed under the following criteria: preservation of natural ecosystems; environmentally friendly nutrient management; significant reduction or absence of harmful chemicals and antibiotics; trophic level efficiency; and farmed fish escape prevention. It would also be beneficial if these systems improved the economies and provided employment in the areas in which they are located (Naylor and Burke, 2005; Costa-Pierce, 2002). In the following sections, we will discuss established and novel saltwater food production systems that most closely follow the listed criteria for an ecosystem-based approach to saltwater-based food production.

The concept of integrated aquaculture production is not a new one and has been practiced in Asian countries for centuries through trial, error, and experimentation (Qian *et al*. 1996; Chan, 1993; Wei, 1990; Li, 1987; Tian *et al*. 1987). Integrated farming, predominantly in fresh and brackish water pond systems, is an ancient practice in China and has become more refined since the implementation of agricultural and rural development policies introduced in 1949. These policies were motivated by the high population growth in China and the need to maximise productivity of available land and water. They were also based on a philosophy of diversified self-reliance of food and raw material production and the use of by-products (i.e. wastes) as an input to produce other resources (Chopin *et al*. 2001; Ruddle and Zhong, 1988). This integrated form of farming is often referred to as polyculture, "the (usually) simultaneous cultivation or growth of two or more compatible plants or organisms (especially crops or fish) in a single area" (Merriam-Webster, 2017). In contrast, the western world tends to focus on high value, intensive monoculture, which has many potential negative outcomes. Unfortunately, many newcomers to the industry from Asia are following this trend, due to the temptation of expeditious financial gains that result from the monocultural production of fish or shrimp (Chopin *et al*. 2001).

Integrated Multi-Trophic Aquaculture (IMTA) combines the cultivation of fed aquaculture species (e.g. finfish, shrimp), with that of organic and inorganic extractive species (e.g. bivalve molluscs, seaweed, and halophytes). It is a practice in which the

wastes from one species are recycled and become the inputs (e.g. fertiliser, food and energy) for another (Barrington *et al*. 2009; Troell *et al*. 2009; Chopin *et al*. 2001). IMTA differs from the traditional practice of aquatic-polyculture in that it incorporates species from different trophic levels, whereas with polyculture, the species tend to be from the same or similar trophic levels, and therefore share the same biological and chemical processes, providing few synergistic benefits (Granada *et al*. 2016; Barrington *et al*. 2009). The principles of IMTA can be applied to saltwater and freshwater operations on land, near the coast or offshore (Klinger and Naylor, 2012; Troell *et al*. 2009). To function well in open-water IMTA systems, the culture of organic extractive species (e.g. shellfish or deposit-feeding invertebrate) and/or inorganic extractive species (e.g. macroalgae) should take place in close-proximity to the cages, usually somewhat downstream to ensure effective uptake of nutrients (Neori *et al*. 2009; Sará *et al*. 2009). Offshore IMTA relies on currents to move nutrient-rich water from fed to extractive species. Coastal and pelagic currents can be difficult to predict and are location and seasonally dependent. Correct positioning of additional crops will require experimental trials and/or modelling (Klinger and Naylor, 2012). The organic extractive species consume particulate organic matter (i.e. uneaten feed/food and faeces) and the inorganic extractive species uptake ammonia, nitrate, phosphorus, and carbon dioxide and release oxygen (Klinger and Naylor, 2012; Sará *et al*. 2009; Neori *et al*. 2004). On land, IMTA usually takes place in tanks, ponds, or as a wetland addition for wastewater treatment. Within the literature, on-land IMTA has been broken down into two additional sub-groups (halophyte wetlands and saltwater aquaponics), both of which include an inorganic extractive species as a component of their integrated, multi-trophic system. These will be discussed in more detail in Sections 1.4.1 and 1.4.2. It is important to note, that an on-land IMTA system that does not contain an inorganic extractive species would not fall into these two sub-groups and is simply referred to as an on-land IMTA or integrated system. A number of potential candidate species have been identified for their inclusion in offshore and on-land IMTA operations, a number of which are detailed as follows.

Inorganic Extractive Species (i.e. Seaweeds & Aquatic Plants)

Intensive seaweed production requires a constant nutrient supply, especially in the summer when warm waters are generally nutrient depleted. Integrating seaweed into fish aquaculture in coastal waters can alleviate the seasonal nutrient depletion by

utilising the constant nutrient supply from fish farms (Zhou *et al*. 2006a; Chopin *et al*. 2001). Seaweeds have a high market value and are sold worldwide for human consumption, and as a source of phycocolloids, feed supplements, agrichemicals, neutrachemicals, and pharmaceuticals. In 2014 alone, the global culture of algae reached approximately 27-28 million tons at an estimated value of US$ 5-6 billion (FAO, 2016; Granada *et al*. 2016; Neori *et al*. 2004).

Gracilaria is one of the most exploited seaweed genera worldwide (Abreu *et al*. 2009) and therefore, one of the most commonly studied candidate species for integration into offshore IMTA systems. A number of at sea trials integrating seaweed with monocultural mariculture operations are detailed below, however, a comprehensive list of references is available in Troell *et al*. (2003), Neori *et al*. (2004), and Granada *et al*. (2016).

Candidate inorganic extractive species for on-land IMTA include seaweeds, halophytes, and low-moderately saline tolerant glycophytes. These will be discussed in more detail in Sections 1.4.1 and 1.4.2.

Studies by Fei *et al*. (2002; 2000) found that when economically important *Gracilaria lemaneiformis* was grown near fish net pens on 5 km of rope, they achieved extremely high levels of growth. The biomass of *G. lemaneiformis* increased from 11.6 to 2025 $g \cdot m^{-1}$ over a 3 month growth period and when Fei et al., (2000; 2002) increased the total length of the seaweed longlines to 80 km, they achieved 4250 $g \cdot m^{-1}$ over the following 4 months. They achieved a final biomass of 240 metric tons (fresh weight {FW}) and attributed this success to its culture in close proximity to the fish cages.

Seaweeds are very effective and efficient at taking up nutrients (i.e. nitrogen and phosphorus), making them an ideal bioremediation tool for aquaculture. (Huo *et al*. 2011; Marinho-Soriano *et al*. 2011; Abreu *et al*. 2009).

Zhou *et al*. (2006a) co-cultivated longlines of *G. lemaneiformis* c. 12 m from black snapper (*Sebastodes fuscescens*) cages and results indicated that this seaweed is a good candidate for seaweed/fish integrated mariculture for bioremediation and economic diversification. *G. lemaneiformis* achieved a maximum growth rate of $11.03 \cdot day^{-1}$ and mean N and P uptake rates of the thalli were estimated at 10.64 and 0.38 $\mu mol \cdot g^{-1} \cdot$dry weight (DW)$\cdot h^{-1}$, respectively. When Zhou *et al*. (2006a) extrapolated these results, they calculated that 1 Ha of *G. lemaneiformis* cultivation in coastal fish farming waters would give an annual harvest of over 70 tons FW (9 tons DW) and 0.22 tons N and 0.03 tons P would be sequestered from the seawater. Buschmann *et al*. (2008) installed a 100

m seaweed longline approximately 100 m from a salmon farm that produces 2500–3000 tons of biomass of fish per annum. The longline was positioned in the main water flow that had an average current speed of 7.6 cm s^{-1} and 2.6 cm s^{-1} during the flood and ebb period, respectively. This 100 m longline contained 50 m of *G. chilensis* and 50 m of *M. pyrifera* at depths of 1 m, 3 m, and 6 m. The growth rate reached an average of 6·day^{-1} and 4·day^{-1} for *M. pyrifera* and *G. chilensis* respectively, equating to an annual production of over 25 kg·m^{-1} of *M. pyrifera* during a 9 month production period and an average of 2.8 kg·m^{-1}·$month^{-1}$ during the spring for *G. chilensis*. Optimal growing conditions occurred in the spring for both species and at a depth of 3 m for *M. pyrifera* and 1m for *G. chilensis*. In the spring, the decrease in nitrate concentration due to uptake by *M. pyrifera* was 11.8 ± 4.5 µM·g(DW)$^{-1}$·h^{-1} compared to 4.9 ± 2.3 µM·g(DW)$^{-1}$·h^{-1}. for *G. chilensis.* However, the annual change in concentration is higher for *G. chilensis* at 1.2–35.6·µM·g(DW)$^{-1}$·h^{-1} in comparison to 3.7–16.9 µM g(DW)$^{-1}$ h^{-1} for *M. pyrifera*. The incorporation of seaweed species with different light requirements to an IMTA system allows for the utilisation of different water column depths and subsequently increases their efficiency and effectiveness as biofilters.

Abreu *et al*. (2009) deployed 3 × 100 m longlines (1 m depth) of *G. chilensis* at a distance of 100 m (L1), 800 m (L2), and 7 km (L3) from salmon cages (production capacity of 1500 tons) in order to receive the main flow of nutrients discharged from the salmon farm during flood tides (average currents: 7.6 cm·s^{-1} and 2.4 cm·s^{-1} during the flood and ebb periods, respectively). A fourth longline cultivation unit (L4) was also set up as a traditional bottom culture in a separate location not impacted by the salmon farm. The two longlines positioned closest to the salmon farm (L1 and L2) performed best in terms of productivity and nitrogen removal. Although the L1 and L2 longlines both had a relative growth rate (RGR) of approximately 4·day^{-1} in the summer and 2·day^{-1} in the autumn, the L2 longline had stronger productivity at c. 1.7 kg·m^{-1}·$month^{-1}$ in comparison to c. 1.48 m^{-1}·$month^{-1}$ for L1. In terms of N removal, L2 removed an average of 9.3 g·m^{-1}·$month^{-1}$, while L1 removed an average of 7.8 g·m^{-1}·$month^{-1}$. The lower levels of production 100 m from the cages (L1) could be attributed to the higher occurrence of epiphyte growth on these seaweeds. Abreu *et al*. (2009) estimated that a 100 Ha *G. chilensis* longline system at a distance of 800 m would effectively remove 100% of the N inputs from a 1500 ton salmon farm.

Organic Extractive Species (i.e. Invertebrates)

Filter-feeding invertebrates filter large volumes of water to meet their food requirements and have a high level of efficiency in retaining small particles, including bacteria (Granada *et al*. 2016; Stabili *et al*. 2006). Several studies have shown that bivalves have enormous potential as bio-controllers of fish farm effluent (Lander *et al*. 2013; Handå *et al*. 2012; MacDonald *et al*. 2011; Reid *et al*. 2010). For example, Reid *et al*. (2010) measured the absorption efficiency of blue and bay mussels (*Mytilus edulis* and *M. trossulus*) feeding on Atlantic salmon feed and faecal particulates and found removal rates of up to 54% of total particulate matter. Macdonald *et al*. (2011) found that the oyster, *Saccostrea commercialis*, is effective at reducing total suspended solids and total N and P released from an Atlantic salmon farm. Studies have also shown significant improvements in the growth of oysters and mussels when co-cultured with salmon (Lander *et al*. 2013; Handå *et al*. 2012; MacDonald *et al*. 2011). Some studies have suggested that bivalves have the potential to act as a reservoir for finfish pathogens. For example, Pietrak *et al*. (2012) demonstrated the capability of *M. edulis* to bioaccumulate *Vibrio anguillarum* in the digestive gland at twice the magnitude found in the water column. If *V. anguillarum* can persist in mussel faecal pellets, it is possible that mussels could generate *Vibrio* reservoirs in sediments and/or faecal matter (Granada *et al.* 2016; Pietrak *et al*. 2012). Other studies, however, have demonstrated that bivalves are not hosts, instead consuming parasites or inactivating pathogens (Molloy *et al*. 2011; Skar and Mortensen, 2007). More research into bivalves' ability to act as pathogen reservoirs is required, however, steps can be taken to minimise the risk of pathogen transmission. Farms should be positioned in locations with sufficient water depth between the bottom of the cage and the benthos at low tide (Granada *et al*. 2016).

In the natural environment, sea cucumbers are detritus feeders that ingest sediment containing animal and plant organic matter and are therefore considered important processors of surface sediment, making them ideal bioremediation candidates for coculture in an IMTA system (Yokoyama, 2013; Slater and Carton, 2009). MacDonald et al. (2013) conducted land-based tank trials and found that the cotton-spinner (*Holothuria forskali*) readily consumed European seabass (*Dicentrarchus labrax*) waste diets at a level that was suitable to process biodeposition beneath commercial sea-bass cages. The grazing by *H. forskali* also reduced the total N content of *D. labrax* waste in a short-term controlled feeding experiment and suppressed total carbon (C)

content in a long-term controlled feeding experiment. *H. forskali* has not yet been utilised on a commercial scale, however, it is a high quality protein source that also has a number of biological features that have potential applications in biotechnology and pharmaceuticals (Bordbar *et al*. 2011; Van Dyck *et al*. 2009; Taboada *et al*. 2003; Rodríguez *et al*. 2000). The Japanese common sea cucumber (*Apostichopus japonicus*) is a valuable species across Asia and studies have demonstrated its potential for integration into an IMTA system. Yokoyama (2013) showed that *A. japonicus* cultured under fish cages exhibited enhanced growth and survival and showed evidence of fish faeces and organic settling matter ingestion. Kang *et al*. (2003) co-cultured *A. japonicus* and charm abalone (*Haliotis discus hannai*) in tanks and found the levels of ammonium nitrogen and nitrite in the water of cocultured groups were lower than the control group (abalone only). Also, the abalone growing in the coculture had significantly better growth and survival, highlighting *A. japonicus*' ability to reduce the levels of inorganic N in the water. Zhou *et al*. (2006b) showed that Chinese scallop (*Chlamys farreri*) lantern nets provide a good habitat for *A. japonicus* and they grew well when in close proximity to these nets. The California sea cucumber (*Parastichopus californicus*) has demonstrated its ability to consume fouling debris such as detritus from shellfish (e.g. oysters), fish faeces, excess fish feed, and algae (Granada *et al*. 2016; Hannah *et al*. 2013; Paltzat *et al*. 2008). Other species of sea cucumber that have been assessed for their potential role in IMTA systems are the orange-footed sea cucumber (*Cucumaria frondosa*) and the Australian brown sea cucumber (*Australostichopus mollis*). *C. frondosa* has demonstrated high absorption efficiency (>80%) of salmon feed and faeces (Nelson *et al*. 2012). *A. mollis* cultured below mussel farms grow rapidly and significantly reduce the accumulation of organic carbon and phytopigments associated with biodeposition from these farms (Handå *et al*. 2012; Zamora and Jeffs, 2012; Zamora and Jeffs, 2011; Slater *et al*. 2009).

Other novel potential additions to IMTA systems include polychaetes and sponges. Polychaetes are highly efficient at filtering, accumulating, and removing waste-associated bacterial groups such as vibrios and potential human pathogens, with high levels of efficiency (Stabili *et al*. 2010; Licciano *et al*. 2005). They can also ingest and assimilate faecal waste from aquaculture farms. One study found that the polychaete *Perinereis nuntia vallata* converted approximately 50% of the nitrogen ingested from Japanese flounder (*Paralichthys olivaceus*) wastewater into body tissue (Honda and Kikuchi, 2002). Another study involving two species of intertidal polychaetes

(*Perinereis helleri* & *Perinereis nuntia*) cultured in sand-beds to remediate wastewater from a prawn farm, revealed that the polychaete filtration process significantly reduced chlorophyll a and suspended solids (Palmer, 2010). Polychaetes have commercial value in the saltwater aquarium industry and a number of species have been shown to have antibacterial properties that have applications in the biotechnology industry (Granada *et al*. 2016; Stabili *et al*. 2009). Like polychaetes, sponges have the ability to utilise bacteria (Stabili *et al*. 2009) and filter organic particles (Osinga *et al*. 2010; Stabili *et al*. 2006; Milanese *et al*. 2003). Stabili *et al*. (2006) showed that Demospongiae (Porifera) non-selectively filter organic particles of 0.1 mm–50 mm in size, retaining up to 80% of suspended solids after processing the water column within 24 h. Organic particles that fall within this size range include: heterotrophic eukaryotes and bacteria, phytoplankton, and detritus. Other studies conducted on Mediterranean sponges (*Dysidea avara*, *Chondrosia reniformis*, *Chondrilla nucula*, and *Spongia officinalis* var. *adriatica*) have shown great filtering efficiency and improved growth when cultured in close proximity to aquaculture farms (Osinga *et al*. 2010; Wijffels, 2008; Milanese *et al*. 2003). Sponges have enormous commercial potential in the areas of biotechnology, pharmaceuticals, and cosmetics (Webster and Taylor, 2012; Koopmans *et al*. 2009; Wijffels, 2008; Sipkema *et al*. 2005).

The majority of recent studies on marine or saltwater IMTA systems in industrialised nations have been conducted on an experimental, small-operation scale, and it can be difficult to extrapolate these results to an industrialised scale (Granada *et al*. 2016; Troell *et al*. 2003). However, on the east coast of Canada, in the Bay of Fundy, a commercial scale IMTA operation has been on-going since 2001. This IMTA system consists of blue mussels (*Mytilus* edulis) and kelps (*Saccharina latissima* & *Alaria esculenta*) in close proximity to salmon cages *(Salmo salar*). An increased growth rate of kelps (46%) and mussels (50%) was seen when cultured in proximity to the fish farms in comparison to reference sites (Chopin and Robinson, 2004; Chopin *et al*. 2004; Lander *et al*. 2004). Over the course of these commercial-scale trials none of the therapeutants used in salmon aquaculture have been detected in kelps and mussels collected from the IMTA sites. Also, levels of heavy metals, arsenic, PCBs, and pesticides have always been below regulatory limits. A taste test of the IMTA mussels in comparison to reference mussels was conducted and showed no discernable difference (Barrington *et al*. 2009; Lander *et al*. 2004). Two attitudinal studies on salmon farming in the area were conducted. The first one found that the general public

were more negative towards current monoculture practices, but feel positive that IMTA would be successful. The second survey found that 65% of participants felt that IMTA had the potential to reduce the environmental impacts of salmon aquaculture, 100% felt it would improve waste management, over 90% believed it would benefit community economics and employment opportunities. All participants felt that seafood produced through IMTA techniques would be safe to eat and 50% were willing to pay 10% more for these products if labelled as such (Barrington *et al*. 2010 Barrington *et al*. 2009).

Culturing species from different trophic levels within the same system, in the right proportions, can help farmers achieve environmental sustainability through bio-mitigation of aquaculture wastes and can also provide the farmer with economic stability through product diversification and risk reduction. Essentially there is the potential to generate revenue from nutrients that would have otherwise been lost (Klinger and Naylor, 2012; Troell *et al*. 2009). Due to filter-feeding organisms' (e.g. bivalves) ability to consume or deactivate potential pathogenic microorganisms and parasites, their inclusion in an IMTA system provides the opportunity to decrease disease outbreaks and control human pathogens. Subsequently the need for antibiotics may be significantly reduced (Granada *et al*. 2016; Klinger and Naylor, 2012; Molloy *et al*. 2011; Barrington *et al*. 2009; Troell *et al*. 2009; Skar and Mortensen, 2007). It must be noted that there is the possibility that bivalves can act as a vector for fish pathogens, however, studies on this issue are limited (Granada *et al*. 2016; Pietrak *et al*. 2012). For larger parasites that may not be ingestible by filter-feeders, other species-integration solutions are available. For example, the use of ballen wrasse (*Labrus berggylta*) and lumpfish (*Cyclopterus lumpus*) for the delousing of cage cultured Atlantic salmon has been demonstrated as very effective (Imsland *et al*. 2014; Skiftesvik *et al*. 2013). As IMTA incorporates ecologically based management practices it has the potential to improve the social acceptability of aquaculture. There is a growing interest amongst consumers in sustainably produced seafood and they are willing to pay a premium for them, particularly if the packaging contains eco-labels. Also, if IMTA operators were to incorporate an eco-tourism venture into their farms, there is the opportunity to further the social acceptability of aquaculture, while also educating the community on food production techniques and ecological principles (Ma *et al*. 2013; Klinger and Naylor, 2012; Roheim *et al*. 2011; Culver and Castle, 2008). As IMTA systems involve a number of different species, farm operators will most likely

need to employ more staff due to the increased workload and need for personnel who are experienced with the cultivation of these additional species.

Unfortunately, there are constraints to the development of IMTA. The economic viability of offshore or on-land IMTA is uncertain. Although IMTA has the potential to provide economic stability through product diversification, the co-culturing of various species from different trophic levels is very complex and the development of a successful IMTA system that produces marketable and profitable biomass of additional crops might be a lengthy process, resulting in economic risk and uncertainty of production (Klinger and Naylor, 2012; Chopin, 2011; Troell *et al*. 2009). Some consumers might be reluctant to purchase seafood cultured in the waste-streams of finfish aquaculture. Therefore, marketing and educational initiatives may need to be developed in order to address or alleviate these concerns. Encouragingly, surveys conducted in the Bay of Fundy, Canada, found that the majority of the general public believes IMTA products are safe to eat (Chopin, 2011; Barrington *et al*. 2010; Bunting and Shpigel, 2009). Some IMTA systems include finfish or shrimp that require aquafeed. To make IMTA truly ecosystem-based, aquafeed producers need to reduce their dependence on fishmeal and fish oil, and consider alternative ingredients that can replace or reduce their need for forage fish (see Section 1.2 for list of alternatives) (Troell *et al*. 2014; Klinger and Naylor, 2012; Bendiksen *et al*. 2011; Naylor *et al*. 2009; Naylor *et al*. 2000). For at-sea IMTA, farmed fish escapes are still a concern. Solutions include the use of stronger net materials, tauter nets that deter sea-mammals (e.g. seals) from grabbing fish, and covers on boat propellers to avoid tears (Naylor *et al*. 2005). The most secure method, however, would be to isolate fish farms from the natural environment in land-based tanks or close-wall sea pens (Naylor *et al*. 2005; Naylor *et al*. 2003).

1.4.1 Halophyte Wetlands (On-Land)

Natural wetlands are an important part of marine, saline, and freshwater ecosystems; holding and recycling nutrients, controlling and buffering natural floods, and providing habitats and breeding and nursery grounds for many wildlife species. Additionally, wetlands can also efficiently remove organic matter, suspended solids and nutrients (N, C, P) through sedimentation, filtration, assimilation, and biological and microbiological absorption (Shpigel *et al*. 2013).

The use of man-made constructed wetlands (CWs) began in the 1970s as a means to provide a habitat for a variety of organisms and to improve water quality. Since then, CWs have been set up to provide flood control, to offset the decline in natural wetlands resulting from agriculture and urban development, to improve water quality, and for food production (Shpigel *et al*. 2013; Kadlec and Knight, 2009). In relation to aquaculture, CWs to date have been mainly used for the rearing of shrimp, crayfish, and commercial fish species and for the treatment of freshwater aquaculture effluent (Buhmann and Papenbrock, 2013a; Schulz *et al*. 2003; Lin *et al*. 2002a; Lin *et al*. 2002b; Tilley *et al*. 2002; Schwartz and Boyd, 1995). Two basic flow regimes have been devised for CWs, free surface flow (SF) and sub-surface flow (SSF). In a SF CW, the water flows above ground and plants are rooted in the sediment layer at the base of the basin or floating in the water. In this system, the water is exposed to the atmosphere and direct sunlight. A SSF CW, on the other hand, consists of a basin filled with an appropriate medium (e.g. coarse rock, gravel, sand, other soils) that is planted with wetland vegetation. A SSF CW is designed so that the water surface remains below the top surface of the medium, preventing odours and insect infestations. These systems are commonly utilised for secondary or tertiary treatment of wastewater (Kadlec and Knight, 2009; Schulz *et al*. 2003). The concept of applying CWs to mariculture systems for wastewater remediation is relatively new, however, a number of trials have already studied the utilisation of halophytes for aquaculture wastewater bioremediation in CWs. A halophyte is a naturally evolved salt-resistant plant that has adapted to grow in saline environments and in some cases they require this exposure to salinity to survive (Singh *et al*. 2014; Ramani *et al*. 2006). Operating halophytes as a plant biofilter of saltwater aquaculture effluent is a low cost opportunity to mitigate potential negative impacts on the environment (Buhmann *et al*. 2015). A recent study by Díaz *et al*. (2013) found that a number of halophytic species (*Salicornia bigelovii*, *Atriplex lentiformis*, *Distichlis spicata*, *Spartina gracilis*, *Allenrolfea occidentalis*, and *Bassia hyssopifolia*) grown under field conditions and irrigated with saline drainage water over a 4 to 6 year period in the San Joaquin Valley of California, grew very successfully and can effectively reduce the volume of saline drainage effluent due to the maintenance of very high levels of evapotranspiration (ET). Under frequent irrigation in drainage lysimeters, daily ET rates for the halophytes were 1.02-1.18 times higher compared to reference ET. Lymbery *et al*. (2006) constructed 16 2.5 m × 0.4 m × 0.3 m SSF wetlands incorporating the estuarine sedge, salt marsh rush (*Juncus kraussii*), and assessed its ability to treat

inland saline aquacultural wastewater. After a 38 day trial, it was found that this CW removed up to 88% of the total phosphorous load and 69% of the total nitrogen load. Although nutrient concentrations didn't have a significant effect on the growth of *J. kraussii* (i.e. plant length and frond number), it was found that higher salinities adversely impacted both growth parameters. Subsequently, Lymbery *et al*. (2006) suggested that *J. kraussii* would be more suited to salinities of up to 20,000 mg/L^{-1} and may not be effective in the treatment of inland, highly saline aquaculture waste, instead, being better suited to the treatment of waste from, for example, low salinity shrimp aquaculture. *J. kraussii* is commonly harvested in South Africa as a source of fibre for craft works and is of significant cultural importance to many Zulu households. For example, for the production of bridal sleeping mats no alternative wetland plant species is acceptable. *J. kraussii* is of significant economic importance to the region, with 97% of *J. kraussii* related income being generated through the sale of craft products and 3% through raw material sales (Traynor, 2008). Shpigel *et al*. (2013) demonstrated that a CW planted with *Salicornia persica* was effective in the removal of N, P, and total suspended solids (TSS) from a 1000 m^3 commercial, intensive, semi-recirculated aquaculture system growing 100 tons of gilt-head seabream (1 g–500 g in size). It was estimated that approximately 10,000 m^2 of wetland planted with *S. persica* would be required to remove nitrogen in wastewater during one year. This study also found that 10,000 m^2 of *S. persica* would be expected to produce an average yield of about 28.8 tons (FW) (2.88 $kg \cdot m^{-2} \cdot year^{-1}$). The upper (edible) part constitutes approximately 80% of the yield, therefore, the marketable yield would be about 23 tons of fresh produce. Both SF and SFF CWs were trialled in this study, and it was found that a SF regime with *S. persica* would likely be more efficient for facilities with low nutrient loads (NL) (e.g. fish hatcheries) and a SFF regime would be more efficient at high NL facilities (e.g. intensive fish farms). Although using CWs for effluent treatment requires a relatively extensive area, a cost-effectiveness analysis conducted by Cardoch *et al*. (2000) found that treatment by wetland costs approximately 75% less to the farmer than conventional on-site treatment. The use of a CW to treat aquaculture wastewater can be even more cost effective if the wetland is planted with a crop that has market demand or potential market demand (Shpigel *et al*. 2013). The commercial application cost of CWs is estimated to be €0.20 per kg of fish produced. Therefore, the cost of the construction and operation of a CW for, for example, 500 tons of fish would be €100,000. With a conservative price of €6 kg^{-1} (FW), the income from 23 tons of *S.*

persica is expected to be €138,000 based on gross calculations (Shpigel *et al*. 2013; Sindilariu *et al*. 2008). Marsh samphire (*S. europaea*) has also been shown to have significant potential in the treatment of aquaculture effluent. Webb *et al.* (2012) constructed a SFF wetland filter bed planted with marsh samphire to evaluate its ability to treat the wastewater from a commercially operated marine fish and shrimp farm. The results demonstrated the effectiveness of a marsh samphire wetland in removing N and P from the wastewater, with 91%–99% of influent dissolved inorganic nitrogen and 41%–88% of influent dissolved inorganic phosphorus removed. A number of species from the genus *Salicornia* have commercial application and potential in the areas of nutrition, medicine, forage crops, and oilseed production (Abdal, 2009; Rhee *et al*. 2009; Price, 2007; Lee *et al*. 2006; Liu *et al*. 2005; Glenn *et al*. 1998; Guil *et al*. 1997; Glenn *et al*. 1991). For example, *S. persica* and *S. europaea* contain compounds with antioxidative properties, such as polyphenols, superoxide dismutases, and peroxidases (Aghaleh *et al*. 2014).

Provided inexpensive land is available, the integration of CWs into on-land aquaculture can be very cost-effective as this only requires moderate capital investment and energy consumption and maintenance expenses are low (Sindilariu *et al*. 2009a; Sindilariu *et al*. 2009b; Lin *et al*. 2005). However, CWs require relatively extensive areas of land, and would not be suitable in locations where land prices are high. The cost of CW operations could, however, be offset by exploiting them as a natural park or tourist attraction (eco-tourism) (Shpigel *et al*. 2013; Sindilariu *et al*. 2008; Cardoch *et al*. 2000). As the maintenance of the CW is low, the farm may not need to employ many (or any) additional staff, however, the construction of the wetland and harvesting of the halophyte crops may provide additional, short-term employment.

1.4.2. Saltwater Aquaponics (On-Land)

For onshore saltwater aquaculture, an integrated solution to the potential negative impacts of aquaculture may lie in a novel concept known as saltwater aquaponics (SA). To be able to explain the concept of SA, we need to first discuss the freshwater origins of this seafood production technique; hydroponics and aquaponics.

Hydroponics

Hydroponics is the technique of growing plants in a nutrient solution (e.g. water containing fertilisers such as chemical salts) with or without the use of an

inorganic/inert (e.g. sand, gravel, coconut coir, perlite, clay balls) medium for mechanical support (Lakkireddy *et al*. 2012; Jones, 2005). When a hydroponic system contains no support medium, it is often referred to as a liquid (non-aggregate) hydroponic system. When a system does contain a medium, it is often referred to as an aggregate hydroponic system (Lakkireddy *et al*. 2012; Jones, 2005; Jensen, 1997). The concept of growing plants in nutrient rich water is centuries old. For example, the Babylonian hanging gardens and the floating gardens of the Aztecs in Mexico were hydroponic in nature (Jones, 2005; Steiner, 1985). The basic concept of hydroponics was established in the 1800s by investigators of plant growth (Lakkireddy *et al*. 2012; Jones, 2005). A number of publications by the Californian scientist, Gericke, popularised the soilless culture of plants in the 1930s (Gericke, 1940; Gericke, 1937; Gericke, 1929). However, it wasn't until the 1980s that hydroponics became a profitable commercial vegetable and flower production method (Jones, 2005). The operation of hydroponic systems in controlled facilities (e.g. greenhouses) was developed by the US army after World War II as an industrial approach to crop production intensification (Love *et al*. 2014; Jones, 2005). Virtually all hydroponic systems in temperate regions operate in greenhouses to: control temperature, reduce evaporative water loss, control diseases and pests, and protect against adverse weather conditions (e.g. wind and rain) (Jensen, 1997). Some common hydroponic systems are detailed below (for information on other hydroponic techniques, please refer to Jones, (2005)).

Deep Flow Technique

The deep flow technique (DFT) for growing leafy vegetables (e.g. heads of lettuce), was developed independently by Jensen, at the University of Arizona, USA, and Massantini, at the University of Pisa Italy, in 1976 (Jensen and Collins, 1985; Gericke, 1929). The production system consists of horizontal, rectangular-shaped tanks lined with plastic. The nutrient medium in the tanks is aerated and recirculated. It is monitored regularly and replenished when required. The plants are placed in floating rafts of expanded plastic (e.g. Styrofoam), which are spread in a single horizontal plane for maximum sunlight interception. The nutrient pools within the rectangular tanks act as a frictionless conveyor belt for planting and harvesting the movable floats. It is also relatively easy to control root temperature by heating or cooling the medium. For example, roots may need to be cooled in order to reduce bolting. This is especially

important if the production system is located in tropical or desert regions (Jensen, 2002; Jensen, 1997). However, one must factor in the costs associated with heating or cooling the medium.

Many of the results from trials establishing DFT were never reported. Nevertheless, this method of hydroponics is becoming increasingly popular due to the systems' ability to control temperature, maximise sunlight exposure, and ease of planting and harvesting (Jensen, 2002; Jensen, 1997). For example, in 2008, Hu *et al*. (2008) treated eutrophic water using *Ipomoea aquatica* Forsskal (swamp cabbage) in a DFT. After 48 h exposure to the plants, the chemical oxygen demand, biochemical oxygen demand, total suspended solids, and chlorophyll *a* were reduced in the effluent by 84.5%, 88.5%, 91.1% and 68.8%, respectively. The concentrations of cadmium, copper, lead, and zinc in the plants all fell within Food and Agriculture Organisation (FAO) and World Health Organisation (WHO) permissible levels. Hu *et al*. (2008) found that cultivating *I. aquatica* in nutrient-rich, eutrophic water, in a DFT system is an effective, low-cost phytoremediation technology to treat water and lower undesirable levels of phosphorus and/or nitrogen. Park and Kurata (2009) introduced a novel aeration technique, microbubbles, to a DFT system growing leaf lettuce (*Lactuca sativa*) and found that the fresh and dry weights of lettuce treated with microbubbles were, respectively, 2.1 times and 1.7 times higher than those of plants treated with standard, macrobubble aeration.

Nutrient Film Technique

The nutrient film technique (NFT) was developed by Dr. Allan Cooper in the late 1960s and refined throughout the 1970s and early 1980s, at the Glasshouse Crops Research Institute, Littlehampton, England. In an NFT system, the plant roots are suspended in a channel, trough, or gully (the term "channel" will be used for the remainder of this NFT section) through which a nutrient solution passes (Jones, 2005; Jensen, 1997; Graves, 1983). The channel containing the plant roots is usually set on a slope (approximately 1%) to allow the nutrient solution added at the top of the channel to flow from the top to the lower end by gravity at a flow rate of approximately 1 L per minute. One potential pitfall to the NFT method is that as the root mat increases in size, the plants at the beginning of the channel restrict the flow of nutrients to those at the further end of the channel. The flowing nutrient solution also tends to move over the top and down the outer edge of the root mat, reducing its contact within the root mass, resulting in poor mixing of the nutrient solution. One solution to these issues is to reduce the length of

the channel and make it wider to accommodate longer-term crops (Jones, 2005). A principle advantage of NFT over other hydroponic systems is that it requires much less nutrient solution. Subsequently, it is easier to heat the solution during winter months, to maintain optimal conditions for the roots, and to cool it during hot summers, particularly necessary for arid or tropical regions. The lower volume of water makes disease control more manageable (Jensen, 1997). Another advantage of NFT systems is the ease of establishment and relative low cost of construction materials (Jones, 2005). Detailed construction information for NFT systems can be found in literature produced by Morgan (1999) and Smith (2004). In most cases, a NFT system is a closed system; the nutrient solution that exits the channel is recovered for reuse. If the system is closed, there is a requirement for the addition of top-up water to replace water lost to evaporation and uptake by the plants and the need to establish procedures for filtering, sterilisation, and reconstitution of the pH and nutrient element content of the medium (Jones, 2005). In an open system, the nutrient solution exiting the channels is discarded, which is costly in terms of water usage, and requires careful disposal of nutrient-rich water (Jones, 2005; Johnson, 2002). Recently, the NFT system has been used for purposes other than the growth of vegetables. Ignatius *et al*. (2014) used an NFT system cultivating *Plectranthus amboinicus*, an aromatic medicinal plant, to treat lead contaminated wastewater. They found that *P. amboinicus* accumulated considerable amounts of lead in the roots and translocation to the leaves and stems was limited to the extent that they could still be used for medicinal purposes.

Aeroponics

In 1942, Carter designed a method of growing plants in water vapour to facilitate the examination of roots. This led to development of air culture growing (Carter, 1942). Today, aeroponics is defined as a technique in which the plant's roots are suspended in mid-air and water and essential nutrients are supplied by means of an aerosol. Mist produced by a water sprinkler bathes the roots, often without a supporting medium; however, the addition of an inorganic/organic medium can sometimes be beneficial (Lakkireddy *et al*. 2012; Jones, 2005; Christie and Nichols, 2004; Jensen, 1997; Barak *et al*. 1996; Nir, 1982).

Oxygen and water are quite often a limiting factor in conventional soil and water media systems, however, as nutrients and water are applied directly to the roots in an aeroponic system, they are in adequate supply (Nir, 1982). The plants are positioned in

the holes of a panel, with the roots suspended in mid-air beneath the panel and enclosed in a spray-box. This ensures that algal growth is prohibited and that the roots are in a humid environment (Jensen, 1997). Although Jensen (1997) suggested that the sprinkler system being turned on for a few seconds every 2–3 minutes is sufficient to keep the roots moist and the nutrient solution aerated, Jones (2005) suggested that continuous exposure of the roots to a fine mist gives better results than intermittent spraying or misting.

With an aeroponic system, the spray-box contains the mister or sprinkler and a reservoir of the nutrient medium. When the roots are long enough, a portion of the roots can gain access to this reservoir and therefore have a continuous supply of water (Steiner, 1985). Although the use of aeroponic techniques is not common for the commercial production of crops, it has considerable potential. As the plants can be cultivated very close to each other, this system is ideal for locations with extreme space and/or weight restrictions. The system is also ideal for locations where water is scarce and/or of poor quality, as aeroponic systems reuse the nutrient solution (the length of time that the nutrient solution can be reused will be dependent on a number of factors, such as: the quantity of nutrients present in the solution, the biomass and type of plants present, temperature) (Nir, 1982). Aeroponics also has potential in the rooting of foliage plant cuttings, as some exporting regulations require that the roots of cuttings be soil-free and the cuttings do not require overhead misting (Lakkireddy *et al*. 2012; Christie and Nichols, 2004). Aeroponics has also shown the ability to achieve higher yields than conventional production techniques and only requires minimal training for the growers already familiar with hydroponic methodologies (Movahedi *et al*. 2012; Nir, 1982).

Movahedi *et al*. (2012) conducted a study comparing aeroponic and conventional soil systems for potato mini-tuber production. The plantlets were grown in both aeroponic and conventional soil systems at a density of 100 plants per m^{-2}. It was found that growing the mini-tubers with an aeroponic system led to an increase in stem length, root length, stem diameter, and yield. The end product was also of better quality when grown in an aeroponic system. These systems can also be run on a continuous basis, apart from some downtime for cleaning or changing the plants (Nir, 1982). Aeroponics can be utilised for both crop production and plant research. For example, Christie and Nichols, (2004) from Massey University (New Zealand) have developed aeroponic systems for growing vegetable crops (e.g. tomatoes, cucumbers, potatoes, and herbs)

and flower crops (e.g. *Zantedeschia* and *Lisianthus*) and for researching crop nutrition, growth analysis, and the gas levels in the root zone.

Hydroponic systems have a number of advantages and disadvantages over traditional crop cultivation methods. Crops can be grown in areas where there is no soil or unsuitable soil (e.g. contaminated with a disease), the labour-intensity of traditional crop production methods (e.g. tilling and watering) is either greatly reduced or eliminated, water and nutrients are conserved, plant diseases are more easily eradicated in closed systems (most hydroponic systems are closed), there is better control over environmental conditions (e.g. root environment, nutrient feeding, irrigation), they are suitable systems for "at-home" vegetable production, and if run successfully, hydroponic systems can operate continuously at maximum yields, making the system attractive in high density and expensive land areas (Jones, 2005). However, hydroponics requires expensive nutrients to feed the plants, initial construction costs are high (even for closed systems), periodic flushing is required which may lead to waste disposal issues, there is a limited availability of plant varieties suitable for controlled growth conditions and more research and development is required. Since, plants react to suitable/unsuitable nutrient conditions quickly, hydroponic systems require constant and careful management, introduced diseases can spread more quickly in a closed system, and the technical aspect of the construction and operation of hydroponic systems requires highly trained staff (Blidariu and Grozea, 2011; Jones, 2005).

Aquaponics

Aquaponics is an on-land, freshwater IMTA system combining the aquacultural production of fish (e.g. fish, crayfish, molluscs, etc.) with the hydroponic production of plants (e.g. vegetables, herbs, fruits, medicinal plants, etc.). The waste produced by the fish provides the nutrients required for plant growth, while the plants remove compounds (e.g. nitrate and phosphorus) resulting from fish excretion, and which may be toxic to organisms such as fish and bivalves (Goddek *et al*. 2016; Shete *et al*. 2016; Love *et al*. 2015; Buzby and Lin, 2014; Love *et al*. 2014; Salam *et al*. 2014). In the majority of cases, aquaponic systems are closed, recirculating systems, which allows for micro-nutrients to be maintained at concentrations sufficient for hydroponic plant production (Tyson *et al*. 2011; Endut *et al*. 2009; Lennard and Leonard, 2006; Rakocy

et al. 2006; Seawright *et al* 1998). Like hydroponics, aquaponic operations commonly take place in a controlled environment (e.g. greenhouses) in an effort to increase crop production yields (Love *et* al. 2014). Aquaponics was also influenced by RAS work conducted in the early 1970s. A major challenge for RASs is the accumulation of nitrogen compounds, which are potentially toxic to fish. A number of investigators experimented with the soilless culture of plants as a fish waste treatment solution for the removal of nitrogen compounds, marking the beginning of aquaponics as we recognise it today (Sutton and Lewis, 1982; Lewis *et al*. 1978; Bohl, 1977; Naegel, 1977; Collins *et al*. 1975; Sneed *et al*. 1975). Since this research was conducted, engineers have developed biofilters that do not rely on plants, however, aquaponic systems improve water quality while producing an additional, potentially profitable crop, distinguishing it from other forms of RAS (Love *et al*. 2014; Timmons and Ebeling, 2010). The development of aquaponics was also influenced by research being conducted on sustainable agriculture (e.g. permaculture) in the 1970s and 1980s. Researchers at the New Alchemy Institute were applying permaculture methods to aquaculture and experimented with the integration of hydroponics and aquaculture (Love *et al*. 2014; Zweig, 1986; Todd, 1980).

Fish in aquaponic systems are usually raised in ponds, tanks, or other forms of containers, while the plants are grown separately in hydroponic tanks. The roots are either submerged in water or, in the case of an aeroponic-style system, exposed to a mist or sprinkling of water. The plants are suspended in gravel, sand, perlite, porous plastic films, or on floating rafts (see beginning of Section 1.4.2 for more detail on hydroponic plant production) (Klinger and Naylor, 2012; Rakocy *et al*. 2006).

All aquaponic systems share the same basic key functions: aquatic animal and plant production, bacterial nitrification (to convert toxic ammonia and nitrite to less toxic nitrate), and suspended solid removal (Diver and Rinehart, 2010). Suspended solids are removed from aquaponic systems in a similar manner to RASs, by passing the wastewater through mechanical filters or using settling ponds to settle the solids out of suspension. These systems can also use organic extractive species in combination with or as a replacement to mechanical methods. Again, like in RASs, ammonia is oxidised to nitrite, and then to nitrate by denitrifying bacteria (see Section 1.3 for more detail) (Rakocy *et al*. 2006). The nitrate and phosphorous rich water is transferred to the hydroponic tanks for absorption by the plants. This nutrient-reduced water is then re-used in the fish tanks/ponds. Due to aquaponics' ability to treat fish wastewater for re-

use in the system, aquaponic operations can achieve fish production densities similar to those achieved in RASs (Tyson *et al*. 2011; Graber and Junge, 2009).

The nutrient removal and water re-use ability fluctuates amongst different aquaponic systems due to a number of variables such as flow rates, the type of plant used, the medium (or lack of) used to grow the plant, and the ratio of plants to fish (Endut *et al*. 2010; Graber and Junge, 2009; Lennard and Leonard, 2006). For example, nitrate and phosphorus removal rates range from 9%-93% to 0%-53%, respectively, while water re-use can reach over 98% (Diver and Rinehart, 2010; Endut *et al*. 2010; Rupasinghe and Kennedy, 2010; Graber and Junge, 2009; Al-Hafedh *et al*. 2008; Lennard and Leonard, 2006). Al-Hafedh *et al*. (2008) compared their recirculating aquaponic system to semi-intensive aquaculture in Saudi Arabia, and found that their system recycled more than 98% of its water and produced more than 40 kg fish/m^3 of water every 6 months, whereas the semi-intensive system exchanged 20%-30% of its water daily and only produced 8-15 kg fish/m^3 over a 6 month period. The most common species of fish currently used in aquaponics include tilapia, perch, carp, barramundi, cod, and trout (Tyson *et al*. 2011). Research has found that plants with low nutrient requirements (e.g. lettuce, herbs, spinach, watercress) perform better in aquaponic systems than more nutrient demanding species (e.g. cauliflower, tomatoes). Lettuce co-cultured with tilapia is the most common aquaponic pairing. The relative proportions of soluble nutrients that the hydroponically grown plants are able to obtain from the fish waste does not mirror the proportion of nutrients normally assimilated by plants growing in a normal manner. A solution to this issue would be to manipulate the nutrient content of the fish diet in such a way that the relative proportions of nutrients excreted by the fish are more similar to the relative proportion of nutrients assimilated by plants, while maintaining optimal nutrition for the fish (Endut *et al*. 2010; Seawright *et al*. 1998). Another option, which is commonly practiced, is to top-up the water supplying the hydroponic plants with nutrients that are in limited supply or are not present in the wastewater (UHAWD, 2016; Somerville *et al*. 2014). Another challenge with an aquaponic system is the dichotomy that exists between the optimum pH for plant nutrient availability in hydroponics (pH 5.5–6.5) and the optimum for nitrifying bacteria in biofilters (pH 7.5–9.0). The recommended pH range for the nutrient solution irrigation water in hydroponics tends to be slightly acidic to avoid precipitation of Fe, Mn, P, Ca, and Mg to insoluble and unavailable salts which occurs when the pH is >7. If aquaponic recirculating water pH is maintained at levels more optimum for nitrifying

bacteria, plant uptake of certain nutrients may become restricted, reducing plant yield (Tyson *et al*. 2011). However, work conducted by Tyson *et al*. (2008a; 2008b) suggests that total yields may be maintained at pH levels above those recommended for the production of plants, when the nutrients constantly bathe the roots.

Saltwater Aquaponics

Although freshwater aquaponics is the most widely described and practiced aquaponic technique, resources of freshwater for food production (agriculture and aquaculture) are becoming increasingly limited and soil salinity is progressively increasing in many parts of the world (FAO, 2016; Fronte *et al*. 2016; Turcios and Papenbrock, 2014; Singh *et al*. 2014; Ventura and Sagi, 2013). This has led to an increased interest and/or move towards alternative water sources (e.g. brackish to highly saline water) and the use of euryhaline or saltwater fish, halophytic plants, seaweed, and low salt tolerant glycophytes (Joesting *et al*. 2016; Nozzi *et al*. 2016; Buhmann and Papenbrock, 2013b; Neori *et al*. 2004; Troell *et al*. 2003; Dufault *et al*. 2001; Dufault and Korkmaz, 2000). Saltwater aquaponics (SA) is an on-land IMTA system combining the aquacultural production of fish (e.g. fish, crustaceans, molluscs, etc.) with the hydroponic production of salt tolerant/resistant or saline plants (e.g. seaweeds, halophytes, salt-tolerant glycophytes etc.) in a range of salinities from low (e.g. brackish water) to high (e.g. seawater) (Fronte *et al*. 2016; Boxman *et al*. 2016; Boxman *et al*. 2015; Waller *et al*. 2015; Pantanella, 2012; Wilson, 2005). The term maraponics (i.e. marine aquaponics) has also been coined for SA systems that utilise seawater. These are mainly located on-land, in coastal locations close to a seawater source (Boxman *et al*. 2016; Fronte *et al*. 2016).

As can be seen in Section 1.4.1, a number of CW studies have shown that halophytes can be successfully irrigated with saline aquacultural wastewater (Díaz *et al*. 2013; Shpigel *et al*. 2013; Webb *et al*. 2012; Sindilariu *et al*. 2008; Lymbery *et al*. 2006; Cardoch *et al*. 2000). The concept of growing halophytes using hydroponic techniques or as part of a SA system is very new. Waller *et al*. (2015) investigated the feasibility of nutrient recycling from a saltwater (16 ppt salinity) RAS for European sea bass (*D. labrax*) through the hydroponic production of three halophyte plants; *Tripolium pannonicum*, *Plantago coronopus*, and *Salicornia dolichostachya*. The hydroponic setup consisted of hydroponic tanks being fed RAS process water at a flow rate of 0.15 $m^3 \cdot h^{-1}$ from 8 am to 8 pm (1.8 $m^3 \cdot day^{-1}$). This flow rate is significantly less than the

flow that would occur through a nitrifying biofilter (15 $m^3 \cdot h^{-1}$ 24 h a day or 360 $m^3 \cdot day^{-1}$). Each plant species grew at a similar specific growth rate (SGR) of 9–9.9 day^{-1}. It was believed that high air temperatures in the greenhouse at the beginning of the experiment may have limited the growth of *P. coronopus* plants. The total production of plant material over the course of the experiment amounted to 6 kg, 4 kg, and 13 kg for *T. pannonicum*, *P. coronopus*, and *S. dolichostachya*, respectively. The plants incorporated a total of 46 g N and 7 g P during the 35 day trial, equivalent to 9% N and 10% P that was introduced with the fish feed. For this system, it was estimated that 189 g of N resulted from fish excretion and if only the best performing halophyte (*S. dolichostachya*) was included, 1128 plants would be needed in a 14.4 m^2 hydroponic area to remove all of this excreted N. During the 35-day trial, the sea bass grew from 32 g to 54 g on average, at a SGR of 1.5 day^{-1} and exhibited an FCR of 0.93. The edible part of the harvested plant material was tested and found to be microbial safe and approved for human consumption. Boxman *et al*. (2016) evaluated the capacity for water treatment and production requirements of two halophytes, sea purslane (*Sesuvium portulacastrum*) and saltwort (*Batis maritima*), when grown in an indoor, bench-scale recirculatory SA system with platy fish (*Xiphophorus* sp.). Two thirty-day trials were carried out at a minimum to maximum salinity of 13.1ppt to 17.1ppt. The first trial assessed nitrate removal rates with either the sea purslane present or absent, and with two different medium types, coconut fibre and expanded clay. Boxman *et al*. (2016) found that the presence of plants significantly contributed to nitrate removal, such that mean nitrate concentrations were 10.1 ± 5.4 mg/L in planted treatments in comparison to 12.1 ± 6.1 mg/L in the unplanted treatments ($p<0.05$). The use of coconut fibre as a medium for the plants resulted in a significantly lower mean level of nitrate in the water (9.78 ± 5.4 mg/L) in comparison to when expanded clay was used (12.4 ± 6 mg/L). Studies utilising scanning electron microscopy have shown that coconut fibre has a high porosity, which corresponds with attachment surfaces for microbial populations (Fornes *et al*. 2003). In addition to surface area, coconut fibre can leach carbonaceous chemical oxygen demand (COD) and provide an organic carbon source for denitrifying bacteria (Weragoda *et al.* 2010). The added COD is important in dilute aquaculture wastewater in which denitrification can be limited by lack of an organic carbon source. Manoj and Vasudevan (2012) treated aquaculture wastewater with coconut coir in a packed column bioreactor and found that it successfully removed nitrate and COD through denitrification. The second trial assessed the impact of flow rate, plant species,

and plant density on nitrogen uptake from the fish tank water. The nitrogen uptake rate was monitored for both sea purslane and saltwort (separately) under the following treatments: high flow rate (1 $L \cdot min^{-1}$) and high density (24 plants/m^2); high flow rate and low density (12 plants/m^2); low flow rate (0.5 $L \cdot min^{-1}$) and high density; low flow rate and low density. It was found that the low flow rate/low density treatment with saltwort had the greatest nitrogen removal rate, ranging from 25% to 172%. However, the mean yield of 0.53 ± 0.09 $kg \cdot m^{-2}$ and 0.32 ± 0.06 $kg \cdot m^{-2}$ for sea purslane and saltwort, respectively, were low and further research into the use of these species in bench-scale units is required. Kong and Zheng (2008) successfully grew *Salicornia bigelovii* hydroponically (in Styrofoam disks floating on nutrient solution) and found that a marketable yield of 1.69 ± 0.21 $kg \cdot m^{-2}$ achieved when grown at high salinities (12 ppt) was significantly higher than the yield achieved at moderate salinities (0.36, 0.48 and 0.6 ppt). Work conducted by Buhmann *et al*. (2015) on the use of halophytes (9 different species) as a biofilter for nutrient-rich saline water found that the use of a hydroponic culture system is more suitable than sand or clay culture if controlled conditions and nutrient cycling are desired. After a 5 week trial, it was shown that at least 10 $mg \cdot L^{-1}$ of nitrate was necessary for reasonable biomass production and 0.3 $mg \cdot L^{-1}$ of phosphate is sufficient, but higher concentration promote the uptake of phosphate. Buhmann *et al*. (2015) found that all tested species have the potential to serve as a biofilter, are a source of valuable co-product, and have potential for integration into a SA systems (species studied in this trial were: *T. pannonicum*; *Atriplex portulacoides*; *S. dolichostachya*; *Plantago coronopus*; *Lepidium latifolium*; and *A. halimus*). As many halophytes have reduced levels of growth at higher salinities, the integration of cultivation of algae into SA is a potential solution for systems that are using seawater levels of salinity (i.e. c. 35 ppt) (Lymbery *et al*. 2006; Wilson, 2005; Reimold and Queen, 1974).

The concept of "saltwater aquaponics (SA)" is very new, an interest in on-land seaweed-based integrated mariculture began to appear in the 1970s, starting from the laboratory-scale and then expanding to outdoor pilot-scale trials. In some of the earliest quantitative studies, Haines (1976) and Langton et al. (1977) studied the growth of the red seaweed, *Hypnea musciformis*, cultured in tanks with shellfish culture effluent. Haines (1976) found that *H. musciformis* grown with the effluent from clam mariculture grew approximately five times faster than growth in unaltered deep water and about three times faster than in surface water. Langton et al. (1977) also grew *H. musciformis*

with clam wastewater and found that it had an ammonia-N uptake rate of up to 70% over a 24 hour period. From the 1980s, the number of studies reporting on the use of algae for integration into on-land aquaculture increased, with *Ulva* spp. and *Gracilaria* spp. being the most frequently studied species. Troell *et al*. (2003), Neori et al. (2004), and Granada et al. (2015) have a comprehensive list of references for these studies, a few examples of which will be discussed below.

Vandermeulen and Gordin (1990) found that *Ulva lactuca* cultured on intensive fishpond wastewater grew very strongly, with a growth rate of over 55 g dry weight (DW)/day^{-1} per 600 L and efficiently removed up to 85% of the ammonium from the wastewater over a 13 day period. Neori *et al*. (1991) cultured *U. lactuca* in effluent from intensive fishponds and found that the specific growth rate and yield were higher for *U. lactuca* grown on enriched fresh seawater. Under wastewater culture conditions, the maximum yield (DW) achieved was 55 g·m^{-2}·day^{-1} and maximum specific growth rate was 18% d^{-1}. Yields achieved through wastewater cultivation were up to 38% higher compared to those on enriched fresh seawater. Through conducting this research, Neori *et al*. (1991) suggested that, for high yield and nitrogen content, *U. lactuca* should be kept at a density of 1–2 kg·m^{-2} and at ammonia fluxes of approximately 0.5 moles·m^{-2}·d^{-1}. Jimenez del Río *et al*. (1996) cultivated *Ulva rigida* in 750 L tanks being fed wastewater from a commercial marine fishpond rearing 40 metric tonnes (Mt) of Gilt-head bream (*Sparus aurata*). The authors determined that maximum yields of *U. rigida* (40g DW·m^{-2}·day^{-1}) were obtained at a seaweed density of 250 g·FW·m^{-2} and a dissolved inorganic nitrogen (DIN) inflow rate of 1.77 g·DIN·m^{-2}. The average annual DIN removal efficiency under these parameters was 2 g DIN·m^{-2}·d^{-1} and it was calculated that 153 m^2 of *U. rigida* tank surface would be needed to recover 100% of the DIN produced by 1 Mt of fish. Buschmann *et al*. (1996) cultivated *Gracilaria chilensis* in four 2500 L raceways that received wastewater from the tank cultivation of coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*O. mykiss*). At its highest, *G. chilensis* production can reach up to 48.9 kg·m^{-2}·year^{-1} and can remove 50% of dissolved ammonium in winter, increasing to 90%–95% in spring. Buschmann *et al*. (1996) also performed an income-analysis model and calculated that the harvesting of *G. chilensis* can provide additional total revenue of over $60,000, representing approximately 10% of the total income. Chow *et al.* (2001) utilised *G. chilensis* as a biofilter in the depuration of effluents from tank cultures of Cabinza grunt (*Isacia conceptionis*), oysters (*Crassostrea gigas*), and sea urchins (*Loxechinus albus*) and

compared its productivity and relative growth rate (RGR) to *G. chilensis* cultivated with seawater. *G. chilensis* was cultivated in 200 L tanks (0.5 m^2 surface area). It was found that productivity was highest in the *G. chilensis* tanks fed with the fish effluent, with a growth rate (FW) of 51.2 $g \cdot m^{-2} \cdot day^{-1}$, in comparison to 23.9, 16.2, and 18.6 $g \cdot m^{-2} \cdot day^{-1}$ for *G. chilensis* tanks fed with oyster effluent, urchin effluent, and control seawater, respectively. Abreu *et al.* (2011) established 12, 1200L (total footprint of 18 m^2) *G. vermiculophylla* tanks at a commercial, land-based intensive aquaculture farm producing 40 tonnes of turbot (*Scophthalmus rhombus*), 5 tons of sea bass (*D. labrax*), and 500,000 Senegalese sole juveniles (*Solea sengalensis*). *G.vermiculophylla* grew best at a stocking density of 3 $kg \cdot WW \cdot m^{-2}$ and water exchange rate of 200 $L \cdot h^{-1}$, producing 0.7 ± 0.05 $kg \cdot DW \cdot m^{-2} \cdot month^{-1}$, while removing 40.54 ± 2.02 $g \cdot m^{-2} \cdot month^{-1}$ of N. They calculated that in one year, this system could produce approximately 156 kg (DW) of seaweed and this biomass level would remove 8.8 kg of N. To attain 100% N removal efficacy, it was calculated that the tank area would need to be increased to 0.36 ha, considering the cultivation conditions are kept the same (i.e. stocking density of 3 $kg \cdot WW \cdot m^{-2}$, 1200 L tanks with a footprint of 1.5 m^{-2}, and a water exchange rate of 200 $L \cdot h^{-1}$). As can be seen from some of the above studies, seaweeds not only grow well when cultivated with effluent water from mariculture, but can grow better than seaweed cultivated with seawater or fertiliser-enriched seawater.

Alternatively, crops that would usually be classed as glycophytes, such as the common tomato (*Lycopersycon esculentum*), the cherry tomato (*Lycopersycon esculentum* var. *Cerasiformee*), and basil (*Ocimum basilicum*) can achieve remarkably successful production levels at up to 4 ppt salinity and are often referred to as having low-moderate levels of salt tolerance (not to be confused with halophytes, which are resistant of high salinities). Other crops that are tolerant of low-moderate salinities include: turnip, radish, lettuce, sweet potato, broad bean, corn, cabbage, spinach, asparagus, beets, squash, broccoli, and cucumber (Fronte *et al*. 2016; Pantanella and Bhujel, 2015; Dufault *et al*. 2001; Dufault and Korkmaz, 2000). Dufault *et al*. (2001) and Dufault and Korkmaz (2000) experimented with shrimp biosolids (SB) (shrimp faecal matter and decomposed feed) as a fertiliser for broccoli (*Brassica oleracea italica*) and bell pepper (*Capsicum annuum*) production, respectively. In both trials, they fertilised the crops with just SB, combined with Oscomote fertiliser (OSM), and just OSM and found that SB does not maximise yields when used alone. For the broccoli trial, the culture system that enhanced yield combined nine MT SB/Ha with 75 kg OSM/Ha, delivering a

combined total of 263, 116, 99, and 99 kg/Ha of N, P, K, and Na, respectively. For the bell pepper trial, the culture system that enhanced yield included the highest rates of both SB and OSM, which delivered a total of 633, 253, and 303 kg/Ha of N, P, and K, respectively. In both trials, however, it was noted that SB contains a high level of sodium and an increase in soil salt concentration could suppress the growth of some crops, especially those that are salt sensitive (e.g. carrots, strawberries, and onions). For this reason, Dufault and Korkmaz (2000) recommend a number of cultural steps when using SB, to reduce the risk of salinity damage. They advise to modify the salinity of the SB by: dilution, blending with other organic matter, leaching SB with irrigation water, or by using SB in soils with high buffering capacity (Dufault *et al*. 2001; Dufault and Korkmaz, 2000). Although the above studies did not use SA techniques, they involved plants that are commonly grown using aquaponic (freshwater) techniques. Therefore, due to their salinity tolerance levels, they have enormous potential as candidate species for use in SA systems using low to medium salinities.

A majority of the SA work conducted so far involves the integration of two trophic levels. An example of a SA system incorporating more than two trophic levels can be seen in an experiment conducted by Neori *et al*. (2000), who designed a 3.3 m^2 system for the intensive land-based culture of Japanese abalone (*Haliotis discus hannai*), seaweeds (*Ulva lactuca* & *Gracilaria conferta*), and pellet-fed Gilt-head bream (*Sparus aurata*). The system design consisted of unfiltered seawater (2400 $L \cdot day^{-1}$) pumped to two abalone tanks, drained through a fish tank, and finally through a seaweed filtration/production unit before being discharged to the sea. The abalone unit consists of two 120 L rectangular bottom drained tanks, which were elevated to allow effluents to drain into the fish tank. A removable screen (1 cm mesh) covered the whole area 10cm above the flat bottom, to retain the abalone while allowing faeces and detritus to drain. The first tank was stocked with 1200 juveniles (mean: 0.23 ± 0.04 g) and the second tank had 251 adults (mean: 15.7 ± 4.6 g). Three hundred sea bream with an average weight of 40 g were stocked in a 600 L (1 m^2 surface area) rectangular aerated tank and fed a 45% protein pellet diet. Stocking density was maintained below 15 $kg \cdot m^3$. *U. lactuca* and *G. conferta* were grown in two 600 L (1 m^2 surface area) tanks. The algae were suspended in the water column by air diffusers situated at the bottom. Total seaweed biomass was kept at approximately 1.5 kg/600L of *U. lactuca* and 5–13 kg/600L of *G. conferta* (excess seaweed was harvested twice a week and fed to the abalone). The fish grew at 0.67% day^{-1}, yielding 28 $kg \cdot m^{-2} \cdot year^{-1}$. The nutrients

excreted by the fish supported high yields of *U. lactuca* (78 $kg \cdot m^{-2} \cdot year^{-1}$) and efficient ammonia filtration (80%), however, *G. conferta* grew poorly. The *Ulva* supported an abalone growth rate of 0.9% day^{-1} and a length increase of 40–66 $\mu m \cdot day^{-1}$ in juveniles and 0.34% day^{-1} and 59 $\mu m \cdot day^{-1}$ in young adults. The total abalone yield was 9.4 $kg \cdot year^{-1}$. Ammonia as a fraction of total feed-N was reduced from 45% in the fish effluents to 10% in the post-seaweed discharge. A surplus of seaweed was created in the system and based on this trials results, a doubling of the abalone:fish ratio form 0.3 to 0.6 is feasible.

SA offers a number of advantages over traditional crop and fish production methods. As SA systems use saline water (brackish to saline) there is a reduced dependence on freshwater, which has become a very limited resource. It is typically practiced in a controlled environment (e.g. a greenhouse; controlled flow-rate tanks) giving a better opportunity for intensive production. Many SA systems are closed RASs with organic and/or mechanical biofilters, subsequently, water reuse is high, wastewater pollution is vastly reduced or eliminated, and contaminants are removed or treated. SA systems that are not RASs significantly reduce the excess nutrients in the wastewater prior to discharge. Also, the occurrence of contaminants in non-RAS SA systems can be reduced or eliminated through the use of water containing low levels of naturally occurring contaminants and the use of alternatives aquafeeds that do not contain dioxins or PCDs (e.g. novel feeds made from macroalgae). This improvement in water quality reduces the potential for disease occurrence and the need for antibiotic use is therefore vastly reduced (Fronte *et al*. 2016; Boxman *et al*. 2015; Klinger and Naylor, 2012; Cole *et al*. 2009; Wilson, 2005; Neori *et al*. 2000; Pantanella and Colla, 2013). Due to SA's versatile configuration and low water requirements, it can be successfully implemented in a wide-variety of settings, from fertile coastal areas to arid deserts, as well as in urban or peri-urban settlements (Pantanella, 2012). Another potential benefit of SA is that many of the species that are suitable for these systems, have a high commercial value. For example, the euryhaline European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) can fetch a market price of €9/kg and €6/kg, respectively. Additionally, edible halophytes tend to have a high market price; with sea-agretto (*Salsola soda*), for example, having a market price of €4/kg–€4.5/kg (Fronte *et al*. 2016; Pantanella, 2012). SA is a dynamic and rapidly growing field that has the potential to provide a number of services to communities. Love *et al*. (2014) conducted an international survey of aquaponic practitioners and found that most were hobbyists,

however, a significant proportion of respondents were educators, non-profit organisations (NGOs), and commercial producers. The main reasons cited for being involved in aquaponics were to grow their own food, to advance environmental sustainability, and improve personal health. As SA shares many of the principles and methodologies of aquaponics, it also has many potential applications for local communities (Love *et al*. 2014). For example, due to the interdisciplinary nature and technological skills required to set-up and run a SA system, they are ideal systems for use as an educational tool. Aquaponic systems, for example, are already in a number of schools across America, allowing students to conduct activities involving chemistry, physics, biology, and sustainability. Also, small to medium scale systems require very little space and can be located in schoolyards, basements, balcony spaces, classrooms, rooftops etc. SA systems could be utilised in the same manner to teach students about these aspects, from a marine/saltwater biology perspective (Hart *et al*. 2013). The ideal pH range for the growth of saltwater fish, halophytes, and saline nitrifying bacteria is approximately 7.5–8.5. Therefore, the issue of a dichotomy between the optimum pH for plant nutrient availability and for nitrifying bacteria that occurs in aquaponics should not be an issue for SA, apart from, perhaps, when salt-tolerant glycophytes are chosen as the plant component (Bioconlabs, 2017; Reimold and Queen, 1974).

Despite these benefits, there are a number of constraints. The hydroponic aspect of SA systems in particular can require a relatively large area of land. For example, Rakocy *et al*. (2006) estimated that, on average, a square meter of plant growth area is required to treat the water for every 60–100 g of fish feed used. There is an increased risk of cross contamination of pathogens (e.g. of the bacteria *Salmonella* and *Escherichia coli*) when growing animals (e.g. fish) near plant produce. However, a number of steps can be taken to prevent any food-safety risks associated with the SA production of food products (refer to Hollyer *et* al. (2009) for more information on on-site freshwater and saltwater aquaponic food safety procedures) (Klinger and Naylor, 2012; Hollyer *et al*. 2009). Due to the novelty of this concept, consumers may be wary of consuming plant produce that was grown with water containing fish faeces. Educational initiatives and careful marketing may help alleviate these concerns (e.g. the use of manure is a common practice in the production of meat and vegetables). Also, if SA develops a strong community-based interest similar to aquaponics, this concern may be reduced further (Love *et al*. 2014; Klinger and Naylor, 2012). As SA is a relatively new concept, there is a lack of large-scale models to base designs off and a lack of trained or

experienced personnel capable of commercial SA management. The development of SA has also been constrained by limited land-based production of saltwater fish species and a limited selection of appropriate edible species that grow in saltwater. Further research is required to identify compatible species of fish and aquatic plants that will thrive in an on-land SA system (Klinger and Naylor, 2012; Boxman *et al*. 2015).

1.5 Conclusions

Integrated multi-trophic aquaculture as a concept is still in its infancy and a large amount of research and development is still required to identify a suitable combination of species, in the correct proportions, that will operate effectively on a site-specific basis. Nevertheless, rethinking aquaculture production with an integrated mind-set is needed to tackle the simultaneous challenges of feed and energy demands, containment of wastes, control of pathogens and disease, escaped fish, land and water requirements, and consumers' increasing preference for sustainably produced food products. Also, as profit margins in aquaculture continue to become smaller, the attractiveness of using wastes as inputs to other profitable crops will continue to grow, as long as food safety issues and the public perception of food produced with water containing fish faeces is effectively dealt with.

1.6 Aims of this thesis

The overall aim of this thesis is to investigate novel concepts and techniques that have the potential to contribute to the improved sustainability of aquaculture.

This overall aim will by achieved by the following objectives:

(1) To assess the biofiltering capacity of *Salicornia europaea* cultivated via aeroponics. Untreated saline aquaculture effluent resulting from current aquaculture practises has the potential to negatively affect the environment, tourism, and other fisheries, therefore effective use of *S. europaea* to filter waste streams could have great utility here.

(2) To assess the stratification of *S. europaea* seeds through the assessment of various methodologies (i.e. various salinities, nutrient addition, and seed sterilisation).

(3) To evaluate the effectiveness of anaesthetising the holothurian, *Holothuria forskali*, and its impact on size measurement variation.

(4) To evaluate the efficacy of PIT tagging of *Holothuria forskali*.

(5) To assess the efficacy of a maraponic system and the utility of fatty acid (FA) analysis to assess the impact of the trial on the FA composition of all species.
(6) To conduct a case study on the viability of establishing IMTA at an existing monocultural aquaculture operation at Bantry Marine Research Station in South-western Ireland.

1.7 References

Abdal, M.S. (2009). *Salicornia* production in Kuwait. *World Applied Sciences Journal,* 6, pp. 1033–1038.

Abreu, M.H., Pereira, R., Yarish, C., Alejandro, H., Buschmann, A.H., and Sousa-Pinto, I. (2011). IMTA with *Gracilaria vermiculophylla*: Productivity and nutrient removal performance of the seaweed in a land-based pilot scale system. *Aquaculture*, 312, pp. 77–87.

Abreu, M.H., Varela, D.A., Henríquez, L., Villarroel, A., Yarish, C., Sousa-Pinto, I., and Buschmann, A.H. (2009). Traditional vs. integrated multi-trophic aquaculture of *Gracilaria chilensis* C.J. Bird, J. McLachlan & E.C. Oliveira: Productivity and physiological performance. *Aquaculture,* 293, pp. 211–220.

Aghaleh, M., Niknam, V., Ebrahimzadeh, H., and Razavi, K. (2014). Antioxidative enzymes in two in vitro cultured *Salicornia* species in response to increasing salinity. *Biologia Plantarum,* 58, pp. 391–394.

Alder, J., Campbell, B., Karpouzi, V., Kaschner, K., and Pauly, D. (2008). Forage fish: From ecosystems to markets. *Annual Review of Environment and Resources,* 33, pp. 153–166.

Al-Hafedh, Y.S., Alam, A., and Beltagi, M.S. (2008). Food production and water conservation in a recirculating aquaponic system in Saudi Arabia at different ratios of fish feed to plants. *Journal of the World Aquaculture Society*, 39, pp. 510–520.

Arthur, R.I., Lorenzen, K., Homekingkeo, P., Sidavong, K., Sengvilaikham, B., and Garaway, C.J. (2010). Assessing impacts of introduced aquaculture species on native fish communities: Nile tilapia and major carps in SE Asian freshwaters. *Aquaculture*, 299, pp. 81–88.

Avnimelech, Y. (1999). Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture*, 176, pp. 227–235.

Axelad, D.A., Bellinger, D.C., Ryan, L.M., and Woodruff, T.J. (2007). Dose-response relationship of prenatal mercury exposure and IQ: An integrative analysis of epidemiologic data. *Environmental Health Perspectives,* 115, pp. 609–615.

Ayer, N.W., and Tyedmers, P.H. (2009). Assessing alternative aquaculture technologies: Life cycle assessment of salmonid culture systems in Canada. *Journal of Cleaner Production*, 17, pp. 362–373.

Azim, M.E., Verdegem, M.C.J., van Dam, A.A., and Beveridge, M.C.M. (2006). *Periphyton: Ecology, Exploitation, and Management*; CABI: Oxfordshire, UK, 319p.

Badiola, M., Mendiola, D., and Bostock, J. (2012). Recirculating aquaculture systems (RAS) analysis: Main issues on management and future challenges. *Aquacultural Engineering,* 51, pp. 26–35.

Bailey, C., Jentoft, S., and Sinclair, P. (1996). *Aquacultural Development: Social Dimensions of an Emerging Industry*; Westview Press: Boulder, CO, USA, p. 300.

Bailey, C., Jentoft, S., and Sinclair, P. (1996). *Aquacultural Development: Social Dimensions of an Emerging Industry*; Westview Press: Boulder, CO, USA, p. 300.

Bailey, C.M. (1988). The social consequences of tropical shrimp Mariculture development. *Ocean and Shoreline Management,* 11, pp. 31–44.

Barak, P., Smith, J.D., Kreuger, A.R., and Peterson, L.A. (1996). Measurement of short-term nutrient uptake rates in cranberry by aeroponics. *Plant, Cell & Environment*, 19, pp. 237–242.

Barrington, K., Ridler, N., Chopin, T., Robinson, S., and Robinson, B. (2010). Social aspects of the sustainability of integrated multi-trophic aquaculture. *Aquaculture International*, 18, pp. 201–211.

Barrington, K., Chopin, T., and Robinson, S. (2009). Integrated multi-trophic aquaculture (IMTA) in marine temperate waters. In *Integrated Mariculture: A Global Review*; Soto, D., Ed.; FAO Fisheries and Aquaculture Technical Paper 529; FAO: Rome, Italy, pp. 7–46.

Bell, J.D., Bartley, D.M., Lorenzen, K., and Loneragan, N.R. (2006). Restocking and stock enhancement of coastal fisheries: Potential, problems and progress. *Fisheries Research*, 80, pp. 1–8.

Bendiksen, E.Å., Johnsen, C.A., Olsen, H.J., and Jobling, M. (2011). Sustainable aquafeeds: Progress towards reduced reliance upon marine ingredients in diets for farmed Atlantic salmon (*Salmo salar* L.). *Aquaculture,* 314, pp. 132–139.

Biocon Labs (2017). Nitrifying Bacteria Facts. Available online: http://www.bioconlabs.com/nitribactfacts.html (accessed on 1st May 2017).

Blanco, S.L., Sobrado, C., Quintela, C., Cabaleiro, S., Gonzalez, J.C., and Vietites, J.M. (2007). Dietary uptake of dioxin (PCDD/PCDFs) and dioxin-like PCBs in Spanish aquacultured turbot (*Psetta maxima*). *Food Additives & Contaminants*, 24, pp. 421–428.

Blidariu, F., and Grozea, A. (2011). Increasing the economical efficiency and sustainability of indoor fish farming by means of aquaponics - Review. *Scientific Papers Animal Science and Biotechnology,* 44(2), pp. 1–8.

Bohl, M. (1977). Some initial aquaculture experiments in recirculating water systems. *Aquaculture*, 11, pp. 323–328.

Boissy, J., Aubin, J., Abdeljalil, D., van der Werf, H.M.G., Bell, G.J., and Kaushik, S.J. (2011). Environmental impacts of plant-based salmonid diets at feed and farm scales. *Aquaculture*, 321, pp. 61–71.

Bordbar, S., Anwar, F., and Saari, N. (2011). High-value components and bioactives from sea cucumbers for functional foods - A review. *Marine Drugs*, 9, pp. 1761–1805.

Boxman, S.E., Nystrom, M., Capodice, J.C., Ergas, S.J., Main, K.L., and Trotz, M.A. (2016). Effect of support medium, hydraulic loading rate and plant density on water quality and growth of halophytes in marine aquaponic systems. *Aquaculture Research,* 48(5), pp. 2463-2477.

Boxman, S., Main, K., Nystrom, M., Ergas, S.J., and Trotz, M.A. (2015). *Aquaponic System Produces Red Drum, Saltwater Vegetable Species*; Global Aquaculture Advocate: Portsmouth, New Hampshire, USA, pp. 58–60.

Boyd, C.E., and Gross, A. (2000). Water use and conservation for inland aquaculture ponds. *Fisheries Management and Ecology*, 7, pp. 55–63.

Brand, L.E., Sunda, W.G., and Guillard, R.R.L. (1986) Reduction of marine phytoplankton reproduction rates by copper and cadmium. *Journal of Experimental Marine Biology and Ecology,* 96, pp. 225–250.

Bridger, C.J., and Garber, A. (2002) Aquaculture escapement, implications, and mitigation: The salmonid case study. In *Ecological Aquaculture: The Evolution of the Blue Revolution*; Costa-Pierce, B.A., Ed.; Blackwell Science: Malden, MA, USA, pp. 77–102.

Brown, N., Eddy, S., and Plaud, S. (2011). Utilization of waste from a marine recirculating fish culture system as a feed source for the polychaete worm, *Nereis virens*. *Aquaculture,*, 322–323, pp. 177–183.

Brown, N. (2002). Flatfish farming systems in the Atlantic region. *Reviews in Fisheries Science,* 10, pp. 403–419.

Buhmann, A.K., Waller, U., Wecker, B., and Papenbrock, J. (2015). Optimisation of culturing conditions and selection of species for the use of halophytes as biofilter for nutrient-rich saline water. *Agricultural Water Management*, 149, pp. 102–114.

Buhmann, A., and Papenbrock, J. (2013a). Biofiltering of aquaculture effluents by halophytic plants: Basic principles, current uses and future perspectives. *Environmental and Experimental Botany,* 92, pp. 122–133.

Buhmann, A., and Papenbrock, J. (2013b). An economic point of view of secondary compounds in halophytes. *Functional Plant Biology*, 40, pp. 952–967.

Bunting, S.W., and Shpigel, M. (2009). Evaluating the economic potential of horizontally integrated land-based marine aquaculture. *Aquaculture*, 294, pp. 43–51.

Bunting, S.W. (2004). Wastewater aquaculture: Perpetuating vulnerability or opportunity to enhance poor livelihoods? *Aquatic Resources Culture and Development*, 1, pp. 51–57.

Burridge, L., Weis, J.S., Cabello, F., Pizarro, J., and Bostick, K. (2010). Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. *Aquaculture,* 306, pp. 7–23.

Buschmann, A.H., Varela, D.A., Hernández-González, M.C., and Huovinen, P. (2008). Opportunities and challenges for the development of an integrated seaweed-based aquaculture activity in Chile: Determining the physiological capabilities of *Macrocystis* and *Gracilaria* as biofilters. *Journal of Applied Phycology*, 20, pp. 571–577.

Buschmann, A.H., Troell, M., Kautsky, N., and Kautsky, L. (1996). Integrated tank cultivation of salmonids and *Gracilaria chilensis* (Gracilariales, Rhodophyta). *Hydrobiologia* , 326/327, pp. 75–82.

Buzby, K.M., and Lin, L. (2014). Scaling aquaponic systems: Balancing plant uptake with fish output. *Aquacultural Engineering,* 63, pp. 39–44.

Cahill, P.L., Hurd, C.L., and Lokman, M. (2010). Keeping the water clean - Seaweed biofiltration outperforms traditional bacterial biofilms in recirculating aquaculture. *Aquaculture*, 306, pp. 153–159.

Cardoch, L., Day, J.W., Rybczyk, J.M., and Kemp, G.P. (2000). An economic analysis of using wetlands for treatment of shrimp processing wastewater - A case study in Dulac, L.A. *Ecological Economics*, 38, pp. 93–101.

Carter, W.A (1942).. A method of growing plants in water vapour to facilitate examination of roots. *Phytopathology*, 32, pp. 623–625.

Carubelli, G., Fanelli, R., Mariani, G., Nichetti, S., Crosa, G., Calamari, D., and Fattore, E. (2007). PCB contamination in farmed and wild sea bass (*Dicentrarchus labrax* L.) from a coastal wetland area in central Italy. *Chemosphere*, 68, pp. 1630–1635.

Chan, G.L. (1993). Aquaculture, ecological engineering: Lessons from China. *AMBIO*, 22(7), pp. 491–494.

Chavez-Crooker, P., and Obreque-Contreras, J. (2010). Bioremediation of aquaculture wastes. *Current Opinion in Biotechnology,* 21, pp. 313–317.

Chopin, T. Progression of the integrated multi-trophic aquaculture (IMTA) concept and upscaling of IMTA systems towards commercialization. *Aquaculture Europe*, 36(4), pp. 5–12.

Chopin, T., and Robinson, S. (2004). Defining the appropriate regulatory and policy framework for the development of integrated multi-trophic aquaculture practises: Introduction to the workshop and positing of the issues. *Bulletin of the Aquaculture Society of Canada,* 104, pp. 4–10.

Chopin, T., Robinson, S., Sawhney, M., Bastarache, S., Belyea, S., Shea, R., Armstrong, W., Stewart, I., and Fitzgerald, P. (2004). The AquaNet integrated multi-trophic aquaculture project: Rationale of the project and development of kelp cultivation as the inorganic extractive component of the system. *Bulletin of the Aquaculture Society of Canada,* 104, pp. 11–18.

Chopin, T., Buschmann, A.H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G.P., Zertuche-González, J.A., Yarish, C., and Neefus, C. (2001). Integrating seaweeds into marine aquaculture systems: A key toward sustainability. *Journal of Phycology,* 37, pp. 975–986.

Chow, F., Macciavello, J., Santa Cruz, S., and Fonck, O. (2001). Utilization of *Gracilaria chilensis* (Rhodophyta: Gracilariaccae) as biofilter in the depuration of effluents from tank cultures of fish, oyster, and sea urchins. *Journal of the World Aquaculture Society*, 32, pp. 214–220.

Christie, C.B., and Nichols, M.A. (2004). Aeroponics—Production system and research tool. *Acta Horticulturae,* 648, pp. 185–190.

Cole, D.W., Cole, R., Gaydos, S.J., Gray, J., Hyland, G., Jacques, M.L., Powell-Dunford, N., Sawhney, C., and Au, W.W. (2009). Aquaculture: Environmental, toxicological, and health issues. *International Journal of Hygiene and Environmental Health*, 212, pp. 369–377.

Collins, M.T., Gratzek, J.B., Shotts, E.B., Jr., Dawe, D.L., Campbell, L.M., and Senn, D.R. (1975). Nitrification in an aquatic recirculating system. *Journal of the Fisheries Resource Board of Canada,* 32, pp. 2025–2031.

Costa-Pierce, B.A. (2002) Ecology as the paradigm for the future of aquaculture. In *Ecological Aquaculture: The Evolution of the Blue Revolution*; Costa-Pierce, B.A., Ed.; Blackwell Science: Malden, MA, USA, pp. 339–372.

Cripps, S.J., and Bergheim, A. (2000). Solids management and removal for intensive land-based aquaculture production systems. *Aquacultural Engineering,* 22, pp 33–56.

Culver, K., and Castle, D. (2008). *Aquaculture, Innovation, and Social Transformation*; Springer Science and Business Media: Berlin, Germany, Volume 17, p. 344.

Dalton, R. (2004). Fishing for trouble. *Nature,* 431, pp. 502–504.

D'Orbcastel, E.R., Blancheton, J.P., and Aubin, J. (2009). Towards environmentally sustainable aquaculture: Comparison between two trout farming systems using life cycle assessment. *Aquacultural Engineering,* 40, pp. 113–119.

Davidson, P., Myers, G.J., Weiss, B., Shamlaye, C.F., and Cox, C. (2006). Commentary: Prenatal methyl mercury exposure from fish consumption and child development: A review of evidence and perspectives from the Seychelles child development study. *Neuro Toxicology,* 27, pp. 1106–1109.

De Schryver, P., Crab, R., and Defoirdt, T. (2008). The basics of bio-flocs technology: The added value for aquaculture. *Aquaculture,* 277, pp. 125–137.

Dewailly, E., Ayotte, P., Lucas, M., and Blanchet, C. (2007). Risk and benefits from consuming salmon and trout: A Canadian perspective. *Food and Chemical Toxicology,* 45, pp. 1345–1349.

Diana, J.S. (2009) Aquaculture production and biodiversity conservation. *BioScience,* 59, pp. 27–38.

Díaz, F.J., Benes, S.E., and Grattan, S.R. (2013). Field performance of halophytic species under irrigation with saline drainage water in the San Joaquin Valley of California. *Agricultural Water Management*, *118*, pp. 59–69.

Diver, S., and Rinehart, L. (2010). Aquaponics - Integration of hydroponics with agriculture. *ATTRA National Sustainable Agriculture Information Service*, 28, pp. 1–28.

Dufault, R.J., Korkmaz, A., and Ward, B. (2001). Potential of biosolids from shrimp aquaculture as a fertiliser for broccoli production. *Compost Science and Utilization,* 9, pp. 107–114

Dufault, R.J., and Korkmaz, A. (2000). Potential of biosolids from shrimp aquaculture as a fertiliser in bell pepper production. *Compost Science and Utilization,* 3, pp. 310–319.

Eagle, J., Naylor, R., and Smith, W. (2004). Why farm salmon outcompete fishery salmon. *Marine Policy,* 28, pp. 259–270.

Easton, M.D.L., Luszniak, D., and Von der Geest, E. (2002). Preliminary examination of contaminant loadings in farm salmon, wild salmon, and commercial salmon feed. *Chemosphere,* 46, pp. 1053–1074.

Endut, A., Jusoh, A., Ali, N., Nik, W.B.W., and Hassan, A. (2010). A study on the optimal hydraulic loading rate and plant ratios in recirculation aquaponic systems. *Bioresource Technology*, 101, pp. 1511–1517.

Endut, A., Jusoh, A., Ali, N., Wan Nik, W.N.S., and Hassan, A. (2009). Effect of flow rate on water quality parameters and plant growth of water spinach (*Ipomoea aquatica*) in an aquaponic recirculating system. *Desalination and Water Treatment,* 5, pp. 19–28.

Fei, X.G., Tseng, C.K., Pang, S.J., Lian, S.X., Huang, R.K., and Chen, W.Z. (2002). Transplant of *Gracilaria lemaneiformis* by raft culture on the sea along fish cages in southern China. In: *Proceedings of the World Aquaculture Society, Baton Rouge, LA, USA, 23–27 April 2002*; p. 219.

Fei, X.G., Bao, Y., and Lu, S. (2000). Seaweed cultivation-traditional way and its reformation. *Oceanologia et Limnologia Sinica,* 31(5), pp. 575–580.

Fleming, I.A., Hindar, K., Mjolnerod, I., Jonsson, B., Balstad, T., and Lamberg, A. (2000). Lifetime success and interactions of farm salmon invading a natural population. *Proceedings of the Royal Society: Biological Sciences*, 267, pp. 1517–1523.

Food and Agriculture Organization of the United Nations (2016). *The State of World Fisheries and Aquaculture 2016: Contributing to Food Security and Nutrition for All*; FAO: Rome, Italy, p. 200.

Food and Agriculture Organisation of the United Nations (2014). *The State of World Fisheries and Aquaculture 2014*; FAO: Rome, Italy, p. 243.

Food and Agriculture Organisation of the United Nations (2010). *The State of World Fisheries and Aquaculture 2010*; FAO: Rome, Italy, p. 197.

Foran, J.A., Carpenter, D.O., Hamilton, M.C., Knuth, B.A., and Schwager, S.J. (2005). Risk-based consumption advice for farmed Atlantic and wild pacific salmon contaminated with dioxins and dioxin-like compounds. *Environmental Health Perspectives,* 113, pp. 552–555.

Ford, J.S., and Myers, R.A. (2008). A global assessment of salmon aquaculture impacts on wild salmonids. *PLoS Biology*, 6, doi:10.1371/journal.pbio.0060033.

Fornes, F., Belda, R.M., Abad, M., Noguera, P., Puchades, R., Maquieira, A., and Noguera, V. (2003). The microstructure of coconut coir dusts for use as alternatives to peat in soilless growing media. *Australian Journal of Experimental Agriculture*, 43, pp. 1171-1179.

Fronte, B., Galliano, G., and Bibbiani, C. (2016). From freshwater to marine aquaponic: New opportunities for marine fish species production. In: *Proceedings of VIVUS conference - Agriculture, Environmentalism, Horticulture and Floristics, Food Production and Processing, and Nutrition, 4th Conference with International Participation on Agriculture, Environmentalism, Horticulture, Floristics, Food Production and Processing and Nutrition, Naklo, Slovenia, 20–21st April 2016*.

Gericke, W.F. (1940). *The Complete Guide to Soilless Gardening*; Prentice-Hall: New York, NY, USA, p. 304.

Gericke, W.F. (1937). Hydroponics—Crop production in liquid culture media. *Science*, 85, pp. 177–178.

Gericke, W.F. (1929). Aquaculture: A means of crop production. *American Journal of Botany*, 16, p. 862.

Gifford, S., Dunstan, R.H., O'Connor, W., Koller, C.E., and MacFarlane, G.R. (2007) Aquatic zooremediation: Deploying animals to remediate contaminated aquatic environments. *Trends in Biotechnology,* 25, pp. 60–65.

Glenn, E.P., Brown, J., and Blumwald, E. (1998). Irrigating crops with seawater. *Scientific American*, 279, pp. 56–61.

Glenn, E.P., O'Leary, J.W., Watson, M.C., Thompson, T.L., and Kuehl, R.O. (1991). *Salicornia bigelovii* Torr.: An oilseed halophyte for seawater irrigation. *Salicornia bigelovii* Torr.: An oilseed halophyte for seawater irrigation. *Science*, 251, pp. 1065–1067.

Goddek, S., Espinal, C.A., Delaide, B., Jijakli, M.H., Schmautz, Z., Wuertz, S., and Keesman, K.J. (2016). Navigating towards decoupled aquaponic systems: A system dynamics design approach. *Water,* 8, pp. 1–29.

Goldburg, R., and Naylor, R. (2005). Future seascapes, fishing and fish farming. *Frontiers in Ecology and the Environment,* 3(1), pp. 21–28.

Gonçalves, A.A., and Gagnon, G.A. (2011). Ozone application in recirculating aquaculture system: An overview. *Ozone: Science and Engineering*, 33, pp. 345–367.

Graber, A., and Junge, R. (2009). Aquaponic systems: Nutrient recycling from fish wastewater by vegetable production. *Desalination*, 246, pp. 147–156.

Granada, L., Sousa, N., Lopes, S., and Lemos, M.F.L (2016). Is integrated multitrophic aquaculture the solution to the sectors' major challenges?—A review. *Reviews in Aquaculture,* 8, pp. 283–300.

Graves, C.J. (1983). The nutrient film technique. *Horticultural Reviews*, 5, pp. 1–44.

Greiner, A.D., and Timmons, M.B. (1998). Evaluation of nitrification rates of microbead and trickling filters in an intensive recirculating tilapia production facility. *Aquacultural Engineering,* 18, pp. 189–200.

Grigorakis, K., and Rigos, G. (2011). Aquaculture effects on the environmental and public welfare—The case of Mediterranean Mariculture. *Chemosphere*, 855, pp. 899–919.

Gross, M.R. One species with two biologies: Atlantic salmon (*Salmo salar*) in the wild and in aquaculture. *Canadian Journal of Fisheries and Aquatic Sciences,* 55, pp. 1–14.

Guil, J.L., Rodriguez-Garcia, I., and Torija, E. (1997). Nutritional and toxic factors in selected wild edible plants. *Plant Foods for Human Nutrition*, 51, pp. 99–107.

Gutierrez-Wing, M.T., and Malone, R.F. (2006). Biological filters in aquaculture: Trends and research directions for freshwater and marine applications. *Aquacultural Engineering,* 34, pp. 163–171.

Haines, K.C. (1976). Growth of the carrageenan-producing tropical red seaweed *Hypnea musciformis* in surface water, 870 m deep water, effluent from a clam mariculture system, and in deep water enriched with artificial fertilizers or domestic sewage. In *10th Symposium on Marine Biology*; Persoone, G., Jaspers, E., Eds.; University Press: Wtteren, Belgium, Volume 1, pp. 207–220.

Handå, A., Ranheim, A., Olsen, A.J., Altin, D., Reitan, K.I., Olsen, Y., Altin, D., Reitan, K.I,; Olsen, Y., and Reinertsen, H. (2012). Incorporation of salmon fish feed and faeces components in mussels (*Mytilus edulis*): Implications for intergrated multi-trophic aquaculture in cool-temperate North Atlantic waters. *Aquaculture*, 370–371, pp. 40–53.

Hannah, L., Pearce, C.M., and Cross, S.F. (2013). Growth and survival of California sea cucumbers (*Parastichopus californicus*) cultivated with sablefish (*Anoplopoma fimbria*) at an integrated multi-trophic aquaculture site. *Aquaculture*, 406–407, pp. 34–42.

Hansen, P., Jacobsen, J.A., and Lund, R.A. (1993). High numbers of farmed Atlantic salmon, *Salmo salar*, observed in oceanic waters north of the Faroe Islands. *Aquaculture Research,* 24(6), pp. 777–781.

Hardy, R.W. (2010). Utilization of plant proteins in fish diets: Effects of global and supplies of fishmeal. *Aquaculture Research,* 41, pp. 770–776.

Hart, E.R., Webb, J.B., and Danylchuk, A.J. (2013). Implementation of aquaponics in education: An assessment of challenges and solutions. *Science Education International,* 24(4), pp. 460–480.

Hastein, T., Hjeltnes, B., Lillehaugh, A., Utne Skare, J., Berntssen, M., and Lundebye, A.K. (2006). Food safety hazards that occur during the production stage: Challenges for fish farming and the fishing industry. *Rev. Sci. Tech.,* 25(2), pp. 607–625.

Hayward, D., Wong, J., and Krynitsky, A.J. (2007). Polybrominated diphenyl ethers and polychlorinated biphenyls in commercially wild caught and farm-raised fish fillets in the United States. *Environmental Research*, 103, pp. 46–54.

Heinen, J.M., Hankins, J.A., and Adler, P.R. (1996). Water quality and waste production in recirculating trout culture system with feeding of a higher energy or a lower energy diet. *Aquaculture,* 27, pp. 699–710.

Helfman, G., Collette, B.B., Facey, D.E., and Bowen, B.W. (2009). *The Diversity of Fishes: Biology, Evolution, and Ecology*, 2nd ed.; Wiley-Blackwell: Chichester, UK, p. 736.

Hites, R.A., Foran, J.A., Carpenter, D.O., Hamilton, M.C., Knuth, B.A., and Schwager, S.J. (2004a). Global assessment of organic contaminants in farmed salmon. *Science,* 303, pp. 226–229.

Hites, R.A., Foran, J.A., Schwager, S.J., Knuth, B.A., Hamilton, M.C., and Carpenter, D.O. (2004b). Global assessment of polybrominated diphenyl ethers in farmed and wild salmon. *Environmental Science & Technology,* 38, pp. 4945–4949.

Hollyer, J., Tamaru, C., Riggs, A., Klinger-Bowen, R., Howerton, R., Okimoto, D., Castro, L., Ron, T.R., Fox, K., Troegner, V., and Martinez, G. (2009). On-farm food safety: Aquaponics. *Food Safety and Technology.* Available online: http://www.ctahr.hawaii.edu/oc/freepubs/pdf/FST-38.pdf (accessed on 14 December 2016).

Honda, H., and Kikuchi, K. (2002). Nitrogen budget of polychaete *Perinereis nuntia vallata* fed on the faeces of Japanese flounder. *Fisheries Science*, 68, pp. 1304–1308.

Hu, M.H., Ao, Y.S., Yang, X.E., and Li, T.Q. (2008). Treating eutrophic water for nutrient reduction using an aquatic macrophyte (*Ipomoea aquatica* Forsskal) in a deep flow technique system. *Agricultural Water Management*, 95, pp. 607–615.

Huo, Y., Wu, H., and Chai, Z. (2012). Bioremediations efficiency of *Gracilaria verrucosa* for an integrated multi-trophic aquaculture system with *Pseudosciaena crocea* in Xiangshan Harbor, China. *Aquaculture*, 326–329, pp. 99–105.

Ignatius, A., Arunbabu, V., Neethu, J., and Ramasamy, E.V. (2014). Rhizofiltration of lead using an aromatic medicinal plant *Plectranthus amboinicus* cultured in a hydroponic nutrient film technique (NFT) system. *Environmental Science and Pollution Research,* 21, pp. 13007–13016.

Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Foss, A., Vikingstad, E., and Elvegård, T.A. (2014). The use of lumpfish (*Cyclopterus lumpus* L.) to control sea lice (*Lepeophtheirus salmonis* Krøyer) infestations in intensively farmed Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 424–425, pp. 18–23.

Jeffery, K.R., Stone, D., Feist, S.W., and Verner-Jeffreys, D.W. (2010). An outbreak of disease caused by *Francisella* sp. in Nile tilapia *Oreochromis niloticus* at a recirculation fish farm in the UK. *Disease of Aquatic Organisms,* 91, pp. 161–165.

Jensen, M.H. (2002). Deep flow hydroponics—Past, present and future. In *Proceedings of the 30th National Agricultural Plastics Congress*, San Diego, CA, USA, 17–19 August 2002; Volume 30, pp. 40–46.

Jensen, M.H. (1997). Hydroponics worldwide—A technical overview. *International Symposium on Growing Media and Hydroponics*, 481, pp. 719–730.

Jensen, M.H., and Collins, W.L. (1985). Hydroponic vegetable production. *Horticultural Reviews*, 7, pp. 1–44.

Jimenez del Río, M., Ramazanov, Z., and García-Reina, G. (1996). *Ulva rigida* (Ulvales, Chlorophyta) tank culture as biofilters for dissolved inorganic nitrogen from fishpond effluents. *Hydrobiologia*, 326/327, pp. 61–67.

Joesting, H.M., Blaylock, R., Biber, P., and Ray, A. (2016). The use of marine aquaculture solid waste for nursery of salt marsh plants *Spartina alterniflora* and *Juncus roemerianus*. *Aquaculture Reports,* 3, pp. 108–114.

Johnson, B. (2002). Greenhouse nutrient management: Regulations and treatment options. *Grow Edge*, 13, pp. 38–43.

Jones, J.B., Jr. (2005). *Hydroponics—A Practical Guide for the Soilless Grower*, 2nd ed.; CRC Press: Boca Raton, FL, USA, p. 440.

Kadlec, R.H., and Knight, R.L. (2009). *Treatment Wetlands*, 2nd ed.; CRS Press: Boca Raton, FL, USA, p. 153.

Kang, K.H., Kwon, J.Y., and Kim, Y.M. (2003). A beneficial co-culture: Charm abalone *Haliotis discus hannai* and sea cucumber *Stichopus japonicus*. *Aquaculture*, 216, pp. 87–93.

Kawarazuka, N., and Béné, C. (2010). Linking small-scale fisheries and aquaculture to household nutritional security: An overview. *Food Security,* 2, pp. 343–357.

Kiljunen, M., Vanhatalo, M., Mantyniemi, S., Peltonen, H., Kuikka, S., Kiviranta, H., Parmanne, R., Tuomisto, J.T., Vuorinen, P.J., Hallikainen, A., Verta, M., Pönni, J., Jones, R.I., and Karjalainen, J. (2007). Human dietary intake of organochlorines from Baltic herring: Implications of individual fish variability and fisheries management. *AMIBO: A Journal of the Human Environment*, 36(2), 257–264.

Klinger, D., and Naylor, R. (2012). Searching for solutions in aquaculture: Charting a sustainable course. *Annual Review of Environment and Resources*, 37, pp. 247–276.

Kolmes, S.A. (2004). Salmon farms and hatcheries. *Environment*, 46, pp. 40–43.

Kong, Y., and Zheng, Y. (2014). Potential of producing *Salicornia bigelovii* hydroponically as a vegetable at moderate NaCl salinity. *HortScience,* 4999, pp. 1154–1157.

Koopmans, M., Matens, D., and Wijffels, R.H. (2009). Towards commercial production of sponge medicines. *Marine Drugs,* 7, pp. 787–802.

Krkosek, M., Ford, J.S., Morton, A., Lele, S., Myers, R.A., and Lewis, M. (2007). Declining wild salmon populations in relation to parasites from farm salmon. *Science*, 318, pp. 1772–1775.

Krkosek, M., Lewis, M.A., Volpe, J.P., and Morton, A. (2006). Fish farms and sea lice infestations of wild juvenile salmon in the Broughton Archipelago - A rebuttal to Brooks (2005). *Fisheries Science,* 14, pp. 1-11.

Lakkireddy, K.K.R., Kasturi, K., and Sambasiva Rao, K.R.S. (2012). Role of hydroponics and aeroponics in soilless culture in commercial food production. *Research and Reviews: Journal of Agricultural Science and Technology*, 1, pp. 26–35.

Langton, R.W., Haines, K.C., and Lyon, R.E. (1977). Ammonia nitrogen produced by the bivalve mollusc *Tapes japonica* and its recovery by the red seaweed *Hypnea musciformis* in a tropical mariculture system. *Helgoländer Wissenschaftliche Meeresuntersuchungen*, 30(1-4), pp. 217–229.

Lander, T.R., Robinson, S.M.C., MacDonald, B.A., and Martin, J.D. (2013). Characterization of the suspended organic particles released from salmon farms and their potential as a food supply for the suspension feeder, *Mytilus edulis* in integrated multi-trophic aquaculture (IMTA) systems. *Aquaculture* 406–407, pp. 160–170.

Lee, S., Kong, D.H., Yun, S.H., Lee, K.P., Franzblau, S.G., Lee, E.Y., and Chang, C.L. (2006). Evaluation of a modified antimycobacterial susceptibility test using Middlebrook 7H10 agar containing 2,3-diphenyl-5-thienyl-(2)-tetrazolium chloride. *Journal of Microbiological Methods,* 66, pp. 548–551.

Lennard, W.A., and Leonard, B.V. (2006). A comparison of three different hydroponic sub-systems (gravel bed, floating and nutrient film technique) in an aquaponic test system. *Aquaculture International,* 14, pp. 539–550.

Lewis, W.M., Yop, J.H., Schramm, H.L., Jr., and Brandenburg, A.M. (1978). Use of hydroponics to maintain quality of recirculated water in a fish culture system. *Transactions of the American Fisheries Society,* 107, pp. 92–99.

Li, S. (1987). Energy structure and efficiency of a typical Chinese integrated fish farm. *Aquaculture,* 65, pp. 105–118.

Licciano, M., Stabili, L., and Giangrande, A. (2005). Clearance rates of *Sabella spallanzanii* and *Branchiomma luctuosum* (Annelida: Polychaeta) on a pure culture of *Vibrio alginolyticus*. *Water Research*, 39, pp. 4375–4384.

Liu, X.G., Xia, Y.G., Wang, F., Sun, M., Jin, Z.J., and Wang, G.T. (2005). Analysis of fatty acid composition of *Salicornia europaea* L. seed oil. *Food Science*, *2*, p. 42.

Love, D.C., Fry, J.P., Genello, L., Hill, E.S., Frederick, J.A., Li, X., and Semmens, K. (2014). An international survey of aquaponics practitioners. *PLoS ONE*, 9, doi:10.1371/journal.pone.0102662.

Lund, E.K. (2013) Health benefits of seafood; is it just the fatty acids? *Food Chemistry*, 140, pp. 413–420.

Le Jeune, A.H., Charpin, M., Deluchat, V., Briand, J.F., Lenain, J.F., Baudu, M., and Amblard, C. (2006). Effect of copper sulphate treatment on natural phytoplanktonic communities. *Aquatic Toxicology*, 80, pp. 267–280.

Levin, P.S., Zabel, R.W., and Williams, J.G. (2001). The road to extinction is paved with good intentions: Negative association of fish hatcheries with threatened salmon. *Proceedings of the Royal Society: Biological Sciences,* 268, pp. 1153–1158.

Lin, Y.F., Jing, S.R., Lee, D.Y., Chang, Y.F., Chen, Y.M., and Shih, K.C. (2005). Performance of a constructed wetland treating intensive shrimp aquaculture wastewater under high hydraulic loading rate. *Environmental Pollution* 134, pp. 411–421.

Lin, Y.F., Jing, S.R., Lee, D.Y., and Wang, T.W. (2002a) Nutrient removal from aquaculture wastewater using a constructed wetland system. *Aquaculture*, 209, pp. 169–184.

Lin, Y.F., Jing, S.R., Lee, D.Y., and Wang, T.W. (2002b) Removal of solids and oxygen demand from aquaculture wastewater with a constructed wetland system in the start-up phase. *Water Environment Research*, 74, pp. 136–141.

Love, D.C., Fry, J.P., Li, X., Hill, E.S., Genello, L., Semmens, K., and Thompson, R.E. (2015). Commercial aquaponics production and profitability: Findings from an international survey. *Aquaculture*, 435, pp. 67–74.

Lymbery, A.J., Doupe, R.G., Bennett, T., and Starcevich, M.R. (2006). Efficacy of a subsurface-flow wetland using the estuarine sedge *Juncus kraussii* to treat effluent from inland saline aquaculture. *Aquaculture Engineering*, 34, pp. 1–7.

Ma, C., Zhang, X., Chen, W., Zhang, G., Duan, H., Ju, M.; Li, H., and Yang, Z. (2013). China's special marine protected area policy: Trade-off between economic development and marine conservation. *Ocean & Coastal Management*, 76, pp. 1–11.

MacDonald, C.L.E. Stead, S.M., and Slater, M.J. (2013). Consumption and remediation of European seabass (*Dicentrarchus labrax*) waste by the sea cucumber *Holothuria forskali*. *Aquaculture International*, 21, pp. 1279–1290.

MacDonald, B.A., Robinson, S.M.C., and Barrington, K.A. (2011). Feeding activity of mussels (*Mytilus edulis*) held in the field at an integrated multi-trophic aquaculture (IMTA) site (*Salmo salar*) and exposed to fish food in the laboratory. *Aquaculture*, 314, pp. 244–251.

Manoj, V.R., and Vasudevan, N. (2012). Removal of nutrients in denitrification system using coconut coir fibre for the biological treatment of aquaculture wastewater. *Journal of Environmental Biology,* 33, pp. 271.

Marinho-Soriano, E., Azevedo, C.A.A., Trigueiro, T.G., Pereira, D.C., Carneiro, M.A.A., and Camara, M.R. (2011). Bioremediation of aquaculture wastewater using macroalgae and *Artemia*. *International Biodeterioration & Biodegradation,* 65, pp. 253–257.

Marsh, L., Subler, S., and Mishra, S. (2005). Suitability of aquaculture effluent solids mixed with cardboard as a feedstock for vermicomposting. *Bioresource Technology* 96, pp. 413–418.

Marshall, D. (2003). *Fishy Business: The Economics of Salmon Farming in BC*; Canadian Centre for Policy Alternative—BC Office: Vancouver, BC, USA, p. 45.

Martins, C.I.M, Eding, E.H., and Verreth, J.A.J. (2011). The effect of recirculating aquaculture systems on the concentration of heavy metals in culture water and tissues of Nile tilapia *Oreochromis niloticus*. *Food Chemistry,* 126, pp. 1001–1005.

Martins, C.I.M., Eding, E.H., Verdegem, M.C.J., Heinsbroek, L.T.N., Schneider, O., Blancheton, J.P., Roque d'Orbcastel, E., and Verreth, J.A.J. (2010). New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. *Aquacultural Engineering,* 43, pp. 83–93.

McGinnity, P., Prodohl, P., Ferguson, A., Hynes, R., Maoileidigh, N.O., Baker, N., Cotter, D., O'Hea, B., Cooke, D., Rogan, G., Taggart, J., and Cross, T. (2003). Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salo salar*, as a result of interactions with escaped farmed salmon. *Proceedings of the Royal Society: Biological Sciences*, 270(1532), pp. 2443–2450.

McGinnity, P., Stone, C., Taggart, J., Cooke, D., Cotter, D., Hynes, R., McCamley, C., Cross, T., and Ferguson, A. Genetic impact of escaped farmed Atlantic salmon (*Salmo salar* L.) on native populations: Use of DNA profiling to assess freshwater performance of wild, farmed, and hybrid progeny in a natural river environment. *ICES Journal of Marine Science*, pp. 998–1008.

McKinnell, S., and Thomson, A.J. (1997). Recent events concerning Atlantic salmon escapees in the Pacific. *ICES Journal of Marine Science,* 54, pp. 1221–1225.

McVicar, A.H. (1997). Disease and parasite implications of the coexistence of wild and culture salmon populations. *ICES Journal of Marine Science,* 54, pp. 1093–1103.

Merriam-Webster. (2017) Available online: http://www.merriam-webster.com/dictionary/polyculture (accessed on 11th May 2017).

Milanese, M., Chelossi, E., Manconi, R., Sará, A., Sidri, M., and Pronzato, R. (2003). The marine sponge *Chondrilla nucla* Schmidt, 1862 as an elective candidate for bioremediation in integrated aquaculture. *Biomolecular Engineering*, 20, pp. 363–368.

Miller, D. (2008). Using aquaculture as a post-mining land use in West Virginia. *Mine Water and the Environment,* 27, pp. 122–126.

Minh, N.H., Minh, T.B., Kajiwara, N., Kunisue, T., Iwata, H., Viet, P.H., Tu, N.P., Tuyen, B.C., and Tanabe, S (2006). Contamination by polybrominated diphenyl ethers and persistent organochlorides in catfish and feed from Mekong River Delta, Vietnam. *Environmental Toxicology and Chemistry,* 25, pp. 2700–2708.

Mirzoyan, N., and Tal. Y.; Gross, A. (2010). Anaerobic digestion of sludge from intensive recirculating aquaculture sytems: Review. *Aquaculture*, 306, pp. 1–6.

Movahedi, Z., Moieni, A., and Soroushzadeh, A. (2012). Comparison of aeroponics and conventional soil systems for potato minituber production and evaluation of their quality characters. *Journal of Plant Physiology and Breeding*, 2, pp. 13–21.

Molloy, S.D., Pietrak, M.R., Bouchard, D.A., and Bricknell, I. (2011). Ingestion of *Lepeophtheirus salmonis* by the blue mussel *Mytilus edulis*. *Aquaculture,* 311, pp. 61–64.

Montory, M., and Barra, R. (2006). Preliminary data on poly-brominated dephenyl ethers (PBDE) in farmed fish tissues (*Salmo salar*) and fish feed in Southern Chile. *Chemosphere*, 63, pp. 1252–1260.

Morgan, L. (1999). Introduction to hydroponic gullies and channels. *Grow Edge*, 106, pp. 67–75.

Naegel, L.C.A. (1977). Combined production of fish and plants in recirculating water. *Aquaculture*, 10, pp. 17–24.

Naylor, R.L., Hardy, R.W., Bureau, P.D., Chiu, A., Elliott, M., Farrell, A.P., Forster, I., Gatlin D.M., Goldburg, R.J., Hua, K., and Nichols, P.D. (2009). Feeding aquaculture in an era of finite resources. *Proceedings of the National Academy of Sciences of the United States of America,* 106, pp. 15103–15110.

Naylor, R., Hindar, K., Fleming, I.A., Goldburg, R., Williams, S., Volpe, J., Whoriskey, F., Eagle, L., Kelso, D., and Mangel, M. Fugitive salmon: Assessing the risks of escaped fish from net-pen aquaculture. (2005). *BioScience*, 55, pp. 427–437.

Naylor, R., and Burke, M. (2005). Aquaculture and ocean resources: Raising tigers of the sea. *Annual Review of Environment and Resources*, 30, pp. 185–218.

Naylor, R., Eagle, J., and Smith, W. (2003). Salmon aquaculture in the Pacific North-west: A global industry with local impacts. *Environment*, 45, pp. 18–39.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., and Troell, M. (2000). Effect of aquaculture on world fish supplies. *Nature,* 405, pp. 1017–1024.

Nelson, E.J., MacDonald, B.A., and Robinson, S.M.C. (2012). The absorption efficiency of the suspension-feeding sea cucumber, *Cucumaria frondosa*, and its potential as an extractive integrated multi-trophic aquaculture (IMTA) species. *Aquaculture*, 370–371, pp. 19–25.

Neori, A., Chopin, T., and Troell, M. (2004). Integrated aquaculture: Rationale, evolution, and state of the art emphasizing seaweed biofiltration in modern Mariculture. *Aquaculture,* 231, pp. 361–391.

Neori, A., Shpigel, M., and Ben-Ezra, D. (2000). A sustainable integrated system for culture of fish, seaweed, and abalone. *Aquaculture*, 186, pp. 279–291.

Neori, A., Cohen, I., and Gordin, H. (1991). *Ulva lactuca* biofilters for marine fish-pond effluents: II. Growth rate, yield, and C:N ratio. *Botanica Marina*, 34(6), pp. 483–489.

Nir, I. (1982). Growing plants in aeroponics growth system. *Acta Horticulturae,* 126, pp. 435-448.

Nozzi, V., Parisi, G., Di Crescenzo, D., Giordano, M., and Carnevali, O. (2016). Evaluation of *Dicentrarchus labrax* meats and the vegetable quality of *Beta vulgaris* var. *cicla* farmed in freshwater and saltwater aquaponic systems. *Water*, 8(423), pp. 1-14.

Osinga, R., Sidri, M., Cerig, E., Gokalp, S.Z., and Gokalp, M. (2010). Sponge aquaculture trials in the East-Mediterranean Sea: New approaches to earlier ideas. *The Open Marine Biology Journal*, 4, pp. 74–81.

Oterhals, A., and Nygard, E (2008). Reduction of persistent organic pollutants in fishmeal: A feasibility study. *Journal of Agricultural and Food Chemistry*, 56, pp. 2012–2020.

Páez-Osuna, F. (2001). The environmental impact of shrimp aquaculture: A global perspective. *Environmental Pollution*, 112, pp. 229–231

Páez-Osuna, F., Guerrero-Galván, S.R., and Ruiz-Fernández, A.C. (1998). The environmental impact of shrimp aquaculture and the coastal pollution in Mexico. *Marine Pollution Bulletin*, 36, pp. 65–75.

Palmer, P.J. (2010). Polychaete-assisted sand filters. *Aquaculture*, 306, pp. 369–377.

Paltzat, D.L., Pearce, C.M., Barnes, P.A., and McKinley, R.S. (2008). Growth and production of California sea cucumbers (*Parastichopus californicus* Stimpson) co-cultured with suspended Pacific oysters (*Crossostrea gigas* Thunberg). *Aquaculture*, 275, pp. 124–137.

Pantanella, E., and Bhujel, C.R. (2015). *Saline Aquaponics—Potential Player in Food, Energy Production*; Global Aquaculture Advocate: Portsmouth, New Hampshire, USA, 2015; pp. 42–43.

Pantanella, E., and Colla, G. (2013). Saline aquaponics opportunities for integrated marine aquaculture. In *Proceedings of the International Aquaponic Conference: Aquaponics and Global Food Security*, University of Wisconsin, Stevens Point, WI, USA, 19–21st June 2013.

Pantanella, E. (2012). *Integrated Marine Aquaculture-Agriculture: Sea Farming out of the Sea*; Global Aquaculture Advocate: Portsmouth, New Hampshire, USA, pp. 70–72.

Park, J.-S., and Kurata, K. (2009). Application of microbubbles to hydroponics solution promotes lettuce growth. *HortTechnology*, 19, pp. 212–215.

Pelletier, N., Audsley, E., Brodt, S., Garnett, T., Henriksson, P., Kendall, A., Kramer, K.J., Murphy, D., Nemecek, T., and Troell, M. (2011). Energy intensity of agriculture and food systems. *Annual Review of Environment and Resources*, 36, pp. 223–246.

Piedrahita, R.H. (2003). Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture*, 226, pp. 35–44.

Pietrak, M.R., Molloy, S.D., Bouchard, D.A., Singer, J.T., and Bricknell, I. (2012). Potential role of *Mytilus edulis* in modulating the infections pressure of *Vibrio anguillarum* 02β on an integrated multi-trophic aquaculture farm. *Aquaculture*, 326–329, pp. 36–39.

Pinto, B., Garritano, S.L., Cristofani, R., Ortaggi, G., Giuliano, A., Amodio-Cocchierri, R., Cirillo, R., DeGiusti, M., Boccia, A., and Reali, D. (2008). Monitoring of polychlorinated biphenyl contamination and estrogenic activity in water, commercial feed, and farmed seafood. *Environmental Monitoring and Assessment,* 144, pp. 445–453.

Price, L.L. (2007). From pedestrian fare to gourmet trend: The case of *Salicornia europaea* L., a traditional gathered wild seashore vegetable. In *Changing Families and Their Lifestyles*; Moerbeek, H.H., Niehof, A., Eds.; Wageningen Academic Publishers: Wageningen, The Netherlands, Volume 2, pp. 201–211.

Primavera, J.H. (2006). Overcoming the impacts of aquaculture on the coastal zone. *Ocean & Coastal Management*, pp. 531–545.

Primavera, J.H. (1997). Socio-economic impacts of shrimp culture. *Aquaculture Research,* 28, pp. 815–827.

Qian, P.Y., Wu, C.Y., Wu, M., and Xie, Y.K. (1996). Integrated cultivation of the red alga *Kappaphycus alvarezii* and the pearl oyster *Pinctada martensi*. *Aquaculture*, 147, pp. 21–35.

Rakocy, J., Masser, M.P., and Losordo, T.M. (2006). *Recirculating Aquaculture Tank Production Systems: Aquaponics—Integrating Fish and Plant Culture*; SRAC Publication: Stoneville, Mississippi, USA, No. 454, p. 16.

Ramani, B., Reeck, T., Debez, A., Stelzer, R., Huchzermeyer, B., Schmidt, A., and Papenbrock, J. (2006). *Ater tripolium* L. and *Sesuvium portulacastrum* L.: Two halophytes, two strategies to survive in saline habitats. *Plant Physiology and Biochemistry,* 44, pp. 395–408.

Read, P., and Fernandes, T. (2003). Management of environmental impacts of marine aquaculture in Europe. *Aquaculture*, 226, pp. 139–163.

Reid, G.K., Liutkus, M., and Bennett, A. (2010). Absorption efficiency of blue mussels (*Mytilus edulis* and *M. trossulus*) feeding on Atlantic salmon (*Salmo salar*) feed and faecal particulates: Implications for integrated mulit-trophic aquaculture. *Aquacultur,* 299, pp. 165–169.

Reimold, R.J., and Queen, W.H. (1974). *Ecology of Halophytes*; Academic Press Inc.: New York, NY, USA; London, UK, 1974, 620p.

Rhee, M.H., Park, H.-J., and Cho, J.Y. (2009). *Salicornia herbacea*: Botanical, chemical, and pharmacological review of halophyte marsh plant. *Journal of Medicinal Plants Research*, 3, pp. 548–555.

Rodríguez, E., González, M., Caride, B., Lamas, M.A., and Taboada, M.C. (2000). Nutritional value of *Holothuria forskali* protein and effects on serum lipid profile in rats. *Journal of Physiology and Biochemistry*, 56, pp. 39–44.

Roheim, C.A., Asche, F., and Santos, J.I. (2011). The elusive price premium for ecolabelled products: Evidence from seafood in the UK market. *Journal of Agricultural Economics*, 62, pp. 655–668.

Ruddle, K., and Zhong, G. (1988). *Integrated Agriculture-Aquaculture in the South of China. The Dike-Pond System in the Zhujiang Delta*; Cambridge University Press: Cambridge, UK, 173p.

Rupasinghe, J.W., and Kennedy, J.O.S. (2010). Economic benefits of integrating a hydroponic-lettuce system into a barramundi fish production system. *Aquaculture Economics and Management,* 14, pp. 81–96.

Russell, M., Robinson, C.D., Walsham, P., Webster, L., and Moffat, C.F. (2011). Persistent organic pollutants and trace metals in sediments close to Scottish marine fish farms. *Aquaculture,* 319, pp. 262–271.

Salam, M.A., Hashem, S, Asadujjaman, M., and Li, F. (2014). Nutrient recovery from in fish farming wastewater: An aquaponic system for plant and fish integration. *World Journal of Fish and Marine Sciences,* 6, pp. 355–360.

Sará, G., Zenone, A., and Tomasello, A. (2009). Growth of *Mytilus galloprovincialis* (Mollusca, Bivalva) close to fish farms: A cases of integrated multi-trophic aquaculture within the Tyrrhenian Sea. *Hydrobiologia*, 636, pp. 129–136.

Schreier, H.J., Mirzoyan, N., and Saito, K. (2010). Microbial diversity of biological filters in recirculating aquaculture systems. *Current Opinion in Biotechnology*, 21, pp. 318–325.

Schroeder, J.P., Croot, P.L., Von Dewitz, B., Waller, U., and Hanel, R. (2011). Potential and limitations of ozone for the removal of ammonia, nitrite, and yellow substances in marine recirculating aquaculture systems. *Aquacultural Engineering*, 45, pp. 35–41.

Schulz, C., Gelbrecht, J., and Rennert, B. (2003). Treatment of rainbow trout farm effluents in constructed wetland with emergent plants and subsurface horizontal water flow. *Aquaculture*, 217, pp. 207–221.

Schwartz, M.F., and Boyd, C.E. (1995). Constructed wetlands for treatment of channel catfish pond effluents. The p*rogressive Fish Culturist,* 57(4), pp. 255–267.

Seawright, D.E., Stickney, R.R., and Walker, R.B. (1998). Nutrient dynamics in integrated aquaculture—Hydroponic systems. *Aquaculture*, 160, pp. 215–237.

Shete, A.P., Verma, A.K., Chadha, N.K., Prakash, C., Peter, R.M., Ahmad, I., and Nuwansi, K.K.T. (2016). Optimization of hydraulic loading rate in aquaponic system with Common carp (*Cyrinus carpio*) and Mint (*Mentha arvensis*). *Aquacultural Engineering,* 72, pp. 53–57.

Shpigel, M., Ben-Ezra, D., Shauli, L., Sagi, M., Ventura, Y., Samocha, T., and Lee, J.J. (2013). Constructed wetland with *Salicornia* as a biofilter for Mariculture effluent. *Aquaculture*, 412–413, pp. 52–63.

Sindilariu, P.D., Reiter, R., and Wedekind, H (2009a). Impact of trout aquaculture on water quality and farm effluent treatment options. *Aquatic Living Resources*, 22, pp. 93–103.

Sindilariu, P.D., Brinker, A., and Reiter, R. (2009b) Factors influencing the efficiency of constructed wetlands used for the treatment of intensive trout farm effluent. *Ecological Engineering*, 35, pp. 711–722.

Sindilariu, P.D., Wolter, C., and Reiter, R. (2008). Constructed wetlands as a treatment method for effluents from intensive trout farms. *Aquaculture*, 277, pp. 179–184.

Singer, A., Parnes, S., Gross, A., Sagi, A., and Brenner, A. (2008). A novel approach to denitrification processes in a zero-discharge recirculating system for small-scale urban aquaculture. *Aquacultural Engineering,* 39, pp. 72–77.

Singh, D., Buhmann, A.K., Flowers, T.J., Seal, C.E., and Papenbrock, J. (2014). *Salicornia* as a crop plant in temperate regions: Selection of genetically characterised ecotypes and optimisation of their cultivation conditions. *AoB Plants*, 6, doi:10.1093/aobpla/plu071.

Sipkema, D., Osinga, R., Schatton, W., Mendola, D., Tramper, J., and Wijffels, R.H. (2005). Large-scale production of pharmaceuticals by marine sponges: Sea, cell, or synthesis. *Biotechnology and Engineering*, 90, pp. 201–222.

Skar, C.K., and Mortensen, S. (2007). Fate of infectious salmon anaemia virus (ISAV) in experimentally challenged blue mussels (*Mytilus edulis*). *Disease of Aquatic Organisms*, 74, pp. 1–6.

Skiftesvik, A.B., Bjelland, R.M., Durif, C.M.F., Johansen, I.S., and Browman, H.I. (2013). Delousing of Atlantic salmon (*Salmo salar*) by cultured vs. wild ballan wrasse (*Labrus bergylta*). *Aquaculture,* 402–403, pp. 113–118.

Slaney, T.L., Hyatt, K.D., Northcote, T.G., and Fielden, R.J. (1996). Status of anadromous salmon and trout in British Columbia and Yukon fisheries. *Fisheries,* 21(1), pp. 20–35.

Slater, M.J., and Carton, A.G. (2009). Effect of sea cucumber (*Australostichopus mollis*) grazing on coastal sediments impacted by mussel farm deposition. *Marine Pollution Bulletin*, 58, pp. 1123–1129.

Slater, M.J., Jeffs, A.G., and Carton, A.G. (2009). The use of waste from green-lipped mussels as a food source for juvenile sea cucumbers, *Australostichopus mollis*. *Aquaculture,* 292, pp. 219–224.

Smith, M.D., Roheim, C.A., Crowder, L.B., Halpern, B.S., Turnipseed, M., Anderson, J.L., Asche, F., Bourillón, L., Guttormsen, A.G., Khan, A., Liguori, L.A., McNevin, A., O'Connor, M.I., Squires, D., Tyedmers, P., Brownstein, C., Carden, K., Klinger, D.H., Sagarin, R., and Selkoe, K.A. (2010). Sustainability and global seafood. *Science*, 327, pp. 784–786.

Smith, B. (2004). A short history of NFT gully design. *Grow Edge*, 15, pp. 79–82.

Sneed, K., Allen, K., and Ellis, J. (1975). Fish farming and hydroponics. *Aquaculture and the Fish Farmer,* 2, pp. 18–20.

Somerville, C., Cohen, M., Pantanella, E., Stankus, A., and Lovatelli, A. *Small-Scale Aquaponic Food Production. Integrated Fish and Plant Farming*; FAO Fisheries and Aquaculture Technical Paper No. 589; FAO: Rome, Italy, 262p.

Stabili, L., Schirosi, R., Licciano, M., Mola, E., and Giangrande, A. (2010). Bioremediation of bacteria in aquaculture waste using the polychaete *Sabella spallanzanii*. *New Biotechnology*, 27, pp. 774–781.

Stabili, L., Schirosi, R., Licciano, M., and Giangrande, A. (2009). The mucus of *Sabella spallanzanii* (Annelida, Polychaeta): Its involvement in chemical defence and fertilisation success. *Journal of Experimental Marine Biology and Ecology*, 374, pp. 144–149.

Stabili, L., Licciano, M., Giangrande, A., Longo, C., Mercurio, M., Marzano, C.N., and Corriero, G. (2006). Filtering activity of *Spongia officinalis* var. adriatica (Schmidt) (Porifera, Demospongiae) on bacterioplankton: Implications for bioremediation of polluted seawater. *Water Research*, 40, pp. 3083–3090.

Steiner, A.A. (1985) The history of mineral plant nutrition until about 1860 as a source of the origin of soilless culture methods. *Soilless Culture,* 1, pp. 7–24.

Subasinghe, R., Soto, D., and Jia, J. (2009). Global aquaculture and its role in sustainable development. *Reviews in Aquaculture,* 1, pp. 2–9.

Sutton, R.J., and Lewis, W.M. (1982) Further observations on a fish production system that incorporates hydroponically grown plants. *The Progressive Fish Culturist, 44, pp. 55–59*.

Taboada, M.C., González, M., and Rodríguez, E. (2003). Value and effects on digestive enzymes and serum lipids of the marine invertebrate *Holothuria forskali*. *Nutrition Research*, 23, pp. 745–758.

Tacon, A.G.J., Hasan, M.R., and Metian, M. (2011). *Demand and Supply of Feed Ingredients for Farmed Fish and Crustaceans*; FAO Fisheries and Aquaculture Technical Paper No. 564; Food Agriculture Organization: Rome, Italy, p. 87.

Tacon, A.G.J., Metian, M., Turchini, G.M., and De Silva, S.S. (2010). Responsible aquaculture and trophic level implications to global fish supply. *Reviews in Fisheries Science*, 18, pp. 94–105.

Tacon, A.G.J., and Metian, M. (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture,* 285, pp. 146–158.

Tacon, A.G.J., Hasan, M.R., and Subasinghe, R.P. (2006). *Use of Fishery Resources as Feed Inputs for Aquaculture Development: Trends and Policy Implications*; FAO Fisheries Circular No. 1018; Food Agriculture Organization: Rome, Italy, p. 99.

Tacon, A.G.J. (1997). Selected developments and trends: Aquafeeds and feeding strategies. In *Review of the State of World Aquaculture*; Shehadeh, Z., Ed.; UN Food Agriculture Organization: Rome, Italy. Available online: http://www.fao.org/docrep/003/W7499E/w7499e16.htm (accessed on 26 August 2016).

Tal, Y., Schreier, H.J., Sowers, K.R., Stubblefield, J.D., and Place, A.R. (2009). Environmentally sustainable land-based marine aquaculture. *Aquaculture*, 286, pp. 28–35.

Tian, Z.P., Gao, F.M., Sun, S., Liu, S.Q., Zhang, Y.L., and Li, L.X. (1987). Effects of the interculture of *Mytilus edulis* and *Laminaria* on the environment condition. *Transactions of Oceanology and Limnology*, 2, pp. 60–66.

Tilley, D.R., Badrinarayanan, H., Rosati, R., and Son, J. (2002). Constructed wetlands as recirculation filters in large-scale shrimp aquaculture. *Aquacultural Engineering*, 26, pp. 81–109.

Timmons, M.B., and Ebeling, J.M. (2010). *Recirculating Aquaculture*; Cayuga Aqua Ventures LLC: Ithaca, NY, USA, p. 948.

Todd, J. (1980). Dreaming in my own backyard. *The Journal of the New Alchemists,* 6, pp. 108–111.

Traynor, C.H. (2008). *Juncus kraussii* harvesting in Umlalazi nature reserve, KwaZulu-Natal, South Africa: Socio-economic aspects and sustainability. *African Journal of Aquatic Science,* 33, pp. 27–36.

Troell, M., Naylor, R.L., Metian, M., Beveridge, M., Tyedmers, P.H., Folke, C., Arrow, K.J., Barrett, S., Crépin, A., Ehrlich, P.R., Gren, Å., Kautsky, N., Levin, S.A., Nyborg, K., Österblom, H., Polasky, S., Scheffer, M., Walker, B.H., Xepapadeas, T., and de Zeeuw, A. (2014). Does Aquaculture add resilience to the global food system? *Proceedings of the National Academy of Science of the United States of America*, 111, pp. 13257–13263.

Troell, M., Joyce, A., Chopin, T., Neori, A., Buschmann, A.H., and Fang, J.G. (2009). Ecological engineering in aquaculture—Potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. *Aquaculture*, 297, pp. 1–9.

Troell, M., Halling, C., Neori, A., Chopin, T., Buschmann, A.H., Kautsky, N., and Yarish, C. (2003). Integrated mariculture: Asking the right questions. *Aquaculture*, 226, pp. 69–90.

Turcios, A.E., and Papenbrock, J. (2014). Sustainable treatment of aquaculture effluents—What can we learn for the past for the future? *Sustainability*, 6, pp. 836–856.

Tyson, R.V., Treadwell, D.D., and Simonne, E.H. (2011). Opportunities and challenges to sustainability in aquaponic systems. *HortTechnology*, 21, pp. 6–13.

Tyson, R.V., Simonne, E.H., Treadwell, D.D., White, J.M., and Simonne, A. (2008a). Reconciling pH for ammonia biofiltration and cucumber yield in a recirculating aquaponic system with perlite biofilters. *HortScience*, 43, pp. 719–724.

Tyson, R.V., Simonne, E.H., Treadwell, D.D., Davis, M., and White, J.M. (2008b). Effect of water pH on yield and nutritional status of cucumber grown in recirculating hydroponics. *Journal of Plant Nutrition, 31, pp. 2018*–2030.

United States Census Bureau (2017). U.S. and World Population Clock. Available online: http://www.census.gov/popclock/ (accessed on 11th April 2017).

University of Hawaii Aquaponics Workforce Development. (2017). Available online: http://www.hawaiiaquaponicsworkforce.com/faqs-2.html (accessed on 13th February 2017).

Van Bussel, C.G.J., Schroeder, J.P., Wuertz, S., and Schulz, C. (2012). The chronic effect of nitrate on production performance and health status of juvenile turbot (*Psetta maxima*). *Aquaculture*, 326–329, pp. 163–167.

Vandermeulen, H., and Gordin, H. (1990). Ammonium uptake using *Ulva* (Chlorophyta) in intensive fishpond systems: Mass culture and treatment of effluent. *Journal of Applied Phycology*, 2, pp. 363–374.

Van Dyck, S., Gerbaux, P., and Flammang, P. (2009) Elucidation of molecular diversity and body distribution of saponins in the sea cucumber *Holothuria forskali* (Echindoermata) by mass spectrometry. *Comparative Biochemistry and Physiology Part B*, 152, pp. 124–134.

Van Rijn, J., Tal, Y., and Schreier, H.J. (2006). Denitrification in recirculating systems: Theory an applications. *Aquacultural Engineering*, 34, pp. 364–376.

Van Rijn, J. (1996). The potential for integrated biological treatment systems in recirculating fish culture: A review. *Aquaculture*, 139, pp. 181–201.

Verdegem, M.C.J., Bosma, R.H., and Verreth, J.A.J. (2006). Reducing water use for animal production through aquaculture. *Internation Journal of Water Resources Development* 22, pp. 101–113.

Volpe, J., Taylor, E., Rimmer, D., and Glickman, B. (2000). Evidence of natural reproduction of aquaculture-escaped Atlantic salmon in a coastal British Columbia river. *Conservation Biology*, 14, pp. 899–903.

Waller, U., Buhmann, A.K., Ernst, A., Hanke, V., Kulakowski, A., Wecker, B., Orellana, J., and Papenbrock, J. (2015). Integrated multi-trophic aquaculture in a zero-exchange recirculation aquaculture system for marine fish and hydroponic halophyte production. *Aquaculture. International*, 23, pp. 1473–1489.

Watten, B.J., and Sirbrell, P.L. (2006). Comparative performance of fixed film biological filters: Application of reactor theory. *Aquacultural. Engineering,* 34, pp. 193–213.

Webb, J.M., Quintã, R., Papadimitriou, S., Norman, L., Rigby, M., Thoma, D.N., and Le Vay, L. (2012). Halophyte filter beds for treatment of saline wastewater from aquaculture. *Water Res*earch, 46, pp. 5102–5114.

Webster, N.S., and Taylor, M.W. (2012). Marine sponges and their microbial symbionts: Love and other relationships. *Environmental Microbiology*, 14, pp. 335–346.

Wei, S.Q. (1990). Study of mixed culture of *Gracilaria tenuistipitata*, *Penaeus penicillatus*, and *Seylla serrata*. *Acta Oceanologica Sinica*, 12, pp. 388–394.

Weragoda, S.K., Tanaka, N., Sewwandi, B.G.N., and Mowjood, M.I.M. (2010). Efficiency of coconut coir-pith as an alternative substrate in the treatment of submerged macrophyte wetland systems in tropical conditions. *Chemistry and Ecology*, 26, pp. 445-452.

Wijffels, R.H. (2008). Potential of sponges and mircoalgae for marine biotechnology. *Trends in Biotechnology*, 26, pp. 26–31.

Wilson, G. (2005). Seaweed is the common denominator in exciting saltwater aquaponics. *Aquaponics Journal*, 36, pp. 12–16.

Winner, R.W., and Owen, H.A (1991). Seasonal variability in the sensitivity of freshwater phytoplankton communities to a chronic copper stress. *Aquatic Toxicology*, 19, pp. 73–88.

Yokoyama, H. (2013). Growth and food source of the sea cucumber *Apostichopus japonicus* cultured below fish cages—Potential for integrated multi-trophic aquaculture. *Aquaculture*, 372–375, pp. 28–38.

Zamora, L.N., and Jeffs, A.G. (2012). The ability of the deposit-feeding sea cucumber *Australostichopus mollis* to use natural variation in the biodesposits beneath mussel farms. *Aquaculture*, 326–329, pp. 116–122.

Zamora, L.N., and Jeffs, A.G. (2011) Feeding, selection, digestion, and absorption of the organic matter from mussel waste by juveniles of the deposit-feeding sea cucumber, *Australostichopus mollis*. *Aquaculture*, 317, pp. 223–228.

Zhang, S., Li, G., Wu, H., Liu, X., Yao, Y., Tao, L., and Liu, H. (2011). An integrated recirculating system (RAS) for land-based fish farming: The effect on water quality and fish production. *Aquacultural Engineering*, 45, pp. 93–102.

Zhou, Y., Yang, H., Hu, H., Liu, Y., Mao, Y., and Zhou, H. (2006a). Bioremediation potential of the macroalga *Gracilaria lemaneiformis* (Rhodophyta) integrated into fed fish culture in coastal waters of north China. *Aquaculture*, 252, pp. 264–276.

Zhou, Y., Yang, H., Liu, S., Yuan, X., Mao, Y., Liu, Y., Xu, X., and Zhang, F. (2006b). Feeding and growth on bivalve biodeposits by the deposit feeder *Stichopus japonicus* Selenka (Echinodermata: Holothuroidea) co-cultured in lantern nets. *Aquaculture*, 256, pp. 510–520.

Zweig, R.D. (1986). An integrated fish culture hydroponic vegetable production systems. *Aquaculture Magazine*, 12(3), pp. 34–40.

Chapter 2

Assessing *Salicornia europaea* (marsh samphire) as a biofilter of marine aquaculture waste using aeroponics

Abstract

As the global demand for seafood increases, natural fish stocks and available coastal areas for aquaculture are dwindling. The release of untreated saline effluent from aquaculture operations can negatively impact upon wildlife, tourism, and fisheries. Therefore, to meet demand while reducing subsequent environmental impacts, it is necessary to develop intensive inland fish cultures with efficient wastewater treatment systems. Effluent is currently treated through mechanical methods in conventional inland farms; and whilst effective, this approach tends to be costly. Recent research into the agricultural development of halophytes is promising, and studies have shown that *Salicornia europaea* has beneficial medicinal properties, is nutrient rich, and has potential as a biofilter of aquacultural wastewater. This study has assessed the biofiltering capacity of *S. europaea* irrigated with wastewater from an oyster hatchery and cultivated via the aeroponic technique, a hydroponic cultivation method where the roots are suspended in mid-air and irrigated by means of mist or a water sprinkler. Up to 97.65%, 97.14%, 99.02%, and 83.34% of Total Ammonia Nitrogen (TAN), nitrite, nitrate, and orthophosphate respectively were removed from the wastewater per week. While the harvestable plant biomass achieved was low, at 0.25 kg/m^2, yet the potential of *S. europaea*, using aeroponic cultivation techniques, for biofiltration of aquacultural wastewater has been effectively demonstrated.

2.1 Introduction

Our planet is currently experiencing a crisis of dwindling freshwater supplies and salinisation of soil and groundwater (Singh *et al*. 2014; Turcios and Papenbrock, 2014; Ventura & Sagi, 2013). Approximately one-third of the global farmed area (approximately 380 million Ha) is affected by salinity and the freshwater shortage is expected to increase in the future due to a growing world population and rise in prosperity (US Census Bureau, 2017; De Vos *et al*. 2010; Ramani *et al*. 2006). With this in mind, it is essential that new crops be developed that have a greater salt resistance than conventional agricultural crops, especially those that can achieve economically viable yields (Ventura and Sagi, 2013; Ventura *et al*. 2011; Yensen 2006; Glenn *et al*. 1999). One option is to increase the salt-resistance of salt sensitive conventional agricultural crops through conventional breeding programs or by developing genetically adapted plants. However, initial attempts to achieve this have been disappointing (Flowers *et al*. 2010; Flowers and Yeo, 1995; Epstein *et al*. 1980).

Another option is to utilise halophytes for commercial crop production. A halophyte is a naturally evolved salt-resistant plant that has adapted to grow in saline environments, and in some cases require this exposure to salinity to survive (Singh *et al*. 2014; Fan *et al*. 2013; Flowers and Colmer, 2008; Ramani, *et al*. 2006; Zhao *et al*. 2002; Flowers *et al*. 1977). Although the agricultural development of halophytes is in its infancy, studies have already highlighted a number of potential applications for a wide variety of halophytes, such as; fodder, phytoremediation, renewable energy (e.g. biofuels), landscaping ornamentals, food for human consumption, and the treatment of saline aquaculture effluent (Ventura and Sagi, 2013; Koyro *et al*. 2011; Ventura *et al*. 2011; Manousaki and Kalogerakis, 2011; El Shaer, 2010; Zia *et al*. 2008; Eganathan *et al*. 2006; Bustan *et al*. 2005; Brown *et al*. 1999; Glenn *et al*. 1998).

Untreated saline aquaculture effluent has the potential to impact negatively upon wildlife, tourism, and fisheries (Granada *et al*. 2016; Buhmann & Papenbrock, 2013; Webb *et al*. 2012; Grigorakis and Rigos, 2011; Primavera 2006; Zhou *et al*. 2006; Brown *et al*. 1999). Although natural fish stocks and eligible coastal areas for aquaculture are decreasing, global seafood demand is increasing. In order to provide for this demand, while reducing subsequent environmental impacts, it is necessary to develop intensive inland fish cultures with efficient systems for wastewater treatment

(FAO, 2016; Troell *et al*. 2014; Turcios & Papenbrock, 2014; Buhmann & Papenbrock, 2013; Klinger and Naylor, 2012). In conventional inland aquaculture, farm effluent is treated through mechanical methods (e.g. recirculating aquaculture systems). Although these methods are effective, they tend to be costly in terms of capital investment, energy consumption, and maintenance requirements (Badiola *et al*. 2012; Klinger and Naylor, 2012; Webb *et al*. 2012; Martin *et al*. 2010; Timmons and Ebeling, 2010). A number of studies have demonstrated the effectiveness or potential of halophytes as plant biofilters of saline aquaculture effluent, grown hydroponically (Boxman *et al*. 2016; Buhmann *et al.* 2015; Waller *et al*. 2015; Kong and Zheng, 2014), in constructed wetlands (Shpigel *et al*. 2013; Webb *et al*. 2012; Lymbery *et al*. 2006), or under field conditions (Díaz *et al*. 2013).

The genus *Salicornia* L. (Chenopodiaceae) has a long history of being used for human consumption as a result of its appealing salty taste and high nutritional value and has gained significant interest globally as a potential halophyte for commercial cultivation (Ventura and Sagi, 2013; Ventura *et al*. 2011; Lu *et al*. 2010; Mudie *et al*. 2005). Studies on a number of species have found that they have a high salt tolerance level (the extent of this salt tolerance is species specific) (Ventura and Sagi, 2013; Ventura *et al*. 2011; Khan and Gul, 2006), contain chemical constituents that have medical importance (Kang *et al*. 2013; Ahn *et al*. 2011; Manikandan *et al*. 2009), have potential as a forage crop (Swingle *et al*. 1996; Glenn *et al*. 1992), have demonstrated their ability to be effective biofilters of aquacultural wastewater (Buhmann *et al*. 2015; Waller *et al*. 2015; Kong and Zheng, 2014; Diaz *et al*. 2013; Shpigel *et al*. 2013), and their seeds have a high oil and protein content (Zerai *et al*. 2010; Glenn *et al*. 1998; O'Leary and Glenn, 1994).

The *Salicornia* genus is widely dispersed in Eurasia, North America, and South Africa, comprising approximately 25-30 species (Singh *et al*. 2014; Kadereit *et at*. 2007). From a European context, *Salicornia* can be found on much of its coastline, from the Arctic to the Mediterranean and on the shores of the Black and Caspian Sea. It can also be found sporadically where inland saline waters occur across Europe. Much of this distribution can be tentatively attributed to *Salicornia europaea* agg. (Davy *et al*. 2001).

The commercial cultivation of *Salicornia* mainly consists of irrigated field production, drip irrigated raised beds, and sub-surface flow through systems, which require a large amount of space and often do not reuse the irrigation water (Ocean Desert Food, 2017; Ein Mor Crops, 2016; Schalke; 2015; Díaz *et al*. 2013; Ventura and Sagi, 2013;

McGrath, 2010; Abdal, 2009; Clark, 1994; O'Leary *et al*. 1985). Consequently, these species may not be suitable for regions with a limited space and/or supply of water. Although *Salicornia* can be planted at high stock densities (up to 10,000 plants/m^2), these methods are limited to horizontal level production (i.e. surface/ground production) (Webb *et al*. 2013; Ventura *et al*. 2011; Ventura *et al*. 2010).

Salicornia europaea (marsh samphire; common glasswort; glasswort) is the most widely distributed species in the *Salicornia* genus across the UK and Ireland (Botanical Society of Britain and Ireland, 2016; Davy *et al*. 2001). *S. europaea* is a succulent, annual, obligate halophyte with extremely reduced leaves (scale-like formations) and a spike-like terminal inflorescence (Singh *et al*. 2014). It stands erect, up to 35 cm, and is fairly branched. It is dark green in colour, becoming yellowish green and ultimately flushed pink or red towards the end of its life-cycle (Davy *et al*. 2001; Ungar, 1979a; Waisel, 1972). Samphire plants produce minute flowers and under natural conditions usually produce them in August and September. There are usually 1-3 flowers per cyme (an arrangement of flowers in a plant inflorescence), with the lateral flowers (located on the spikes) one to two-thirds as large as the central flower (Devlin, 2015; Singh et al. 2014; Davy *et al*. 2001). *S. europaea* can be found at all levels (low to high) of sandy and/or muddy saltmarshes, in the transitional area of saltmarsh to sand dunes, dune-slacks inundated with the tide, in channels and pans, mudflats, sandflats, and, on occasion, in open saline areas (e.g. behind sea-walls) (National Parks and Wildlife Service, 2014 & 2013; Davy *et al*. 2001; Jefferies *et al*. 1981). In intertidal habitats marsh samphire grows on a range of marine sediments; from silts to fine clays and in gravels and shelly-sand. On occasion, where marsh samphire is found in inland saline environments, the substrates can vary from fine clays to coarse sands. These substrates tend to be saline, brackish, or alkaline (Davy *et al*. 2001) (Figure 1).

Figure 1: *Salicornia europaea* growing on a saltmarsh (outlined in red), Fota Island, Cork, Ireland (*original photo by Gunning*)

Much like other species of the *Salicornia* genus, *S. europaea* has a long history of being used for human consumption (Tuan *et al*. 2015; Kim *et al*. 2012; Price, 2007). Studies have shown it has beneficial medicinal properties (Rad *et al*. 2014; Essaidi *et al*. 2013; Wu *et al*. 2012; Rhee *et al*. 2009; Kong *et al*. 2008; Im *et al*. 2006), oilseed with a high nutritional and medicinal value (Liu *et al*. 2005; Austenfeld, 1986), is nutrient rich (e.g. high levels of Vitamin C, proteins, sugars, and fatty acids) (Essaidi *et al*. 2013; Guil *et al*. 1997) and has potential as a forage crop (Abdal, 2009; Shimizu, 2000). A limited number of studies have also demonstrated that *S. europaea* has potential as a biofilter of aquaculture wastewater (Quintã *et al*. 2015; Webb *et al*. 2013; Webb *et al*. 2012). Constructed wetlands planted with halophytes, including *Salicornia* species, as a means to treat saline aquaculture wastewater are becoming increasingly popular (Webb *et al*. 2013; Webb *et al*. 2012; Buhmann and Papenbrock, 2013; Shpigel *et al*. 2013; Ventura and Sagi, 2013; Calheiros *et al*. 2012; Sousa *et al*. 2011; Lymbery *et al*. 2006; Brown *et al*. 1999; Brown and Glen, 1999). However, CWs require relatively extensive areas of land (only horizontal production is feasible), and would not be suitable in locations where available land is unavailable or expensive. Provided inexpensive land is available, the integration of CWs into on-land aquaculture can be very cost-effective as

they only require moderate capital investment and have low energy consumption and maintenance expenses. They also tend to recirculate the wastewater (Webb *et al*. 2013; Webb *et al*. 2012; Lin *et al*. 2005; Sindilariu *et al*. 2009a; Sindilariu *et al*. 2009b). Although there are studies that utilise hydroponic techniques for the biofiltration of saline aquaculture wastewater and/or cultivation of halophytes, including *Salicornia* species (Boxman *et al*. 2016; Buhmann *et al*. 2015; Waller *et al*. 2015; Kong and Zheng; 2014; Ventura *et al*. 2011; Ventura *et al*. 2010), there are none that specifically utilise aeroponics. Aeroponics is a cultivation technique in which the plant's roots are suspended in mid-air and water and essential nutrients are supplied by means of an aerosol mist or water sprinkler bathing the roots, which facilitates the oxidation of ammonia to nitrite and nitrate. Aeroponic systems reuse the nutrient solution and allow for horizontal and/or vertical production, subsequently, these systems are ideal for locations with a scarce water supply and/or space limitations (Lakkireddy *et al*. 2012; Ghaly *et al*. 2005; Jones, 2005; Christie and Nichols, 2004; Barak *et al*. 1996; Nir, 1982). Aeroponic systems have also shown the ability to achieve higher yields than conventional production methods for non-halophytic plants (Movahedi *et al*. 2012; Nir, 1982).

The aim of this study was to: (1) Assess the suitability of aeroponics as a cultivation technique for *S. europaea;* (2) Determine what medium is suitable for cultivating *S. europaea* in an aeroponic propagator; (3) Assess the effectiveness of *S. europaea* as a biofilter of aquaculture waste using aeroponics as the cultivation technique.

2.2 Methods and Materials

In Trial 1 the use of four different medium types for cultivating *S. europaea* in an aeroponic propagator were assessed. Two of these mediums are commonly utilised in hydroponic systems (coconut coir and clay pebbles) and two were conventional mediums (a medium consisting of a mixture of sand and soil at a ratio of 50:50 and a 100% soil medium). Each medium type utilised in the aeroponic propagators was compared to a control (i.e. the same medium in combination with conventional manual irrigation methods). In trial 2, the growth success and biofiltering capabilities of *S. europaea* cultivated in aeroponic systems irrigated with varying salinities (0, ~11, & ~31 ppt) of wastewater from an Irish oyster hatchery, were evaluated.
S. europaea seeds for both trials were sourced from Victoriana Nursery Gardens, Kent, England (https://www.victoriananursery.co.uk/). For each trial, seeds were considered germinated when the radicle was at least 2 mm long (Zhao *et al*. 2016; Boestfleisch *et al*. 2014; Jha *et al*. 2012; Rueda-Puente *et al*. 2003).

Trial 1: Cultivation of *Salicornia europaea* using aeroponics, a preliminary trial assessing various medium types

Prior to the commencement of the aeroponic trial, the germination of *S. europaea* was attempted on three occasions before a successful methodology was found. It was discovered that a stratification treatment was required for the successful germination of the *S. europaea* seeds that were obtained for this trial (source: Victoriana Nursery, Kent, England) (see appendix Chapter 2 for more detail on these germination attempts).
Following the identification of a successful germination method for *S. europaea,* trial 1 took place at University College Cork (UCC) from 16th May to 5th September 2014.

Pre trial seed development

Pre germination stratification

S. europaea seeds (n=50) were distributed equally amongst five 90 mm petri dishes (n=10 per dish) containing 90 mm filter paper. A 0.5 l mist-spray watering bottle was used to dampen the filter paper (approximately 0.5 ml of freshwater was required per

petri dish). The lids of the petri dishes were taped shut and the petri dishes were then placed in a dark refrigerator at 5 ± 0.45 °C. The seeds were checked on a daily basis. If mould was present on the seeds, it was gently removed with a small paintbrush. If a large amount of mould had formed within the petri dish, the mould was removed and the filter paper was replaced. The filter paper was kept damp throughout the stratification period and required dampening approximately every 3-4 days. The stratification stage lasted 30 days (Keiffer and Ungar, 1997; Keiffer *et al*. 1994; Philipupillai & Ungar, 1984; Grouzis *et al*. 1976).

Early germination

Following removal from the fridge, the petri dishes were kept indoors at the plant biology laboratory, UCC, under natural light and ambient temperature conditions (approximately 15.5 h days/8.5 h nights; mean temperature: 20 ± 1.3 °C) for 10 days. Salinity was introduced at this stage by dampening the filter paper with approximately 0.5 ml of 10 ± 0.34 ppt saltwater (30:70 seawater:freshwater) (Webb *et al*. 2013; Webb *et al*. 2012; Lv *et al.* 2012; Aghaleh *et al*. 2009; Keiffer *et al*. 1994). The petri dishes were checked daily for mould formation and to see if their respective medium required more saline solution. If mould was developing, it was removed with a small paintbrush. Each petri dish was kept damp by adding 0.5 ml of the above solution when the filter paper appeared dry (required every 3-4 days for the duration of this stage).

Seedling development

This stage took place in a greenhouse at UCC (natural light - approximately 15.5 h days/8.5 h nights; ambient temperature - mean 21.06 ± 2.74 °C). The seedlings were transferred with a fine brush from the petri dishes to two seed trays (36.5cm x 22.8 cm x 5.3 cm) (n=25 per tray) containing a 50:50 sand:soil medium (Shamrock® multi-purpose compost & Hortland® horticultural sand). Each seed tray was placed on top of a watering tray. The watering trays were filled with a 10 ± 0.5 ppt saltwater (30:70 seawater:freshwater)/phostrogen (1ml/l) (Bayer CropScience Ltd; NPK: 14:10:27) solution every 3-4 days (the watering tray was refilled when the seed tray was no longer in contact with the water in the watering tray). The seedling development stage lasted for 30 days (Webb *et al*. 2013; Webb *et al*. 2012; Lv *et al.* 2012; Aghaleh *et al*. 2009; Keiffer *et al*. 1994).

Experiment: Seedling on-growing in aeroponic propagators

The on-growing stage took place in a greenhouse (not temperature or light controlled) at UCC (natural light - approximately 14h day/10h night; ambient temperature - mean 21.4 ± 2.3 °C) for 42 days, until the plants began to produce flowers and cease growth.

General setup

The most successful seedlings (n=24; mean height: 9.16 ± 1.05 cm) were selected and divided evenly into eight treatments based on medium type and cultivation method as follows:

Treatment 1-4 (aeroponic cultivation):

- Treatment 1: coconut coir (Jiffy® coco pellets; Figure 2)
- Treatment 2: 50:50 sand:soil mixture (Shamrock® multi-purpose compost & Hortland® horticultural sand)
- Treatment 3: clay pebbles (Gold Label®)
- Treatment 4: 100% soil (Shamrock® multi-purpose compost)

Control 1-4 (cultivation in pots on watering trays):

- Control 1: coconut coir (Jiffy® coco pellets)
- Control 2: 50:50 sand:soil mixture Shamrock® multi-purpose compost & Hortland® horticultural sand)
- Control 3: clay pebbles (Gold Label®)
- Control 4: 100% soil (Shamrock® multi-purpose compost)

The seedlings (n=24) were removed from the seed trays by pushing a blunt wooden stick through the medium adjacent to each seedling until it reached the bottom of the tray. The stick was then manoeuvred under the roots of the seedling, and pushed upwards in order to carefully remove the seedling, with roots intact. The roots of each seedling were gently rinsed with 10 ppt saltwater solution to remove the medium.

For the aeroponic treatments (T1-4), seedlings (n=12) were then transplanted into individual plastic net pots (50 mm height; 50 mm ID; 55 mm OD; Figure 2) containing one of four different medium types (n=3 seedlings per medium). Each pot was randomly placed (randomised with Excel) in a slot of the supporting tray of the

Nutriculture® X-stream 12 plant site aeroponic propagator (59 x 49 x 51 cm; Figure 3, 4, and 5a).

For the control treatments (C1-4), seedlings (n=12) were transplanted into individual plastic flower pots (9 cm height; 9 cm top diameter; 6.5 cm base diameter) containing one of four different medium types (n=3 seedlings per medium) and placed, randomly (randomised with Excel), on a water tray (Figure 5b). A small hole was dug into the centre of the respective medium in each treatment with the blunt end of pen/small paintbrush, and the roots gently lowered in.

For both the aeroponic and control treatments, pots that contained the 50:50 sand:soil mixture or the soil, were lined with 1.3 mm polyethylene mesh to prevent sand or soil entering the water reservoir of the propagator. For pots that contained coconut coir, a small hole was created in the coir netting at the bottom of the medium to ensure the roots could pass through.

Figure 2: a) Coconut coir discs prior to hydration (right) and fully hydrated (left); b) clay pebbles; and c) plastic net pot (50mm height; 50mm ID; 55mm OD) (*original photos by Gunning*)

a)

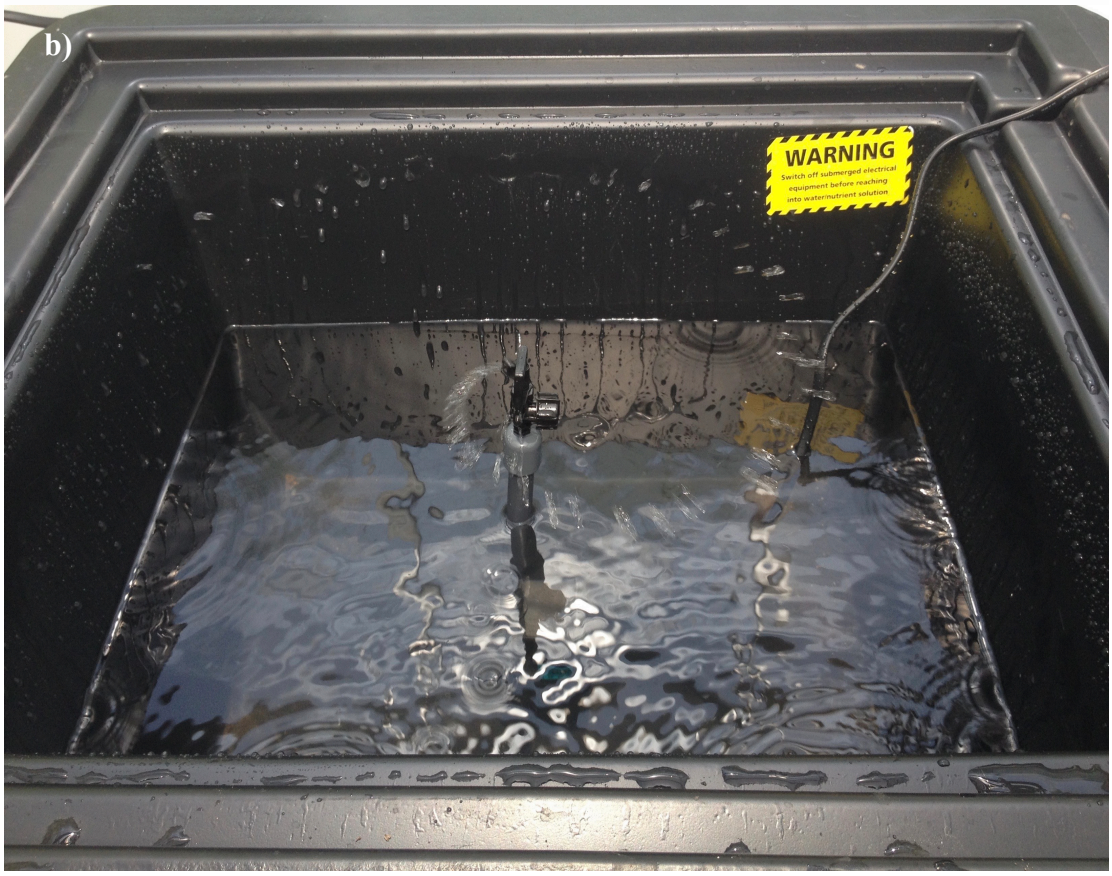


Figure 3: a) Nutriculture® X-stream 12 plant site aeroponic propagator (59cm x 49 cm x 51 cm) (*photo courtesy of Britcropshydroponics Ltd*); b) water sprinkler system in water reservoir of aeroponic propagator (*photo courtesy of Gunning*)

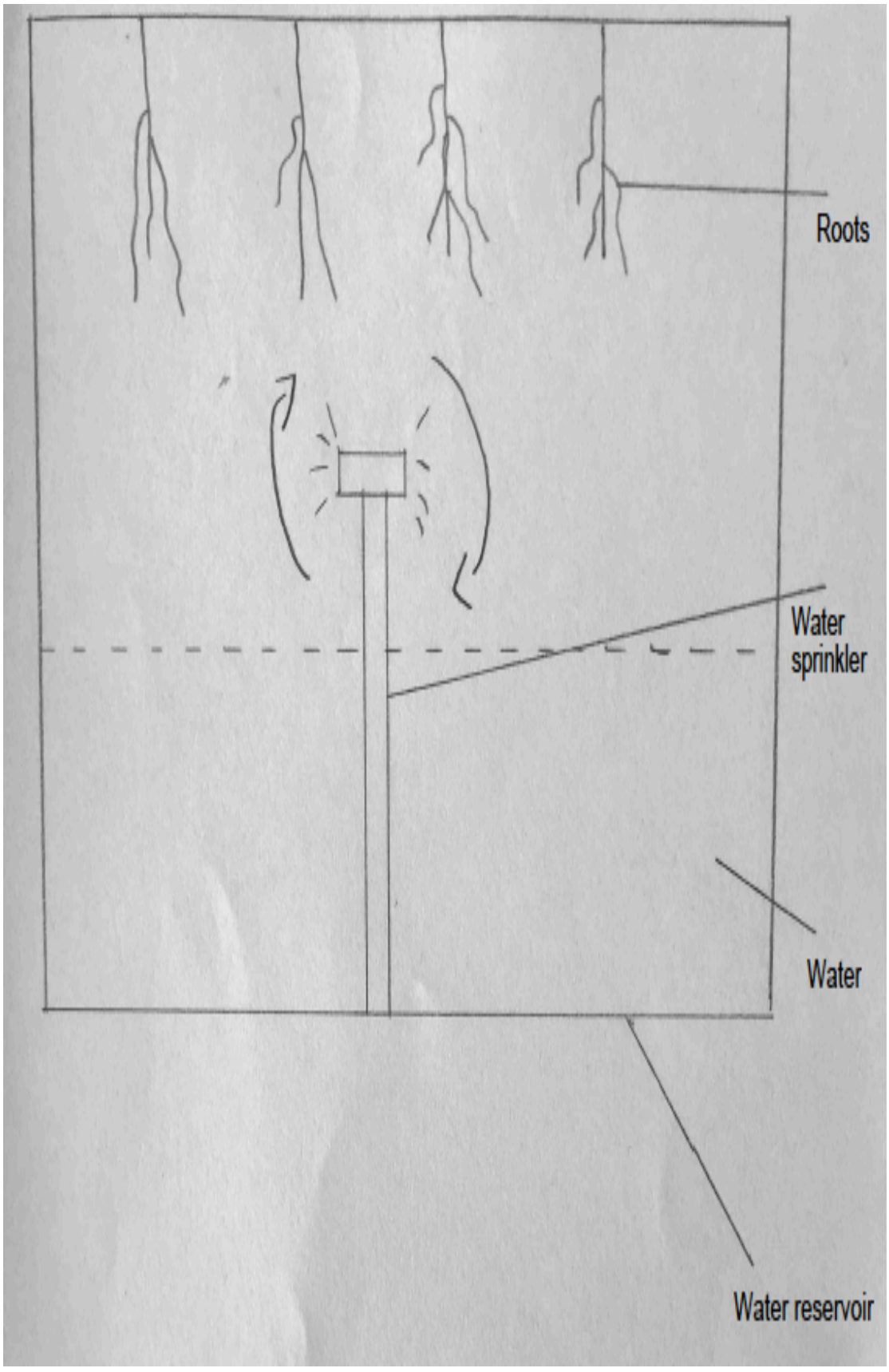


Figure 4: Schematic of the aeroponic propagator's water reservoir and water sprinkler system

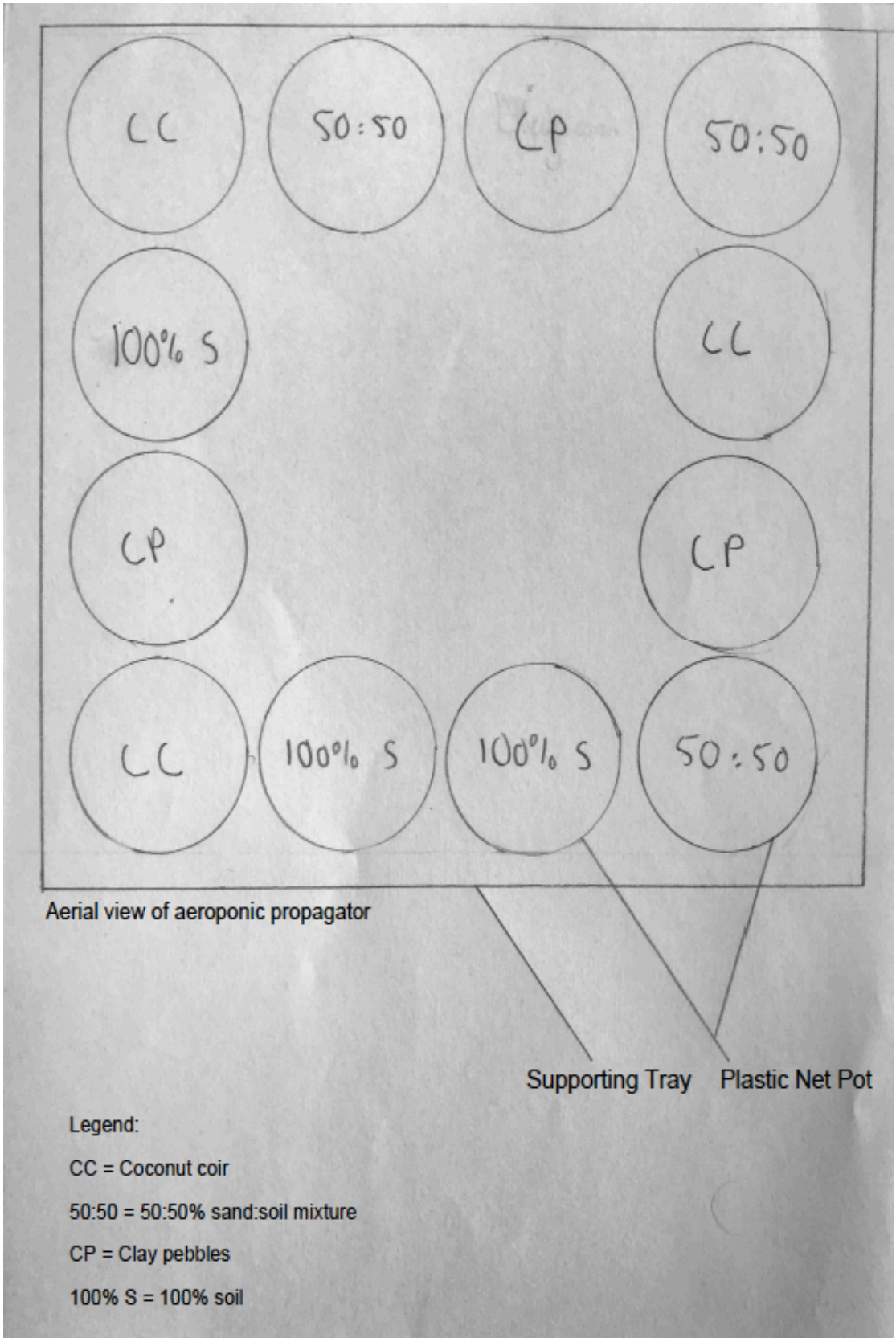


Figure 5a: Schematic of aerial view of aeroponic propagator (aeroponic treatments) detailing the randomised positioning of the four medium types

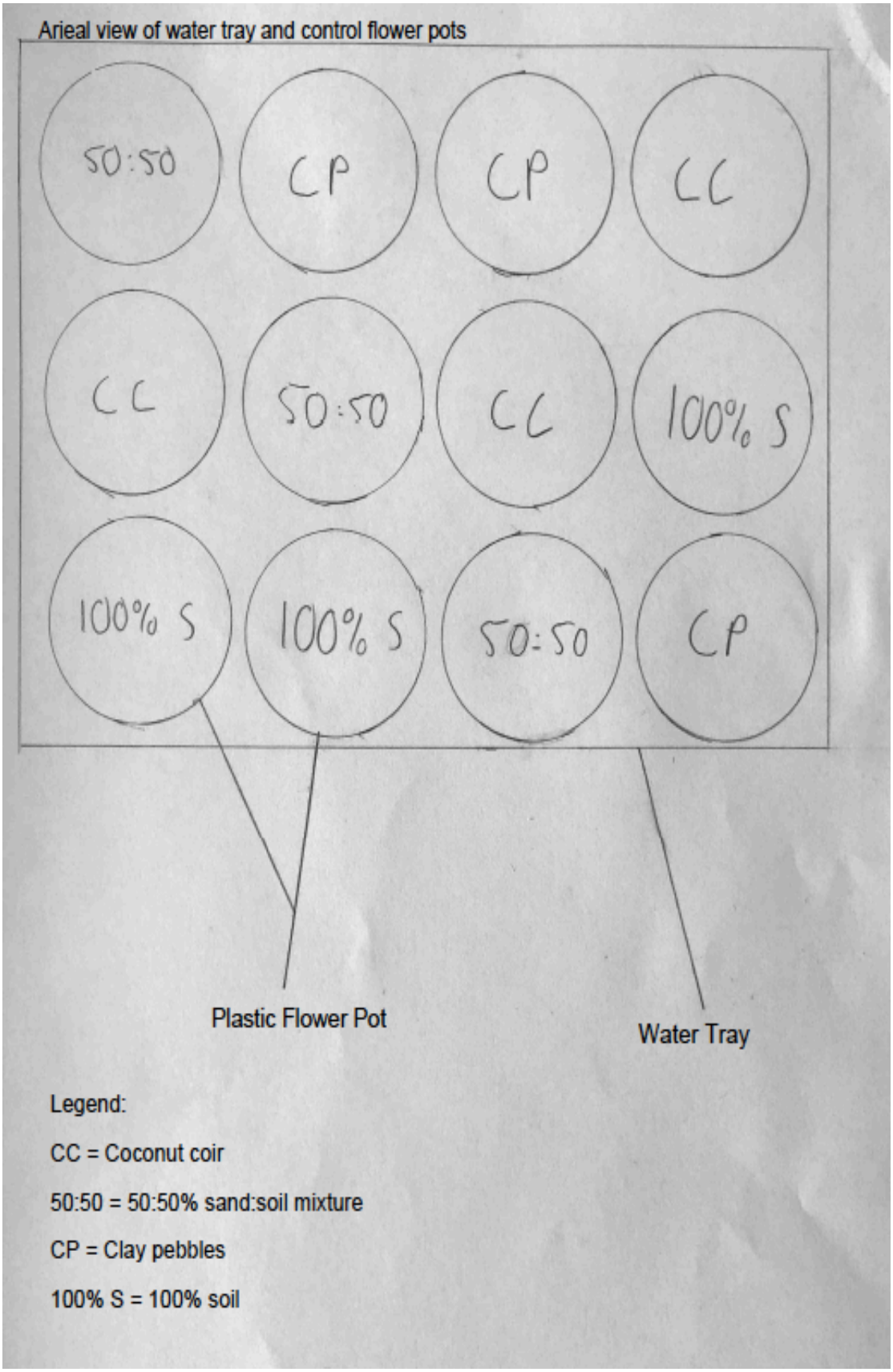


Figure 5b: Schematic of aerial view of watering tray (control treatments) detailing the randomised positioning of the four medium types

Irrigation regime

(1) Aeroponic treatment: the reservoir tank (below supporting tray) was filled with 15l of 10 ± 1.87 ppt saltwater/phostrogen (1 ml/l; NPK: 14:10:27) solution. Additional phostrogen plant feed was added once per week. A complete water change of the tank occurred once, halfway through this on-growing stage.

(2) Control treatment: the water tray was filled with a 10 ± 0.56 ppt saltwater/phostrogen (1 ml/l; NPK: 14:10:27) solution which was replaced every 3-4 days (when the water in the gardening tray was no longer in contact with the bottom of the flower pots).

Growth monitoring

The height (cm) and number of nodes and branches of each plant was recorded every 4-7 days throughout this stage.

Trial 2: Aeroponic cultivation and biofiltering assessment of *Salicornia europaea* irrigated with oyster hatchery wastewater

Trial 2 took place from the 13th May to the 18th September 2015 at the Tralee Bay Oyster Hatchery, Co. Kerry.

Pre germination stratification

S.europaea seeds (n=900) were distributed equally amongst 36 90 mm petri dishes (n=25 per dish) and given a 30 day stratification treatment at 5 ± 0.56 °C (Keiffer and Ungar, 1997; Keiffer *et al*. 1994; Philipupillai and Ungar, 1984; Ungar, 1979b; Grouzis *et al*. 1976).

Early germination

After the 30 day stratification stage, the petri dishes were moved indoors (oyster hatchery water assessment laboratory) and kept under natural light and ambient temperature conditions (approximately 18h days and 6h nights; mean temperature: 17 °C ± 0.54). The filter paper from each petri dish was replaced and any mould formation removed. Salinity was introduced at this stage by dampening the filter papers with approximately 0.5 ml of 10.13 ± 1.2 ppt saltwater using a 0.5 L spray bottle (30:70 seawater:freshwater) (Webb *et al*. 2013; Webb *et al*. 2012; Lv *et al.* 2012; Aghaleh *et al*. 2009; Keiffer *et al*. 1994). For a period of two weeks the petri dishes were checked daily for mould and to see if the filter paper required dampening. If mould formation was occuring it was removed with a small paintbrush. Each petri dish was kept damp by adding 0.5ml of the above solution when the filter paper appeared dry (required every 3-4 days for the duration of this stage). After two weeks, 675 seedlings (25% of the seeds did not germinate) were evenly distributed amongst three different treatment groups (9 petri dishes per treatment, with 25 seeds per dish):

- Treatment 1: 33.33% saline wastewater : 66.66% freshwater (salinity: 10.5 ± 0.50 ppt)
- Treatment 2: 66.66% saline wastewater : 33.33% freshwater (salinity: 20.78 ± 0.69 ppt)
- Treatment 3: 100% saline wastewater: 0% freshwater (salinity: 30.90 ± 1.01 ppt)

These treatments were chosen to assess what percentage of wastewater (i.e. nutrient content) and salinity level *S. europaea* would grow most successfully at. For one week

each treatment was given their respective solution (c.0.5 ml) when the filter paper appeared to be dry.

Seedling development

The seedlings from each treatment were then transferred evenly, using a fine brush, in to 36.5x22.8x5.3 cm seed trays (3 seed trays per treatment), with approximately 2-3cm distance between each seedling. The three seed trays from each treatment were placed in their own 79x41x4.6 cm garden tray containing the respective treatment solution. For this development stage all treatment trays were moved to a mobile polyethylene greenhouse (mean temperature: 21.79 ± 4.55 °C; Figure 6) that had a natural light cycle of approximately 15-17 h light and 7-9 h dark for the duration of the stage (28 days). The seedlings were checked on a daily basis, and the respective treatment solution added to the garden tray if required (when the water is no longer in contact with the base of the seed tray). Mortality rates and measurements (height {cm}, number of nodes and branches) of successful seedlings were monitored on the final day of this stage.

Figure 6: Polyethylene mobile greenhouse (natural light and ambient temperature) used for seedling development stage (*original photos by Gunning*)

On-growing (aeroponics)

One hundred and twenty seedlings from each treatment were transferred to individual Nutriculture® X-stream 120 plant site aeroponic propagators (one propagator per treatment) (115 cm x 64.5 cm x 46 cm; Figure 7) which were positioned outside (Each propagator had a lid to protect the seedlings from the elements). For each treatment, transferred seedlings were equivalent to the size distribution (mean height ± SD) of all seedlings present at the end of the seedling development stage.

For each treatment, individual seedlings were transferred to plastic net pots (50 mm height; 50 mm ID; 55 mm OD; Figure 2) containing coconut coir. The positioning of each seedling within the aeroponic propagator was completely randomised with Microsoft Excel.

The bottom reservoir of the aeroponic propagators contained 60 L of the following:

Treatment 1: 33.33% saline wastewater: 66.66% freshwater (salinity: 11.1 ± 0.2 ppt)
Treatment 2: 66.66% saline wastewater: 33.33% freshwater (salinity: 21.5 ± 1.3 ppt)
Treatment 3: 100% saline wastewater: 0% freshwater (salinity: 31.6 ± 0.8 ppt)

The treatment solution in each propagator was replaced every 7 days. Triplicate samples of the treatment solution from the bottom reservoir of each propagator were taken from when it was first added to the propagators and again after 7 days (before the water was replaced) on four separate occasions (week 3, 4, 5, and 6 of this stage). These samples were sent to the Aquatic Services Unit, Environmental Research Institute, UCC (EPA accredited) for total ammonia nitrogen (TAN), nitrite, nitrate, and orthophosphate analysis. The aeroponic propagators (mean temperature: 24.32 ± 7.24 °C) were positioned outside, so received natural sunlight (c. 12.5-15 h light and 9-11.5 h dark). Growth parameters (height {cm}, number of nodes, and number of branches) were measured for each seedling of each treatment after 8, 27, 42, and 49 (final day of trial) days. On the last day of the trial, the weight (g) of each plant was measured. The lid remained on the propagators for the duration of the trial, only being removed when measuring the seedlings, checking on their condition, and replacing the water. The butterfly flap in the cover of the system was left open during the day to allow for greater air circulation (closed when raining) and was closed at night to limit the reduction in temperature. During periods of heavy rain, the gap between the lid and the propagator

was sealed with waterproof tape to ensure rainwater did not enter the reservoir. The trial was ended after 49 days when the plants had begun to flower.

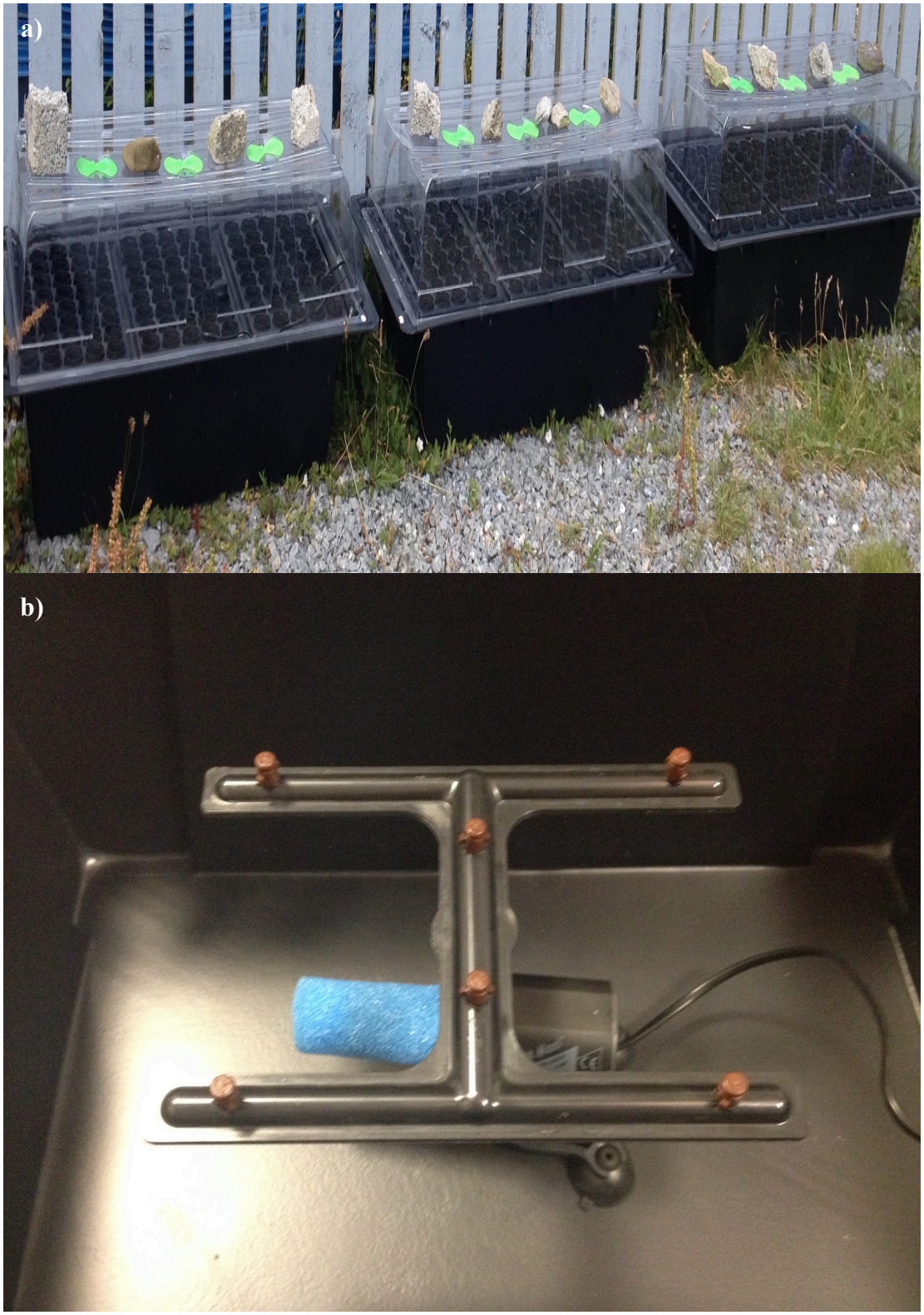


Figure 7: a) 120-slot aeroponic propagators (115cm x 64.5cm x 46cm) positioned outdoors at the Tralee Oyster Hatchery, Co. Kerry; and b) the water sprinkling system in the water reservoir of each 120-slot propagator (*original photos by Gunning)*

Statistical analysis

All statistical analysis was compiled using SPSS software (IBM) version 23. All data was tested for normal distribution and homogeneity of variance with the Shapiro-Wilk test and Levene's test, respectively ($p>0.05$). P-values less than 0.05 were considered statistically significant.

(a) Trial 1

One-way ANOVAs were used to test the significance of differences in the number of nodes and branches and height between aquaponic medium treatments and between control treatments. When equal variance could not be assumed, a Welch test was used. Tukey HSD post-hoc analyses were conducted when ANOVAs gave a statistically significant result ($p<0.05$). For data that was not normal, Kruskal-Wallis tests were used.

Independent t-tests were used to test the significance of differences in the number of nodes and branches and height between each individual aquaponic medium treatment and its respective control treatment. If the groups' variance were unequal, an adjustment was made to the degrees of freedom using the Welch-Satterthwaite method. For data that was not normally distributed, Kruskal-Wallis tests were used.

(b) Trial 2

Kruskal-Wallis tests were used to test the significance of difference in the number of nodes and branches and height (cm) between treatments during the greenhouse and aeroponic stages of the trial. Kruskal-Wallis tests were also used to test the significance of difference of the mean weight (g) between treatments on the last day of the trial. Paired t-tests were used to test the significance of difference in water parameters (i.e. TAN, nitrite, nitrate, and orthophosphate) at the beginning and end of each monitored week for each individual treatment. For data that was not normal, a Wilcoxon signal-rank test was used.

2.3 Results

Trial 1

Aeroponic treatments (Treatment 1-4):

Over the 42 days of this trial, there was no significant difference between the number of nodes and branches of plants grown aeroponically in four different mediums (T1-4) ($p>0.05$) (Figure 8). In terms of height, there was a significant difference on day 7 between the coconut coir (T1) and clay pebble (T3) mediums (T1 > T3; $p<0.05$) and between the coconut coir (T1) and 100% soil (T4) mediums (T1 > T4; $p<0.05$), on day 14 between all treatments ($p<0.05$, $p<0.01$, and $p<0.001$), on day 21 between the coconut coir (T1) and clay pebble (T3) (T1 > T3; $p<0.05$), coconut coir (T1) and 100% soil (T4) (T1 > T4; $p<0.01$), and 50:50 sand:soil (T2) and 100% soil (T4) (T2 > T4; $p<0.05$) mediums, and on day 25 between the coconut coir (T1) and 100% soil (T4) (T1 > T4; $p<0.01$), 50:50 sand:soil (T2) and 100% soil (T4) (T2 > T4; $p<0.05$), and clay pebble (T3) and 100% soil (T4) (T3 > T4; $p<0.05$) mediums (Figure 8).

On the final day of the trial (day 42 of on-growing) there was no significant difference between the number of nodes, number of branches, and height of plants grown aeroponically on different media types ($p>0.05$) (Figure 8).

Control treatments (Control 1-4):

There was a mortality rate of 33% (1 plant) for treatment 7 (clay pebbles). After this plant died (15th August), the average of the remaining two plants was reported. Over the 42 days of this trial, there was no significant difference between the number of nodes, number of branches and height of plants grown in each control treatment ($p>0.05$) (Figure 9).

Aeroponic vs. control treatments:

In the majority of cases, there was no significant difference ($p>0.05$) between aeroponic and control (cultivated in hand-watered pots) treatments when comparing the number of nodes and branches. However, there was a significant difference in the number of nodes and branches on day 42 between plants cultivated aeroponically and in pots

(control) in the case of 50:50 sand:soil (T2 > C2; p,0.05) and soil (T4 > C4; $p<0.05$) mediums, respectively.

In relation to height, there was a significant difference between the aeroponic and control cultivation of plants grown in coconut coir (T1 > C1) on day 14, 25, 32, 38, and 42 ($p<0.05$ and $p<0.01$), between plants cultivated in 50:50 sand:soil (T2 > C2) on day 25, 32, 38, and 42 ($p<0.05$), between plants cultivated in clay pebbles, (T3 > C3) and plants cultivated in 100% soil (T4 > C4) on day 32 and 42 ($p<0.05$ and $p<0.01$).

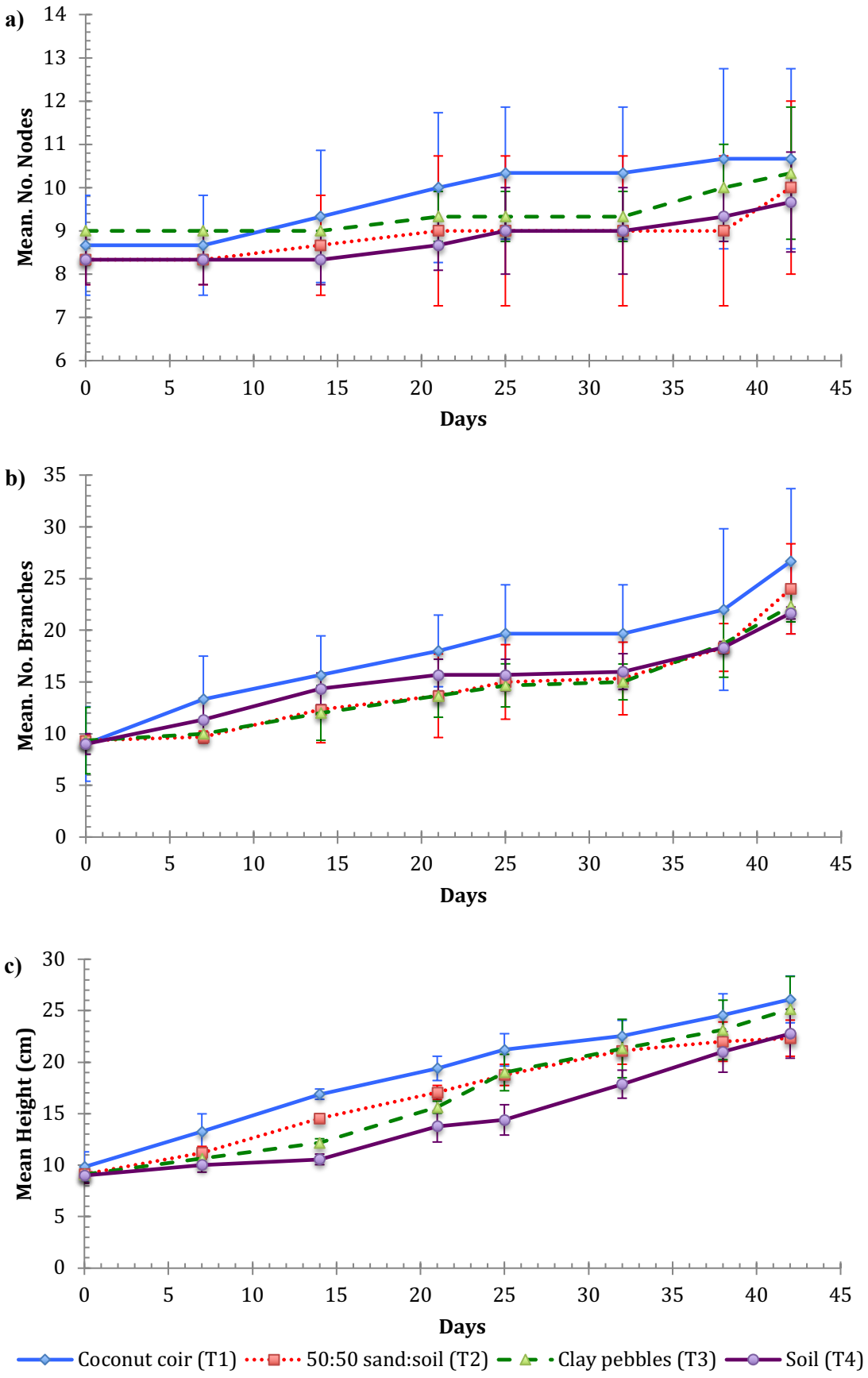


Figure 8: Mean; a) number of nodes; b) number of branches; and c) height of plants from the aeroponic treatments (T 1-4) (mean ± SD; Trial 1)

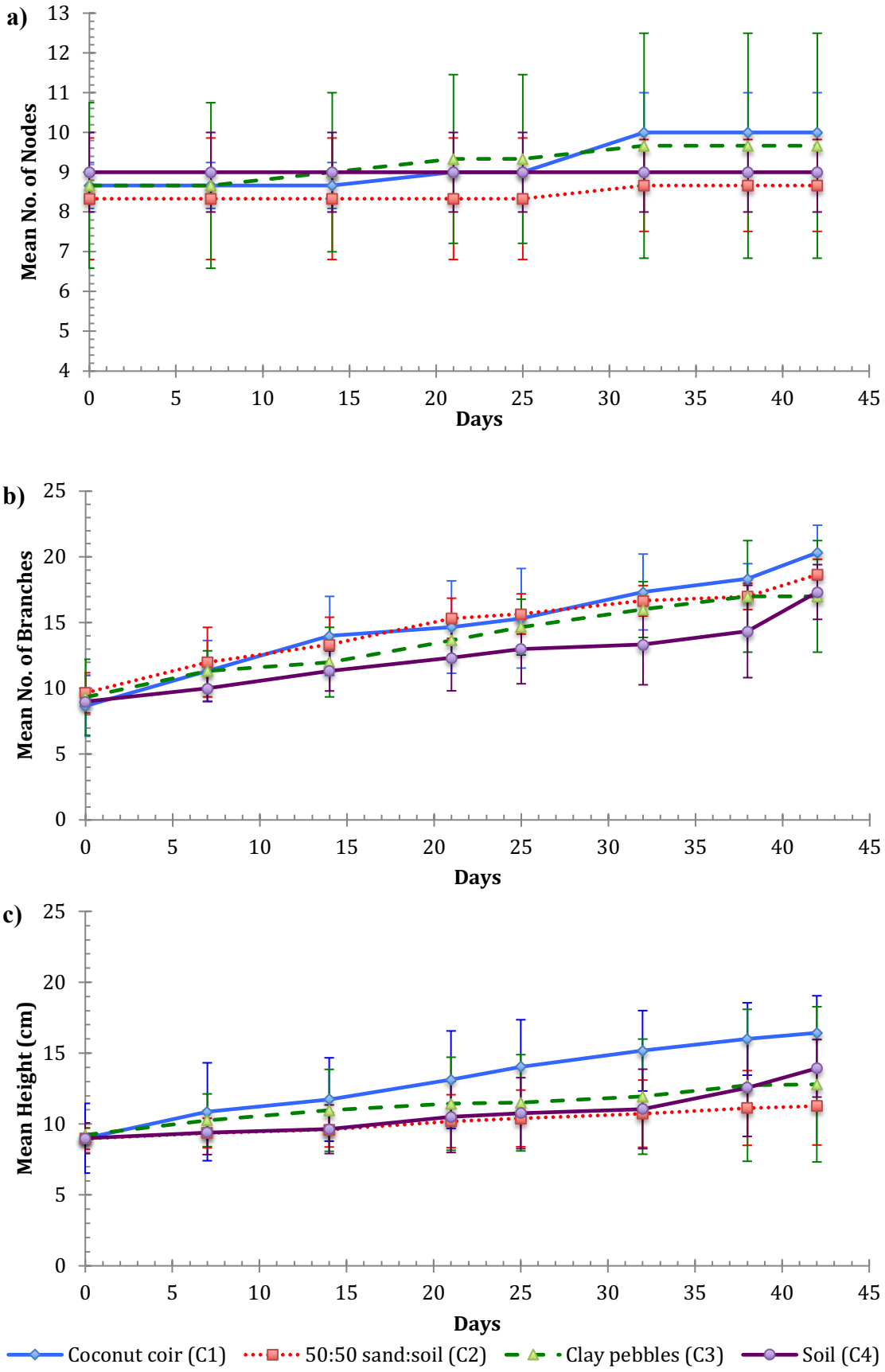


Figure 9: Mean; a) number of nodes; b) number of branches; and c) height of plants from the control treatments (C 1-4) (mean ± SD; Trial 1)

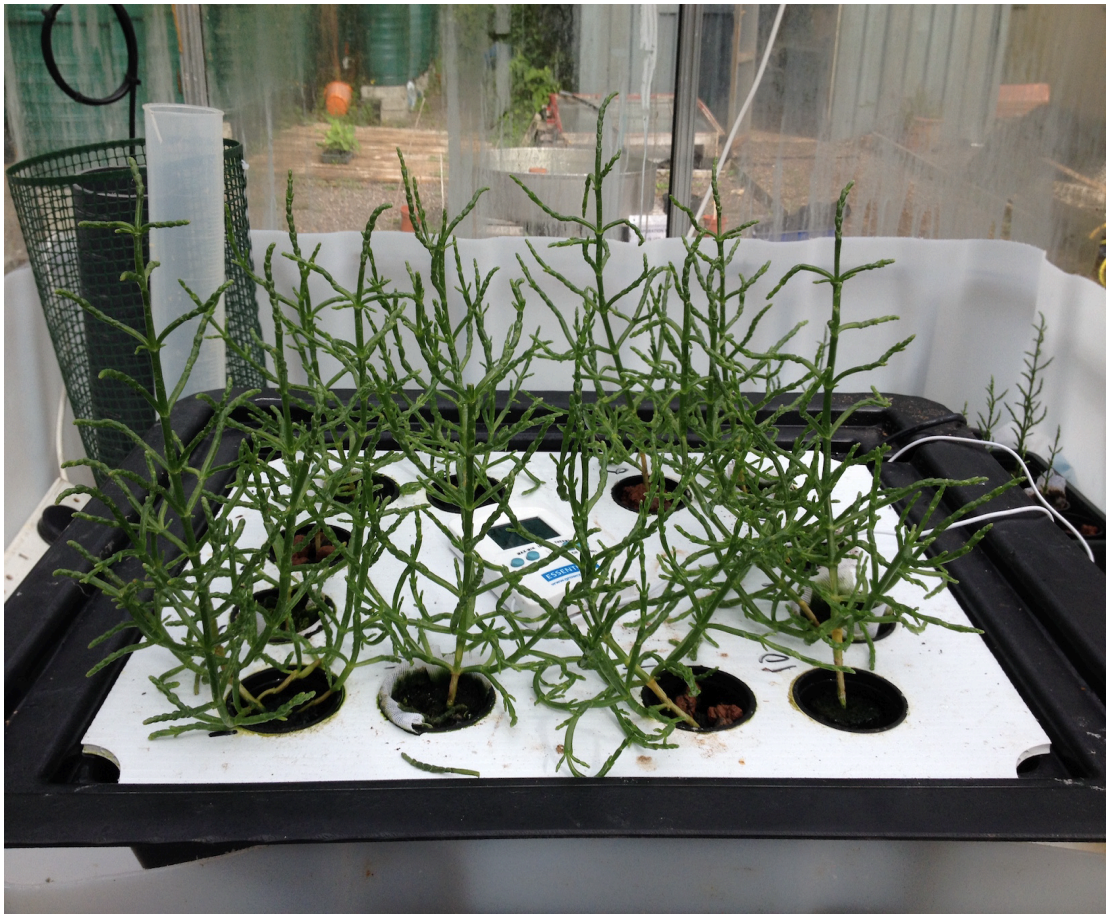


Figure 10: *S. europaea* plants (aeroponic unit) at the end of the 42 day trial (*original photo by Gunning*)

Trial 2

Plant growth

Seedling development stage

By the end of the seedling development stage, treatment 1 (33.33% saline wastewater: 66.66% freshwater; 11.1 ± 0.2 ppt), treatment 2 (66.66% saline wastewater: 33.33% freshwater; 21.5 ± 1.3 ppt), treatment 3 (100% saline wastewater: 0% freshwater; salinity: 30.90 ± 1.01 ppt) had a mortality rate of 8%, 15.6%, and 29.3%, respectively (mortalities were excluded from the mean data). The mean number of nodes and branches and mean height (2.74 ± 1.29; 1.87 ± 1.55; 1.89 ± 1.17 cm) for treatment 1 were significantly larger than treatment 2 (2.47 ± 0.9; 0.26 ± 0.68; 1.35 ± 0.82 cm; $p<0.01$ or $p<0.001$) and treatment 3 (2.06 ± 0.7; 0.06 ± 0.3; 0.84 ± 0.51 cm; $p<0.001$). The difference between the mean number of nodes and branches and mean height of treatment 2 and 3 was also significant ($p<0.001$).

On-growing (aeroponics) stage

There was a significant difference in growth measurements between the following treatments on the following days:

Nodes:

- Treatment 1 vs. 2: day 8 (T1 > T2; $p<0.001$).
- Treatment 1 vs. 3: day 0 (T1 > T3; $p<0.05$), day 8 (T1 > T3; $p<0.05$), day 27 (T1 > T3; $p<0.001$), day 42 (T1 > T3; $p<0.001$), and day 48 (T1 > T3; $p<0.001$).
- Treatment 2 vs. 3: day 8 (T2 > T3; $p<0.001$), day 27 (T2 > T3; $p<0.001$), day 42 (T2 > T3; $p<0.001$), and day 48 (T2>T3; $p<0.001$).

Branches:

- Treatment 1 vs. 2: day 0 (T1 > T2; $p<0.05$), day 8 (T1 > T2; $p<0.05$), and day 27 (T1 > T2; $p<0.001$).
- Treatment 1 vs. 3: day 0 (T1 > T3; $p<0.001$), day 8 (T1 > T3; $p<0.001$, day 27 (T1 > T3; $p<0.001$), day 42 (T1 > T3; $p<0.001$), and day 48 (T1 > T3; $p<0.001$).
- Treatment 2 vs. 3: day 0 (T2 > T3; $p<0.001$), day 8 (T2 > T3; $p<0.001$), day 27

(T2 > T3; p<0.001), day 42 (T2 > T3; p<0.001), and day 48 (T2 > T3; p<0.001).

Height:

- Treatment 1 vs. 2: day 0 (T1 > T2; p<0.05), day 8 (T1 > T2; p<0.01), and day 27 (p<0.001).
- Treatment 1 vs. 3: day 0 (T1 > T3; p<0.001), day 8 (T1 > T3; p<0.001), day 27 (T1 > T3; p<0.001), day 42 (T1 > T3; p<0.001), and day 48 (T1 > T3; p<0.001).
- Treatment 2 vs. 3: day 0 (T2 > T3; p<0.001), day 8 (T2 > T3; p<0.001), day 27 (T2 > T3; p<0.001), day 42 (T2 > T3; p<0.001), and day 48 (T2 > T3; p<0.001).

By the end of the aeroponic stage (end of trial), the mean number of nodes of plants on treatment 1 (6.12 ± 2.18) was significantly larger compared to those on treatment 3 (4.46 ± 1.72; p<0.001). The mean number of branches of plants on treatment 1 (10.78 ± 5.02) was significantly larger than for those on treatment 3 (6.97 ± 4.60; p<0.001). The mean height of plants on treatment 1 (9.71 ± 4.39 cm) was also significantly larger than for plants on treatment 3 (6.06 ± 3.37 cm; p<0.001) (Figure 11). Treatment 1 gave a significantly larger mean harvestable (mortalities excluded; roots excluded) biomass (1.91 ± 0.63 g/plant) than treatment 2 (1.65 ± 0.51 g/plant; p<0.01) and treatment 3 (0.83 ± 0.27 g/plant; p<0.001) (note: T2 vs, T3; p<0.001) (Figure 12). By the end of this stage/trial treatment 1, 2, and 3 had a mortality rate of 19.2%, 9.2%, and 34.2%, respectively (the mean number of nodes and branches and mean height of mortalities were included in the data post mortality as the last level reached prior to expiring).

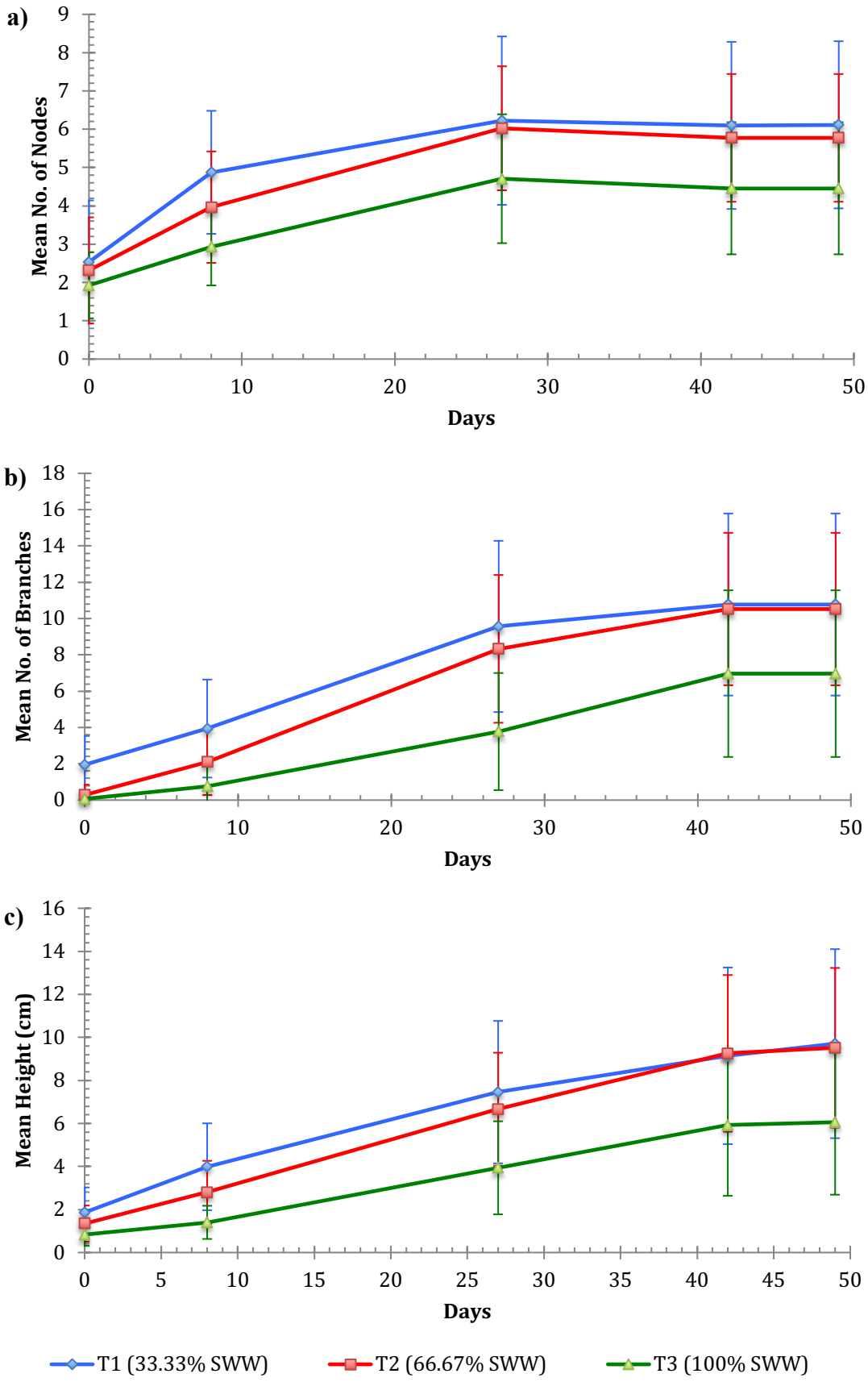


Figure 11: Mean; a) number of nodes; b) number of branches; and c) height of plants over the course of the aeroponic growth stage (mean ± SD; SWW = saline wastewater; Trial 2)

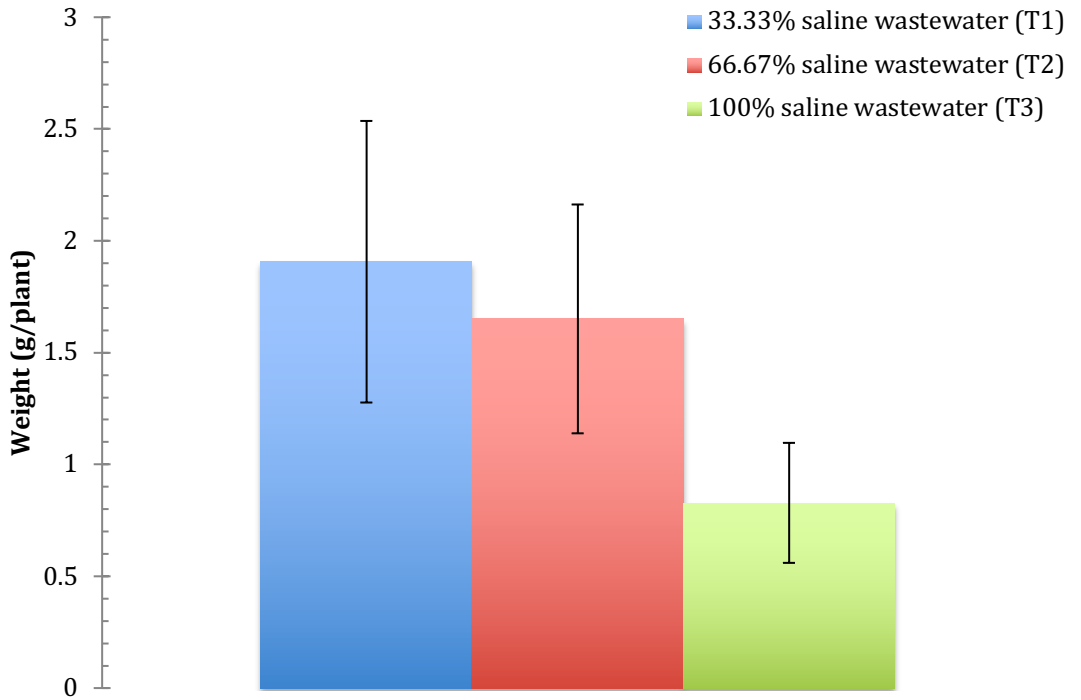
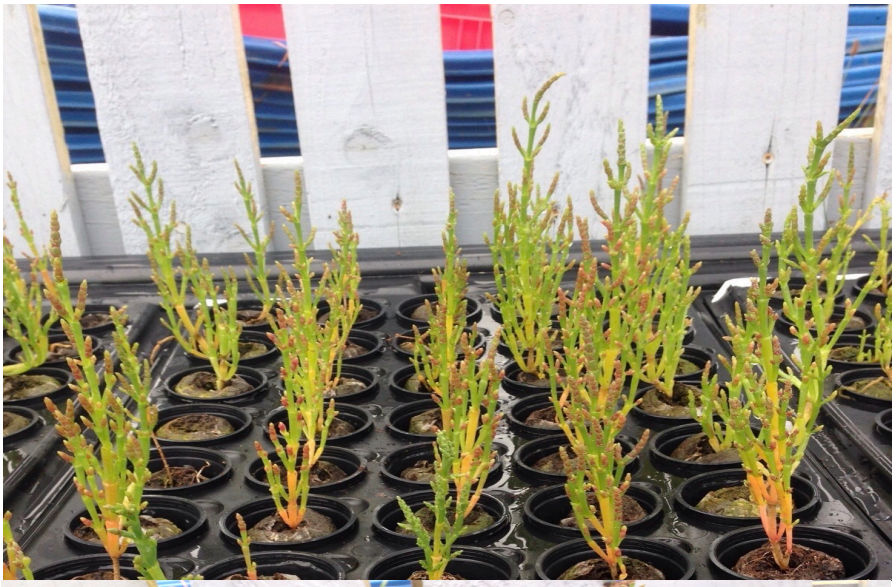
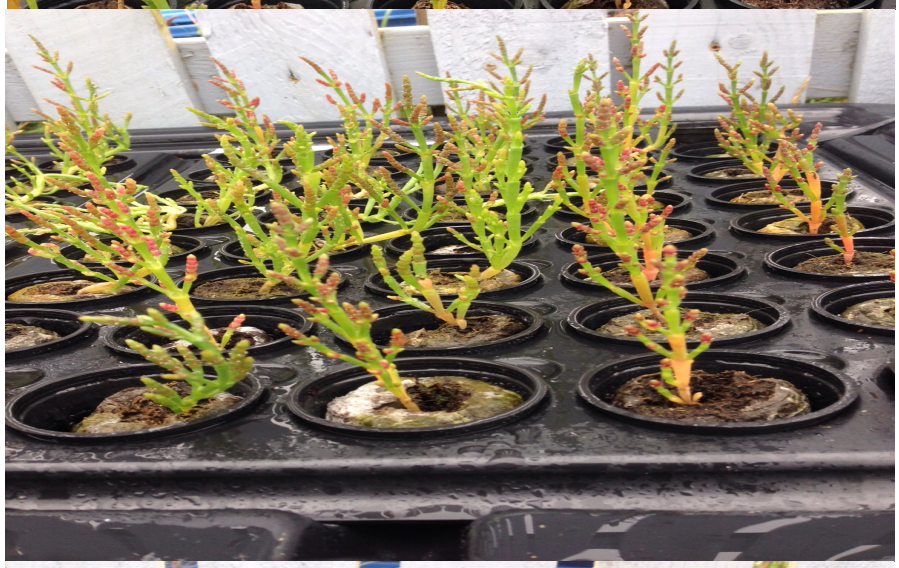


Figure 12: Mean harvestable biomass at the end of the aeroponics growth stage (mean ± SD; Trial 2)

a)



b)



c)

Figure 13: Examples of *S. europaea* plants at the end of the trial for a) treatment 1; b) treatment 2; and c) treatment 3 (Trial 2) (*original photo by Gunning*)

Water parameters – aeroponic stage

In the majority of cases, the level of TAN, nitrite, and nitrate in each of the treatment waters was reduced after 1 week in the aeroponic propagators. The level of phosphate present after one week was more variable, being reduced in the aeroponic propagators on 50% of occasions for all treatments. However, for week 3, 5, and 6 of this stage, the level of nutrients in the wastewater being added to the aeroponic propagators was low and the change by the end of the week was only minor. On week 4, however, the nutrient levels in the hatchery wastewater were a lot higher than other weeks, reflecting the variation in levels found in the wastewater that the hatchery releases. On week 4, for treatment 1, 2, and 3, respectively, the TAN concentration was reduced by 2.28, 2.65, and 2.69 mg/L to 0.11 ± 0.004 ($p<0.01$), 0.06 ± 0.01 ($p<0.01$), and 0.5 ± 0.06 ($p<0.001$) mg/L, nitrite was reduced by 0.05, 0.04, and 0.02 mg/L to 0.01 ± 0.001 ($p<0.01$), 0.001 ± 0.001 ($p<0.05$), and 0.11 ± 0.01 ($p<0.05$) mg/L, nitrate was reduced by 3.04, 2.82, and 2.93 mg/L to 0.34 ± 0.02 ($p<0.01$), 0.03 ± 0.001 ($p<0.001$), and 0.52 ± 0.01 ($p<0.01$) mg/L, and orthophosphate was reduced by 2, 2.4, and 2.27 mg/L to 0.4 ± 0.01 ($p<0.01$), 0.54 ± 0.08 ($p<0.01$), and 0.53 ± 0.03 ($p<0.01$) mg/L (Figures 14 - 17).

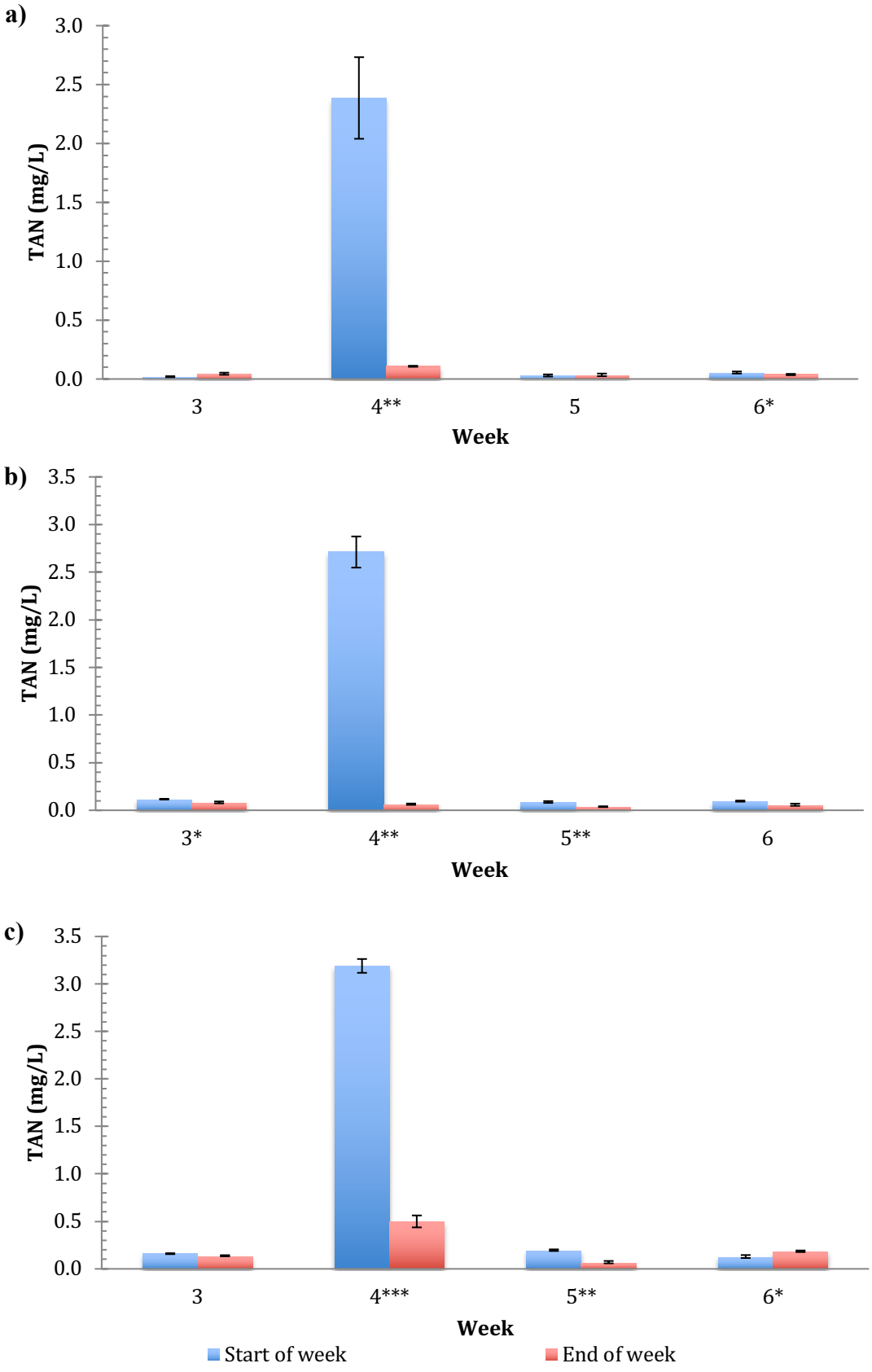


Figure 14: TAN levels of: a) 33.33% wastewater (T1); b) 66.66% wastewater T2), & c) 100% wastewater (T3) at the beginning and end of four monitored weeks (Trial 2) (* = p<0.05; ** = p<0.01; *** = p<0.001)

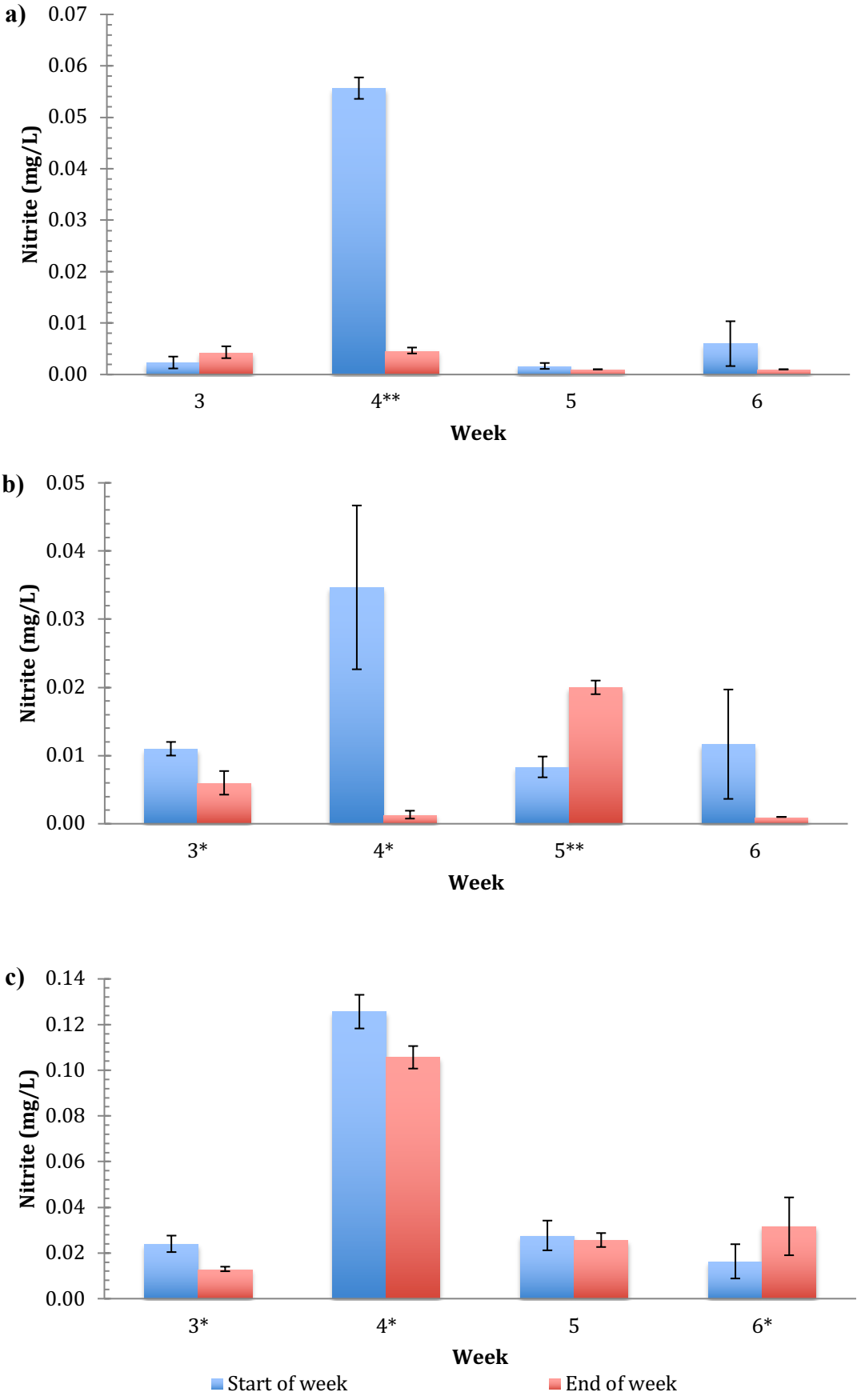


Figure 15: Nitrite levels of: a) 33.33% wastewater (T1); b) 66.66% wastewater (T2), & c) 100% wastewater (T3), at the beginning and end of four monitored weeks (Trial 2) (* = p<0.05; ** = p<0.01; *** = p<0.001)

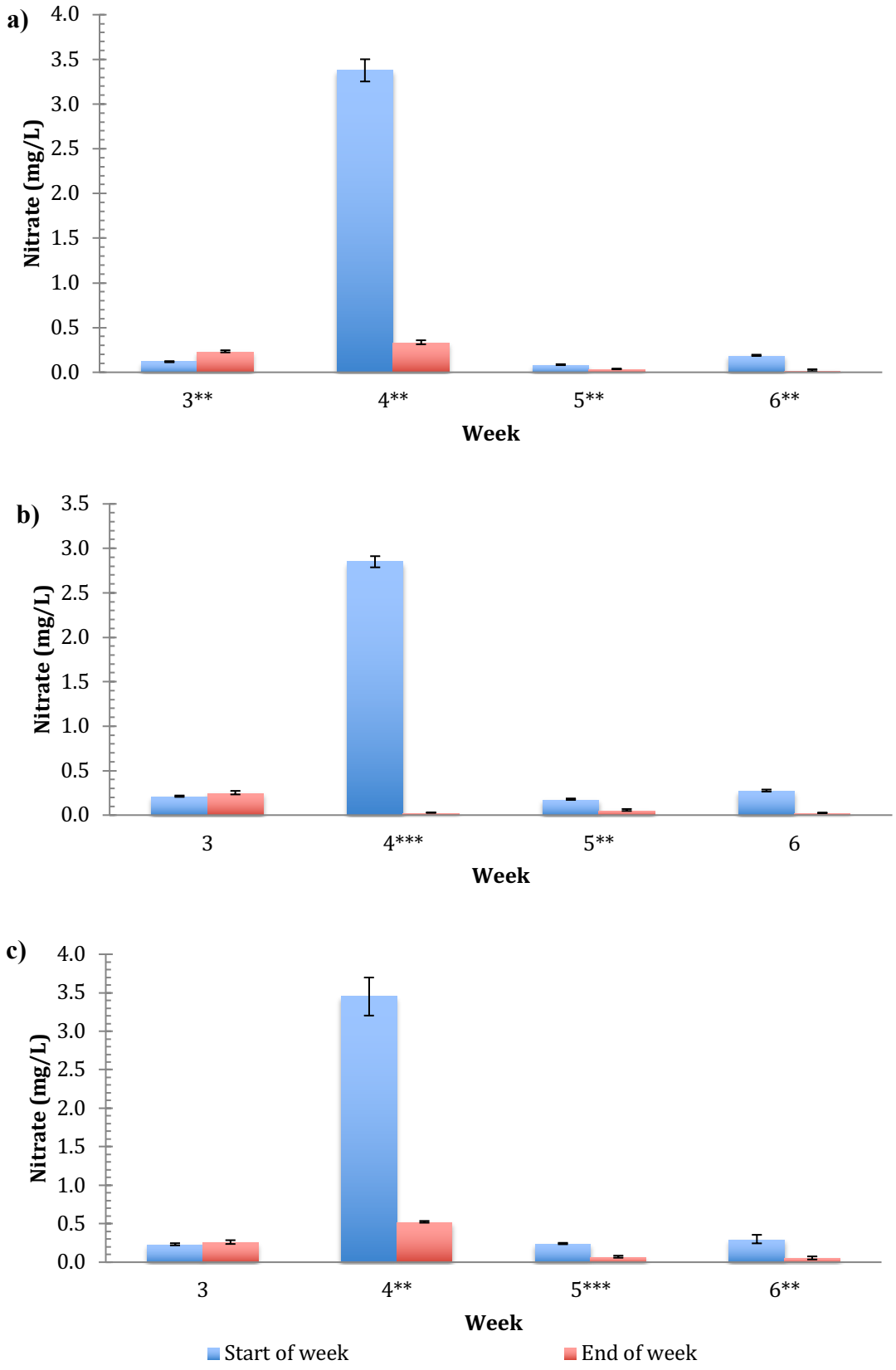


Figure 16: Nitrate levels of: a) 33.33% wastewater (T1); b) 66.66% wastewater (T2), & c) 100% wastewater (T3), at the beginning and end of four monitored weeks (Trial 2) (* = p<0.05; ** = p<0.01; *** = p<0.001)

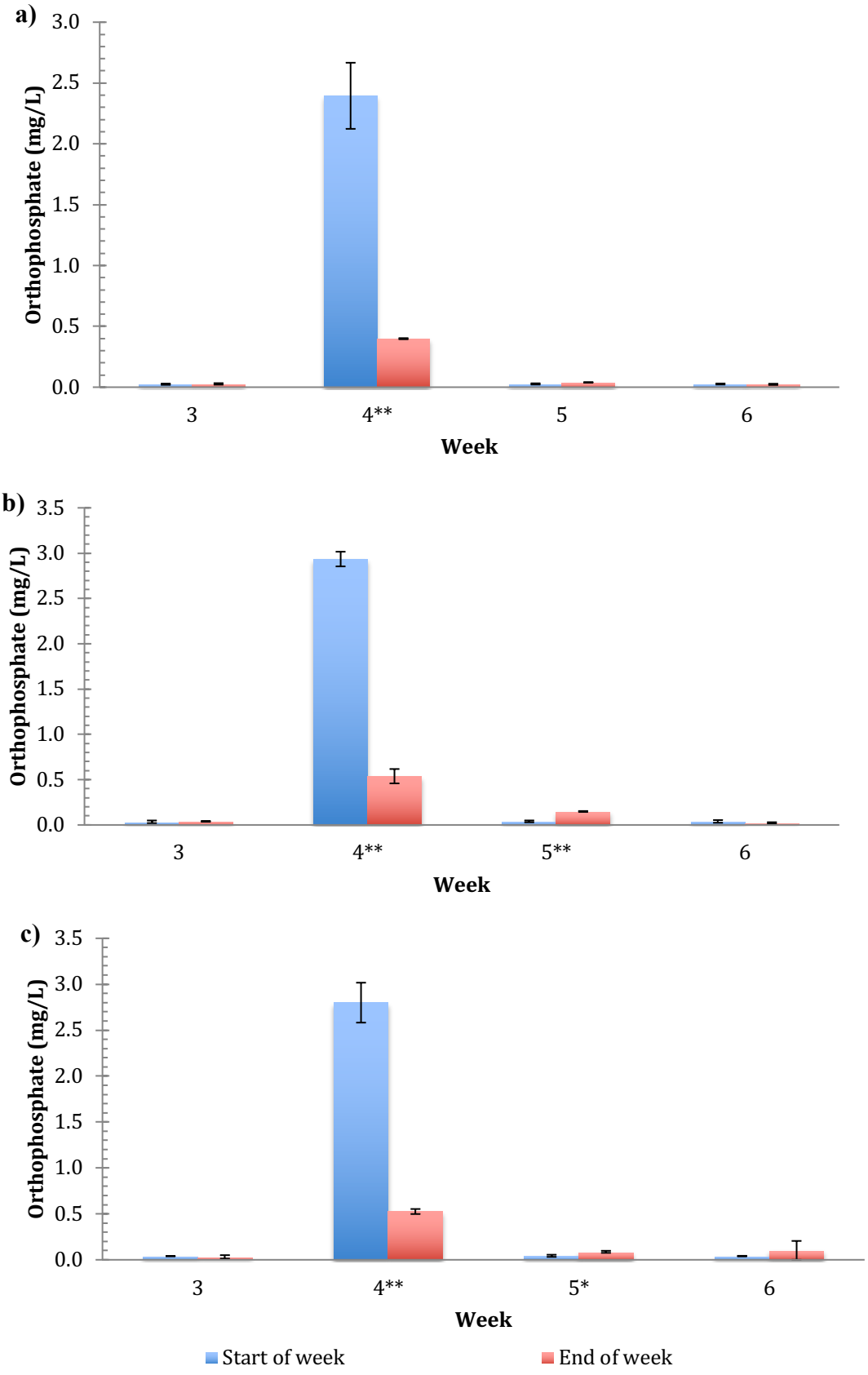


Figure 17: Orthophosphate levels of: a) 33.33% wastewater (T1); b) 66.66% wastewater (T2), & c) 100% wastewater (T3), at the beginning and end of four monitored weeks (Trial 2) (* = p<0.05; ** = p<0.01; *** = p<0.001)

2.4 Discussion

Cultivation of Salicornia europaea with aeroponics

Aeroponics is a cultivation method that reuses the nutrient solution and allows for high stocking densities and horizontal and vertical cultivation. It is an ideal cultivation system when space or water supply limitations are a major factor. Also, due to its flexibility, smaller units can be constructed, which are ideal for educational purposes and small-scale private 'backyard' production (Lakkireddy *et al*. 2012; Jones, 2005; Christie and Nichols, 2004; Barak *et al*. 1996; Nir, 1982). Although there are studies that utilise hydroponic techniques for the cultivation of *Salicornia* species (Waller *et al*. 2015; Buhmann *et al*. 2015; Kong and Zheng; 2014; Ventura *et al*. 2011; Ventura *et al*. 2010), there are none that specifically utilise the aeroponic technique.

No significant difference was found in the number of nodes and branches between aeroponic and control treatments for most comparisons, however the height of plants in the aeroponic treatments was significantly higher than for the control treatments on many of the days assessed. Keiffer *et al*. (1994), while measuring the effect of salinity on the growth and survival of *S. euroapaea*, grew plants in pots sitting in a watering tray containing freshwater or c.10 ppt saline water and half strength Hoagland's nutrient solution (both the aeroponic and control treatments for trial 1 were irrigated with 10 ± 1.87 ppt phostrogen nutrient solution). Over an 11 week growth period (controlled temperature and light), the best growth was achieved for those plants receiving the 10 ppt salinity treatment, with mean height increasing from approximately 1.9 cm to 16.30 ± 1.21 cm, mean number of nodes from approximately 0.9 to 9.71 ± 0.69, and the mean number of branches from 0 to 15.14 ± 1.58 cm. In comparison, and notwithstanding the lack of fully controlled conditions, the growth of *S. europaea* in the aeroponic propagator in the current trials was considered successful.

Lv *et al*. (2012) assessed the salt tolerance of *S. europaea* by irrigating pot cultivated plants (medium: vermiculite) with half-strength modified Hoagland's solution and found that after 21 days, shoot height (cm) had reached approximately 12 cm, 13 cm, and 13.2 cm for plants exposed to salinities of 11.6 ppt, 17.4 ppt, and 23.2 ppt, respectively. Shoot height decreased as the salinity increased above 23.2 ppt. It is important to note that plants were 30 days old prior to the beginning of the growth trial, however, the authors did not specify their starting size (Lv *et al*. 2012).

In terms of what medium was most suitable, it was found that *S. europaea* cultivated in coconut coir had the highest number of nodes, branches and height for both the aeroponic and control treatments when compared against the other mediums. Coconut coir was also chosen as the aeroponic medium for the oyster hatchery wastewater trial (trial 2) due to its ease of use and successful growth.

Salicornia europaea cultivated with oyster hatchery wastewater

After a 7 week growth trial (trial 2), the mean number of nodes and branches, height, and harvestable biomass (g/plant) was the highest for treatment 1 (11.1 ppt saline wastewater), but was very similar to treatment 2 (21.5 ppt saline wastewater) ($p>0.05$ for all aspects of growth monitored, except weight – $p<0.01$). Growth (number of nodes/branches, height, harvestable biomass) for treatment 3 (31.6 ppt saline wastewater) was substantially less than both treatment 1 and 2 ($p<0.001$). By the end of the 7 week aeroponic growth trial, treatment 1, 2, and 3 had a mortality rate of 19.17%, 9.17%, and 34.17%, respectively. These results correspond with other studies on *S. europaea* and other species of *Salicornia* that have found growth may be restricted by salinities approaching full sea water strength (i.e. 30-35 ppt) and beyond (Lv *et al*. 2012; Ventura *et al*. 2011; Aghaleh *et al*. 2009).

The strongest growth in terms of weight for this trial was achieved with treatment 1, which, by the end of the 7 week growth period, resulted in a harvestable biomass of 1.91 ± 0.63 g/plant (total harvestable biomass; 184.93 g). Taking into consideration the size of the aeroponic propagator (approximately 0.74 m^2; stocking density: 120 plants/0.74 m^2), this would equate to 0.25 kg/m^2 at a stocking density of approximately 162 plants/m^2. A study by Webb *et al*. (2013) treated the wastewater from a commercially operating intensive recirculating marine aquaculture facility growing marine shrimp (*Litopenaeus vannamei*) with constructed wetlands planted with *S. europaea* at density of 10,000 plants/m^2 (high density treatment) and 200 plants/m^2 (low density treatment). Over a 6 week period they achieved a biomass of approximately 5 kg/m^2 (high density) and 4 kg/m^2 (low density) (Webb *et al*. 2013). Webb *et al*. (2012) conducted a similar trial, treating the wastewater from a commercially operating intensive recirculating marine aquaculture facility growing marine shrimp (*L. vannamei*), sole (*Solea solea*), and turbot (*Scophthalmus maximus*), with a constructed wetland planted with *S. europaea* at a density of 90 plants/m^2. They

achieved a biomass of 1.8kg/m^2 over a 6 week period (Webb *et al*. 2013; Webb *et al*. 2012).

There are a number of potential reasons why harvestable biomass in the current study was low in comparison to previously reported studies over a similar time period (7 weeks): (1) *Plant flowering*: *S. europaea* plants flower in August or September, after which time they die (Devlin, 2015; Singh *et al*. 2014; Davy *et al*. 2001). The current trial started quite late into the year, with no artificial control over temperature and light conditions. Subsequently, the plants began to flower by week 5-6, and therefore, growth began to reduce. (2) The oyster hatchery did not have the facilities required to maximise the growth potential of *S. europaea* (e.g. greenhouses, nursery facilities etc.). Therefore, the early stages of growth (prior to aeroponic on-growing) took place in a mobile polyethylene greenhouse and the aeroponic propagators for the on-growing stage were positioned outside. A sturdy glass greenhouse may have provided increased protection from the elements (e.g. wind) and improved control of light and temperature, which may have had an impact on the level of growth achieved. (3) *Aeroponic propagator "dry zones"*: It was noted during trial 2 that certain areas of the aeroponic propagators (most frequently at the corner areas of the trays) did not get a sufficient level of spray from the spray bar ("dry zones"). For treatment 1, 2, and 3, respectively, 87.0%, 90.9%, and 58.5% of mortalities occurred in these "dry zones" (example of "dry zones" can be found in the top left and bottom right corner of Figure 13c). Of those plants that did not die in these zones, a reduced level of growth for each treatment was noted in the majority of cases. The potential for "dry-zones" should be considered when purchasing aeroponic propagators or when designing a bespoke propagator. The spray bar should be altered or designed in such a manner that all seedlings will receive a sufficient level of spray to facilitate maximum growth. (4) *Low levels of nutrients in wastewater*: The levels of TAN, nitrite, nitrate, and phosphate were quite low in the hatchery's wastewater during most of the trial. For treatment 1 and 2, this wastewater was diluted with freshwater to acquire the respective salinity level, further reducing the level of available nutrients. To improve the harvestable biomass of *S. europaea* irrigated with low nutrient wastewater, additional nutrients and/or elements could be added, such as nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, copper, boron, manganese, zinc, iron, and molybdenum (Kong and Zheng, 2014; Ventura *et al*. 2010).

For the majority of weeks that nutrient analysis was conducted (weeks 3, 5, and 6), nutrient (i.e. TAN, nitrite, nitrate, and orthophosphate) levels from the oyster hatchery were low, subsequently, the change in nutrient levels after 1 week in the aeroponic units, for each treatment, was quite low. However, on week 4, the levels of nutrients leaving the hatchery were far higher. For treatment 1, 2, and 3, respectively, TAN was reduced by 95.47% ($p<0.01$), 97.64% ($p<0.01$), and 84.33% ($p<0.001$), nitrite was reduced by 91.07% ($p<0.01$), 97.14% ($p<0.05$), and 15.87% ($p<0.05$), nitrate was reduced by 90.05% ($p<0.01$), 99.02% ($p<0.001$), and 84.82% ($p<0.01$), and orthophosphate was reduced by 83.34% ($p<0.01$), 81.67% ($p<0.01$), and 81.21% ($p<0.01$). It is important to note that TAN levels, particularly on week 4, were very similar at the start of the week (when the saline wastewater was added to each treatment tank) despite the difference in wastewater salinities between treatments. The reason for this anomaly was unknown.

Webb *et al*. (2013) found that constructed wetlands planted with *S. europaea* removed 62 ± 34.6 mmol N m^{-2} d^{-1} and 18.3 ± 5 mmol P m^{-2} d^{-1} from aquaculture wastewater. Webb *et al*. (2012) also planted a constructed wetland with *S. europaea* to treat aquaculture wastewater, and found that over the 88 days of the study, cumulative nitrogen removal was 1.28 mol m^{-2}, of which 1.09 mol m^{-2} was retained in plant tissue. This equated to a plant uptake rate ranging from 2.4 to 27 mmol N g^{-1} dry weight d^{-1}. The cumulative dissolved inorganic phosphate (DIP) was 0.11 ± 0.01 mol m^{-2}, with up to 75% of DIP removed retained in the plant tissue. During periods of high nutrient loading, mean daily removal reached a high of 6.6 ± 3.05 mmol m^{-2} d^{-1} (Webb *et al*. 2012). Both studies by Webb demonstrate the effectiveness of *S. europaea* as a biofilter of wastewater from land-based intensive marine aquaculture farms (Webb *et al.* 2013; Webb *et al*. 2012). Although our study demonstrated the biofiltering capacity of *S. europaea*, it is important to note that the wastewater of the oyster farm was not rich in nutrients. Further studies should take place on a larger scale, assessing the effectiveness of treating the wastewater from an intensive on-land aquaculture farm with *S. europaea* via the aeroponic technique. There are currently no other studies that have assessed the biofiltering potential of *S. europaea*, however, other studies have demonstrated the effectiveness or potential of other *Salicornia* species as biofilters of aquaculture wastewater (Buhmann *et al*. 2015; Waller *et al*. 2015; Kong and Zheng, 2014; Diaz *et al*. 2013; Shpigel *et al*. 2013).

In light of these results, when deciding upon what salinity of wastewater is most suitable

for the cultivation of *S. europaea*, one must consider the trade-off between the volume of wastewater that can be treated (i.e. the need to dilute the wastewater to reduce the salinity) and the quantity of harvestable *S. europaea* that is achievable. For instance, in the majority of cases *S. europaea* growing in treatment 3 solution (30.9 ppt saline wastewater) was effective at filtering the wastewater, but suffered poor growth and high mortalities (exception: on week 4, nitrite levels were 0.126 mg/l, which were only reduced by 0.02 mg/L {15.87%}). If wastewater treatment was the only goal, this would be the most suitable salinity, as a higher volume of wastewater could be treated. However, if a marketable and profitable biomass of *S. europaea* is also the goal, a lower salinity is necessary. *S. europaea* growing in the treatment 2 solution (20.78 ppt saline wastewater) had a lower final harvestable biomass than treatment 1 (10.5 ppt saline wastewater) (184.93 g vs. 179.96 g; $p<0.01$). Therefore, with treatment 2's salinity (20.78 ppt) a lower percentage of the wastewater needed to be diluted, resulting in a larger amount of wastewater that can be treated at any one time, with a minimal reduction in harvestable biomass. It is important to note that other studies that treated aquacultural wastewater with *S. europaea* used full-salinity (c. 30-35 ppt) wastewater without having a negative impact on growth (Webb *et al*. 2013; Webb *et al*. 2012). Further studies on the use of aeroponics as a cultivation method for the biofiltering of aquaculture wastewater with *S. europaea*, which address the limitations discussed above (in particular the low level of nutrients of the wastewater in trial 2), are required.

2.5 Conclusion

This study has demonstrated both the potential of aeroponics as a cultivation technique for *S. europaea*, and the capacity of *S. europaea* to effectively biofilter low-nutrient aquacultural wastewater. Future studies should assess the effectiveness of *S. europaea* as a biofilter of wastewater from an intensive aquaculture farm using the aeroponic technique. These studies should also address the limitations of this study, in particular; the design flaws of the aeroponic propagators used and the lack of suitable facilities for *S. europaea* cultivation (e.g. a greenhouse).

The main impediment to the large-scale cultivation of halophytes, including *Salicornia,* has been the prevalence of undesirable crop characteristics (e.g. non-uniform flowering and ripening) in wild germplasm. Therefore, there is a need to improve upon these

undesirable traits through selective breeding. The wild accessions of *S. bigelovii*, for example, differ significantly in plant size, biomass, seed yield, days to flowering, and days to harvesting. Hence the wild germplasm exhibits sufficient genotypic diversity and a favourable flowering system to support a breeding program. Improvements to lines have resulted in 33-44% higher seed and biomass yields since breeding programmes on *S. bigelovii* began (Zerai, *et al.* 2010). Inoculation of crop plants with plant-growth-promoting bacteria (PGPB) is a contemporary agricultural practise used to improve crop yields. A study by Bashan *et al.* (2000) found that *S. bigelovii* inoculated with PGPBs significantly increased plant height and dry weight (Bashan *et al.* 2000). Selective breeding and PGPB studies has not yet been conducted on *S. europaea*, however, such research could greatly improve its development as a commercial produced halophytic crop.

2.6 Acknowledgements

Rory Nunan (Cork Institute of Technology) assisted in the setting up of trial 1. Laura Barrett (Institute of Technology, Tralee) assisted in the setting up and monitoring of trial 2. Prof. Gavin Burnell gave advise on the design of these trials and provided feedback on the chapter. Thank you to the staff of Tralee Bay Hatchery for their assistance throughout trial 2.

2.7 References

Abdal, M.S. (2009). *Salicornia* production in Kuwait. *World Applied Sciences Journal*, 6, pp. 1033-1038.

Aghaleh, M., Niknam, V., Ebrahimzadeh, H., and Razavi, K. (2009). Salt stress on growth, pigments, proteins, and lipid peroxidation in *Salicornia persica* and *S. europaea*. *Biologia Plantarum*, 53(2), pp. 243-248.

Ahn, B., Kim, R., Choi, D., and Kim, Y. (2011). Effect of *Salicornia bigelovii* extract on the activities of whitening and anti-wrinkle. *Applied Chemistry for Engineering,* 22(1), pp. 55-60.

Austenfeld, F. (1986). Nutrient reserves of *Salicornia europaea* seeds. *Physiologia Plantarum*, 68, pp. 446-450.

Badiola, M., Mendiola, D., and Bostock, J. (2012). Recirculating aquaculture systems (RAS) analysis: Main issues on management and future challenges. *Aquacultural Engineering,* 51, pp. 26–35.

Barak, P., Smith, J.D., Kreuger, A.R., and Peterson, L.A. (1996) Measurement of short-term nutrient uptake rates in cranberry by aeroponics. *Plant Cell and Environment*, 19, pp. 237–242.

Bashan, Y., Moreno, M., and Troyo, E. (2000). Growth promotion of the seawater-irrigated oilseed halophyte *Salicornia bigelovii* inoculated with mangrove rhizosphere bacteria and halotolerant *Azospirillum* spp. *Biology and Fertility of Soils*, 32(4), pp. 265-272.

Boestfleisch, C., Wagenseil, N.B., Buhmann, A.K., Seal, C.E., Wade, E.M., Muscolo, A., and Papenbrock, J. (2014). Manipulating the antioxidant capacity of halophytes to increase their cultural and economic value through saline cultivation. *AoB Plants,* 6, DOI: https://doi.org/10.1093/aobpla/plu046.

Botanical Society of Britain and Ireland (2015). Available online: http://bsbi.org/maps?taxonid=2cd4p9h.30r. Date Visited: 07/11/2016.

Boxman, S.E., Nystrom, M., Capodice, J.C., Ergas, S.J., Main, K.L., and Trotz, M.A. (2016). Effect of support medium, hydraulic loading rate and plant density on water quality and growth of halophytes in marine aquaponic systems. *Aquaculture Research,* pp. 1-15, DOI: 10.1111/are.13083.

Brown, J.J., Glenn, E.P., Fitzsimmons, K.M., and Smith, S.E. (1999). Halophytes for the treatment of saline aquaculture effluent. *Aquaculture*, 175, pp. 255-268.

Brown, J.J., and Glenn, E.P. (1999). Reuse of highly saline aquaculture effluent to irrigate a potential forage halophyte, *Suada esteroa. Aquacultural Engineering*, 20, pp. 91-111.

Buhmann, A. K., Waller, U., Wecker, B., and Papenbrock, J. (2015). Optimization of culturing conditions and selection of species for the use of halophytes as biofilter for nutrient-rich saline water. *Agricultural Water Management*, 149, pp. 102-114.

Buhmann, A., and Papenbrock, J. (2013). Biofiltering of aquaculture effluents by halophytic plants: basic principles, current uses and future perspectives. *Environmental and Experimental Botany*, 92, pp. 122-133.

Bustan, A., Pasternak, D., Pirogova, I., Durikov, M., deVries, T.T., Meccawi, S., and Degen, A.A. (2005). Evaluation of saltgrass as a fodder crop for saline environments. *Journal of the Science of Food and Agriculture*, 85, pp. 2077-2084.

Calheiros, C.S.C., Quitério, P.V.B., Silva, G., Crispim, L.F.C., Bix, H., Moura, S.C., Castro, P.M.L. (2012). Use of a constructed wetland system with *Arundo* and *Sarcocorina* for polishing high salinity tannery water. *Journal of Environmental Management*, 95, pp. 66-71.

Christie, C.B, and Nichols, M.A. (2004). Aeroponics - A Production system and research tool. *Acta Horticulturae*, 648, pp. 185–190.

Clark, A. (1994). Samphire – From sea to shining seed. Aramco World, 45(6). Available online: http://archive.aramcoworld.com/issue/199406/samphire-from.sea.to.shining.seed.htm. Date Visited: 1/12/16.

Davy, A. J., Bishop, G. F., and Costa, C. S. B. (2001). *Salicornia* L. (*Salicornia pusilla* J. Woods, *S. ramosissima* J. Woods, *S. europaea* L., *S. obscura* P.W. Ball & Tutin, *S. nitens* P.W. Ball & Tutin, *S. fragilis* P.W. Ball & Tutin and *S. dolichostachya* Moss). *Journal of Ecology*, 89, pp. 681-707.

Devlin, Z. (2015). Information on Glasswort, agg, Wildflowers of Ireland. Available online:http://www.wildflowersofireland.net/plant_detail.php?id_flower=475&wildflower=Glassworts. Date Visited: 14/11/16.

De Vos, A. C., Broekman, R., Groot, M. P., and Rozema, J. (2010). Ecophysical response of *Crambe maritima* to airborne and soil-borne salinity. *Annals of Botany*, 105, pp. 925-937.

Díaz, F. J., Benes, S. E., and Grattan, S. R. (2013). Field performance of halophytic species under irrigation with saline drainage water in the San Joaquin Valley of California. *Agricultural Water Management*, 118, pp. 59-69.

Eganathan, P.S.R., Subramanian, H.M., Latha, R., and Srinivasa Rao, C. (2006). Oil analysis in seeds of *Salicornia brachiate*. *Industrial Crops and Products*, 23, pp. 177-179.

Ein Mor Crops (2016). *Salicornia* by Ein Mor Crops. Available online: http://www.tradekey.com/product-free/Salicornia-5032009.html. Date Visited: 21/7/17.

El Shaer, H.M. (2010). Halophytes and salt-tolerant plants as potential forage for ruminants in the Near East region. *Small Ruminant Research*, 91, pp. 3-12.

Epstein, E., Norlyn, J.D., Rush, D.W., Kingsbury, R.W., Kelley, D.B., Cunningham, G.A., and Wrona, A.F. (1980). Saline culture of crops: a genetic approach. *Science*, 210, pp. 399-404.

Essaidi, I., Brahmi, Z., Snoussi, A., Koubaier, H.B.H., Casabianca, H., Abe, N., Omri, A.E., Chaabouni, M.M., and Bouzouita, N. (2013). Phytochemical investigation of Tunisian *Salicornia herbacea* L. antioxidant, antimicrobial and cytochrome P450 (CYPs) inhibitory activities of its methanol extract. *Food Control*, 32(1), pp. 125-133.

Fan, P., Nie, L., Jiang, P., Feng, J., Lv, S., Chen, X., Bao, H., Guo, J., Tai, F., Wang, J., Jia, W., and Li, Y. (2013). Transcriptome analysis of *Salicornia europaea* under saline conditions revealed adaptive primary metabolic pathways as early events to facilitate salt adaption. *PLoS*, 8(11), e80595, doi: 10.1371/journal.pone.0080595.

Flowers, T.J., Galal, H.K., and Bromham, L. (2010). Evolution of halophytes: multiple origins of salt tolerance in land plants. *Functional Plant Biology,* 37, pp. 604-612.

Flowers, T.J., and Colmer, T.D. (2008). Salinity tolerance in halophytes. *New Phytologist*, 179(4), pp. 945-963.

Flowers, T.J., and Yeo, A.R. (1995). Breeding for salinity resistance in crop plants: where next? *Australian Journal of Plant Physiology*, 22, pp. 875-884.

Flowers, T.J., Troke, P.F., and Yeo, A.R. (1977). Mechanism of salt tolerance in halophytes. *Annual Review of Plant Physiology and Plant Molecular Biology*, 28, pp. 89-121.

Food and Agricultural Organization of the United Nations (2016). *The State of World Fisheries and Aquaculture 2016: Contributing to Food Security and Nutrition for All*. FAO, Rome, Italy, p. 200.

Ghaly, A.E., Kamal, M., and Mahmoud, N.S. (2005). Phytoremediation of aquaculture wastewater for water recycling and production of fish feed. *Environment International,* 31(1), pp. 1-13.

Glenn, E.P., Brown, J.J., and Blumwald, E. (1999). Salt tolerance and crop potential of halophytes. *Critical Reviews in Plant Sciences*, 18, pp. 227-255.

Glenn, E.P., Brown, J.J., and O'Leary, J.W. (1998). Irrigating crops with seawater. *Scientific American*, 279, pp. 76-81.

Glenn, E.P., Coates, W., Riley, R., and Swingle, S. (1992). *Salicornia bigelovii* Torr.: a seawater-irrigated forage for goats. *Animal Feed Science and Technology*, 40, pp. 21-30.

Granada, L., Sousa, N., Lopes, S., and Lemos, M.F.L. (2016). Is integrated multitrophic aquaculture the solution to the sectors' major challenges? - A review. *Reviews in Aquaculture*, 8, pp. 283–300.

Grigorakis, K., and Rigos, G. (2011). Aquaculture effects on the environmental and public welfare—The case of Mediterranean Mariculture. *Chemosphere*, 855, pp. 899–919.

Grouzis M., Berger, A., and Heim, G. (1976). Polymorphisme et germination des grains chez trois espèces annuelles du genre *Salicornia*. *Oecologia Plantarum*, 11(1), pp. 41-52.

Guil, J. L., Rodriguez-Garcia, I., and Torija, E. (1997). Nutritional and toxic factors in selected wild edible plants. *Plant Foods for Human Nutrition*, 51, pp. 99-107.

Im, S., Kim, K., and Lee, C. (2006). Immunomodulatory activity of polysaccharides isolated from *Salicornia herbacea*. *International Immunopharmacology*, 6(9), pp. 1451-1458.

Jefferies, R. L., Davy, A. J., and Rudmik, T. (1981). Population biology of the salt marsh annual *Salicornia europaea* agg. *Journal of Ecology*, 69, pp. 17-31.

Jha, B., Gontia, I., and Hartmann, A. (2012). The roots of the halophyte *Salicornia brachiate* are a source of new halotolerant diazotrophic bacteria with plant growth-promoting potential. *Plant Soil*, 356, pp. 265-277.

Jones Jr, J.B. (2005). Hydroponics—A Practical Guide for the Soilless Grower, 2nd ed.; CRC Press: Boca Raton, FL, USA, p. 440.

Kadereit, G., Ball, P., Beer, S., Mucina, L., Sokoloff, D., Teege, P., Yaprak, A.E., and Freitag, H. (2007). A taxonomic nightmare comes true: phylogeny an biogeography of glassworts (*Salicornia* L. Chenopodiaceae). *Taxon*, 56, pp. 1143-1170.

Kang, H., Koppula, S., and Park, T. (2013). *Salicornia bigelovii* Torr attenuates neuro-inflammatory responses in lipopolysaccharide-induced BV-2 microglia by regulation of NF-kappa B signaling. *Tropical Journal of Pharmaceutical Research*, 12(6), pp. 897-903.

Keiffer, C.H., and Ungar, I.A. (1997). The effect of extended exposure to hypersaline conditions on the germination of five inland halophyte species. *American Journal of Botany*, 84(1), pp. 104-111.

Keiffer, C.H., McCarthy, B.C., and Ungar, I.A. (1994). Effect of salinity and waterlogging on growth and survival of *Salicornia europaea* L., and inland halophyte. *Ohio Journl of Science,* 94(3), pp. 70-73.

Khan, M.A., and Gul, B. (2006). Halophyte seed germination. In: Khan, M.A., and Weber, D.J. (eds.), *Ecophysiology of High Salinity Tolerant Plants.* Springer Birhäuser Verlag, The Netherlands, pp. 11-30.

Kim, J.Y., Kong, C-S., Im Lee, J., Kim, H., Park, H.Y., Lee, H-S., Lee, C., and Seo, Y. (2012). Evaluation of novel antioxidant trterpenoid saponins from the halophyte *Salicornia herbacea*. *Bioorganic and Medicinal Chemistry Letters*, 22, pp. 4318-4322.

Klinger, D., and Naylor, R. (2012). Searching for solutions in aquaculture: Charting a sustainable course. *Annual Review of Environment and Resources,* 37, pp. 247–276.

Kong, Y., and Zheng, Y. (2014) Potential of producing *Salicornia bigelovii* hydroponically as a vegetable at moderate NaCl salinity. *HortScience,* 4999, pp. 1154–1157.

Kong, C., Kim, Y.A., Kim, M., Park, J., Kim, J., Kim, S., Lee, B., Nam, T.J., and Seo, Y. (2008). Flavonoid glycosides isolated from *Salicornia herbacea* inhibit matrix metalloproteinase in HT1080 cells. *Toxicology in Vitro*, 22(7), pp. 1742-1748.

Koyro, H.W., Khan, M.A., and Leith, H. (2011). Halophytic crops: a resource for the future to reduce the water crisis? *Emirates Journal of Food and Agriculture*, 23, pp. 1-16.

Lakkireddy, K.K.R., Kasturi, K., Sambasiva Rao, K.R.S. (2012). Role of hydroponics and aeroponics in soilless culture in commercial food production. *Research and Reviews: Journal of Agricultural Science and Technology*, 1(1), pp. 26–35.

Lin, Y.F., Jing, S.R., Lee, D.Y., Chang, Y.F., Chen, Y.M., and Shih, K.C. (2005). Performance of a constructed wetland treating intensive shrimp aquaculture wastewater under high hydraulic loading rate. *Environmental Pollution*, 134, pp. 411–421.

Liu, X. G., Xia, Y. G., Wang, F., Sun, M., Jin, Z. J., and Wang, G. T. (2005). Analysis of fatty acid composition of *Salicornia europaea* L. seed oil. *Food Science*, 2, p. 42.

Lu, D., Zhang, M., Wang, S., Cai, J., Zhou, X., and Zhu, C. (2010). Nutritional characterization and changes in quality of *Salicornia bigelovii* Torr. during storage. *LWT – Food Science and Technology* , 43, 519-524.

Lv, S., Jiang, P., Chen, X., Fan, P., Wang, X., and Li, Y. (2012). Multiple compartmentalization of sodium conferred salt tolerance in *Salicornia europaea*. *Plant Physiology and Biochemistry*, 51, pp. 47-52.

Lymbery, A.J., Doupe, R.G., Bennett, T., and Starcevich, M.R. (2006). Efficacy of a subsurface-flow wetland using the estuarine sedge *Juncus kraussii* to treat effluent from inland saline aquaculture. *Aquacultural Engineering,* 34(1), pp. 1-7.

Manikandan, T., Neelakandan, T., and Rani, G.U. (2009). Antibacterial activity of *Salicornia brachiata*, a halophyte. *Journal of Phytology*, 1(6), pp. 441-443.

Manousaki, E., and Kalogerakis, N. (2011). Halophytes present new opportunities in phytoremediation of heavy metals and saline soils. *Industrial and Engineering Chemistry Research*, 50, pp. 656-660.

Martins, C.I.M., Eding, E.H., Verdegem, M.C.J., Heinsbroek, L.T.N., Schneider, O., Blancheton, J.P., Roque d'Orbcastel, E., and Verreth, J.A.J. (2010). New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. *Aquacultural Engineering*, 43, pp. 83–93.

McGrath, C. (2010). Energy: Planting new seeds for the take-off. *Inter Press Service News Agency*. Available at: http://www.ipsnews.net/2010/02/energy-planting-new-seeds-for-the-take-off/. Accessed on: 23/11/16.

Movahedi, Z., Moieni, A., and Soroushzadeh, A. (2012). Comparison of aeroponics and conventional soil systems for potato minituber production and evaluation of their quality characteristics. *Journal of Plant Physiology and Breeding*, 2, pp. 13-21.

Mudie, P.J., Greer, S., Brakel, J., Dickson, J.H., Schinkel, C., Peterson-Welsh, R., StevensM., Turner, N.J., Shadow, M., and Washington, R. (2005). Forensic palynology and ethnobotany of *Salicornia* species (Chenopodoaceae) in northwest Canada and Alaska. *Canadian Journal of Botany*, 83, pp. 111-123.

National Parks and Wildlife Service (2014). Site synopsis: Dundalk Bay SAC. Available online: https://www.npws.ie/sites/default/files/protected-sites/synopsis/SY000455.pdf. Accessed on: 04/11/16.

National Parks and Wildlife Service (2013). Site synopsis: Tramore Dunes and Backstrand SAC. https://www.npws.ie/sites/default/files/protected-sites/synopsis/SY000671.pdf. Accessed on: 04/11/16.

Nir, I. (1982). Growing plants in aeroponics growth system. *Acta Horticulturae*, 126, pp. 435–448.

Ocean Desert Food (2017). *Salicornia* Fresh Tips. Available online: http://www.oasefoundation.eu/project_sub/163. Accessed on: 31/6/17.

O'Leary, J.W., and Glenn, E.P. (1994). Global distribution and potential for halophytes. In: Squires, V.R., and Ayoub, A.T. (eds.). *Halophytes as a Resource for Livestock and for Rehabilitation of Degraded Lands.* Kluwer Academic Publishers, pp. 7-17.

O'Leary, J.W., Glenn, E.P., and Watson, M.C. (1985). Agricultural production of halophytes irrigated with seawater. *Plant and Soil*, 89, pp. 311-321.

Philipupillai, J., and Ungar, I. A. (1984). The effect of seed dimorphism on the germination and survival of *Salicornia europaea* L. populations. *American Journal of Botany*, 71(4), pp. 542-549.

Price, L.L. (2007). From pedestrian fare to gourmet trend: the case of *Salicornia europaea* L., a traditional gathered wild sea shore vegetable. In: Moerbeek., H. H. S., and Niehof., A. (eds.). *Changing Families and their lifestyles*, Vol 2, Wageningen Academic Publishers, The Netherlands, pp. 201-211.

Primavera, J.H. (2006). Overcoming the impacts of aquaculture on the coastal zone. *Ocean and Coastal Management*, 49(9-10), pp. 531-545.

Quintã, R., Santos, R., Thomas, D.N., and Le Vay, L. (2015). Growth and nitrogen uptake by *Salicornia europaea* and *Aster tripolium* in nutrient conditions typical of aquaculture wastewater. *Chemosphere*, 120, pp. 414-421.

Rad, J.S., Alfatemi, S.M.H., and Rad, M.S. (2014). *In vitro* assessment of antibacterial activity of *Salicornia herbacea* L. seed extracts againsts multidrug resistant gram-positive and gram-negative bacteria. *International Journal of Biosciences*, 4(6), pp. 217-222.

Ramani, B., Reeck, T., Debez, A., Stelzer, R., Huchzermeyer, B., Schmidt, A., and Papenbrock, J. (2006). *Aster tripolium* L. and *Sesuvium portulacastrum* L.: two halophytes, two strategies to survive in saline habitats. *Plant Physiology and Biochemistry*, 44, pp. 395-408.

Rhee, M.H., Park, H.J., and Cho, J.Y. (2009). *Salicornia herbacea*: botanical, chemical, and pharmacological review of halophyte marsh plant. *Journal of Medicinal Plants Research*, 3, pp. 548-555.

Rueda-Puente, E., Castellanos, T., Troyo-Diéguez, E., Díaz de León-Alvarez, J.L., and Murillo-Amador, B. (2003). *Journal of Agronomy and Crop Science*, 189, pp. 323-332.

Schalke, D. (2015). *Salicornia* in Dutch greenhouses. *Fresh Plaza.* Available online: *http://www.freshplaza.com/article/145667/Salicornia-in-Dutch-greenhouses*. Date visited: 12/7/17.

Shimizu, K. (2000). Effects of salt treatment on the production and chemical composition of salt wort (*Salicornia herbacea* L.), rhodesgrass and alfalfa. *Japanese Journal of Tropical Agriculture*, 44(1), pp. 61-67.

Shpigel, M., Ben-Ezra, D., Shauli, L., Sagi, M., Ventura, Y., Samocha, T., and Lee, J. J. (2013). Constructed wetland with *Salicornia* as a biofilter for mariculture effluents. *Aquaculture*, 412-413, pp. 52-63.

Sindilariu, P.D., Reiter, R., and Wedekind, H. (2009a). Impact of trout aquaculture on water quality and farm effluent treatment options. *Aquatic Living Resources*, 22, 93–103.

Sindilariu, P.D., Brinker, A., and Reiter, R. (2009b). Factors influencing the efficiency of constructed wetlands used for the treatment of intensive trout farm effluent. *Ecological Engineering*, 35, 711–722.

Singh, D., Buhmann, A. K., Flowers, T. J., Seal, C. E., and Papenbrock, J. (2014). *Salicornia* as a crop plant in temperate regions: selection of genetically characterised ecotypes and optimization of their cultivation conditions. *AoB PLANTS*, 6(plus071); doi:10.1093/aobpla/plu071.

Sousa, W.T.Z., Panitz, C.M.N., and Thomaz, S.M. (2011). Performance of pilot-scale vertical flow constructed wetlands with and without the emergent macrophyte *Spartina alterniflora* treating mariculture effluent. *Brazilian Archives of Biology and Technology*, 54(2), pp. 405-413.

Swingle, R.S., Glenn, E.P., and Squires, V. (1996). Growth performance of lambs fed mixed diets containing halophyte ingredients. *Animal Feed Science and Technology*, 63(1-4), pp. 137-148.

Timmons, M.B., and Ebeling, J.M. (2010). *Recirculating Aquaculture*; Cayuga Aqua Ventures LLC: Ithaca, NY, USA, p. 948.

Troell, M., Naylor, R.L., Metian, M., Beveridge, M., Tyedmers, P.H., Folke, C., Arrow, K.J., Barrett, S., Crépin, A., Ehrlich, P.R., Gren, Å., Kautsky, N., Levin, S.A., Nyborg, K., Österblom, H., Polasky, S., Scheffer, M., Walker, B.H., Xepapadeas, T., and de Zeeuw, A. (2014). Does Aquaculture add resilience to the global food system? *Proceedings of the National Academy of Sciences of the United States of America,* 111, pp. 13257–13263.

Tuan, N.Q., Lee, W., Oh, J., Kulkarni, R.R., Gény, C., Jung, B., Kang, H., Bae, J-S., and Na, M. (2015). Flavanones and chromones from *Salicornia herbacea* mitigate septic lethality via restoring vascular barrier integrity. *Journal of Agricultural and Food Chemistry*, 63(46), pp. 10121-10130.

Turcios, A. E., and Papenbrock, J. (2014). Sustainable treatment of aquaculture effluents – what can we learn from the past for the future? *Sustainability*, 6, pp. 836-856.

Ungar, I.A. (1979a). Seed bank investigations with soils from an inland saltpan. *Bulletin of the Ecological Society of America*, 60, p. 134.

Ungar, I.A. (1979b). Seed dimorphism in *Salicornia europaea* L. *Botanical Gazette*, 140(1), pp. 102-108.

United States Census Bureau. U.S. and World Population Clock (2017). Available online: http://www.census.gov/popclock/ (accessed on 1st February 2017).

Ventura, Y., and Sagi, M. (2013). Halophyte crop cultivation: The case for *Salicornia* and *Sarcocornia*. *Environmental and experimental botany*, 92, pp. 144-153.

Ventura, Y., Wuddineh, W.A., Myrzabayeva, M., Alikulov, Z., Khozin-Goldberg, I., Shpigel, M., Samocha, T. M., and Sagi, M. (2011). Effect of seawater concentration on the productivity and nutritional value of annual *Salicornia* and perennial *Sarcocornia* halophytes as leafy vegetable crops. *Scientia Horticultura*, 128, pp. 189-196.

Ventura, Y., Wuddineh, W.A., Ephrath, Y., Shpigel, M., and Sagi, M. (2010). Molybdenum as an essential element for improving total yield in seawater-grown *Salicornia europaea* L. *Scientia Horticulturae*, 126, pp. 395-401.

Waisel, Y. (1972). *Biology of halophytes*. Academic Press, New York, p. 410.

Waller, U., Buhmann, A.K., Ernst, A.; Hanke, V., Kulakowski, A., Wecker, B., Orellana, J., and Papenbrock, J. (2015). Integrated multi-trophic aquaculture in a zero-exchange recirculation aquaculture system for marine fish and hydroponic halophyte production. *Aquaculture International*, 23, pp. 1473–1489.

Webb, J.M., Quintã, R., Papadimitriou, S., Norman, L., Rigby, M., Thomas, D.N., and Le Vay, L. (2013). The effect of halophyte planting density on the efficiency of constructed wetlands for the treatment of wastewater from marine aquaculture. *Ecological Engineering*, 61, pp. 145-153.

Webb, J.M., Qunit., R., Papadimitriou, S., Norman, L., Rigby, M., Thomas, D.N., and Le Vay, L. (2012). Halophyte filter beds for treatment of saline wastewater from aquaculture. *Water Research*, 46, pp. 5102-5114.

Wu, Y., Yang, R., Chen, M., and Liao, X. (2012). Antitumor activity of conjugated linoleic acid converted from linoleic acid in *Salicornia herbacea* seed oil. *Food Science*, 33(23), p. 318-322.

Yensen, N.P. (2006). *Hal*ophyte uses for the twenty-first century. In: Khan, M.A., and Weber, D.J. (eds). *Ecophysiology of High Salinity Tolerant Plants.* Springer Verlag, pp. 367-397.

Zerai, D.B., Glenn, E.P., Chatervedi, R., Lu, Z., Mamood, A.N., Nelson, S.G., and Ray, D.T. (2010). Potential for the improvement of *Salicornia bigelovii* through selective breeding. *Ecological Engineering*, 36, pp. 730-739.

Zhao, S., Zhou, N., Zhao,, Z-Y., Zhang, K., Wu, G-H., and Tian, C-Y. (2016). Isolation of endophytic plant growth-promoting bacteria associated with the halophyte *Salicornia europaea* and evaluation of their promoting activity under salt stress. *Current Microbiology*, 73(4), pp. 574-581.

Zhao, K.F., Fan, H., and Ungar, I.A. (2002). Survey of halophyte species in China. *Plant Science*, 163, pp. 491-498.

Zhou, Y., Yang, H., Hu, H., Liu, Y., Mao, Y., and Zhou, H. (2006). Bioremediation potential of the macroalga *Gracilaria lemaneiformis* (Rhodophyta) integrated into fed fish culture in coastal waters of north China. *Aquaculture*, 252, pp. 264–276.

Zia, S., Egan, T.P., and Khan, M.A. (2008). Growth and selective ion transport of *Limonium stocksii* Plumbaginacea under saline conditions. *Pakistan Journal of Botany*, 40, pp. 697-709.

Chapter 3

Improvements to *Salicornia europaea* seed germination: an assessment of stratification treatments

Abstract

Many families of halophytes have physiological dormancy and, generally, will not germinate until this dormancy is broken. Such dormancy can be broken by exposure to wet and cold conditions, referred to as stratification. Although studies have shown that a stratification treatment can enhance the germination success of *S. europaea* seeds, studies that include a stratification pre-treatment stage often lack detail, not indicating, for example, the salinity and/or the methodology utilised for the stage. The aim of this study was to assess various methodologies for stratification of *S. europaea* seeds, e.g. at salinities ranging from 0-34.21 ppt, for different time periods, effects of nutrient addition, or seed sterilisation prior to stratification. It was found that a 2-week stratification treatment at salinities of approximately 10-18 ppt is most effective for *S. europaea* seeds, while nutrient addition did not appear to significantly improve germination success. An improvement in germination and early growth (i.e. shoot and root emergence) of *S. europaea* seeds was also observed when seeds were sterilised prior to stratification.

3.1 Introduction

The germination of halophytes under natural conditions is regulated by variations in soil salinity, light availability, and an ambient thermo-period (El-Keblawy and Al-Rawai, 2005; Khan and Ungar, 1997; Ungar, 1991). The soils in which halophytes grow tend to become more saline during summer months as a result of rapid water evaporation. Subsequently, seed germination in such soil tends to take place during spring when soil salinity is lower due to higher levels of precipitation (Al-Hawija *et al*. 2012; Li and XiMing, 2007; Khan and Gul, 1998; Khan and Ungar, 1998). Studies have shown that the germination success of halophytes is reduced as salinity increases (Qu *et al*. 2008a; Qu *et al*. 2008b; Khan and Gulzar, 2003).

For the purpose of experimentation and commercial cultivation, seeds may need to be incubated at conditions (e.g. temperature, light, salinity) simulating those of their natural habitat (Al-Hawija *et al*. 2012; Baskin and Baskin, 2001). Many families of halophytes have physiological dormancy and, generally, will not germinate until this dormancy is broken. Such dormancy can be broken by stratification. Stratification is the process of pre-treating seeds with wet and cold conditions that simulate the natural conditions that a seed must endure before germination (Al-Hawija *et al*. 2012; Baskin and Baskin, 2004; Baskin and Baskin, 2001; Keiffer and Ungar, 1997; Keiffer *et al*. 1994; Philipupillai & Ungar, 1984; Ungar, 1979; Ungar, 1978; Grouzis *et al*. 1976).

Although the ecology, morphology, and biology of *S. europaea* has been studied extensively (Singh *et al*. 2014; Aghaleh *et al*. 2009; Davy *et al*. 2001; Ellison, 1987a; Ellison, 1987b; Ungar, 1987; Riehl and Ungar, 1982; Jefferies *et al*. 1981, Ungar *et al*. 1979; Ungar, 1979), research into its cultivation is limited (Ventura and Sagi, 2013; Webb *et al*. 2013; Webb *et al*. 2012; O'Leary *et al*., 1985). One such limitation relates to the stratification requirements of the seeds. Although studies have shown that a stratification treatment can enhance the germination success of *S. europaea* (Keiffer and Ungar, 1997; Philipupillai & Ungar, 1984), studies that do include a stratification pre-treatment stage for *S. europaea* seeds are often vague in detail, not indicating, for example, the salinity and/or the methodology utilised for the stage (Davy *et al*. 2001; Keiffer and Ungar, 1997; Keiffer *et al*. 1994; Philipupillai & Ungar, 1984; Ungar, 1979).

From previous experience of germinating *S. europaea* seeds (Chapter 2; Trial 1), it was found that a stratification, pre-germination, treatment was required for the successful germination of seeds.

The aim of this study was to assess, the success of: (1) stratification at different salinity levels; (2) stratification of varying durations, with or without nutrient addition and; (3) seed sterilisation prior to stratification.

3.2 Methods and Materials

Trial 1 assessed the germination success of seeds that underwent four weeks of stratification at five levels of salinity (0, ~10, ~18, ~24, & ~34 ppt respectively). Trial 2 examined the germination success of seeds that underwent stratification of varying durations (7, 14, & 21 days) on agar with or without nutrient addition. Trial 3 assessed the impact of sterilising seeds prior to stratification on germination success. The seeds were disinfected for 1, 5, 10, and 15 minutes prior to the commencement of stratification and compared to a control group that were not sterilised prior to stratification.

S. europaea seeds for trials 1 and 2 were sourced from Victoriana Nursery Gardens, Kent, England (https://www.victorianananursery.co.uk/) and for trial 3 from Alsa Gardens, West France (http://www.alsagarden.com/en/). For each trial, seeds were considered germinated when the radicle was at least 2 mm long (Zhao *et al*. 2016; Boestfleisch *et al*. 2014; Jha *et al*. 2012; Rueda-Puente *et al*. 2003).

Trial 1: Stratification at different salinity levels

Trial 1 took place at University College Cork (UCC) from 12th April to 22nd May 2015. *S. europaea* seeds were subjected to a 30 day stratification (dark refrigerator; mean temperature: 5 ± 0.34 °C) treatment (Figure 1) followed by 10 days post-stratification light exposure, at 5 different salinity levels (treatments 1-5) (note: the salinity for each treatment was maintained post-stratification). There were four replicates per treatment (n=25 seeds per replicate):

- Treatment 1: 100% distilled freshwater (salinity: 0 ppt)
- Treatment 2: 30% seawater: 70% distilled freshwater (salinity: 10.41 ± 0.56 ppt)
- Treatment 3: 50% seawater: 50% distilled freshwater (salinity: 17.89 ± 0.72 ppt)
- Treatment 4: 70% seawater: 30% distilled freshwater (salinity: 24.33 ± 0.25 ppt)
- Treatment 5: 100% seawater (salinity: 34.21 ± 0.31 ppt)

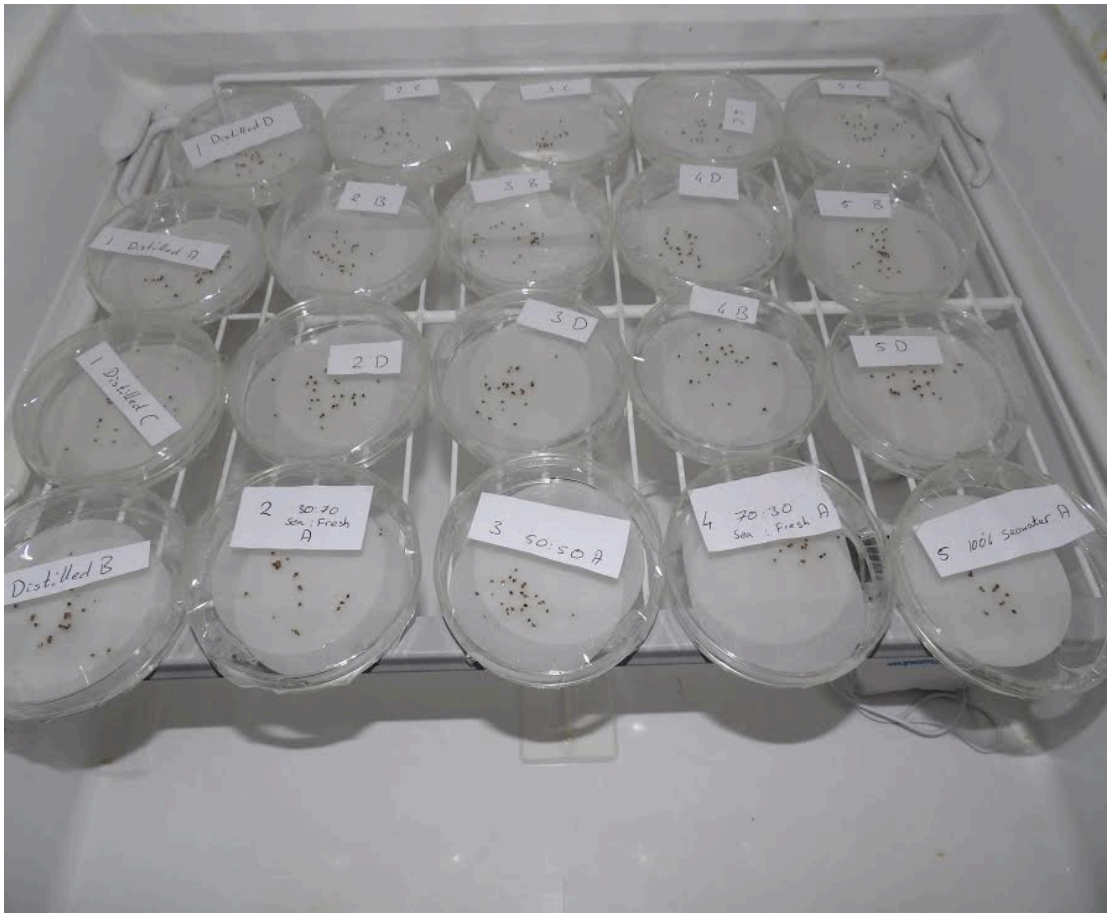


Figure 1: Stratification treatments (*photo courtesy of Earl*)

For each treatment seeds (n=25) were scattered evenly over 90 mm filter paper within 90 mm petri dishes. There were four petri dishes (i.e. 4 replicates) per treatment (n=100 seeds per treatment). The 90 mm filter paper was made damp by spraying with approximately 0.5 ml of the respective treatment solution using a 1 L spray-bottle and the lids of the petri dishes were taped shut. The petri dishes were then placed in a dark refrigerator at 5 ± 0.51 °C for four weeks. The seeds were checked daily and the filter paper dampened if required. The filter paper required dampening every 3-4 days with the respective treatment solution. If mould was present, it was not removed. This was done to assess the impact of salinity on mould growth and the impact of mould on germination.

After stratification, the petri dishes were transferred to a controlled temperature and light growth room (mean temperature: 19.35 ± 1.34 °C) for ten days under a light of 5.3 ± 2.3 $\mu mol\ m^{-2}\ s^{-1}$ at a day length of 16 h light/8 h dark (light was measured with a Skye® PAR meter). The filter papers needed dampening every 3-4 days with the respective treatment solution.

After 10 days the number of seedlings that had germinated in each petri dish was recorded and tested for photosynthetic activity (Fv/Fm) (Maxwell and Johnson, 2000; Butler, 1978; Kitajima and Butler, 1975) with ImagingWin® Version 2.41a software (Walz©, Germany).

As no seeds germinated from treatment 5 (100% seawater), each replicate petri-dish from this treatment was exposed to one of the other treatment solutions (i.e. 100% freshwater {T1}, 30% {T2}, 50% {T3}, or 70% {T4} seawater; n=1 petri-dish/replicate per treatment solution; n=25 seeds per treatment solution) for a further 10 days to assess their viability.

Trial 2: Stratification of varying duration, with or without nutrient addition

Trial 2 took place at UCC from 17th June to 29th July 2015. The germination of *S. europaea* seeds was tested at three different stratification periods (1, 2, & 3 week(s)), with and without the addition of Murashige and Skoog (MS) nutrients to agar (Sigma-Adlrich® A1296). As with trial 1, stratification took place in a dark refrigerator at 5 ± 0.21 °C. For all treatments, seeds were placed on 0.6% agar with 17.34 ± 0.87 ppt saltwater (50:50 seawater:distilled water) solution in 90 mm petri dishes. Prior to being placed in the fridge, the petri-dishes were taped shut. No additional water was required throughout the stratification stage. This salinity level was chosen as 10-day old germinated seeds, which had a stratification pre-treatment at this salinity in the previous trial, had a higher level of photosynthetic activity. Treatments 1-3 had no additional nutrients added and treatment 4-6 had 4.4g/L (pH 5.8) of MS added to the agar. There were 3 replicates per treatment (n=10 seeds per replicate):

- Treatment 1 - MS and 1 week stratification period
- Treatment 2 - MS and 2 weeks stratification period
- Treatment 3 - MS and 3 weeks stratification period
- Treatment 4 – No added nutrients and 1 week stratification period
- Treatment 5 - No added nutrients and 2 weeks stratification period
- Treatment 6 - No added nutrients and 3 weeks stratification period

After each respective stratification period the petri dishes were transferred to a growth room (mean temperature: 19 ± 1.30 °C) for 3 weeks under a light of 5.3x10 $\mu mol\ m^{-2}\ s^{-1}$ at a day length of 16 h light/8 h dark. The number of seeds that had germinated in each petri dish after 1, 2, 3, 6, 7, 8, 10, 13, 15, and 21 days in the growth room was recorded. After 21 days it was also noted if the germinated seedlings had developed shoots.

Trial 3 – Seed sterilisation prior to stratification

Trial 3 took place at UCC from 31st July to 5th September 2015. Firstly, 160 *S. europaea* seeds were wrapped in muslin (n=20 seeds per muslin) and immersed in an 80% ethanol: 20% distilled water solution for 30 seconds (Amiri *et al.* 2010; Ozawa *et al*. 2007). Secondly, the seeds were washed in a 0.5% sodium hypochlorite solution (90% distilled water: 10% bleach {5% Sodium Hypochlorite}) (Khan & Weber, 1986; Philipupillai & Ungar 1984) for 1, 5, 10, or 15 minutes (n=40 seeds per immersion time) (treatment 1-4). Thirdly, all sterilised seeds (n=160) were vigorously rinsed in a 500L beakers (n=40 seeds per beaker) containing distilled water, for 10 seconds, a total of 3 times. For each rinsing, the seeds were transferred to a new 500L beaker containing fresh distilled water. No time elapsed between each rinsing. Forty seeds did not go through any of this sterilisation procedure prior to stratification (treatment 5; control). There were four replicates per treatment (n=10 seeds per replicate):

- Treatment 1: Sterilisation for 1 minute prior to stratification
- Treatment 2: Sterilisation for 5 minutes prior to stratification
- Treatment 3: Sterilisation for 10 minutes prior to stratification
- Treatment 4: Sterilisation for 15 minutes prior to stratification
- Treatment 5 (control): No sterilisation prior to stratification

For each treatment group, seeds were then transferred to two 90 mm petri dishes (n=10 seeds per dish/replicate) containing 0.4% agar and two 90 mm petri dishes containing 0.5% agar (n=10 seeds per dish/replicate) enriched with 4.4 g/L MS (pH 5.8; 17.34 ± 0.27 ppt saltwater {50:50 seawater:distilled water}). The agar was modified over trial 4, as 0.6% was too firm and the seedlings could not penetrate the agar effectively. The seeds were stratified (as per methods described on pg. 126) for 2 weeks (i.e. until signs of root emergence was recorded in at least one petri dish per replicate). The petri dishes were then positioned under natural light conditions at approximately 15h light/9 h dark (mean temperature: 19 ± 1.23 °C). Germination and root emergence (%) on the final day of stratification and shoot and open shoot emergence (%) over 21 days post stratification, were recorded.

Note that there was no significant difference ($p>0.05$) in the germination and root emergence of seeds placed on the 0.4% and 0.5% agar. Subsequently, in the results section, mean data of each treatment is a mean of both agar types. It was noted, however, that the 0.4% agar did not hold its shape and it would be advised to use 0.5% agar in future studies.

Statistical analysis

All statistical analysis was performed using SPSS software (IBM) version 23. Prior to statistical analysis, percentage data were transformed using the arcsine transformation. All data was tested for normal distribution and homogeneity of variance with the Shapiro-Wilk test and Levene's test, respectively ($p>0.05$). Values less than 0.05 were considered statistically significant.

(a) Trial 1

A One-way ANOVA was used to test the significance of difference of the mean germination (%) of each stratification salinity treatment. Kruskal-Wallis tests were used to test the significance of difference of the Fv/Fm of each stratification salinity treatment.

(b) Trial 2

Independent t-tests were used to test the significance of difference of the mean germination (%) of treatment 1 vs. 4, 2 vs. 5, and 3 vs. 6 (i.e. nutrients vs. no nutrients over 1, 2, and 3 week stratification). For data that was not normal, Kruskal-Wallis tests were used. One-way ANOVAs were used to test the significance of difference of the mean germination of treatments 1 vs. 2 vs. 3 and 4 vs. 5 vs. 6 (i.e. 1 vs. 2 vs. 3 week stratification duration for nutrient and no nutrient addition). For data that was not normal, Kruskal-Wallis tests were used.

Kruskal-Wallis tests were used to test the significance of difference of the mean germination of each treatment (i.e. treatment 1-6) over each sampling date (day 6 excepted). A Welch test was used to test the significance of difference of the mean germination of each treatment (i.e. treatment 1-6), 6 days post stratification (equal variance could not be assumed). A Dunnett's T3 post-hoc analyses were conducted as the Welch test gave a statistically significant result ($p<0.05$).

A one-way ANOVA was used to test the significance of difference of the mean shoot emergence (%) between each treatment (treatment 1 and 4 excluded due to lack of variance).

(c) Trial 3

One-way ANOVAs were used to test the significance of difference of the mean germination and root emergence between each treatment (i.e. treatment 1-5) immediately post a 2 week stratification period.

One-way ANOVAs were used to test the significance of difference of the mean shoot emergence between each treatment over 21 days post stratification. When equal variance could not be assumed, a Welch test was used. Tukey HSD post-hoc analyses were conducted when ANOVAs gave a statistically significant result ($p<0.05$). For data that was not normal, Kruskal-Wallis tests were used.

3.3 Results

Trial 1

Ten days post stratification the mean germination (note: seeds were considered germinated when the radicle was at least 2mm long) was 44 ± 13%, 58 ± 14%, 53 ± 15%, 27 ± 20%, and 0% for treatment 1 (100% distilled freshwater; 0 ppt), 2 (30% seawater: 70% distilled freshwater; 10.41 ± 0.56 ppt), 3 (50% seawater: 50% distilled water; 17.89 ± 0.72 ppt), 4 (70% seawater: 30% distilled water; 24.33 ± 0.25 ppt), and 5 (100% seawater; 34.21 ± 0.31 ppt), respectively ($p>0.05$) (Figure 2).

For those seeds from treatment 5 that did not germinate, exposure to treatment 1's (100% freshwater), treatment 2's (30% seawater), treatment 3's (50% seawater) and treatment 4's (70% seawater) solution for a further 10 days, resulted in a mean germination (%) (recovery germination) of 64%, 44%, 44%, and 36%, respectively (n=25 per treatment solution; n=1 replicate per treatment).

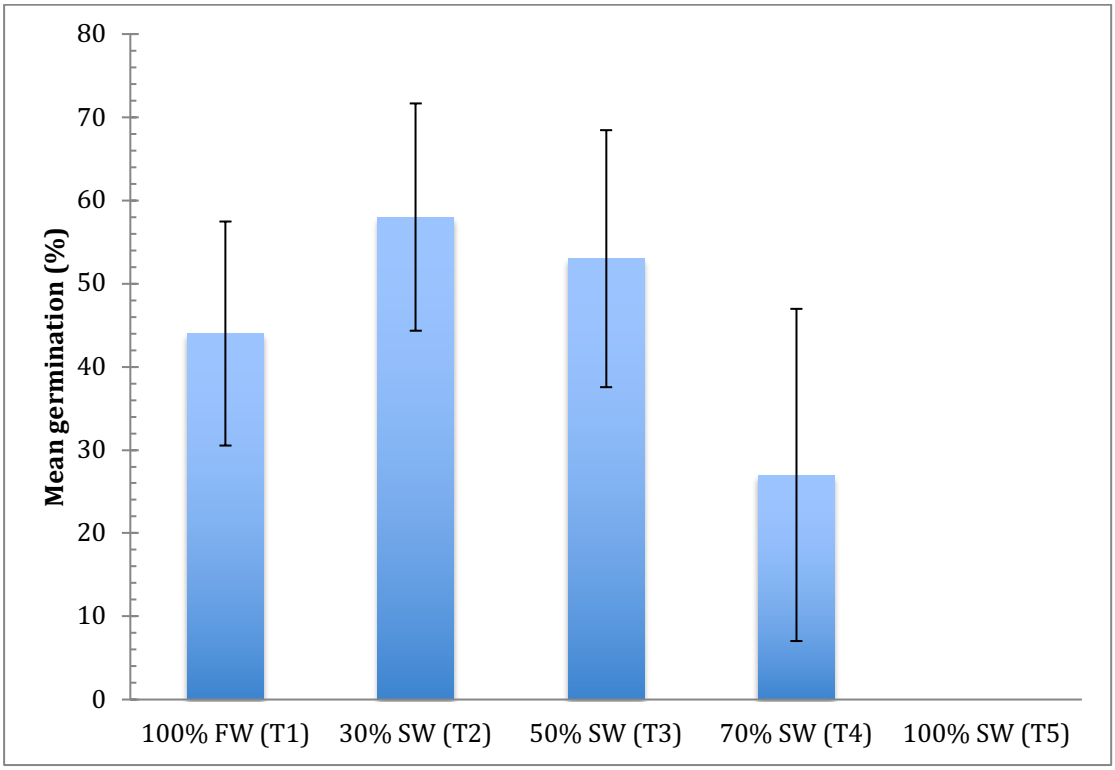


Figure 2: Mean germination 10 days post stratification (Trial 1) (mean ± SD; FW = distilled freshwater; SW = seawater)

By the end of the stratification period mould was present on 52 ± 6% and 40 ± 15% of seeds from treatment 1 and 2, respectively. This percentage did not change 10 days post stratification. Of those seeds that did not germinate in treatment 1 and 2, 79 ± 9% and 83.84 ± 14.64%, respectively, were covered in mould. No mould was present in any petri-dish from treatments 3, 4, and 5 by the end of the stratification or 10 day post stratification.

The 10 day old germinated seedlings from treatments 1-4 were tested for maximum efficiency (PS II). Fv/Fm was highest for seedlings from treatment 3 (50% seawater) (Figure 3). Fv/Fm was significantly higher for treatment 3 in comparison to treatment 1 (100% freshwater) ($p<0.01$), for treatment 1 in comparison to treatment 4 (70% seawater) ($p<0.05$), and for treatment 3 in comparison to treatment 4 ($p<0.01$). Nevertheless, there was not a large difference in the health of germinated seedlings between treatments.

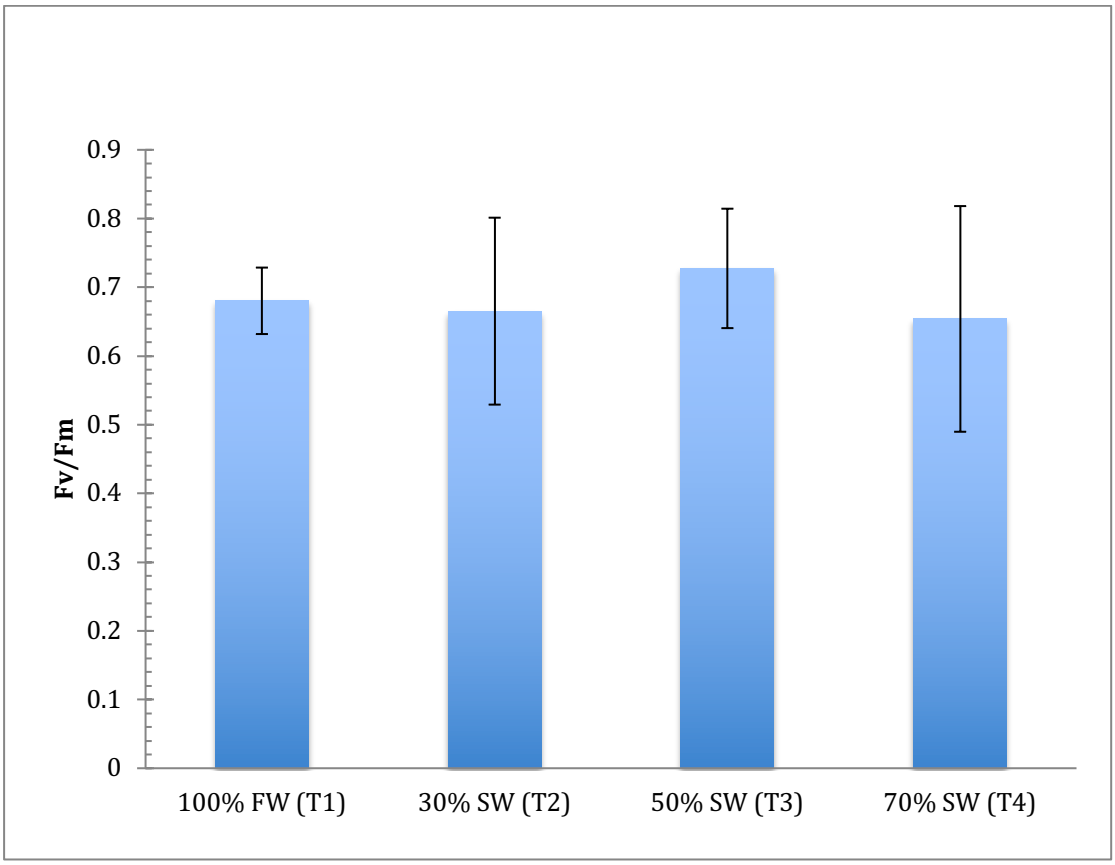


Figure 3: Maximum efficiency of photosystem II (Fv/Fm) of seedlings 10 days post stratification (Trial 1) (mean ± SD; FW = distilled freshwater; SW = seawater)

Trial 2

Nutrients

There was little difference in germination between treatments that had MS nutrients present and those that had no nutrients present for each stratification period assessed (1, 2, and 3 weeks) over the duration of the trial. However, there was a significant difference on day 6 between MS nutrient (T1) and no nutrient (T4) treatments exposed to a 1 week stratification period ($T1 > T4$; $p<0.05$) and on day 3 between MS nutrient (T3) and no nutrient (T6) treatments exposed to a 3 week stratification period ($T1 > T4$; $p<0.05$) (Figure 4).

Stratification time

There was no significant difference ($p>0.05$) in the germination of MS nutrient treatments (T1-3) when comparing treatments that had 1 (T1), 2 (T2), and 3 (T3) weeks stratification (Figure 5).

There was only a significant difference in the germination of no nutrient treaments (T4-6) when comparing treatments that had 2 (T5) and 3 (T6) weeks stratification ($T5> T6$; $p<0.05$) (Figure 5).

When all treatments (1-6) were compared against each other, after 21 days post stratification, the largest germination percentage was found for those seeds that had a 2 week stratification period and were exposed to MS agar (treatment 2; 80 ± 10%). However, there was no significant difference between each individual treatment ($p>0.05$) [Note: when all treatments were compared with each other across all sampling dates, there was a significant difference between treatment 1 and 6 and 2 and 6 on day 6 ($p<0.05$)].

By the end of the stratification period, mould was present on 13.33 ± 5.78%, 23.33 ± 15.28%, 10 ± 0%, 6.67 ± 5.77%, 6.67 ± 11.55%, and 3.33 ± 5.77% of seeds from treatment 1-6, respectively. By 21 days post stratification mould was present on 20 ± 10%, 26.67 ± 11.55%, 20 ± 10%, 16.67 ± 11.55%, 20 ± 20%, and 16.67 ± 5.77% of seeds from treatment 1-6, respectively. Of those seeds that did not germinate, 43.61 ± 14.35%, 66.67 ± 57.74%, 44.44 ± 13.88%, 41.11 ± 8.39%, 26.67 ± 46.19%, and 61.11 ± 34.70% from treatment 1-6, respectively, were covered in mould.

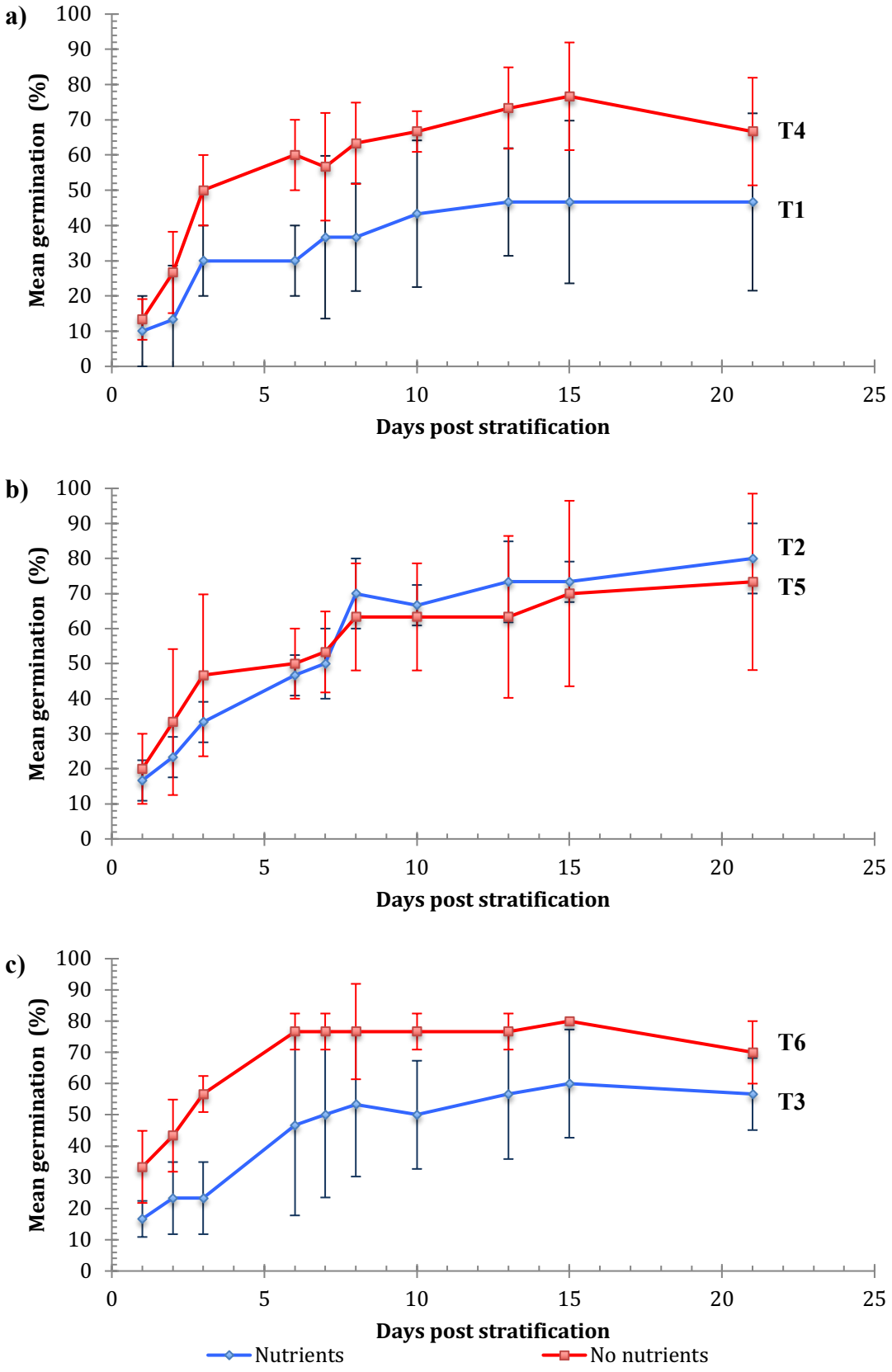


Figure 4: Mean germination percentage of seeds on agar with: (a) MS nutrients (T1) vs. no nutrients (T4) (1 week post stratification); (b) MS nutrients (T2) vs. no nutrients (T5) (2 week post stratification); (c) MS nutrients (T3) vs. no nutrients (T6) (1 week post stratification) (mean ± SD; Trial 2)

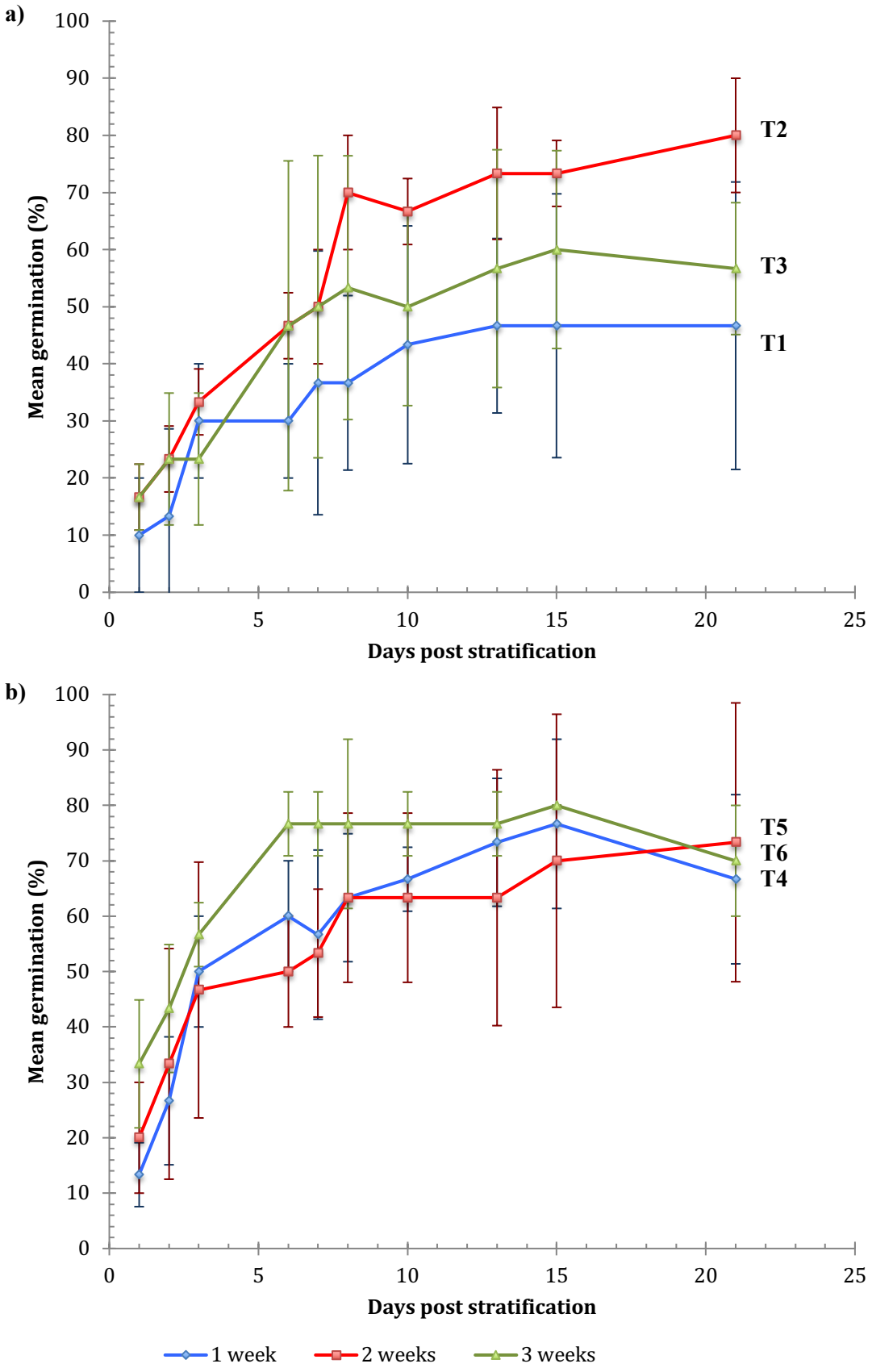


Figure 5: Mean germination percentage of seeds on agar with (a) MS nutrients and 1 week (T1); 2 weeks (T2); 3 week (T3) stratification & (b) no nutrients and 1 week (T4); 2 week (T5), 3 week (T6) stratification (mean ± SD; Trial 2)

At 21 days post stratification, those seeds that had 3 weeks of stratification on non-MS agar had the largest shoot emergence (32.22 ± 13.31%) (Figure 6). There was no significant difference ($p>0.05$) between treatments 2, 3, 5, and 6 (treatments 1 and 4 were excluded from statistical analysis due to a lack of variance).

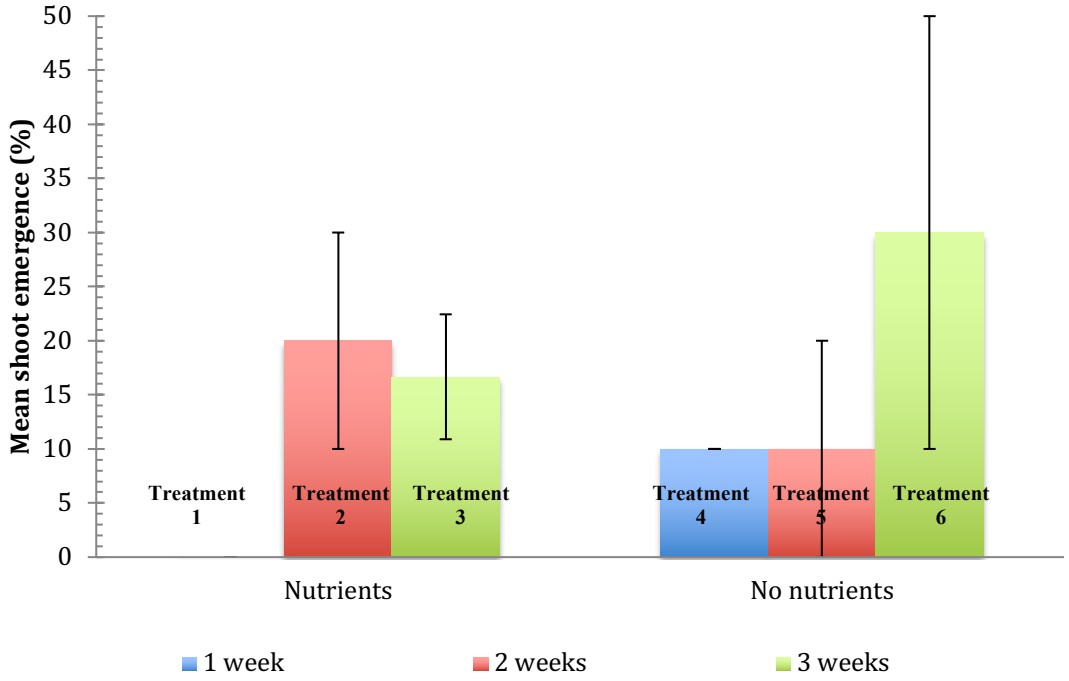


Figure 6: Mean shoot emergence 21 days post – 1, 2, & 3 week stratification with (Treatment 1-3) and without (treatment 4-6) nutrient addition (mean ± SD; Trial 2)

Trial 3

Immediately after the 2 week stratification, mean germination (%) for seeds sterilised for 5 minutes prior to stratification (treatment 2) was 77.5 ± 12.6%, however, seeds sterilised for 1 (treatment 1), 10 (treatment 3), 15 (treatment 4), and 0 (treatment 5; control) minutes prior to stratification had a germination percentage only 2.5%, 10%, 5%, and 5% lower than treatment 2, respectively (Figure 7a). Root emergence for treatment 1 was 62.5 ± 17.1% with treatments 2, 3, 4, and 5 (control) being 7.5%, 12.5%, 2.5%, and 17.5% lower, respectively (Figure 7b). There was no significant difference ($p>0.05$) between any treatments for mean germination and root emergence (see appendix, Table 1 and 2, pg. 362, for mean germination and root emergence based on agar type).

Mould was present on 8 ± 5% of seeds from treatment 5 (control) by the end of the stratification period and on 10 ± 8%, and 22.5 ± 17.1% of seeds from treatment 3 and 5, respectively 21 days post stratification. Of those seeds that did not germinate, 58 ± 50% and 75 ± 22% from treatment 3 and 5, respectively, were covered in mould. No mould grew on any seed from treatment 1, 2 and 4 at any stage.

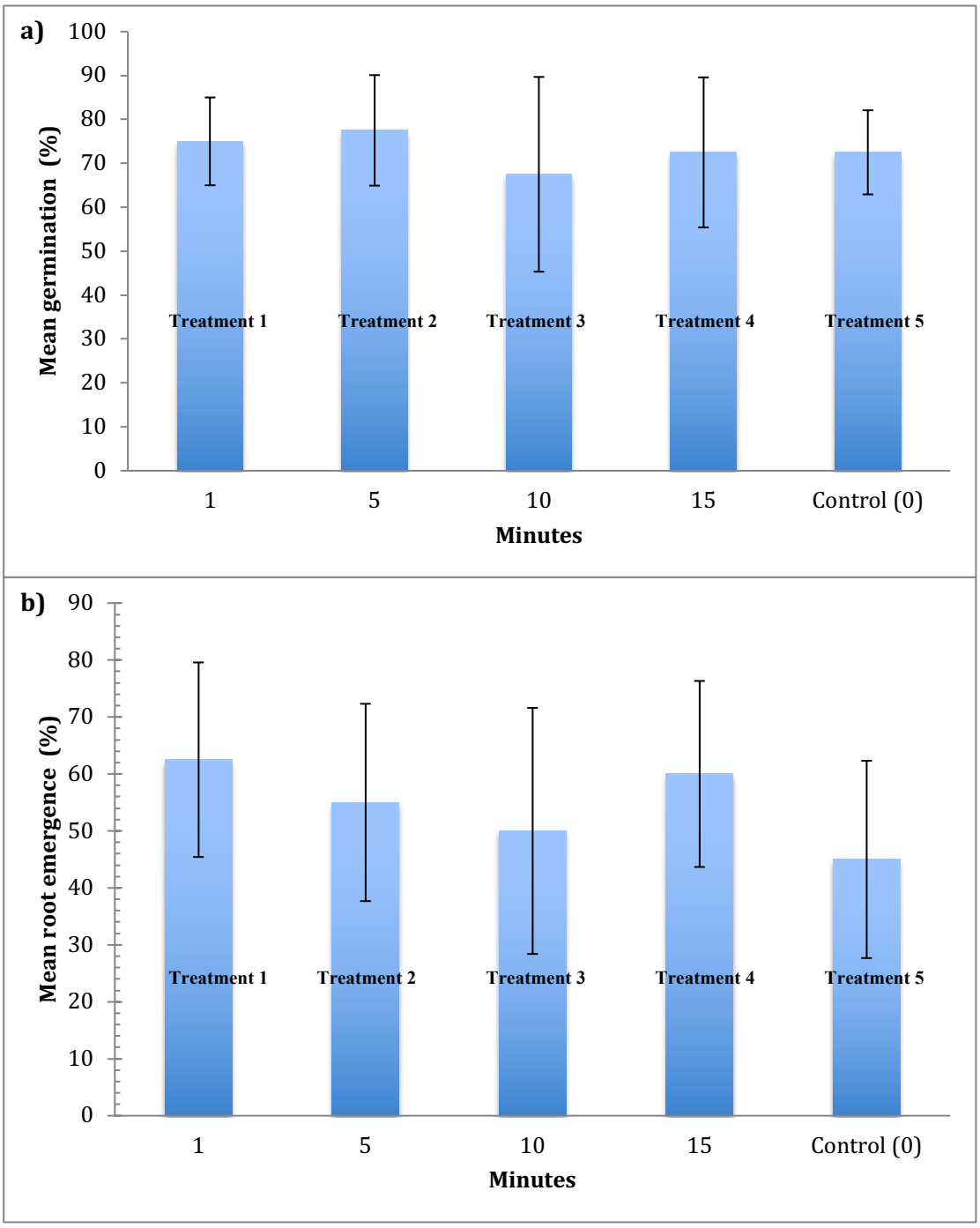


Figure 7: a) Mean germination percentage and b) mean root emergence percentage post 2 week stratification of seeds disinfected for 1, 5, 10, 15, and 0 (control) minutes prior to stratification (mean ± SD; Trial 3)

There was no significant difference in shoot emergence (%) between all treatments over the 21 days post stratification. By the end of the trial (21 days post stratification), the shoot emergence of treatment 1 was 90 ± 12%, followed by treatment 4 (15 minutes disinfection), 2 (5 minutes), 3 (10 minutes), and the control (0 minutes), at 83 ± 5%, 77.5 ± 17.1%, 75 ± 17%, and 65 ± 17%, respectively ($p>0.05$) (Figure 8).

There was no significant difference ($p>0.05$) over the monitored 21 days post stratification between any treatments from day 7 onwards. On day 1, there was a significant difference between treatment 1 and 5 ($p<0.05$). On day 3 there was a significant difference between treatment 1 and 3, 4, and 5 ($p<0.05$), between treatment 3 and 5 ($p<0.05$) and between treatment 4 and 5 ($p<0.05$). On day 4 and 5 there was a significant difference between treatment 5 and 1, 2, 3, and 4 ($p<0.05$).

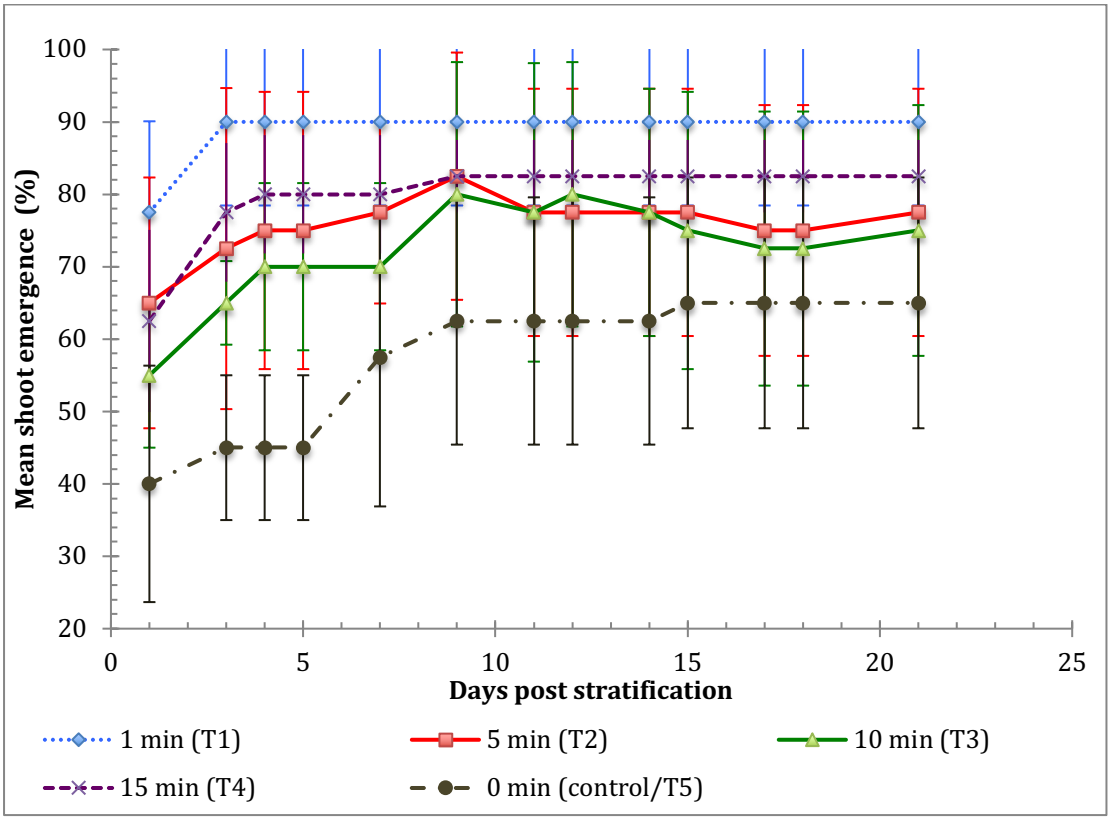


Figure 8: Mean shoot emergence post 2 week stratification of seeds disinfected for 1, 5, 10, 15, and 0 (control) minutes prior to stratification (mean ± SD)

3.4 Discussion

Annual *Salicornia* species are generally highly salt-resistant, but response to salinity during the germination process can be highly variable (Ventura and Sagi, 2013; Ventura *et al*. 2011; Khan and Gul, 2006; Khan *et al*. 2000). Although studies have shown that a stratification treatment can enhance the germination success of *S. euroapaea* (Keiffer and Ungar, 1997; Philipupillai & Ungar, 1984), it was unclear from the literature whether this step is commonly utilised for the germination of *Salicornia* in an experimental or commercial setting. Those studies that do include the pre-germination stratification stage often do not report sufficient detail, for example, not indicating the salinity and/or the methodology utilised for the stage (Davy *et al*. 2001; Keiffer and Ungar, 1997; Keiffer *et al*. 1994; Philipupillai & Ungar, 1984; Ungar, 1979).

In trial 1, the aim was to determine the impact of a 30 day stratification treatment, at various salinities, on the germination of *S. europaea* seeds 10 days post stratification (note: the salinity for each treatment was maintained post-stratification). Although there was no significant difference in germination between the salinity levels assessed, seeds germinated at a salinity of 17.89 ppt were significantly healthier (Fv/Fm level) than those germinated at a salinity of 0 ppt and 24.33 ppt ($p<0.05$). For this reason, a salinity of 17 ppt was chosen for trial 2. Of those seeds that did not germinate in treatment 1 (0 ppt) and 2 (10.41 ppt), 79 ± 9% and 83.84 ± 14.64%, respectively, were covered in mould. An increase in salinity appeared to control mould growth, as no mould was present on any seeds from treatment 3-5 (17.98, 24.33, and 34.21 ppt). The 100% seawater treatment resulted in a germination of 0%, however, 64%, 44%, 44%, and 36% of seeds germinated when transferred to salinities of 0 ppt, 10.41 ppt, 17.89 ppt, and 24.33 ppt.

A study by Ungar (1979) assessed the recovery germination of small (1.1 ± 0.1 mm) and large (1.8 ± 0.1 mm) *S. europaea* seeds by immersing them in distilled water for 42 days following exposure to salt stress (50 and 100 ppt) for 56 and 100 days (note: recovery germination refers to germination of seeds after failure to germinate at one set of parameters and exposed to a new set of parameters). They found that the recovery germination was 16 ± 4.3% and 16 ± 1.6% for small seeds who initially underwent 50 ppt salt-stress for 56 and 100 days, respectively, and 14 ± 1.2% and 18 ± 3.5% for those that underwent 100 ppt salt-stress for 56 and 100 days, respectively. The recovery

germination was 91 ± 2% and 77 ± 2% for large seeds who underwent 50 ppt salt-stress for 56 and 100 days, respectively, and 82 ± 5% and 67 ± 1% for those that underwent 100 ppt salt-stress for 56 and 100 days, respectively.

Pujol *et al*. (2000) found that when *S. ramosissima* seeds where exposed to iso-osmotic stress, the percentage of un-germinated seeds that recovered (i.e. germinated) when they were transferred to distilled water did not differ significantly from the percentage of seeds that germinated in distilled water (controls). There is limited literature available on what salinities are most effective for the germination of *S. europaea*, and those that are available differ in their findings. Keiffer and Ungar (1997) found that the mean germination percentage 30 days after a 30 days stratification treatment at 5°C for *S. europaea* was 53 ± 11%, 39 ± 8%, 39 ± 8%, and 41 ± 10 at salinities of 0 ppt, 10 ppt, 20 ppt, and 30 ppt, respectively (it was assumed that the stratification pre-treatment occurred with freshwater. It was not explicitly expressed, however, it was noted that the step took place *before* salinity treatments).

Philipupillai and Ungar, (1984) assessed the impact of a 4 week stratification pre-treatment at 4°C and subsequent exposure to various salinities, on the germination success of small (<1.4 mm) and large (≥1.5 mm) *S. europaea* seeds (note: it was unclear whether the various salinities that the seeds were exposed to was also assessed during the stratification period or if the stratification period was conducted with freshwater for all treatments). After 5 weeks, they found that germination was enhanced with a stratification pre-treatment for both large and small seeds and that large seeds had a greater tolerance to higher salinities. The most successful germination of small seeds occurred post-stratification when exposed to a salinity of 0 ppt (41 ± 9%) and 10 ppt (32 ± 5%) at a temperature of 5-15 °C. By comparison, the best germination for small seeds that did not undergo stratification was 3 ± 1% and 3 ± 2%, when exposed to 0 ppt, at a temperature of 5-25°C and 15-25 °C, respectively, post-stratification. The most successful germination of large seeds occurred post-stratification when exposed to a salinity of 0 ppt (89 ± 2%) and 30 ppt (82 ± 4%) at a temperature of 5-15°C. By comparison, the best germination for large seeds that did not undergo stratification was 48 ± 4% and 40 ± 3%, when exposed to 0 ppt, at a temperature of 5-15 °C and 15-25 °C, respectively, post-stratification. It was also noted that large seeds obtained a germination of 43 ± 11% when exposed to a salinity of 50 ppt, whereas small seeds did not germinate at 50 ppt and obtained a germination of 10 ± 4% at 30 ppt, post stratification (Philipupillai and Ungar, 1984).

Ungar (1967) found that the germination percentages of *S. europaea* were 50.5%, 28.5%, 20.75%, 11.5%, and 10% at a salinity of 0 ppt, 5 ppt, 10 ppt, 30 ppt, and 50 ppt, respectively. A stratification pre-treatment did not take place prior to this germination trial. Khan and Gul, (2006) found that the germination percentage of *S. europaea* seeds reduced from 75-100% to 10% or less when exposed to a salinity of 850 mM (i.e. 51 ppt). It was not clear from this study if a stratification pre-treatment took place. Although the number of studies that compare different germination salinities for *S. europaea* is limited, the majority of studies utilising *S. europaea* expose the seeds to freshwater or low-medium salinity (i.e. <20 ppt) until germination occurs (with or without a stratification pre-treatment) (Quintã *et al*. 2015; Webb *et al*. 2013; Lv *et al*. 2012; Webb *et al*. 2012; Ventura *et al*. 2010; Keiffer *et al*. 1994).

In trial 2, the impact of stratification duration (1, 2, or 3 weeks) and the presence or absence of nutrients at this stage, were assessed (salinity: 17.34 ppt; based on results of trial 1). It was noted in trial 1 that germination had begun for a number of seeds (c. 10-35 %) by the 4th week of stratification, indicating that 4 weeks may not be necessary to break dormancy. Therefore, for trial 2 seeds were not subjected to 4 weeks of stratification. It was found that seeds exposed to 2 weeks of stratification on MS nutrient agar (treatment 2) and non-MS nutrient (no additional nutrients) agar (treatment 5) had the highest germination at 21 days post stratification (seeds exposed to natural light) at 80 ± 10% and 73.33 ± 25.17%, respectively ($p>0.05$). Although there was not a large difference between germination based on nutrient addition (treatment 2 vs. 5) 21 days post stratification, it was noted that seedlings from treatment 5 (non-MS agar) were developing red roots, a sign of nutrient deficiency.

A study conducted by Ungar (1979) found that the germination of *S. europaea* seeds was not promoted by treatment with nutrient solutions (half-strength Hoagland or Arnon no. 2). It would appear from this trial (trial 2) that *S. europaea* seeds only need a 2 week stratification period for successful germination to occur. However, it is important to note that this is just one trial utilising one source of seeds. A study conducted by Davy *et al*. (2001) found that a stratification treatment (c. 3°C) of up to 5 weeks was necessary to break the dormancy of seeds produced in early autumn, while a number of studies utilised a 30 day or 4 week period at 4-5 °C (Keiffer and Ungar, 1997; Keiffer *et al*. 1994; Philipupillai and Ungar, 1984). The use of agar instead of filter paper made it easier to keep the seeds damp throughout the trial. Of those seeds that did not germinate, 43.61 ± 14.35%, 66.67 ± 57.74%, 44.44 ± 13.88%, 41.11 ±

8.39%, 26.67 ± 46.19%, and 61.11 ± 34.70% from treatment 1-6, respectively, were covered in mould, indicating that agar, regardless of nutrient presence, facilitated the growth of mould, which may have been responsible for the non germination of some seeds.

The aim of trial 3 was to assess the impact of sterilising seeds for 0 (control), 1, 5, 10, and 15 minutes prior to stratification. For this trial, only a 2 week stratification period was necessary, as a large number of seeds from all treatments began to germinate (67.5 ± 22.2% - 77.5 ± 12.6%) and undergo root emergence (45 ± 17% - 62.5 ± 17.1%) within this period of time. It was found that there was no significant difference in the germination and mean root emergence of seeds sterilised for 0, 1, 5, 10, or 15 minutes prior to stratification. However, those seeds sterilised for 1 minute prior to stratification did have significantly higher ($p<0.05$) shoot emergence than those that underwent other sterilisation durations 1-5 days post stratification (Figure 8). Of those seeds that did not germinate, 58 ± 50% and 75 ± 22% from treatment 3 and 5, respectively, were covered in mould, and this may have been responsible for these seeds not germinating. As all sterilisation treatments, with the exception of treatment 3 (sterilised for 10 minutes), did not have mould growth on seeds, it would appear that sterilisation of seeds prevents mould formation. These results suggest that sterilising seeds prior to stratification does not have a significant impact on germination success and root emergence, and a limited significant impact on shoot emergence (i.e. no significant difference from day 7 onwards).

Nevertheless, the seeds from the control treatment (no sterilisation) still had a high germination and root emergence while undergoing stratification (2 weeks), indicating that this early onset of growth cannot be attributed to the impact of sterilisation alone. It is possible that the new seed source for trial 5 (the Atlantic coast of West France) may have been a less dormant ecotype and did not require more than 2 weeks to break dormancy. Also, although the seeds were not measured, it was noted that these seeds were bigger than those used in trial 1 and 2 of this study, and trial 1 and 2 of chapter 2 (source: Kent, England) (Jessica Earl, personal observation, 2015). As discussed above, previous studies have found that larger *S. europaea* seeds germinate more easily/quickly, and may not require stratification (Philipupillai & Ungar, 1984; Ungar, 1979b). Philipupillai and Ungar (1984) also sterilised (with 0.53% sodium hypochlorite for one minute followed by repeated distilled water rinsing) *S. europaea* seeds prior to germination trials, however, they did not directly assess the impact of this procedure on

germination success. Nevertheless, sterilisation did not appear to have a negative impact on germination (Philipupillai and Ungar, 1984).

3.5 Conclusion

This study has shown that, although there was no significant difference in germination based on salinity level during stratification, *S. europaea* seeds exposed to salinities of c.17 ppt were significantly healthier in comparison to those exposed to freshwater (distilled) or to those exposed to brackish water of c.24 ppt salinity. No significant improvement in germination success resulted from the addition of nutrients during stratification, while sterilising seeds prior to stratification did not significantly improve the germination and root emergence of *S. europaea* seeds, and had a limited improvement to shoot emergence.

It was also noted during the course of this study that seeds of *S. europaea* vary in size from approximately 1 to 2 mm, with larger seeds perhaps having a higher germination success and salinity tolerance (Philipupillai and Ungar, 1984; Ungar, 1979). Subsequently, future studies could assess these approaches to stratification on seed size classes.

3.6 Acknowledgements

Jessica Earl (UCC) conducted the experimental work for this chapter with the assistance of Daryl Gunning. Prof. Gavin Burnell and Dr. Marcel Jansen gave advice on the design of these trials and provided feedback on the chapter.

3.7 References

Aghaleh, M., Niknam, V., Ebrahimzadeh, H., and Razavi, K. (2009). Salt stress on growth, pigments, proteins, and lipid peroxidation in *Salicornia persica* and *S. europaea*. *Biologia Plantarum*, 53(2), pp. 243-248.

Al-Hawija, B.N., Partzsch, M., Hensen, I. (2012). Effects of temperature, salinity, and cold stratification on seed germination in halophytes. *Nordic Journal of Botany*, 30, pp. 627-634.

Amiri, B., Assareh, M. H., Jafari, M., Rasuoli, B., Arzani, H., and Jafari, A. A. (2010). Effect of salinity on growth, ion content and water status of glasswort. *Caspian Journal of Environmental Sciences,* 8(1), pp. 79–87.

Baskin, C.C., and Baskin, J.M. (2004). A classification system for seed dormancy. *Seed Science Research*, 14, pp. 1-16.

Baskin, C.C., and Baskin, J.M. (2001). *Seeds: ecology, biogeography and evolution of dormancy and germination.* Elsevier, Amsterdam, The Netherlands, p. 666.

Butler, W.L. (1978). Energy distribution in the photochemical apparatus of photosynthesis. *Annual Review of Plant Physiology*, 29, pp. 345–378.

Davy, A. J., Bishop, G. F., and Costa, C. S. B. (2001). *Salicornia* L. (*Salicornia pusilla* J. Woods, *S. ramosissima* J. Woods, *S. europaea* L., *S. obscura* P.W. Ball & Tutin, *S. nitens* P.W. Ball & Tutin, *S. fragilis* P.W. Ball & Tutin and *S. dolichostachya* Moss). *Journal of Ecology*, 89, pp. 681-707.

El-Keblawy, A., and Al-Rawai, A. (2005). Effects of salinity, temperature, and light on germination of invasive *Prosopis juliflora* (Sw.) D.C. *Journal of Arid Environments*, 61(4), pp. 555-565.

Ellison, A.M. (1987a). Effects of competition, disturbance, and herbivory on *Salicornia europaea. Ecology*, 68(3), pp. 576-586.

Ellison, A.M. (1987b). Density-dependent dynamics of *Salicornia europaea* monocultures. *Ecology*, 68(3), pp. 737-741.

Grouzis M., Berger, A., and Heim, G. (1976). Polymorphisme et germination des grains chez trois espèces annuelles du genre *Salicornia*. *Oecologia Plantarum*, 11(1), pp. 41-52.

Jefferies, R. L., Davy, A. J., and Rudmik, T. (1981). Population biology of the salt marsh annual *Salicornia europaea* agg. *Journal of Ecology*, 69, pp. 17-31.

Keiffer, C.H., and Ungar, I.A. (1997). The effect of extended exposure to hypersaline conditions on the germination of five inland halophyte species. *American Journal of Botany*, 84(1), pp. 104-111.

Keiffer, C.H., McCarthy, B.C., and Ungar, I.A. (1994). Effect of salinity and waterlogging on growth and survival of *Salicornia europaea* L., and inland halophyte. *Ohio Journl of Science,* 94(3), pp. 70-73.

Khan, M.A., and Gul, B. (2006). Halophyte seed germination. In: Khan, M.A., and Weber, D.J. (eds.), *Ecophysiology of High Salinity Tolerant Plants.* Springer Birhäuser Verlag, The Netherlands, pp. 11-30.

Khan, M.A., and Gulzar, S. (2003). Light, salinity, and temperature effects on the seed germination of perennial grasses. *American Journal of Botany*, 90, pp. 131-134.

Khan, M.A., Gul, B., and Weber, D.J. (2000). Germination response of *Salicornia rubra* to temperature and salinity. *Journal of Arid Environments*, 45, pp. 207-214.

Khan, M.A., and Gul, B. (1998). High salt tolerance in germinating dimorphic seeds of *Arthrocnemum indicum*. *International Journal of Plant Sciences*, 159(5), pp. 826-832.

Khan, M.A., and Ungar, I.A. (1998). Seed germination and dormancy of *Polygonum aviculare* L. influenced by salinity, temperature, and gibberellic acid. *Seed Science and Technology*, 26, pp. 107-117.

Khan, M.A., and Ungar, I.A. (1997). Effects of thermoperiod on recovery of seed germination of halophytes from saline conditions. *American Journal of Botany*, 84, pp. 279-283.

Khan, M.A., and Weber, D.J. (1986). Factors influencing seed germination in *Salicornia pacifica* var. *utahensis*. *American Journal of Botany*, *73*(8), pp. 1163–1167.

Kitajima, M., and Butler, W.L. (1975). Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. *Biochimica et Biophysica Acta - Bioenergetics*, 376(1), pp. 105-115.

Li, L., and XiMing, Z. (2007). Germination strategies of two halophytes in salt desert of northwestern China. *Science in China Series D: Earth Sciences*, 50(1), pp. 115-121.

Lv, S., Jiang, P., Chen, X., Fan, P., Wang, X., and Li, Y. (2012). Multiple compartmentalization of sodium conferred salt tolerance in *Salicornia europaea*. *Plant Physiology and Biochemistry*, 51, pp. 47-52.

Maxwell, K., and Johnson, G. N. (2000). Chlorophyll fluorescence – a practical guide. *Journal of Experimental Botany,* 51(345), pp. 659-668.

O'Leary, J.W., Glenn, E.P., and Watson, M.C. (1985). Agricultural production of halophytes irrigated with seawater. *Plant and Soil*, 89, pp. 311-321.

Ozawa, T., Wu, J., and Fujii, S. (2007). Effect of inoculation with a strain of *Pseudomonas pseudoalcaligenes* isolated from the endorhizosphere of *Salicornia europaea* on salt tolerance of the glasswort. *Soil Science and Plant Nutrition*, 53, pp. 12-16.

Philipupillai, J., and Ungar, I. A. (1984). The effect of seed dimorphism on the germination and survival of *Salicornia europaea* L. populations. *American Journal of Botany*, 71(4), pp. 542-549.

Qu, X-X., Huang, Z-Y., Baskin, J.M., and Baskin, C.C. (2008a). Effect of temperature, light and salinity on seed germination and radicle growth of the geographically widespread halophyte shrub *Halocnemum strobilaceum*. *Annals of Botany,* 101(2), pp. 293-299.

Qu, X-X., Baskin, J.M., Wang, L., and Huang, Z. (2008b). Effects of cold stratification, temperature, light and salinity on seed germination and radicle growth of the desert halophyte shrub, *Kalidium caspicum*. *Plant Growth Regulation*, 54(3), pp. 241-248.

Quintã, R., Santos, R., Thomas, D.N., and Le Vay, L. (2015). Growth and nitrogen uptake by *Salicornia europaea* and *Aster tripolium* in nutrient conditions typical of aquaculture wastewater. *Chemosphere*, 120, pp. 414-421.

Riehl, T.E., and Ungar, I.A. (1982). Growth and ion accumulation in *Salicornia europaea* under saline field conditions. *Oecologia*, 54(2), pp. 193-199.

Singh, D., Buhmann, A. K., Flowers, T. J., Seal, C. E., and Papenbrock, J. (2014). *Salicornia* as a crop plant in temperate regions: selection of genetically characterised ecotypes and optimization of their cultivation conditions. *AoB PLANTS*, 6(plus071); doi:10.1093/aobpla/plu071.

Ungar, I.A. (1991). *Ecophysiology of vascular halophytes*. CRC press, Boca Raton, Florida, USA, p. 221.

Ungar, I.A. (1987). Population characteristics, growth, and survival of the halophyte *Salicornia europaea*. *Ecology*, 68(3), pp. 569-575.

Ungar, I.A., Benner, D.K., and McGraw, D.C. (1979). The distribution and growth of *Salicornia europaea* on an inland salt pan. *Ecology*, 60(2), pp. 329-336.

Ungar, I.A. (1979). Seed dimorphism in *Salicornia europaea* L. *Botanical Gazette*, 140(1), pp. 102-108.

Ungar, I.A. (1978). Halophyte seed germination. *The Botanical Review*, 44(2), pp. 233-264.

Ungar, I.A. (1967). Influence of salinity and temperature on seed germination. *Ohio Journal of Science*, 67(2), pp. 120-123.

Ventura, Y., and Sagi, M. (2013). Halophyte crop cultivation: The case for *Salicornia* and *Sarcocornia*. *Environmental and experimental botany*, 92, pp. 144-153.

Ventura, Y., Wuddineh, W.A., Myrzabayeva, M., Alikulov, Z., Khozin-Goldberg, I., Shpigel, M., Samocha, T. M., and Sagi, M. (2011). Effect of seawater concentration on the productivity and nutritional value of annual *Salicornia* and perennial *Sarcocornia* halophytes as leafy vegetable crops. *Scientia Horticultura*, 128, pp. 189-196.

Ventura, Y., Wuddineh, W.A., Ephrath, Y., Shpigel, M., and Sagi, M. (2010). Molybdenum as an essential element for improving total yield in seawater-grown *Salicornia europaea* L. *Scientia Horticulturae*, 126, pp. 395-401.

Webb, J.M., Quintã, R., Papadimitriou, S., Norman, L., Rigby, M., Thomas, D.N., and Le Vay, L. (2013). The effect of halophyte planting density on the efficiency of constructed wetlands for the treatment of wastewater from marine aquaculture. *Ecological Engineering*, 61, pp. 145-153.

Webb, J.M., Qunit., R., Papadimitriou, S., Norman, L., Rigby, M., Thomas, D.N., and Le Vay, L. (2012). Halophyte filter beds for treatment of saline wastewater from aquaculture. *Water Research*, 46, pp. 5102-5114.

Chapter 4

Improving the size measurement accuracy of the holothurian, *Holothuria forskali*, through the use of anaesthesia

Abstract

With a body shape that is quite plastic, and a body weight (BW) that can vary considerably depending on the amount of water in the respiratory trees, gathering consistent size measurements of holothurians can be challenging. Nevertheless, consistent and accurate size measurements are important to the development of sea cucumber aquaculture and fishery industries in relation to the determination of growth rates, stock enhancement, and conservation efforts. This study evaluated the effectiveness of three anaesthetics ($MgCl_2$; $MgSO_4$; KCl) at various concentrations on the holothurian, *Holothuria forskali*, and their potential in reducing size measurement (BW & body length {BL}) variation. Concentrations of 0.05%, 0.1%, 0.5%, and 1% $MgSO_4$, 0.5% and 1% KCl, and 0.5% $MgCl_2$, were ineffective at anaesthetising *H. forskali*, while 2% and 4% $MgSO_4$ and 1%, 1.5%, and 2% $MgCl_2$ were effective. The most efficient anaesthetic was 2% $MgCl_2$, with the quickest combined anaesthesia relaxation and recovery time of 38.75 ± 10.73 minutes. BW measurement variability (% CV) was reduced post anaesthesia for all effective anaesthetics with the greatest reduction obtained by the 2% $MgCl_2$ treatment (84.45%; $p<0.05$). A reduction in BL variability was seen for all effective anaesthetics, with 4% $MgSO_4$ having the greatest reduction (50.96%; $p<0.05$). Both 1.5% and 2% $MgCl_2$ had a similar reduction at 50.69% and 49.31%, respectively ($p<0.05$). This study found that long periods of exposure to anaesthetic or handling of *H. forskali* can lead to morphological and physiological stress. Care should be taken to limit exposure and handling duration as much as possible to eliminate or greatly reduce the level of stress caused.

4.1 Introduction

For centuries, sea cucumbers (class: Holothuroidea) have been a popular luxury food item in Asian seafood markets, commonly being sold as bêche-de-mer (dry body wall) (Rodríguez-Barreras *et al*, 2016; Gianasi *et al*, 2015; Santos *et al*. 2015; Purcell *et al*. 2013; Anderson *et al*. 2011; Raison, 2008). The capture fishery industry of popular species (i.e. tropical and Asian) is declining due to overexploitation and poor management of many of these fisheries. Studies on breeding, aquaculture, restocking, and stock enhancement methods of holothurian species have become more common in recent years, with mariculture in particular emerging as a viable prospect to subsidise these waning captures (Gianasi *et al*, 2015; Wantanabe *et al*. 2012; Santos *et al.* 2015; Purcell *et al*. 2014; Purcell *et al*. 2012a; Bell *et al*. 2007; Purcell *et al*. 2006; Hamel *et al*. 2001; Conand and Byrne, 1993). New non-target species from the northern hemisphere are also being fished and traded to the Asian market to meet demand, such as the Mediterranean species: *Holothuria arenicola*, *H.tubulosa*, *H.polii*, and *H. mammata* (MacDonald *et al*. 2013; Nelson *et al*. 2012; Sicuro and Levine, 2011; Conand, 2004).

Despite the increased interest in sea cucumber hatchery and aquaculture techniques, there is a methodological problem with the evaluation and monitoring of holothurian size. It is difficult to measure the length of holothurians consistently as their body can change drastically and frequently by contraction and extension. Holothurian body weight (BW) can also vary considerably, depending on the amount of water in the respiratory trees when weighed (Watanabe, *et al*. 2012; Yamana *et al.* 2005; Battaglene *et al.* 1999; Sewell, 1990). Various methods have been trialled to reduce this BW variation, from removing additional fluid from the respiratory tree by applying gentle pressure to the anterior or posterior end of the sea cucumber to blotting the sea cucumbers dry with paper towel, prior to weighing (Zamora and Jeffs, 2012; Zamora and Jeffs, 2011; Slater and Jeffs, 2010; Slater *et al*. 2009; Slater and Carton, 2007; Sewell 1990 and 1987). However, of these studies, only Slater and Carton (2007) assessed how their methods impacted measurement variability (reduced variability to <±5%). There are currently no standardised methods for size measurement of holothurians. The ability to obtain accurate size measurements would be of considerable benefit to the development of the sea cucumber aquaculture industry (e.g.

for the determination of growth rates and stock enhancement) and for the study of wild population structures for fishery management and conservation efforts.

One possible solution to this lack of accurate body size and weight measurements is the use of anaesthesia. It has been reported that menthol-ethanol is an effective anesthetiser of *Apostichopus japonicus* that improves size measurement accuracies (Yamana and Hamano, 2006; Yamana *et al*. 2005; Hatanaka and Tanimura, 1994). Other trials have successfully anaesthetised holothurians with magnesium sulphate ($MgSO_4$), magnesium chloride ($MgCl_2$), and potassium chloride (KCl) (Purcell *et al*. 2012b; Guzman and Guevara, 2002; Yanagisawa, 1998; Chao *et al*. 1993; Tuwo and Conand, 1992), however, these anaesthetic agents have either been for lethal sampling experiments and/or have not assessed the impact on size measurement variation. Watanabe *et al*. (2012) assessed the impact of anaesthesia (0.5% KCl; 0.05% $MgSO_4$; 2% & 4% menthol-ethanol) on variations in weight and length measurements of *Holothuria scabra* and found that 2% menthol-ethanol significantly reduced the coefficient of variation of the mean body length (BL) and BW by 68% and 43%, respectively. Echinoderms have a unique open circulatory system mediated by the circulation of coelomic fluid filled within the perivisceral coelom. The coelomocytes in the coelomic fluid are considered to be involved in digestion, gas exchange, excretion of waste products, and transportation and storage of nutrients. It is through entering this open circulatory system that it is believed the anaesthetic agents have their affect upon the sea cucumbers (Watanabe *et al*. 2012; Xing *et al*. 2008; Eliseikina and Magarlamov, 2002).

Holothuria forskali (cotton spinner) is a surface-feeding aspidochirote holothurian commonly found in the Atlantic and Mediterranean (MacDonald *et al*. 2013, Tuwo and Conand, 1992; Pérez-Ruzafa and López-Ibor, 1988). Although *H. forskali* has been documented below 300 m in the Canary Islands (Pérez-Ruzafa, *et al*. 1987) it is generally considered a littoral species that is characteristic of rocky bottoms and sea-grass beds (Tuwo and Conand, 1992). It is relatively large (up to 25 cm in length) and has the ability to release cuvierian tubules as a defence mechanism against predators. It is generally light to dark brown or black; however, the underside (location of tube feet) often has a yellowish mottling (DeMoor *et al*. 2003; Vandenspielgel *et al*. 2000; Tuwo and Conand, 1992). *H. forskali* has not yet been utilised commercially, however, it is a high-quality protein source (Taboada *et al.* 2003; Rodríguez *et al.* 2000) with a ω3/ω6 ratio within the range recommended by the World Health Organisation (Santos *et al*.

2015). It also has a number of biological features that have potential applications in biotechnology and pharmaceuticals (MacDonald *et al*, 2013; Bordbar *et al*. 2011; Van Dyck *et al*. 2009; Rodríguez *et al*. 1991) and is being explored as a species for Integrated Multi-Trophic Aquaculture (IMTA) (Zamora *et al*. 2016; MacDonald *et al*. 2013; Deudero *et al*. 2011).

The aim of this study was to assess the:

(1) The efficacy of the anaesthetic agents, $MgSO_4$, KCl, and $MgCl_2$, in anaesthetising *H. forskali*.

(2) Impact of these anaesthetic agents on *H. forskali* body length and width measurement variation.

Anaesthetics were chosen due to their reported success in previous literature, eco-friendly constituents (chemical elements found naturally in the environment, as opposed to synthetic anaesthetic agents such as: pentobarbital, chloral hydrate, urethane, and benzocaine (Culloty and Mulcahy, 1992)), and low cost.

4.2 Methods and Materials

In this study, three trials were conducted on *Holothuria forskali*. The first (preliminary) trial assessed the efficacy of varying concentrations of anaesthetic agent ($MgSO_4$; KCl; $MgCl_2$) on *H. forskali*. The second trial assessed the impact of successful anaesthetic agents on body measurement variability by comparing the pre and post anaesthesia measurements for each anaesthetic treatment. The third trial (main trial) also assessed the impact of successful anaesthetic agents on body measurement variability, this time by comparing body measurement variability of anaesthetic treatments against a control treatment (no anaesthesia prior to body measurements).

Trial 1: Preliminary assessment of anaesthetic agent efficacy

Specimen collection, feeding, and storage

Forty-eight *H. forskali* specimens (mean wet weight {WW}: 163.45 ± 51.87 g) were wild-caught by divers off the coast of Castletownbere, Beara peninsula, County Cork, Ireland on the 1st November 2013 and transported to the Aquaculture and Fisheries Development Centre (AFDC), University College Cork (UCC) in polystyrene boxes containing damp *Laminaria digitata*. Specimens were held in four 400 L tanks (12 specimens per tank; Figure 1) and allowed to acclimatise for a period of 5 days before the first trial began. No mortalities occurred during the acclimatisation period.

Following each treatment, the sea cucumbers were transferred to plastic mesh baskets (55 cm x 40 cm x 14 cm; Figure 2) which were divided into four equal sized sections with plastic mesh screens (n=4 specimens per basket; n=1 specimen per section; n=1 basket per treatment) that were held in 400 L tanks (n=3 baskets per tank; Figure 1) to allow for long-term recovery monitoring. The temperature of each tank was maintained with a PSA Aquaclim 10 reversible heatpump/chiller and had continuous water circulation throughout the acclimatisation and recovery periods (1000 L sump filled with fresh sea water every 3 days) (temperature: 14 ± 1.0 °C; pH: 8.0 ± 0.05; DO: 8.0 ± 0.42 mg/L; salinity: 34.5 ± 0.05 ppt) and specimens were fed a powdered seaweed mix (80% *Ascophyllum nodosum* : 20% *Fucus serratus*) *ab libitum.* Seawater was sourced from Fastnet Mussels, Gearhies, Bantry Bay, Co. Cork.

Figure 1: Acclimatisation/recovery tank setup (*photo courtesy of Cipriano-Maack*)

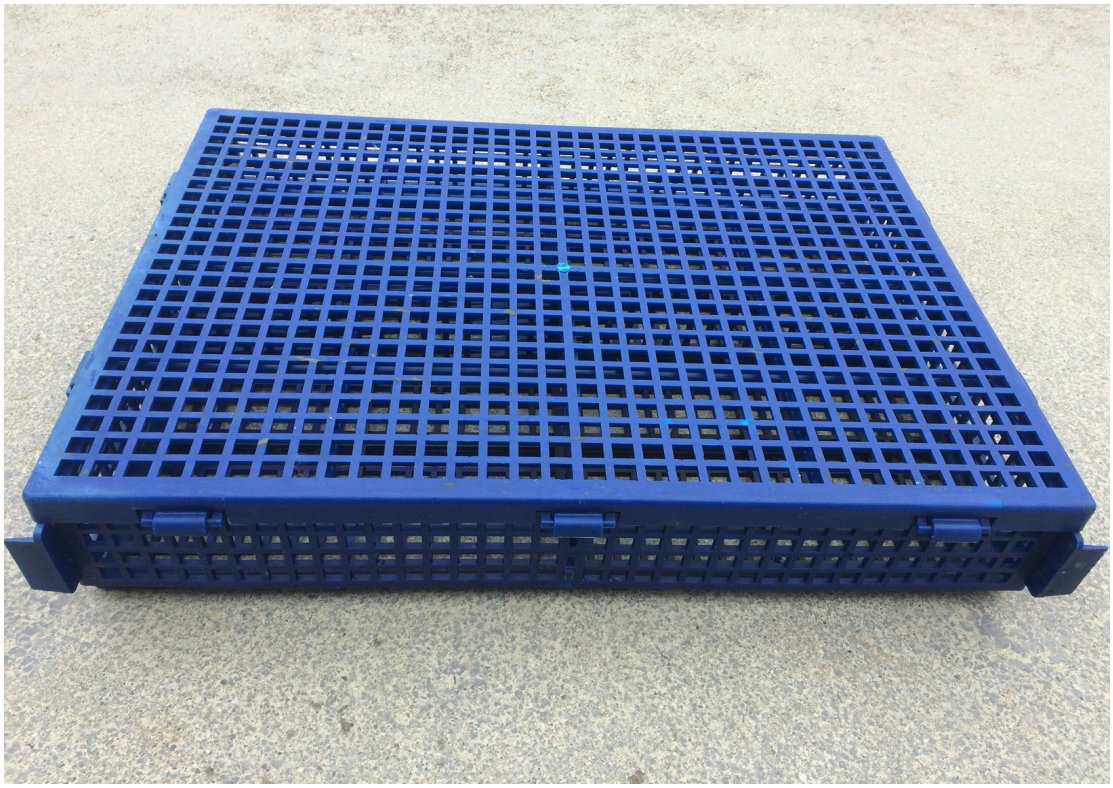


Figure 2: Plastic mesh basket (55 cm x 40 cm x 14 cm) used for long-term recovery monitoring (*original photo by Gunning*)

Treatments

Treatments 1-6 (n=4 per treatment) tested each anaesthetic at the following concentrations: 0.05% (T1) and 0.1% (T2) $MgSO_4$; 0.5% (T3) and 1% (T4) KCl; and 0.5% (T5) and 1% (T6) $MgCl_2$. These concentrations were based on previous trials conducted on holothurians, echinoderms, and bivalves (Watanabe *et al*. 2012; Hickman *et al*. 2004; Guzman and Guevara, 2002; Yanagisawa, 1998; Culloty and Mulcahy, 1992). Following from the findings of these treatments, the following concentrations were also tested: 0.5% (T7), 1% (T8), 2% (T11), and 4% (T12) $MgSO_4$ and 1.5% (T9) and 2% (T10) $MgCl_2$ (n=4 per treatment) (Table 1).

Table 1: Trial 1 anaesthetic treatments

Treatment (n=4 per treatment)	Date	Anaesthetic	Concentration (%)
1	06/11/13	$MgSO_4$	0.05
2			0.1
3	07/11/13	KCl	0.5
4			1
5	08/12/13	$MgCl_2$	0.5
6			1
7	11/11/13	$MgSO_4$	0.5
8			1
9	12/11/13	$MgCl_2$	1.5
10			2
11	13/11/13	$MgSO_4$	2
12			4

Anaesthesia methodology & assessment

For each treatment, specimens were simultaneously placed into 4 L aerated tanks (n=1 per tank; Figure 3) containing their respective anaesthetic solution (anaesthetic dissolved in 1 L seawater) and the length of time it took for the sea cucumbers to be fully anaesthetised (relaxation time) was recorded. The specimen was considered to be fully anaesthetised when each of the following criteria were met; 1) body relaxation (i.e. cessation of crawling movements); 2) failure of tentacles to react to prodding (i.e. the tip of the tentacles were touched with the tip of a forceps); and 3) the inability to anchor firmly (i.e. lack of tube feet attachment to the tank surface). If not all criteria were met after two hours, the anaesthesia was considered a failure. After anaesthesia assessment specimens were then placed in individual 4 L aerated tanks containing 1 L seawater (temperature: 14 ± 0.59 °C; pH: 8.04 ± 0.06; DO: 8.9 ± 0.33 mg/L; salinity:

34.5 ± 0.02 ppt) to monitor anaesthesia recovery (i.e. how quickly they came out of anaesthesia). Full anaesthesia recovery was considered when: 1) crawling movement began; 2) tentacles reacted to prodding; and 3) the majority of tube-feet began to attach. Specimens were kept at this anaesthesia recovery stage for a minimum of 1 hour or until the specimen had recovered from anaesthesia.

Note: to minimise handling stress, body measurements of specimens were not taken before the commencement of trial 1. However, care was taken to ensure each treatment group had an equal spread of small, medium, and large individuals (selected by eye).

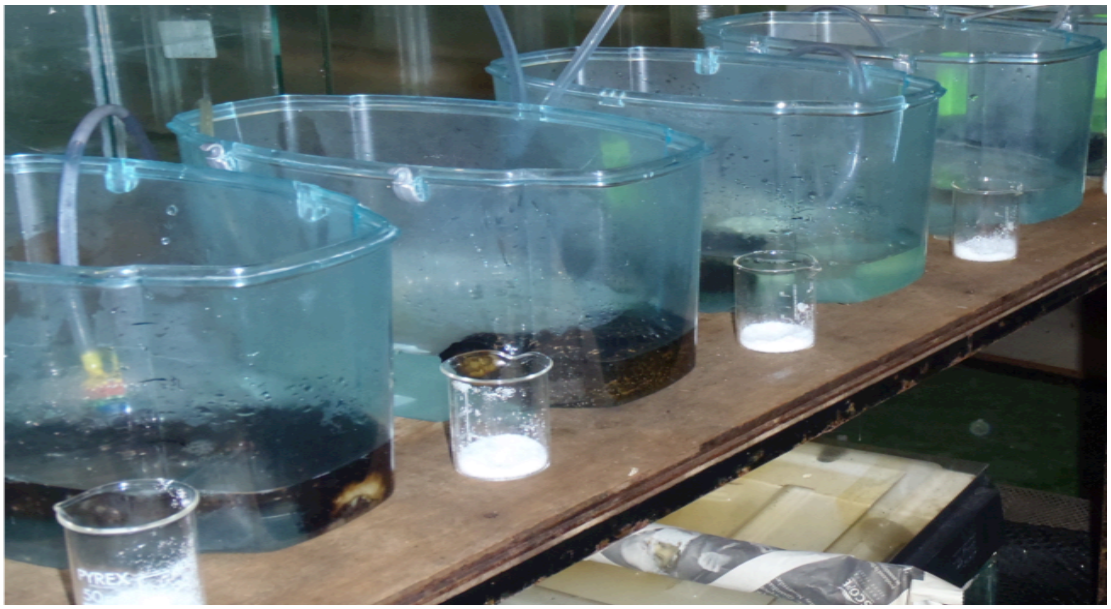


Figure 3: *H. forskali* in individual 4 L tanks with 1 L of filtered seawater prior to addition of anaesthetic (*original photo by Gunning*)

Water parameters

Salinity, pH, DO, and temperature of the seawater in each 4L tank was monitored pre and post anaesthetics addition (after anaesthetic was fully dissolved) for each treatment. (Note: the seawater for the anaesthesia and recovery tanks was from the same source as the acclimatisation and long-term recovery tanks).

Stress and recovery monitoring

To monitor any potential negative impacts of exposing *H. forskali* to anaesthetics, morphological (skin lesions) and physiological (release of cuvierian tubules; evisceration; swelling) indicators of stress were monitored for each individual specimen

during: 1) the treatment and 2) the anaesthesia recovery stage (1 hour duration). Specimens were visually inspected for the presence of skin lesions twice a week, for four weeks after the final treatment was completed (long-term recovery monitoring) (Table 2).

Table 2: Indicators of sea cucumber stress

Stress Indicators	**Description**	**Severity level**	**Stress severity criteria**
Morphological			
Skin lesions	Tissue damage visible as different colouration than surrounding tissue	0	No visual indication of skin lesion presence
		1	<10% body coverage
		2	10-50% body coverage
		3	>50% body coverage
Physiological			
Stress Indicators	**Description**		
Release of cuvierian tubules	Release of defensive threads		
Evisceration	Total or partial extrusion of internal organs		
Swelling	Abnormal enlargement of the body into a balloon shape		

Trial 2: Preliminary assessment of anaesthetic agent impact on body measurement variation

Specimen collection, feeding, and storage

Twenty *H. forskali* specimens (mean wet weight {WW}: 149.73 ± 43.85 g) were wild-caught by divers off the coast of Castletownbere, Beara peninsula, County Cork, Ireland on the 5th December 2013 and transported to the AFDC, UCC in polystyrene boxes containing damp *Laminaria digitata*. Specimens were held in two 400 L tanks (10 specimens per tank) and allowed to acclimatise for a period of 5 days before the first trial began (Figure 1). No mortalities occurred during the acclimatisation period.

Following each treatment, the sea cucumbers were transferred to plastic mesh baskets (55 cm x 40 cm x 14 cm; Figure 2) divided into four equal sized sections with plastic mesh screens (n=4 specimens per basket; n=1 specimen per section; n=1 basket per treatment) that were held in five individual 400 L tanks (n=1 baskets per tank; figure 1) to allow for long-term recovery monitoring (Figure 1). Throughout the acclimatisation and experiment duration each tank's temperature was maintained with the same system as detailed in trial 1 (temp: 14 ± 1.4 °C; pH: 8.0 ± 0.08; DO: 8.12 ± 0.45 mg/L; salinity: 34.51 ± 0.08 ppt) and specimens were fed a powdered seaweed mix (80% *Ascophyllum nodosum* : 20% *Fucus serratus*) *ab libitum.* Specimens were unfed for 48 hours prior to each treatment commencement to ensure the gut was fully evacuated. Seawater was sourced from Fastnet Mussels, Gearhies, Bantry Bay, Co. Cork.

Treatments

The following anaesthetic agents from trial 1 were effective in anaesthetising *H. forskali* and their impact on body measurement variation was assessed: 1%, 1.5%, 2% $MgCl_2$, and 2%, 4% $MgSO_4$ (Table 3).

Table 3: Trial 2 anaesthetic treatments

Treatment (n=4 per treatment)	Date	Anaesthetic
1	09/12/13	1% $MgCl_2$
2	09/12/13	1.5% $MgCl_2$
3	10/12/13	2% $MgCl_2$
4	10/12/13	2% $MgSO_4$
5	11/12/13	4% $MgSO_4$

Anaesthesia methodology & assessment

For each treatment (n=4 per treatment) the same methodology utilised in trial 1 was employed in trial 2. The anaesthesia recovery tanks had the following mean parameters - temperature: 15 ± 0.3 °C; pH: 8.09 ± 0.04; DO: 8.7 ± 0.48 mg/L; salinity: 34.5 ± 0.04 ppt.

Body measurements

For each treatment, specimens were first placed in separate 4 L aerated experimental tanks (n=1 per tank) containing 1000ml of filtered seawater (Figure 3). Size measurements (BL and BW) of each specimen were measured five times, being returned to the tanks for 5 minutes between measurements. After all pre anaesthesia measurements were taken each sea cucumber was transferred to individual aerated 4 L holding tanks (n=1 per tank) containing seawater while the anaesthetic agent was added to the experimental tanks. The specimens were transferred to their respective experimental tank once the anaesthetic solution was ready. As soon as an individual specimen was fully anaesthetised the above pre anaesthesia measurement procedure was also conducted on the anaesthetised individual. BL was measured to the nearest 1 mm using a calliper and BW was measured to the nearest 0.01 g using a digital microbalance after a gentle pressure was applied to the posterior half of the specimen while blotting dry on paper towel to remove as much excess water as possible. Specimens were out of water/anaesthetic solution for approximately 30 seconds during the measurement process (pre/post anaesthesia). The effect of anaesthesia on the coefficient of variation (CV) of BW and BL measurements of *H. forskali* (Watanabe *et al*. 2012) was calculated using the following formula:

CV (%) of BW/BL measurements = 100 x (SD / $X_{BW/BL}$), in which SD stands for standard deviation and $X_{BW/BL}$ for average body weight/body length

Water parameters

The same water parameters were monitored via the same methodology as already reported for trial 1 (Note: the seawater for the anaesthesia, holding, and recovery tanks was from the same source as the acclimatisation and long-term recovery tanks).

Stress and recovery monitoring

Morphological (skin lesions) and physiological (release of cuvierian tubules; evisceration; swelling) indicators of stress were monitored for each individual specimen during the body measurement procedure pre and post anaesthesia, during anaesthesia (prior to repeated body measurements), and during the anaesthesia recovery stage (1 hour duration). All specimens were also monitored for the presence of skin lesion twice per week for four weeks post trial (long-term recovery monitoring) (Table 2).

Trial 3: Assessment of anaesthetic agent efficacy and impact on body measurement variation

Specimen collection, feeding, and storage

Sixty *H. forskali* specimens (mean w.w.: 95.36 ± 28.54 g) were wild-caught by a diver from Kenmare Bay, County Kerry, Ireland on the 22nd of November 2016 and transported to the Bantry Marine Research Station (BMRS) in polystyrene boxes containing damp *Laminaria digitata*. Upon arrival at BMRS, they were transferred to an 8000 L flow-through tank (flow rate: 22.8 L/min; mean temp: 10 ± 1.2 °C; mean DO: 8.67 ± 0.60; mean salinity: 33.01 ± 0.39 ppt; Figure 4). The seawater was from Bantry Bay, Co. Cork and was passed through a 60µm drum filter before entering the tank. In order to acclimate the sea cucumbers, they were held for 7 days prior to the commencement of trial 3. No sea cucumber mortalities were recorded during the acclimation period. Following each treatment, specimens were transferred to a plastic mesh basket (n=2 or 4 specimens per basket; n=1 specimen per section; n=2 baskets per treatment; Figure 2) and returned to the 8000 L tank used for specimen acclimatisation for long-term recovery monitoring. Throughout the acclimatisation and experiment duration, the specimens were fed a powdered seaweed mix (80% *Ascophyllum nodosum* : 20% *Fucus serratus*) *ad libitum*. Specimens were unfed for 48 hours prior to each treatment commencement to ensure the gut was fully evacuated.

Figure 4: Acclimatisation and recovery tank (8000 L) (*original photo by Gunning*)

Treatments and control

Trial 3 was conducted from 29th November to 8th December 2016 and consisted of 6 treatments (n=10 specimens per treatment), measuring BW and BL measurement variability (CV) after being anaesthetised with 2% and 4% $MgSO_4$ and 1%, 1.5%, and 2% $MgCl_2$ (treatment 1-5), and with no anaesthesia prior to measurement (control) (Table 4).

Table 4: Trial 3 anaesthetic treatments and control

Treatment (n=10 per treatment)	Date	Anaesthetic
Control	29/11/16	None
1	29 – 30/11/16	2% $MgSO_4$
2	30/11/16 – 1/12/16	4% $MgSO_4$
3	5 – 6/12/16	1% $MgCl_2$
4	6 – 7/12/16	1.5% $MgCl_2$
5	7 – 8/12/16	2% $MgCl_2$

Anaesthesia assessment

For treatments 1-5 (n=10 per treatment) the same methodology as utilised in trial 1 & 2 was employed.

Body measurements

BW and BL measurement variability (CV) were assessed through the same methodology that was employed in trial 2. However, in this trial (trial 3), pre anaesthesia body measurements were not made for each treatment, instead, a control group was used to assess BW and BL measurements with no prior anaesthesia. The anaesthesia treatments (treatments 1-5) assessed body measurements post-anaesthesia, using the respective anaesthetic agent, only. In trial 3, specimens were dabbed dry with cloth rather than paper towels. It was noted in trial 2 that paper towels stuck to the body of the specimens and may have contributed to skin lesion damage.

Water parameters

Salinity, pH, DO, and temperature of the seawater were monitored in each 4 L tank of the control and of each treatment pre and post anaesthetics addition (Note: the seawater for the anaesthesia, holding, and recovery tanks was from the same source as the acclimatisation and long-term recovery tanks).

Stress and recovery monitoring

Morphological (skin lesions) and physiological (release of cuvierian tubules; evisceration; swelling) indicators of stress were monitored for each individual specimen during BW/BL measurements and for 1 hour post measurement for the control and anaesthesia treatments (note: for the anaesthesia treatments, the 1 hour post measurement monitoring began at the start of the anaesthesia recovery stage) and during anaesthesia (prior to repeated body measurements) for the anaesthesia treatments. All specimens were also monitored for the presence of skin lesion twice per week for four weeks post trial (long-term recovery monitoring).

Statistical analysis

All statistical analysis was compiled using SPSS software (IBM) version 23. Data was tested for normal distribution and homogeneity of variance with the Shapiro-Wilk and Levene's test, respectively ($p>0.05$). Values less than 0.05 were considered statistically significant.

(a) Trial 1

One-way ANOVAs were used to test the significance of difference of relaxation and recovery times between each anaesthetic treatment. Tukey HSD post-hoc analyses were conducted when ANOVAs gave a statistically significant result ($p<0.05$).

Paired t-tests were used to test the significance of difference of water parameters pre and post anaesthesia addition for each treatment. For data that was not normal, a Wilcoxon signal-rank test was used.

(b) Trial 2

One-way ANOVAs were used to test the significance of difference of relaxation and recovery times between each anaesthetic treatment. For both relaxation and recovery times homogeneity of variance was not observed; therefore, a Welch test was applied. Dunnett T3 post-hoc analyses were conducted when the Welch test gave a statistically significant result ($p<0.05$).

Paired t-tests were used to test the significance of difference of water parameters pre and post anaesthesia addition for each treatment.

Paired t-tests were used to test the significance of differences between pre and post anaesthesia BW and BL measurement variability (CV) for each individual treatment. For data that was not normal, a Wilcoxon signal-rank test was used.

One-way ANOVAs were used to test the significance of differences in pre anaesthesia BW and BL variability and post anaesthesia BW and BL variability, between all treatment groups. Tukey HSD post-hoc analyses were conducted when ANOVA's gave a statistically significant result ($p<0.05$). For data that was not normal, individual Kruskal-Wallis tests were used.

Each individual treatment's post anaesthesia BW and BL variability (CV) was measured against all treatments' pre anaesthesia measurements using an independent t-

test. If the groups' variance were unequal, an adjustment was made to the degrees of freedom using the Welch-Satterthwaite method.

(c) Trial 3

One-way ANOVAs were used to test the significance of difference of relaxation and recovery times between anaesthetic treatments. Tukey HSD post-hoc analyses were conducted when ANOVAs gave a statistically significant result ($p<0.05$). For data that was not normal, individual Kruskal-Wallis tests were used.

Paired t-tests were used to test the significance of difference of water parameters pre and post anaesthesia addition for each treatment. For data that was not normal, a Wilcoxon signal-rank test was used.

Each anaesthetic treatments' BW and BL variability (CV) were individually measured against the control's (no anaesthesia) BW and BL variability using independent t-tests. If the groups' variance were unequal, an adjustment was made to the degrees of freedom using the Welch-Satterthwaite method. For data that was not normally distributed, Kruskal-Wallis tests were used.

4.3 Results

Trial 1: Preliminary assessment of anaesthetic agent efficacy

Anaesthetic agent efficacy

Concentrations of 0.05%, 0.1%, 0.5%, and 1% $MgSO_4$, and 0.5% $MgCl_2$ were ineffective in anaesthetising *H. forskali*. None of the criteria for anaesthesia were met by specimens exposed to 0.05%, 0.1%, 0.5%, and 1% $MgSO_4$. Although a cessation of movement and an inability to anchor firmly with the tube feet was noted, after two hours in 0.5% $MgCl_2$, the tentacles of all specimens at this concentration were still reacting to prodding. Both 0.5% and 1% KCl were irritating and stressful to the specimens, which resulted in the experiments being terminated prematurely. A concentration of 2% $MgCl_2$ resulted in the quickest relaxation time (9.5 ± 1.73 mins) and 1% $MgCl_2$ had the quickest recovery time (10.25 ± 2.87 mins) (Table 5). The relaxation time of specimens exposed to 2% $MgCl_2$ was significantly lower than for *H. forskali* exposed to 1% $MgCl_2$, 1.5% $MgCl_2$, and 2% $MgSO_4$ ($p<0.05$). The anaesthesia recovery time of specimens exposed to 1% $MgCl_2$ was significantly lower than for animals exposed to1.5% $MgCl_2$ and 4% $MgSO_4$ ($p<0.05$). All other differences in relaxation or recovery time between anaesthetic agents were not significant ($p>0.05$).

Table 5: Relaxation and recovery time of effective anaesthetic agents (Trial 1) (mean ± SD)

Anaesthetic conc. (%)	Relaxation time (mins)	Recovery time (mins)
$MgSO_4$		
2	40.5 ± 7.77	19.75 ± 3.30
4	28 ± 11.80	25 ± 5.60
$MgCl_2$		
1	44.75 ± 14.77	10.25 ± 2.87
1.5	36.5 ± 6.61	23.5 ± 6.76
2	9.5 ± 1.73	20 ± 6.78

Water parameters

For each anaesthetic treatment, except for 0.05% $MgSO_4$, 0.1% $MgSO_4$, 0.5% KCl, and 1% KCl treatments which experienced a small decrease, the mean temperature of the seawater post anaesthetic addition increased by a larger amount as the concentration of

anaesthetic added got higher. The largest increase in temperature occurred for the 4% $MgSO_4$ treatment. The mean salinity of the seawater for each anaesthetic treatment, except for 0.5% $MgSO_4$, which experienced a small decrease, also increased by a larger amount as the concentration got higher. The largest increase in salinity occurred for the 2% $MgCl_2$ treatment (increase of 19.36 ppt). The mean pH of the seawater for each anaesthetic trialled, except for 2% $MgSO_4$, 4% $MgSO_4$ and 0.5% $MgCl_2$, increased after the addition of an anaesthetic agent, however, an increase in concentration did not always result in a larger change in pH. The largest change in pH post anaesthesia addition occurred for the 0.05% $MgSO_4$ and 1% KCl treatments (increase of pH 0.37) (Table 6). For all treatments, pre and post anaesthesia addition, the DO remained relatively constant (8.4 ± 0.62 mg/L).

Table 6: Water parameters pre and post anaesthesia addition (Trial 1) (mean ± SD; same subscript post anaesthesia = not significant; different subscript post anaesthetic = significantly different [comparison is pre vs post for each individual anaesthetic])

Anaesthetic (%)	Temp (°C)		Salinity (ppt)		pH	
$MgSO_4$	***Pre***	***Post***	***Pre***	***Post***	***Pre***	***Post***
0.05	14±0.33^a	14±0.33^b	34.45±0.17^a	34.83±0.09^b	8.14±0.13^a	8.51±0.15^b
0.1	14±0.05^a	14.±0.08^a	34.43±0.16^a	34.95±0.10^b	8.07±0.04^a	8.43±0.18^b
0.5	15±0.23^a	15±0.22^b	34.50±0.22^a	36.34±0.19^b	8.05±0.06^a	8.30±0.27^a
1	14.±0.14^a	15±0.12^a	34.39±0.10^a	37.86±0.04^b	8.08±0.5^a	8.15±0.07^a
2	15±0.05^a	16±0.21^a	34.45±0.09^a	40.34±0.02^b	8.10±0.02^a	7.91±0.21^b
4	15±0.10^a	19±0.14^b	34.62±0.32^a	44.57±0.07^b	8.10±0.04^a	7.71±0.14^b
KCl						
0.5	14±0.08^a	14±0.04^b	34.47±0.10^a	40.09±0.08^b	8.09±0.05^a	8.33±0.10^a
1	14±0.05^a	14±0.05^b	34.59±0.39^a	45.28±0.05^b	8.11±0.07^a	8.48±0.10^b
$MgCl_2$						
0.5	14±0.12^a	16±0.10^b	34.43±0.15^a	39.35±0.03^b	8.09±0.05^a	8.08±0.03^a
1	14±0.70^a	17±0.80^b	34.44±0.20^a	44.08±0.12^b	8.09±0.10^a	8.11±0.80^a
1.5	14±0.14^a	17±0.23^b	34.58±0.28^a	49.20±0.08^b	8.13±0.08^a	8.14±0.05^a
2	14.0.30^a	18±0.29^b	34.40±0.18^a	53.76±0.71^b	8.14±0.11^a	8.18±0.06^b

Stress and recovery monitoring

<u>$MgSO_4$</u>

No morphological (skin lesions) or physiological signs of stress were noted during anaesthesia and no physiological signs of stress were noted during the anaesthesia and long-term recovery stages for all treatments. During the anaesthesia recovery stage, 25%, 25%, and 50% of individuals exposed to 1%, 2% and 4% $MgSO_4$, respectively, had severity-level 1 (<10% body coverage) skin lesion damage. By the end of the long-

term recovery period (4 weeks), only 25% of organisms treated with 4% $MgSO_4$ had level skin lesion damage. This damage was only slightly visible.

$MgCl_2$

No morphological or physiological signs of stress were noted during anaesthesia and no physiological signs of stress were noted during the anaesthesia recovery and long-term recovery stage for all treatments. During the anaesthesia recovery stage, 25% of individuals exposed to 1.5% and 2% $MgCl_2$ had level 1 skin lesion damage. By the end of the long-term recovery period, all specimens from the $MgCl_2$ treatments had fully recovered from skin lesion damage (Figure 5).

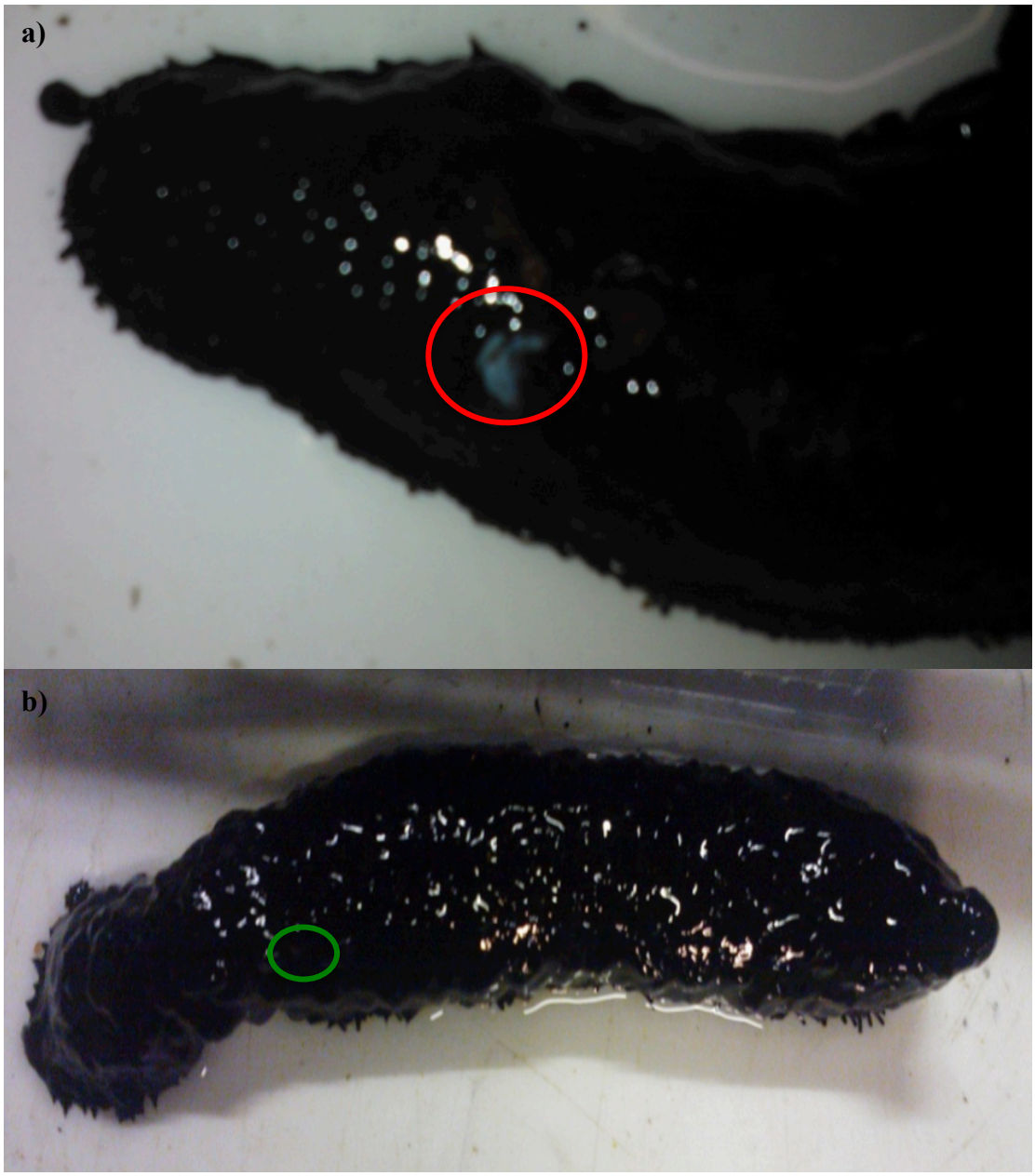


Figure 5: Example of: a) level 1 skin lesion damage to specimen following anaesthesia trial with 2% $MgCl_2$ (circled in red); b) specimen showing no damage by the end of the recovery stage (green circle indicates approximate location of previous damage) (*original photos by Gunning*)

KCl

Within 5 minutes of being exposed to 0.5% KCl, 25% of individuals had level 1 skin lesion damage, 50% eviscerated, and 25% released cuvierian tubules and eviscerated. Also, within 5 minutes of being exposed to 1% KCl, 25% and 50% of individuals had level 1 and 2 skin lesion damage, respectively, 50% eviscerated, and 25% released cuvierian tubules and eviscerated (Figure 6). No change in skin lesion damage was seen during the anaesthesia recovery stage and no specimen experienced physiological signs of stress. However, there was a mortality rate of 50% at this stage for the 1% KCl treatment. By the third week of the long-term recovery period, 25% specimens from the 0.5% KCl treatment had died and the mortality rate of specimens from the 1% KCl treatment increased to 75%. The remaining specimens from both treatments had level 1 skin lesion damage.

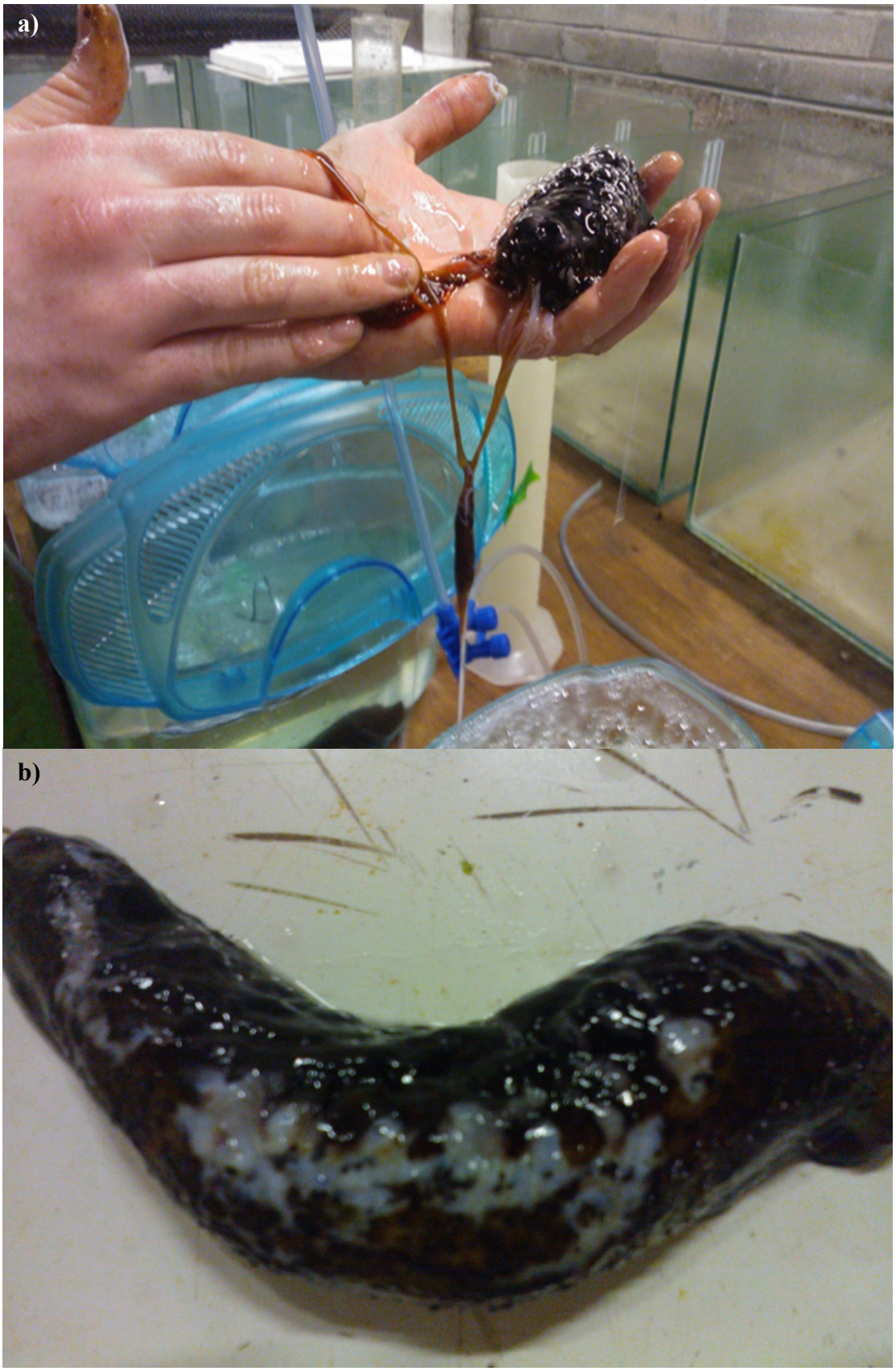


Figure 6: Example of: a) specimen eviscerating and discharging cuvierian tubules following exposure to KCl and b) level 2 skin lesion damage immediately after exposure to KCl (*original photos by Gunning*)

Trial 2: Preliminary assessment of anaesthetic agent impact on body measurement variation

Anaesthetic agent efficacy

As was the case with trial 1, a treatment with 2% $MgCl_2$ gave the quickest relaxation time (9 ± 0.82 min) and 1% $MgCl_2$ had the quickest recovery time (10.5 ± 3.11 min) (Table 7). The relaxation time of specimens exposed to 2% $MgCl_2$ was significantly lower than for exposure to 1.5% $MgCl_2$ ($p<0.01$), and 2% $MgSO_4$ ($p<0.01$). Only the recovery time of specimens exposed to 1% $MgCl_2$ was significantly lower ($p<0.05$) than that for 4% $MgSO_4$. All other differences in relaxation or recovery time between anaesthetic agents were not significant ($p>0.05$).

Table 7: Relaxation and recovery time of anaesthetic agents (Trial 2) (mean ± SD)

Anaesthetic conc. (%)	Relaxation time (min)	Recovery time (min)
$MgSO_4$		
2	41.75 ± 6.24	28.25 ± 22.04
4	29.5 ± 13.82	25 ± 4.55
$MgCl_2$		
1	45.5 ± 17.45	10.5 ± 3.11
1.5	36.75 ± 3.30	24 ± 7.35
2	9 ± 0.82	18.75 ± 2.36

Water parameters

For each anaesthetic treatment, the mean temperature of the seawater increased by the largest amount post anaesthetic addition, as the concentration of anaesthetic added got higher. The largest increase in temperature occurred for the 4% $MgSO_4$ treatment. The mean salinity of the seawater for each anaesthetic treatment also increased by a larger amount as the concentration got higher. The largest increase in salinity occurred for the 2% $MgCl_2$ treatment (increase of 19.88 ppt). The mean pH of the seawater for each anaesthetic treatment increased after the addition of an anaesthetic agent for the 1.5% and 2% $MgCl_2$ treatments and decreased for the 2% $MgSO_4$, 4% $MgSO_4$, and 1% $MgCl_2$ treatments. The largest change in pH post anaesthesia addition occurred for the 2% $MgSO_4$ treatment (decrease of pH 0.62) (Table 8). As was the case with trial 1, for

all treatments, pre and post anaesthesia addition, the DO remained relatively constant (8.31 ± 0.81 mg/L).

Table 8: Water parameters pre and post anaesthesia addition (Trial 2) (mean ± SD; same subscript post anaesthesia = not significant; different subscript post anaesthetic = significantly different [comparison is pre vs post for each individual anaesthetic])

Anaesthetic (%)	Temp (°C)		Salinity (ppt)		pH	
$MgSO_4$	***Pre***	***Post***	***Pre***	***Post***	***Pre***	***Post***
2	15 ± 0.05^a	16 ± 0.38^b	34.47 ± 0.40^a	40.95 ± 0.67^b	8.09 ± 0.10^a	7.47 ± 0.24^b
4	15 ± 0.09^a	17 ± 0.21^b	34.49 ± 0.53^a	43.72 ± 0.98^b	8.19 ± 0.17^a	7.60 ± 0.32^a
$MgCl_2$						
1	14 ± 0.06^a	16 ± 0.72^b	34.69 ± 0.42^a	42.56 ± 1.37^b	8.21 ± 0.17^a	8.16 ± 0.11^a
1.5	14 ± 0.12^a	16 ± 0.26^b	34.64 ± 0.29^a	48.42 ± 0.41^b	8.07 ± 0.07^a	8.09 ± 0.07^a
2	14 ± 0.27^a	16 ± 0.07^b	34.40 ± 0.18^a	54.28 ± 0.92^b	8.12 ± 0.12^a	8.22 ± 0.08^a

Body Measurements

For all anaesthesia treatments, variation in BW measurements (CV) reduced post anaesthesia, with variation following 1%, 1.5% and 2% $MgCl_2$ treatments reducing significantly (Table 9). The largest reduction was seen for 2% $MgCl_2$ (reduction of 90.93%), closely followed by 1% and 1.5% $MgCl_2$ (reduction of 87.43% and 85.92%, respectively). However, it is important to note that there was a substantial difference in pre anaesthesia BW measurement variation between the treatments, with the largest difference seen between the 1% $MgCl_2$ and 2% $MgSO_4$ treatments (a difference of 81.09%; $p<0.05$). This difference had an impact on the percentage reduction in BW measurements post anaesthesia seen for each treatment and is not a clear indication of which anaesthetic performed most effectively. The treatment with the lowest post-anaesthesia BW variation was 2% $MgCl_2$ (CV: 0.39%), with the highest occurring for the 1% $MgCl_2$ treatment (CV: 1.13%) (Table 9). There was no significant difference ($p>0.05$) between any treatment's post-anaesthesia BW variability. When the post-anaesthesia BW variation of each individual treatment (n=4) was compared with the pre anaesthesia BW variation data from all treatments (n=20) the percentage reduction in BW measurement variation was: 83.60%, 83.15%, 74.61%, 84.49%, and 91.24% for 2% $MgSO_4$, 4% $MgSO_4$, 1% $MgCl_2$, 1.5% $MgCl_2$, and 2% $MgCl_2$, respectively, with all treatments resulting in a significant reduction ($p<0.05$) apart from 1% $MgCl_2$ ($p>0.05$).

Table 9: Difference in variability (CV) of mean BWs pre and post anaesthesia (Trial 2) (n=4 per anaesthetic treatment; NS = not significant)

Anaesthetic	CV (%)		% Change	P-value
	Pre	***Post***		
2% $MgSO_4$	1.70	0.73	↓ 57.06	NS
4% $MgSO_4$	2.34	0.75	↓ 67.95	NS
1% $MgCl_2$	8.99	1.13	↓ 87.43	<0.05
1.5% $MgCl_2$	4.90	0.69	↓ 85.92	<0.05
2% $MgCl_2$	4.30	0.39	↓ 90.93	<0.01

There was no significant difference ($p>0.05$) in post-anaesthesia BL variation for any anaesthetic agent tested. The treatment with the lowest post-anaesthesia BL variation was 2% $MgCl_2$ (CV: 5.88%), with the highest occurring for the 1% $MgCl_2$ treatment (CV: 11.18%) (Table 10). There was no significant difference ($p>0.05$) between any treatment's post-anaesthesia BL variability. When the post-anaesthesia BL variation of each individual treatment (n=4) was compared with the pre anaesthesia BL variation data from all treatments (n=20) the percentage increase in BL measurement variation was: 22.51%, 0.65%, and 44.63% for 2% $MgSO_4$, 4% $MgSO_4$, and 1% $MgCl_2$, respectively, while the percentage decrease was: 22.25% and 23.93% for 1.5% and 2% $MgCl_2$, respectively. All differences were not significant ($p>0.05$) apart from the reduction seen for 2% $MgCl_2$ ($p<0.05$).

Table 10: Difference in variability (CV) of mean BLs pre and post anaesthesia (Trial 2) (n=4 per anaesthetic treatment; NS = not significant)

Anaesthetic	CV (%)		% Change	P-value
	Pre	***Post***		
2% $MgSO_4$	9.41	9.47	↑ 0.63	NS
4% $MgSO_4$	5.75	7.78	↑ 26.09	NS
1% $MgCl_2$	7.64	11.18	↑ 31.66	NS
1.5% $MgCl_2$	4.64	6.01	↑ 22.80	NS
2% $MgCl_2$	11.23	5.88	↓ 47.64	NS

Anaesthetisation increased significantly the mean BW for 1% $MgCl_2$ and 2% $MgSO_4$ treated specimens and the mean BL for 1% $MgCl_2$, 2% $MgCl_2$, 2% $MgSO_4$, and 4% $MgSO_4$ treated specimens (Figure 7 & 8) and decreased significantly the mean BW for 2% $MgCl_2$ and 4% $MgSO_4$ (Figure 7) (see Appendix, Table 3 and 4, for mean BW and BL of repeated measurements for each specimen per treatment, pre and post anaesthesia).

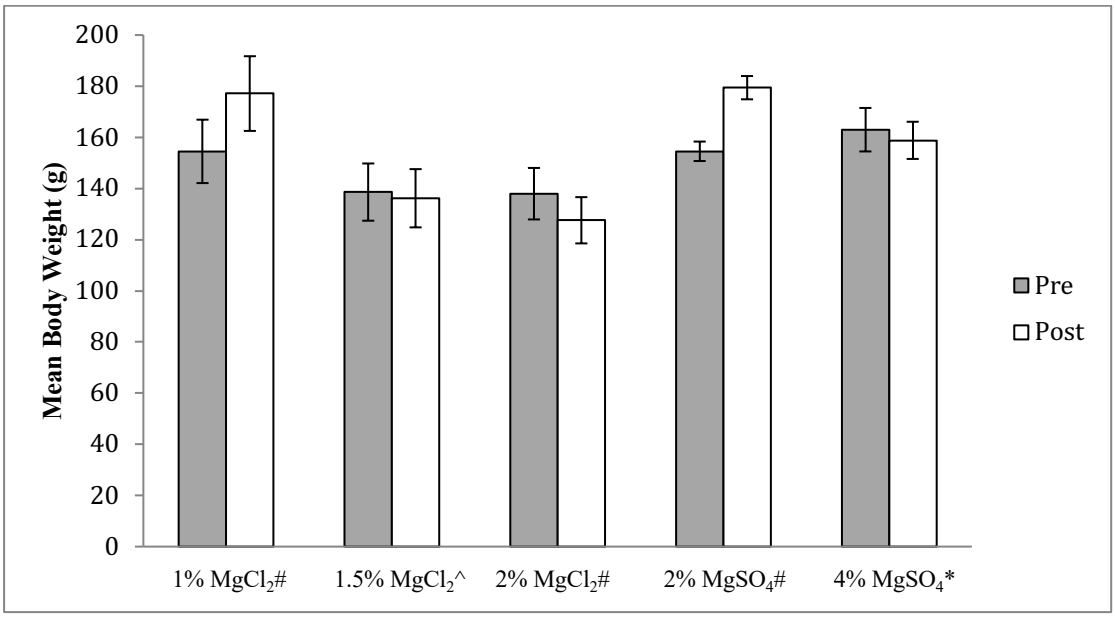


Figure 7: Mean BW (g) pre and post anaesthesia (Trial 2) (*P<0.05; #P<0.01; ^NS; ± SD)

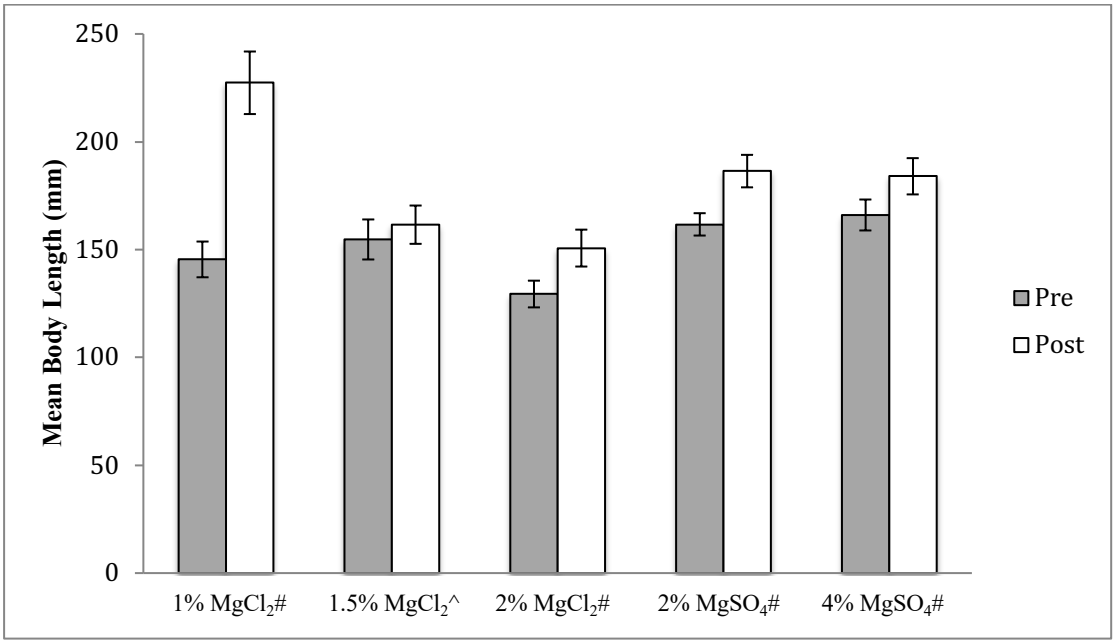


Figure 8: Mean BL (mm) pre and post anaesthesia (Trial 2) (*P<0.05; #P<0.01; ^NS; ± SD)

Stress and recovery monitoring

$MgSO_4$

(a) Pre anaesthesia measurement

By the end of pre anaesthesia repeated measurements, 50% and 25% of specimens from the 2% and 4% $MgSO_4$ treatments, respectively, had severity-level 1 skin lesion damage. During the drying process for pre anaesthesia BW measurements of the 4% $MgSO_4$ treatment, 25% of individuals released cuvierian tubules on one occasion.

(b) Anaesthesia exposure & post-anaesthesia measurement

After 3 minutes in the 4% $MgSO_4$ solution, 25% of specimens released cuvierian tubules. During anaesthesia (prior to repeated measurements) and post anaesthesia measurements no physiological signs of stress were noted for the 2% $MgSO_4$ treatment and no further morphological signs of stress were noted for both treatments. During the anaesthesia recovery stage, 50% and 75% of individuals from the 2% and 4% $MgSO_4$ treatments, respectively, had severity-level 1 skin lesions. No physiological signs of stress occurred during this stage for both treatments. By the end of the four-week recovery period, only 25% of individuals from the 4% $MgSO_4$ treatment had level 1 skin lesions.

$MgCl_2$

(a) Pre anaesthesia measurement

By the end of pre anaesthesia repeated measurements, 25%, 25%, and 50% of specimens from the 1%, 1.5%, and 2% $MgCl_2$ treatments, respectively, had level 1 skin lesion damage. During the drying process for BW measurement of the 1% $MgCl_2$ treatment, 25% of individuals released cuvierian tubules on one occasion. During the handling of specimens for BL measurement of the 2% $MgCl_2$ treatment, 25% of individuals released cuvierian tubules on one occasion.

(b) Anaesthesia exposure & post-anaesthesia measurement

For all concentrations of $MgCl_2$ trialled, no physiological or further morphological signs of stress were noted during anaesthesia (prior to repeated measurements) or post-

anaesthesia measurements and no physiological signs of stress were noted during the anaesthesia recovery and recovery stage. During the anaesthesia recovery stage, 25% of individuals exposed to 1% $MgCl_2$ and 50% of individuals exposed to 1.5% and 2% $MgCl_2$ had level 1 skin lesion damage. By the end of the long-term recovery period, only 25% of 1% and 2% $MgCl_2$ individuals had level 1 skin lesion damage.

Trial 3: Assessment of anaesthetic agent efficacy and impact on body measurement variation

Anaesthetic agent efficacy

The 2% $MgCl_2$ treatment resulted in the quickest relaxation time (9.6 ± 2.33 min) and 1% $MgCl_2$ had the quickest recovery time (15.3 ± 4.92 min) (Table 11). The relaxation time of specimens exposed to 2% $MgCl_2$ and 1.5% $MgCl_2$ were significantly lower than 1% $MgCl_2$ ($p<0.01$) while those exposed to 1% $MgCl_2$ had significantly lower relaxation times than those on 2% $MgSO_4$ ($p<0.05$). The recovery time of specimens exposed to 1% $MgCl_2$, 1.5% $MgCl_2$, and 2% $MgSO_4$ were significantly lower than 2% $MgCl_2$ ($p<0.001$, $p<0.05$, and $p<0.05$, respectively) and specimens exposed to 1% $MgCl_2$, 1.5% $MgCl_2$, and 2% $MgSO_4$, were significantly lower than 4% $MgSO_4$ ($p<0.01$). All other differences in relaxation or recovery time between anaesthetic agents were not significant ($p>0.05$).

Table 11: Relaxation and recovery time of anaesthetic agents (Trial 3) (mean ± SD)

Anaesthetic conc. (%)	Relaxation time (min)	Recovery time (min)
$MgSO_4$		
2	10.1 ± 6.92	21 ± 5.44
4	13.95 ± 7.54	40.85 ± 11.57
$MgCl_2$		
1	19.3 ± 3.86	15.3 ± 4.92
1.5	12.7 ± 3.06	19.65 ± 10.18
2	9.6 ± 2.33	32.85 ± 9.42

Water parameters

For each anaesthetic treatment the mean temperature of the seawater increased, post anaesthetic addition by the largest amount, as the concentration of anaesthetic added got higher. The largest increase in temperature occurred for the 2% $MgCl_2$ treatment. The mean salinity of the seawater for each anaesthetic treatment also increased by a larger amount as the concentration got higher. The largest increase in salinity occurred for the 4% $MgSO_4$ treatment (increase of 24.8 ppt). The mean pH of the seawater for each anaesthetic treatment increased after the addition of an anaesthetic agent for the 1%, 1.5% and 2% $MgCl_2$ treatments and decreased for the 2% and 4% $MgSO_4$ treatments. The largest change in pH post anaesthesia addition occurred for the 2% $MgSO_4$ treatment (decrease of pH 0.25) (Table 12). All pre and post anaesthesia water parameters for each treatment were significantly different ($p<0.05$ or $p<0.01$). As was the case with trial 1 and 2, for all treatments, pre and post anaesthesia addition, the DO remained relatively constant (8.11±0.51 mg/L).

Table 12: Water parameters pre and post anaesthesia addition (Trial 3) (mean ± SD) same subscript post anaesthesia = not significant; different subscript post anaesthetic = significantly different [comparison is pre vs post for each individual anaesthetic])

Anaesthetic (%)	Temp (°C)		Salinity (ppt)		pH	
Control	10.36 ± 0.25		32.9 ± 0.54		8.23 ± 0.09	
$MgSO_4$	***Pre***	***Post***	***Pre***	***Post***	***Pre***	***Post***
2	10±0.33^a	13±0.18^b	32.5±0.97^a	53.3±0.95^b	8.20±0.02^a	7.95±0.02^a
4	10±0.61^a	14±0.82^b	33.1±0.32^a	57.9±0.74^b	8.20±0.03^a	7.83±0.01^a
$MgCl_2$						
1	11±0.57^a	14±0.69^a	32.8±0.42^a	43.8±0.42^b	8.25±0.04^a	8.39±0.02^b
1.5	10±0.17^a	15±0.20^b	32.7±0.48^a	50.4±1.08^b	8.24±0.02^a	8.38±0.01^a
2	11±0.59^a	16±0.43^b	32.5±0.85^a	56.6±1.27^b	8.27±0.01^a	8.43±0.02^b

Body measurements

The BW variability (CV) of all anaesthetic treatments was lower than that of the control, with 2% $MgCl_2$ having the largest difference (84.45% lower) ($p<0.05$). The 1% and 1.5% $MgCl_2$ treatments also resulted in a big difference in BW variation in comparison to the control, being 74.56% and 79.51% lower, respectively ($p<0.05$). Both 2% and 4% $MgSO_4$ treatments gave lower BW variation than the control, but the difference was

smaller than the $MgCl_2$ treatments, with a reduction of 46.29% and 32.51%, respectively ($p>0.05$) (Table 13).

Table 13: Difference in control vs. anaesthesia treatment mean BW variability (CV) (Trial 3) (n=10 per anaesthetic treatment/control; NS = not significant)

Treatment	CV (%)	% Change	P value
Control	***2.83***		
2% $MgSO_4$	1.52	↓ 46.29	NS
4% $MgSO_4$	1.91	↓ 32.51	NS
1% $MgCl_2$	0.72	↓ 74.56	<0.05
1.5% $MgCl_2$	0.58	↓79.51	<0.05
2% $MgCl_2$	0.44	↓84.45	<0.05

The mean BL variability (CV) of all anaesthetic treatments was lower than that of the control, with 4% $MgSO_4$ resulting in the largest difference (50.96% lower) ($p<0.05$). The 1%, 1.5%, and 2% $MgCl_2$ treatments gave a similar reduction in BL variation, being 43.99%, 50.69%, and 49.31%, respectively, lower than the control's ($p<0.05$). The BW variation of the 2% $MgSO_4$ treatment was only 7.25% lower than the control ($p>0.05$) (Table 14).

Table 14: Difference in control vs. anaesthesia treatment mean BL variability (CV) (Trial 3) (n=10 per anaesthetic treatment/control; NS = not significant)

Treatment	CV (%)	% Change	P value
Control	***10.89***		
2% $MgSO_4$	10.10	↓ 7.25	NS
4% $MgSO_4$	5.34	↓50.96	<0.05
1% $MgCl_2$	6.10	↓43.99	<0.05
1.5% $MgCl_2$	5.37	↓50.69	<0.05
2% $MgCl_2$	5.52	↓49.31	<0.05

Stress and recovery monitoring

Control

At the end of the body measurement procedure, 50% of control individuals had level 1 skin lesion damage. Cuvierian tubule release occurred for 40% of individuals, with

specimens releasing tubules 2-4 times during BW and/or BL measurements. Severity-level 1 skin lesion damage increased to 60% of individuals during the short-term post measurement stress-monitoring period (1 hour). There were no physiological signs of stress during this period for any control individuals. By week 2 of the 4-week recovery period (long-term recovery), all specimens had recovered from skin lesion damage.

2% $MgSO_4$

Prior to repeated body measurements, 10% of individuals had level 1 skin lesion. Swelling occurred for 10% of individuals, with the specimen swelling for 1 minute after 2 minutes of being in the anaesthetic solution. At the end of the body measurement procedure, 60% of individuals had level 1 skin lesion damage. Cuvierian tubule release occurred for 30% of individuals, with specimens releasing tubules 1-3 times during BW measurements. During the anaesthesia recovery period, level 1 skin lesion damage had increased to 100% of individuals, while 10% of individuals swelled once for 20 seconds. By the end of the long-term recovery period, 30% of individuals still had level 1 skin lesion damage, however, the damage was only slightly visible.

4% $MgSO_4$

Prior to repeated body measurements, 30% of individuals had level 1 skin lesion. Swelling occurred for 30% of individuals, with specimens swelling 1-3 times for 30-120 seconds immediately after being added to the anaesthetic solution and/or within 5 minutes of being added. Cuvierian tubule release occurred for 20% of individuals immediately after and/or within 5 minutes of being added to the anaesthesia solution. At the end of the body measurement procedure, 50% of individuals had level 1 skin lesion damage. Cuvierian tubule release occurred for 10% of individuals, with specimens releasing tubules 1-2 times during BW measurements. During the anaesthesia recovery period, level 1 skin lesion damage was noted on 70% of individuals, while 10% of individuals had level 2 damage. Swelling occurred during this period for 20% of individuals, occurring 3 times for one individual (30-120 second duration) and once for the other (30 seconds duration). Both occurrences of swelling took place within the first 15 minutes of the recovery stage. By the end of the long-term recovery period, 40% of individuals still had level 1 skin lesion damage, with damage only slightly visible for 30% of these individuals.

1% $MgCl_2$

Prior to repeated body measurements, no individuals had level 1 skin lesion or physiological damage. By the end of the body measurement procedure, 20% of individuals had level 1 skin lesion damage. During the anaesthesia recovery period, incidences of level 1 skin lesion damage had increased to 50% of individuals. There were also no physiological signs of stressing during repeated measurements or the anaesthesia recovery period. By the 3rd week of the long-term recovery period, all specimens had recovered from their skin lesion damage.

1.5% $MgCl_2$

Immediately after being placed in the anaesthetic solution, 10% of individuals eviscerated slightly. Internal organs were spotted extruding from the specimens body, however, they were pulled back into the body during the recovery stage. Also immediately after being placed in the anaesthetic solution, 10% of individuals swelled for 1 minute. Prior to repeated measurements, 10% of individuals had level 1 skin lesion damage. By the end of the body measurement procedure, 40% of individuals had level 1 skin lesion damage. During the anaesthesia recovery period, level 1 skin lesion damage was noted on 70% of individuals. There were no physiological signs of stressing during repeated body measurements and anaesthesia recovery. By the 3rd week of the long-term recovery period, all specimens had recovered from their skin lesion damage.

2% $MgCl_2$

Prior to repeated body measurements, 30% of individuals had level 1 skin lesion damage. Cuvierian tubule release occurred for 20% of individuals after 30 seconds and 8 minutes in the anaesthetic solution. Swelling occurred at this stage for 20% of individuals. For one individual it occurred once for 30 seconds, immediately after being added to the solution. For the other specimen, it occurred 3 times, for 50 seconds immediately after being added, and on two more occasions lasting approximately 90 seconds, 4 and 5 minutes after being added to the solution. By the end of the body measurement procedure, 60% of 2% $MgCl_2$ individuals had level 1 skin lesion damage. During the anaesthesia period, level 1 skin lesion damage was noted on 90% of individuals, while 10% had level 2 damage. There were no physiological signs of stressing during repeated body measurements and anaesthesia recovery. By the end of

the long-term recovery period, only 10% of individuals had visible signs of skin lesion damage.

4.4 Discussion

Due to the plastic nature of a sea cucumber body shape, body length (BL) can vary considerably by contraction and elongation (Watanabe *et al*. 2012; Yamana *et al*. 2005; Battaglene *et al*. 1999). The body weight (BW) can also show considerable variation depending on the intestinal content and/or the amount of water in the respiratory trees (Sewell, 1990). Obtaining accurate size measurements is vital in determining the growth rate of organisms and analysing population dynamics, information that is necessary for aquaculture and stock management research. Despite this, no standardised method for obtaining accurate size measurements of sea cucumbers currently exists.

Two studies attempted to reduce the weight variability of *Australostichopus mollis* measurements in the field by weighing at times when the gut is most likely to be empty (time ranged from between 11.30 and 15.30 and 09.30 and 17.30) and by removing additional fluid from the respiratory trees by applying gentle pressure to the anterior end of the sea cucumber prior to weighing (Slater and Jeffs, 2010; Slater and Carton, 2007). Depending on the operational procedure of the farm or fishery, however, weighing at specific times may not always be practical. In addition to these methods, Slater and Jeffs (2010) blotted the sea cucumbers dry before weighing. In a laboratory setting, Zamora and Jeffs (2012 and 2011) removed excess water from the respiratory tree of *A. mollis* by squeezing the posterior half of each animal and blotting the external wall dry prior to weighing. The animals were unfed for 48 hours prior to being weighted to ensure gut evacuation (Zamora and Jeffs, 2012 and 2011). Slater *et al.* (2009) allowed 24 hours for *A. mollis* individuals to evacuate their gut and then took the weight measurements after blotting them dry on unbleached tissue. Only the study conducted by Slater and Carton (2007) reported the impact their measurement technique had on BW variability, stating that it was reduced by <±5%.

In this study, *Holothuria forskali* specimens were unfed for 48 hours and gentle pressure was applied to the posterior half, while blotting dry with paper towel, prior to being weighed. The BW variability (% CV) achieved by applying these methods ranged from 1.70% to 10.89% (trial 2 & 3 non-anaesthetised individuals).

A BW measurement after the removal of internal organs and coelomic fluid is reported to be the most accurate size index in *Apostichopus japonicas*, however, this methodology is not widely used as it requires a long period of time to prepare a sample

and it is a lethal sampling method, which makes it unsuitable for any studies that require the continuous monitoring of specimens over time (Watanabe *et al.,* 2012).

The goal of this study was to find an anaesthetic that effectively and efficiently anaesthetised *Holothuria forskali* and to evaluate the impact of anaesthesia on body measurement accuracy.

It was found that treatments with 1%, 1.5%, and 2% $MgCl_2$ and 2% and 4% $MgSO_4$ were successful in anaesthetising *H. forskali* (Trial 1-3), while 0.5% $MgCl_2$, 0.05%, 0.1%, 0.5%, and 1% $MgSO_4$, and 0.5% and 1% KCl were unsuccessful (Trial 1). In a study by Guzman and Guevara (2002), *Isostichopus badionotus* and *Holothuria Mexicana* were successfully anaesthetised with 0.05% and 0.1% $MgSO_4$. However, Wantanabe *et al*. (2012), found 0.05% $MgSO_4$ to be unsuccessful in anaesthetising *Holothuria scabra* (Jaeger) juveniles. Juvenile *Apostichopus japonicus* (<20mm BL) were found to be successfully anaesthetised with 0.035-0.5% KCl, however, KCl was ineffective for larger specimens (Yanagisawa, 1998). Wantanabe *et al*. (2012) also found 0.5% KCl to be ineffective in anaesthetising *H. scabra* juveniles. Trials by Purcell *et al*. (2012b) and Tuwo and Conand, (1992) successfully anaesthetised holothurians (including *H. forskali*) with $MgCl_2$, however, this was for lethal sampling experiments. Both concentrations of KCl tested (0.5% and 1%) in this study caused severe stress to the specimens (cuvierian tubule release, gut evisceration, and skin lesions) resulting in a 25% and 75% mortality rate for 0.5% and 1% KCl, respectively (Trial 1). Despite this adverse reaction, the change in water parameters (i.e. salinity, temperature, pH) post anaesthesia addition was larger for a number of the other anaesthetic treatments, the exposure to which caused minimal stress to the specimens (Table 6) (note: the impact on water parameters was concentration and anaesthetic agent dependent). Therefore, it would appear that the specimens were reacting adversely to the anaesthetic itself, and not to the impact it was having on water parameters. This was surprising, considering its safe use in other studies (Watanabe *et al*. 2012; Yanagisawa, 1998). The results of this and other studies would suggest that anaesthesia success is species specific.

Although a number of the anaesthetic agents trialled in this study significantly improved the measurement BW and BL measurement accuracy of *H. forskali*, the most successful anaesthetic agent trialled was 2% $MgCl_2$, with a mean BW variability of 0.39-0.44% and BL variability of 5.52-5.88%. By comparison, the variability of mean BW and BL measurements of non-anaesthetised specimens from this study was 1.70-

8.99% and 4.64-10.89%, respectively. Although a substantial reduction in BW variability was seen after anaesthesia with 2% $MgCl_2$, the variability in BL measurements was still relatively high. This was also the case for all other anaesthetic agents trialled, with the lowest mean BL variability post-anaesthesia achieved for 4% $MgSO_4$, at 5.34%. Consequently, it is recommended that only BW should be used (post-anaesthesia) as an accurate assessment of *H. forskali* growth, at least until a methodology that significantly reduces BL measurement variability is found. Watanabe *et al*. (2012) found that the BW and BL measurement variability of *H. scabra* was significantly reduced after anaesthetisation with 2% menthol-ethanol. BW variability was reduced from 6.5% to 3.7% and BL variability from 8.25 to 2.65% (Watanabe *et al*. 2012). A lower BL variability was achieved by Watanabe *et al*. (2012) and future studies should assess the effectiveness of methanol-ethanol on *H. forskali*. However, the BW variability achieved after anaesthesia with 2% $MgCl_2$ was substantially lower in this study.

The most efficient anaesthetic agent, in terms of combined relaxation and recovery time, was also 2% $MgCl_2$, at 38.75 ± 10.73 minutes. Although there are no studies available which assess the efficacy of anaesthetising holothurians with $MgCl_2$, studies on oysters, sea anemones and cephalopods found it to be the most effective and efficient anaesthetic agent (Culloty and Mulcahy, 1992; Moore, 1989; Messenger *et al*. 1985).

$MgSO_4$ at a concentration of 0.05%, 0.1%, and 0.5%, and $MgCl_2$ at a concentration of 0.5%, caused no morphological or physiological stress to *H. forskali*, however, they were ineffective at these concentrations. The successful anaesthetic agents (i.e. 2% and 4% $MgSO_4$ and 1%, 1.5%, and 2% $MgCl_2$), however, caused signs of low level stress. In trial 1 (no repeated measurements; only exposure to anaesthetic agents) 25-50% (n=4 per treatment) of individuals experienced low skin lesion damage (<10% body coverage) by the end of the trial. However, after 4 weeks recovery, almost all specimens (only 1 specimen treated with 4% $MgSO_4$) had fully recovered from skin lesion damage. No specimen experienced physiological signs of stress (i.e. cuvierian tubule release, evisceration, swelling) during exposure to these anaesthetic agents in trial 1.

It was also apparent from this study that *H. forskali* is stressed by repeated handling. In trial 3, 60% of individuals from the control treatment (i.e. repeated body measurements with no anaesthesia; n=10) had low skin lesion damage by the end of the short-term recovery period (despite the change from cloth {trial 1} to paper towels for drying; cloth towels were thought to be exacerbating skin lesion damage), while 40% of

individuals released cuvierian tubules during repeated body measurements. Despite this, all control specimens fully recovered from skin lesion damage 2 weeks post trial. Also, in trial 2, 25-50% (n=4 per treatment) of anaesthetised specimens experienced low skin lesion damage and 25% of specimens from the 4% $MgSO_4$, 1% $MgCl_2$, 2% $MgCl_2$ treatments experienced physiological stress, during pre anaesthesia body measurements. Therefore, stress noted post anaesthesia might have been caused or exacerbated by pre anaesthesia measurements. Nevertheless, it was evident from this study that long-term exposure to anaesthetics (i.e. 20 minutes post successful anaesthesia to allow for repeated measurements) and repeated handling increases the incidences of low level skin lesion damage. Although the incidence of skin lesion damage of anaesthetised individuals increased during repeated body measurements, it must be noted that specimens were exposed to the anaesthetic solutions for an additional 20 minutes after the point at which the specimens were fully anaesthetised and handled an additional four times, to facilitate these repeated measurements. Also, the majority of skin damage resulting from repeated handling and/or exposure to $MgCl_2$ or $MgSO_4$ healed fully within 4 weeks. In a situation where anaesthetics would be used for *H. forskali* size measurements in a hatchery or aquaculture setting, the specimens would only be exposed to the anaesthetic solution for the duration it took to become fully anaesthetised and be handled for the duration it would take to complete body measurements once. It is likely that with reduced handling and exposure to anaesthetics, incidences of skin damage would be minimal or non-existent. However, to err on the side of caution it is recommended that size measurements of *H. forskali* under anaesthesia be limited to once every 4 weeks. Future studies should assess the level of stress experienced by *H.forskali* specimens after following one post-anaesthesia BW and BL measurement.

It is also important to note that the addition of $MgSO_4$ and $MgCl_2$ to seawater caused a large increase in temperature (Note: the addition of $MgCl_2$ to water causes an exothermic reaction, however, the addition of $MgSO_4$ causes an endothermic reaction. The reason for the increase in water temperature, in most cases, following the addition of $MgSO_4$ to water was unknown). As specimens were added to the anaesthesia solution as soon as they were ready, they were exposed to this sudden change in temperature. There is a possibility that this exposure may have resulted in some of the stress experienced during this study. A commonly utilised method for the non-harmful induction of spawning in holothurians involves a heat shock treatment of 3-5 °C. This

involves transferring the specimens between tanks that are 3-5°C lower or higher in temperature or rapidly increasing or decreasing the temperature of the tank that the specimens are contained in (Dabbagh *et al*. 2011; Morgan, 2009; Laxminarayana, 2005). As part of spawning trials conducted at the Bantry Marine Research Station (BMRS), Bantry, Co. Cork, *H. forskali* specimens were transferred from a tank at c.10.3 °C to a tank at c. 15.5 °C, in an effort to induce spawning. The method was successful and had no negative impact on the health of the specimens (Gunning and Evans, personal observation, 2013). Also, as was noted above, specimens exposed to KCl demonstrated significant levels of stress (and some mortalities), yet, the change in temperature was small, with a mean decrease of 0.30 °C and 0.28 °C for 0.5% and 1% KCl, respectively. Nevertheless, future studies should allow the anaesthetic solution to return to (or close to) the temperature of the seawater that the sea cucumbers are in prior anaesthesia treatment to assess the impact on sea cucumber health and anaesthetic agent efficacy.

4.5 Conclusion

Anaesthetising *H. forskali* with 2% $MgCl_2$ significantly increases the accuracy of body measurements, and resulted in the greatest reduction in BW variability, and the quickest combined anaesthesia relaxation and recovery time of those anaesthetic agents assessed in this study. Due to the repeated measurements that were necessary for these experiments, exposure to anaesthetics and handling of sea cucumbers was higher than would be necessary for the BW and BL measurements post-anaesthesia in a commercial aquaculture or fishery scenario. Care should be taken to limit handling duration and exposure time to anaesthetics to reduce or eliminate the possibility of morphological and/or physiological stress occurring.

4.6 Acknowledgements

Benjamin Horgan (UCC) assisted in the setting up and monitoring of trial 1 and 2. Prof. Gavin Burnell gave advise on the design of these trials and provided feedback on the chapter. A big thank you to Dean Murphy (Shellfish Ireland, Ltd.) and Declan Sullivan (Marine Harvest Ltd.) who dived for the sea cucumbers used in these trials.

4.7 References

Anderson, S.C., Flemming, J.M., Watson, R., and Lotze, H.K. (2011). Serial exploitation of global sea cucumber fisheries. *Fish and Fisheries,* 12(3), pp. 317-339.

Battaglene S. C., Seymour J. E., and Ramofa, C. (1999). Survival and growth of cultured juvenile sea cucumbers, *Holothuria scabra*. *Aquaculture,* 178, pp. 293-322.

Bell, J. D., Agudo, N. N., Purcell, S. W., Blazer, P., Simutoga, M., Pham, D., and Patrona, L. D. (2007). Grow-out of sandfish *Holothura scabra* in ponds shows that co-culture with shrimp *Litopenaeus stylirostris* is not viable. *Aquaculture*, 273, pp. 509-519.

Bordbar, S., Anwar, F., and Saari, N. (2011). High-value components and bioactives from sea cucumbers for functional foods – a review. *Marine Drugs*, 9, pp. 1761-1805.

Chao, S. M., Chen, C. P., and Alexander, P. S. (1993). Reproductive periodicity of a tropical dendrochirote holothurian, *Phyrella fragilis* (Echinodermata: Holothuroidea), in Taiwan. *Bulletin of the Institute of Zoology Academia Sinica (Taipei)*, 32, pp. 111-119.

Conand, C. (2004). Present status of world sea cucumber resources and utilization: an international overview. In: Lovatelli, A., Conand, C., Purcell, S., Uthicke, Hamel, J-F., and Mercier, A. (eds.). Advances in sea cucumber aquaculture and management, *FAO, Rome, Fisheries Technical Paper, Food and Agriculture Organisation*, 463, pp. 13-24.

Conand, C., and Byrne, M. (1993). A review of recent development in the world sea cucumber fisheries. *Marine Fisheries*, 55, pp. 1-13.

Culloty, S.C., and Mulcahy, M.F. (1992). An evaluation of anaesthetics for *Ostrea edulis* (L.). *Aquaculture*, 107, pp. 249-252.

Dabbagh, A-R., Sedaghat, M.R., Rameshi, H., and Kamrani, E. (2011). Breeding and larval rearing of the sea cucumber *Holothuria leucospilota* Brandt (*Holothuria vegabunda* Selenka) from the northern Persian Gulf, Iran. *SPC Beche-de-mer Information Bulletin*, 31, pp. 35-38.

DeMoor, S., Herbert Waite, J., Jangoux, M., and Flammang, P. (2003). Characterization of the Adhesive from Cuvierian Tubules of the Sea Cucumber *Holothuria forskali* (Echinodermata, Holothuroidea). *Marine Biotechnology*, 5, pp. 45-57.

Deudero, S., Tor, A., Alomar, C., Valencia, J.M., Sarriera, P., and Blanco, A. (2011). Integrated multitrophic aquaculture: filter feeders bivalves as efficient reducers of wastes derived from coastal aquaculture assessed with stable isotope analyses. In: Sladonja, B. (ed.). Aquaculture and the Environment – A Shared Destiny, *InTech Europe, Eijeka, Croatia*, p. 246.

Eliseikina, M.G., and Magarlamov, T.Y. (2002). Coelomocyte morphology in the holothurians *A. japonicus* (Aspidochirota: Stichopodidae) and *Cucumaria japonica* (Dendrochirotida: Cucumariidae). *Russian Journal of Marine Biology,* 28, pp. 197-202.

Gianasi, B. L., Verkaik, K., Hamel, J., and Mercier, A. (2015). Novel use of PIT tags in sea cucumbers: promising results with the commercial species *Cucumaria frondosa*. *PloS one*, 10(5), DOI: 10.1371/journal.pone.0127884.

Guzman, H.M., and Guevara, C.A. (2002). Population structure, distribution and abundance of three commercial species of sea cucumber (Echinodermata) in Panama. *Caribbean Journal of Science*, 38, pp. 230-238.

Hamel, J.F., Conand, C., Pawson, D.L., and Mercier, A. (2001). The sea cucumber *Holothuria scabra* (Holothuroidea: Echinodermata): its biology and exploitation as beche-de-mer. *Advances in Marine Biology*, 41, pp. 129-202.

Hatanaka, H., and Tanimura, K. (1994). Application of menthol as an anesthetizer for body size measuring of sea-cucumber juveniles. *Aquaculture Science*, 42, pp. 221-225.

Hickman, C.P., Kats, L., and Hickman, C. (2004). Appendix C: Narcotizing methods. In: Laboratory Studies in Integrated Principles of Zoology, 12th Edition. *McGraw-Hill Global Education Holdings, LLC*.

Laxminarayana, A. (2005). Induced spawning and larval rearing of the sea cucumber *Bohadschia marmorata* and *Holothuria atra* in Mauritius. *SPC Beche-de-mer Information Bulletin*, 22, pp. 48-51.

MacDonald, C. L. E., Stead, S. M., and Slater, M. J. (2013). Consumption and remediation of European Seabass (*Dicentrarchus labrax*) waste by the sea cucumber *Holothuria forskali*. *Aquaculture International,* 21(6), pp. 1279-1290.

Messenger, J.B., Nixon, M., and Ryan, K.P. (1985). Magnesium chloride as an anaesthetic for cephalopods. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology,* 82(1), pp. 203-205.

Moore, S.J. (1989). Narcotising sea anemones. *Journal of the Marine Biological Association of the United Kingdom*, 69, pp. 803-911.

Morgan, A.D. (2009). Spawning of the temperate sea cucumber *Australostichopus mollis* (Levin). *Journal of the World Aquaculture Society*, 40(3), pp. 363-373.

Nelson, E. J., MacDonald, B. A., and Robinson, S. M. C. (2012). A review of the Northern sea cucumber *Cucumaria frondosa* (Gunnerus, 1767) as a potential aquaculture species. *Reviews in Fisheries Science*, 20, pp. 212-219.

Pérez-Ruzafa, A., and López-Ibor, A. (1988). Echinoderm fauna from the south-western Mediterranean. Biogeographic relationships. In: R. D. Burke, P. V. Mladenov, P. L. Lambert and R. L. Parsley (eds.). Echinoderm Biology, *A. A. Balkema*, *Rotterdam*, pp. 355-362.

Pérez-Ruzafa, A., Marcos, C., and Bacallado, J.J. (1987). Presencia de *Holothuria* (*Panningothuria*) *forskali* (Echinodermata: Holoturidea) En Ias Islas Canarias. *Vieraea: Folia scientarum biologicarum canariensium*, 17, pp. 361-368.

Purcell, S.W., Polidoro, B.A., Hamel, J-F., Gamboa, R.U., and Mercier, A. (2014). The cost of being valuable: Predictors of extinction risk in marine invertebrates exploited as luxury seafood. *Proceedings of the Royal Society B: Biological Sciences,* 281, 20133296, DOI: 10.1371/journal.pone.0095075.

Purcell, S.W., Mercier, A., Conand, C., Hamel J-F., Toral-Granda, M.V., Lovatelli, A., and Uthicke, S. (2013). Sea cucumber fisheries: Global review of stock status, management measures and drivers of overfishing. *Fish and Fisheries,* 14, pp. 34-59.

Purcell, S.W., Hair, C.A., and Mills, D.J. (2012a). Sea cucumber culture, farming and sea ranching in the tropics: Progress, problems and opportunities. *Aquaculture*, 368-369, pp. 68-81.

Purcell, S. W., Samyn, Y., and Conand, C. (2012b). Commercially important sea cucumbers of the world. *FAO Species Catalogue for Fishery Purposes, FAO Fish Finder, Food and Agriculture organization of the United Nations, Rome*, 6, pp. 1-223.

Purcell, S. W., Patrois, J., and Fraisse, N. (2006). Experimental evaluation of co-culture of juvenile sea cucumbers, *Holothuria scabra* (Jeager), with juvenile blue shrimp, *Litopenaeus stylirostris* (Stimpson). *Aquaculture Research*, 37, pp. 515-522.

Raison, C., M. (2008). Advances in sea cucumber aquaculture and prospects for commercial cultivation of *Holothuria scabra*. *CAB reviews,* 3, pp. 1-15.

Rodríguez-Barreras, R., López-Morell, J., and Sabat, A.M. (2016). Effectiveness of two tagging devices in the sea cucumber *Holothuria (Halodeima) grisea. Marine and Freshwater Research,* DOI: http://dx.doi.org/10.1071/MF15473.

Rodríguez, E., González, M., Caride, B., Lamas, M. A., and Taboada, M. C. (2000). Nutritional value of *Holothuria forskali* protein and effects on serum lipid profile in rats. *Journal of Physiology and Biochemistry*, 56, pp. 39-44.

Rodríguez, J., Castro, R., and Riguera, R. (1991). Holothurinosides: new antitumour non sulphated triterpenoid glycosides from the sea cucumber *Holothuria forskali*. *Tetrahedron*, 47(26), pp. 4753-4762.

Santos, R., Dias, S., Pinteus, S., Silva, J., Alves, C., Tecelão, Pedrosa, R., and Pombo, A. (2015). Sea cumber *Holothuria forskali*, a new resource for aquaculture? Reproductive biology and nutraceutical approach. *Aquaculture Research*, 47, pp. 2307-2323.

Sewell, M. A. (1990). Aspects of the ecology of *Stichopus mollis* (Echinodermata: Holothuroidea) in north-eastern New Zealand. *New Zealand Journal of Marine and Freshwater Research*, 24, pp. 97-103.

Sewell, M. A. (1987). The reproductive biology of *Stichopus mollis* (Hutton). *Department of Zoology, University of Auckland, Auckland*, pp. 98.

Sicuro, B., and Levine, J. (2011). Sea cucumbers in the Mediterranean: a potential species of aquaculture in the Mediterranean. *Review in Fisheries Science*, 19, pp. 299-304.

Slater, M. J., and Jeffs, A. G. (2010). Do benthic sediment characteristics explain the distribution of juveniles of the deposit-feeding sea cucumber *Australostichopus mollis*? *Journal of Sea Research*, 64, pp. 241-249.

Slater, M. J., Jeffs, A. G., and Carton, A. G. (2009). The use of the waste from green-lipped mussels as a food source for juvenile sea cucumber, *Australostichopus mollis*. *Aquaculture*, 292, pp. 219-224.

Slater, M.J., and Carton, A.G. (2007). Survivorship and growth of the sea cucumber *Australostichopus (Stichopus) mollis* (Hutton 1872) in polyculture trials with green-lipped mussel farms. *Aquaculture,* 272, pp. 389-398.

Taboada, M.C., González, M., and Rodríguez, E. (2003). Value and effects on digestive enzymes and serum lipids of the marine invertebrate *Holothuria forskali*. *Nutrition Research*, 23, pp. 1661-1670.

Tuwo, A., and Conand, C. (1992). Reproductive biology of the Holothurian *Holothuria Forskali* (Echinodermata). *Journal of the Marine Biological Association of the United Kingdom,* 72, pp. 745-758.

Vandenspiegel, D., Jangoux, M., and Flammang, P. (2000). Maintaining the line of defense: regeneration of cuvierian tubules in the sea cucumber *Holothuria forskali* (Echinodermata, Holothuridea). *Biology Bulletin*, 198, pp. 34-49.

Van Dyck, S., Gerbaux, P., and Flammang, P. (2009). Elucidation of molecular diversity and body distribution of saponins in the sea cucumber *Holothuria forskali* (Echinodermata) by mass spectrometry. *Comparative Biochemistry and Physiology, Part B,* 152, pp. 124-134.

Watanabe, S., Zarate, J.M., Sumbing, J.G., Lebata-Ramos, M.J.H., and Nievales, M.F. (2012). Size measurement and nutritional condition evaluation methods in sandfish (Holothuria scabra Jaeger). *Aquaculture Research*, 43, pp. 940-948.

Xing, K., Yang, H.S., and Chen, M.Y. (2008). Morphological and ultrastructural characterisation of the coelomocytes in *Apostichopus japonicas*. *Aquatic Biology*, 27, pp. 85-92.

Yamana, Y., and Hamano, T. (2006). New size measurement for the Japanese sea cucumber *Apostichopus japonicus* (Stichopodidae) estimated from the body length and body breadth. *Fisheries Science*, 72(3), pp. 585-589.

Yamana Y., Hamano T., and Yamamoto, K. (2005), Anesthetizer of the adult sea cucumber *Apostichopus japonicus. Nippon Suisan Gakkaishi,* 71, pp. 299- 306.

Yanagisawa, Y. (1998). Chiiki tokusan zoushoku gijutsu kaihatsu jigyou houkokusho, kyokuhirui (Stock enhancement technology development project report for regional resources, echinoderms). *Aichi Prefectural Government*, pp. 36-39.

Zamora, L.N., Yuan, X., Carton, A.G., and Slater, M.J. (2016). Role of deposit-feeding sea cucumbers in integrated multitrophic aquaculture: progress, problems, potential, and future challenges. *Reviews in Aquaculture*, pp. 1-18, DOI: 10.1111/raq.12147.

Zamora, L. N., and Jeffs, A. G. (2012). The ability of the deposit-feeding sea cucumber *Australostichopus mollis* to use natural variation in the biodeposits beneath mussel farms. *Aquaculture*, 326-329, pp. 116-122.

Zamora, L. N., and Jeffs, A. G. (2011). Feeding, selection, digestion and absorption of the organic matter from mussel waste by juveniles of the deposit-feeding sea cucumber *Australostichopus mollis*. *Aquaculture*, 317(1), pp. 223-228.

Chapter 5

An assessment of Passive Integrated Transponder (PIT) tag retention in the holothurian, *Holothuria forskali*

Abstract

The tagging of holothurians is notoriously difficult due to a lack of hard tissue and the plastic nature of the body wall. The majority of tagging or marking techniques tested to date (e.g. T-bar tags, skin scratches/branding, coded wires, chemical tags) have had a limited rate of success and/or major drawbacks (e.g. only suitable for batch identification, requires the sacrifice of the specimen or complex analysis, causes substantial morphological or physiological stress, mortality). Studies on the effectiveness of passive integrated transponder (PIT) tags for holothurians are limited and reveal limited success with the application of these tags. This study evaluated the efficacy of 1.4 mm x 8 mm PIT tags, injected in three different locations (dorsal body wall, ventral body wall, and through the aquapharyngeal bulb). A comparison was made of two different sized PIT tags (1.4 mm x 8 mm & 2.12 mm x 11.5 mm) injected into the dorsal body wall and aquapharyngeal bulb of *Holothuria forskali* specimens anaesthetised or not anaesthetised prior to tagging. PIT tag retention for all treatments dropped below 50% within 15 days (range: 1-15 days) post tagging and reached 0% within 50 days (range: 8-50 days). Tagging ventrally caused severe stress and mortality to 25% of specimens. For all other treatments, morphological and physiological stress as a direct result of tagging had a low (1-25%) to medium (25-50%) rate of incidence, low severity, and no long-term effects on the health of specimens. The poor tag retention achieved in this study suggests that PIT tags are not an effective tagging method for *H. forskali.*

5.1 Introduction

Long-term studies are an important tool for monitoring the survival, recruitment, growth, behaviour, genetics, and population dynamics of marine species (Lauzon-Guay and Scheibling 2008; Mowat and Strobeck, 2000; Pradel, 1996). Tagging specimens has proven useful for the individual monitoring of fish, invertebrates, birds, reptiles and mammals for such studies, in both field and controlled environments (Nicolaus *et al*. 2008; Duggan and Miller, 2001; Steyermark *et al*. 1996; Schooley *et al*. 1993; Prentice *et al*. 1990; Emery and Wydoski 1987).

Numerous studies have been conducted on holothurian ecology in the context of conservation and management efforts (Mercier and Hamel, 2013; MacTavish *et al*. 2012; Anderson *et al*. 2011; So *et al*. 2011; So *et al*. 2010; Mercier and Hamel, 2009; Hamel and Mercier, 1996a; Hamel and Mercier, 1996b). These studies were largely in response to the overexploitation and poor management of many holothurian fisheries (Gianasi *et al*. 2015; Santos *et al*. 2015; Purcell *et al*. 2014; Anderson *et al*. 2011; Hamel *et al*. 2001; Carpenter and Niem, 1998). Studies have also been conducted on the development of commercial-scale aquaculture of valuable, overfished tropical species and of culture methods for several other species (Purcell *et al*. 2012; Agudo, 2006; Ivy and Giraspy, 2006; Mercier *et al*. 2004). However, the lack of a reliable technique to tag individual holothurians has hindered capture-recapture and tracking studies, which provide essential information on holothurian biology, ecology, and reproduction. This lack of knowledge may inhibit the future development of a sustainable sea cucumber fishery and aquaculture industry worldwide (Gianasi *et al*. 2015; Santos *et al*. 2015; Cieciel *et al*. 2009; Shiell, 2006).

An important criterion for the efficacy of any tag is that it does not adversely affect the tagged individual and has a high retention (Rodríguez-Barreras *et al*. 2016; Rodríguez-Barreras and Sabat, 2015; Lauzon-Guay and Scheibling, 2008). Due to the plastic nature of the body wall, a high likelihood of foreign material expulsion, a lack of hard tissue, and the common occurrence of infection and necrosis around the tagged area, holothurians have proven very difficult to tag (Gianasi *et al*. 2015; Shiell, 2006; Conand, 1991). The majority of techniques trialled so far have yielded limited success and/or have considerable drawbacks.

External tags, such as T-bar or anchor tags, which are inserted through the body wall using a tagging gun, have shown relatively high retention for some species of holothurian over a short time period (i.e. < 3 months) (Rodríguez-Barreras *et al*. 2016; Cieciel *et al*. 2009; Kirshenbaum *et al*. 2006; Conand, 1991). However, other studies have displayed low retention over short and medium to long (i.e. > 3 months) time periods (Xu *et al*. 2017; Rodríguez-Barreras *et al*. 2016; Rodríguez-Barreras *et al*. 2014; Cieciel *et al*. 2009; Purcell *et al*. 2008; Purcell *et al*. 2006; Kirshenbaum *et al*. 2006; Reichenbach, 1999; Conand, 1991). Additionally, specimens in each of these studies experienced one or more of the following side effects: damage to the internal organs, localised necrosis, infection, evisceration, mortality, increased mobility in the field, open sores, skin sloughing, and reduced growth. Although retention has been high over short durations (i.e. 10-60 days), drawbacks include: necrosis, reduced growth, increased mobility in the field, and the disappearance of marks within weeks (Shiell, 2006; Mercier *et al*. 2000; Reichenbach, 1999; Ramofafia *et al*. 1997).

Chemical tags (e.g. fluorochromes), although inexpensive, simple, and long-lasting, do not provide a unique identifier, require a microscope for viewing, may be toxic to juveniles, or unsuitable for cold-temperate and polar species due to the temperature dependency of flurochrome uptake (Purcell and Blockmans, 2009; Purcell *et al*. 2008; Kirshenbaum *et al*. 2006; Purcell *et al*. 2006). Genetic markers are also effective; however, they are expensive, impractical for short-term studies, time-consuming, require extensive analytical skills, and are unsuitable for field monitoring (Uthicke and Purcell, 2004; Uthicke *et al*. 2004; Uthicke and Benzie, 2002). Coded wire tags (internal) are unsuitable for capture-recapture studies, as they must be excised for identification, usually resulting in the specimen being sacrificed (Cieciel *et al*. 2009; Purcell *et al*. 2006; Lokani, 1992). Studies have also identified sea cucumbers (*Australostichopus mollis* & *Stichopus mollis*) in controlled and field conditions through photo identification (Slater and Jeffs, 2010; Slater *et al*. 2009; Slater and Carton, 2007; Raj, 1997). However, a major disadvantage of this method is the potential for misidentification due to human error, especially with those species that do not have obvious differences in natural markings, patterns etc.

Passive integrated transponder (PIT) tags are small, inert microchips with an electromagnetic coil encapsulated in biocompatible glass, which are inserted under the animal's skin (e.g. into the muscle or body cavity) through surgical incision, or more commonly, with a needle. Each tag is programmed with a unique identification number

that is read with a microchip reader, allowing for non-invasive identification of individuals. The glass casing protects the electronic components and reduces the potential for tissue irritation (Gibbons and Andrews, 2004; Rogers *et al*. 2002). Since the mid-1980s, the use of PIT tags has grown rapidly for behavioural, physiological, conservation, management, and commercial harvesting studies across a broad range of taxa (mainly vertebrates), where increased recapture rates and long-term identification of individuals is necessary (Wilson *et al*. 2011; Eymann *et al*. 2006; Mueller *et al*. 2006; Low *et al*. 2005; Skov *et al*. 2005; Gibbons and Andrews, 2004; Galimberti *et al*. 2000; Jehle and Hödl, 1998). Studies of their efficacy in marine invertebrates have shown varying degrees of success (Rodríguez-Barreras and Wangensteen, 2016; Rodríguez-Barreras and Sabat, 2015; Cipriano *et al*. 2014; Lauzon-Guay and Scheibling, 2008; Kurth *et al*. 2007; Woods, 2005; Bubb *et al*. 2002; Caceci *et al*. 1999; Hagen, 1996).
The majority of studies evaluating PIT tags in holothurians (*Holothuria grisea, H. Mexicana, H. whitmaei*, & *Actinopyga miliaris*) have reported poor retention (Rodríguez-Barreras *et al*. 2016; Rodríguez-Barreras *et al*. 2014; Purcell *et al*. 2008). However, it must be noted that there has been a limited number of studies conducted and one study found that *Cucumaria frondosa* tagged with PIT tags had retention of up to 92% after 30 days and 68% after 300 days (Gianasi *et al*. 2015). As discussed in the previous chapter, the sea cucumber *Holothuria forskali* has commercial potential in the biotechnology, pharmaceutical, fishery, and aquaculture industries (Zamora *et al*. 2016; MacDonald *et al*. 2013; Bordbar *et al*. 2011; Deudero *et al*. 2011; Van Dyck *et al*. 2009; Taboada *et al*. 2003; Rodríguez *et al*. 2000; Rodríguez *et al*. 1991). The aim of this study was to determine if PIT tags could be used as a reliable and innocuous marking technique for *H. forskali*.

5.2 Methods and Materials

The first trial (preliminary) assessed retention of a 1.4 mm x 8 mm PIT tag at three different body locations: mid-dorsal wall, mid-ventral wall, and into the aquapharyngeal bulb. The second trial assessed retention of a 1.4 mm x 8 mm and a 2.12 mm x 11.5 mm PIT tag at two body locations, the mid-dorsal wall and through the aquapharyngeal bulb (Figure 1). The mid-ventral location was excluded due to the level of stress and mortality recorded in trial 1. The impact of pre-tagging anaesthesia on tag retention was also assessed.

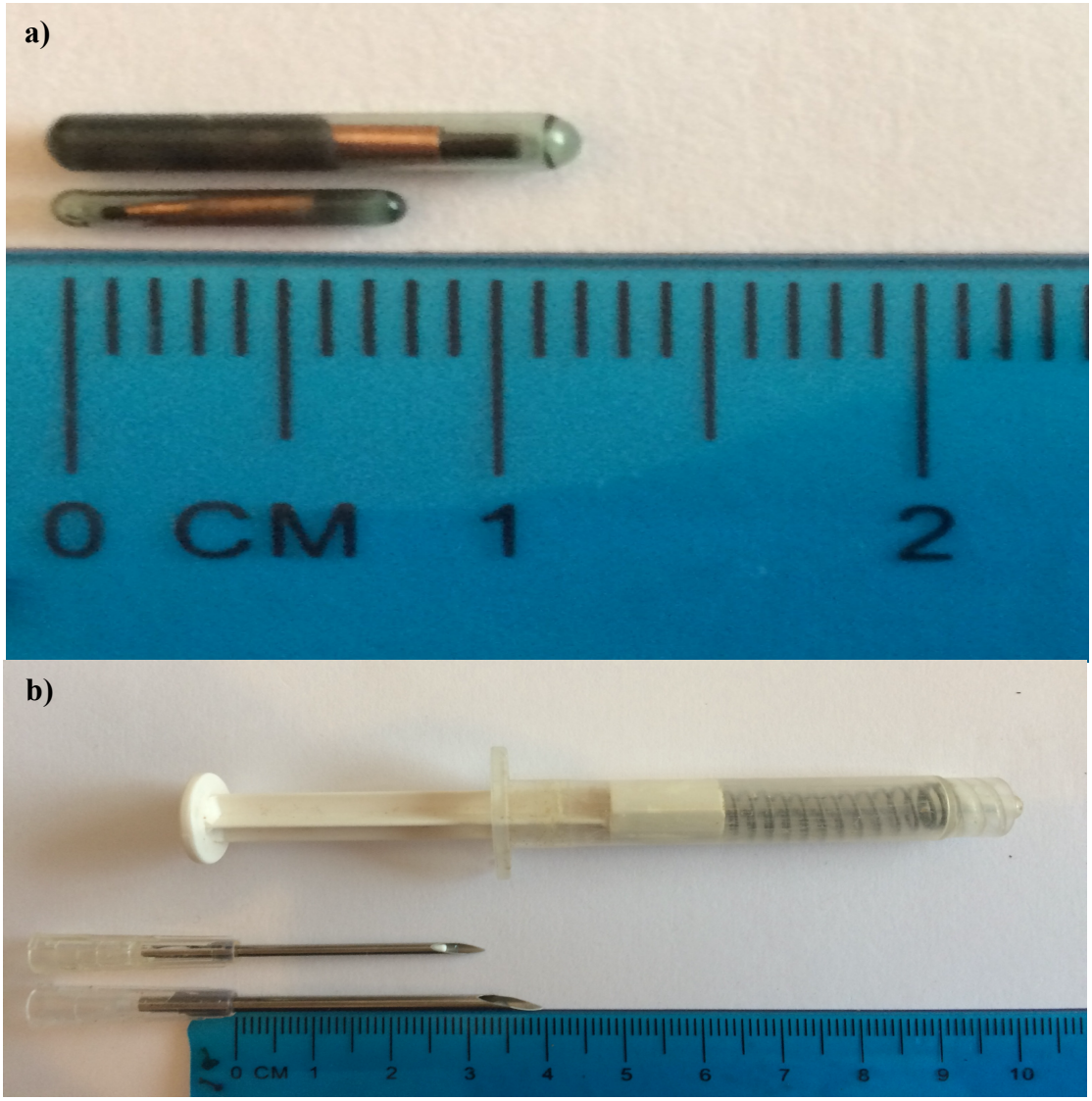


Figure 1: a) Small (1.4 mm x 8 mm) and large (2.12 mm x 11.5 mm) PIT tags & b) small (2 mm x 33 mm) and large (2.7 mm x 40 mm) syringe (bottom left) and PIT tag syringe implanter device (top) (*original photos by Gunning*)

Trial 1: Preliminary assessment of PIT tag effectiveness at three body locations

Animal collection and experimental conditions

Sixty *H. forskali* specimens (mean w.w.: 132.29 ± 43.22 g) were wild-caught by a diver off the coast of Castletownbere, Beara peninsula, County Cork, Ireland on the 23rd of January 2014 and transported to the Aquaculture and Fisheries Development Centre, University College Cork in polystyrene boxes containing damp *Laminaria digitata*. Upon arrival, each specimen was blotted dry with paper towel to remove excess water and weighed to the nearest .00 g. Specimens were then distributed across twelve plastic mesh baskets (55 cm x 40 cm x 14 cm; see Figure 2) (n=5 specimens per basket) ensuring as similar a spread of weights (g ± SD) per basket as possible. These baskets were then randomly placed in four 400 L tanks (see Figure 1) (n=3 baskets per tank) and assigned a treatment. Each basket per tank was assigned treatment 1 (tagged mid-dorsally), treatment 2 (tagged mid-ventrally), or treatment 3 (tagged through the aquapharyngeal bulb) with each tank containing one basket from each treatment. The specimens were allowed to acclimatise for 7 days prior to trial commencement. No sea cucumber mortalities were recorded during the acclimation period.

On the 30th January 2014 specimens were tagged as per their treatment (n=20 per treatment). Following the tagging procedure and short-term stress monitoring (see below for details), the specimens were returned to their respective basket/tank.

Throughout the acclimatisation and experimental phases, each tank's temperature was maintained with a PSA® Aquaclim 10 reversible heatpump/chiller and had continuous water circulation (1000 L sump filled with fresh sea water every 3 days) (temperature: 14.2 ± 1.3 °C; DO: 8.4 ± 0.3 mg/L; pH: 8.3 ± 0.06; salinity: 34.0 ± 0.7 ppt). The specimens were fed a powdered seaweed mix (80% *Ascophyllum nodosum* : 20% *Fucus serratus*) *ad libitum*. Seawater for these tanks was sourced from Fastnet Mussels, Gearhies, Bantry Bay, Co. Cork.

Treatments

(a) Treatment 1 - Dorsal body wall

A PIT tag was inserted into the mid-dorsal wall of *H. forskali* individuals (n = 20, mean w.w.: 137.24 ± 41.42 g). As previously published research had shown low tag retention when injected directly into the coelom (Rodríguez-Barreras *et al*. 2016; Rodríguez-Barreras *et al*. 2014; Purcell *et al*. 2008), the syringe was inserted into the body wall

mid dorsally at an angle of approximately 10-15 ° in an attempt to lodge the tag within the boy wall (body wall depth: approximately 2-6 mm). Extreme care was taken while attempting to inject the tag into the body wall as inserting the syringe too deep could result in the tag being released into the coelomic cavity and being rejected within a relatively short period of time (days to weeks) (Rodríguez-Barreras *et al*. 2016; Rodríguez-Barreras *et al*. 2014; Purcell *et al*. 2008). Also, if the tag was injected too superficially, it could pass back through the hole that the syringe created (Rodríguez-Barreras *et al*. 2016; personal observation, 2016, 2014; Gianasi *et al*. 2015).

(b) Treatment 2 - Ventral body wall

Twenty individuals (mean w.w.: 128.30 ± 43.99 g) were tagged mid-ventrally via the same methodology applied in treatment 1. The thickness of the ventral wall is also c. 2-6 mm, and the same concern about tag loss during the injection procedure detailed in treatment 1, also applied here.

(c) Treatment 3 - Aquapharyngeal bulb

Twenty individuals (mean w.w: 131.63 ± 44.83 g) were tagged in the aquapharyngeal bulb by inserting the syringe approximately 0.1-0.5 cm posterior to the oral cavity, at an angle of approximately 45 °. The tag was released once a second puncture was felt, indicating that the syringe had passed through the body wall and had reached the aquapharyngeal bulb.

PIT tagging methodology

The tagging procedure took approximately 5 seconds for tagging dorsally and ventrally, and approximately 5-10 seconds for tagging through the aquapharyngeal bulb. For each body region, the PIT tag (1.4 mm x 8 mm) was applied using a sterile syringe (2 mm x 32 mm) (Figure 1).

Each PIT tag was scanned with a portable universal microchip reader (RealTrace® RT100) prior to injection to ensure the tags were functional and immediately after being implanted to ensure the tags were being read within the injected location (Figure 2). Each PIT tag had a unique 12-digit identification code.

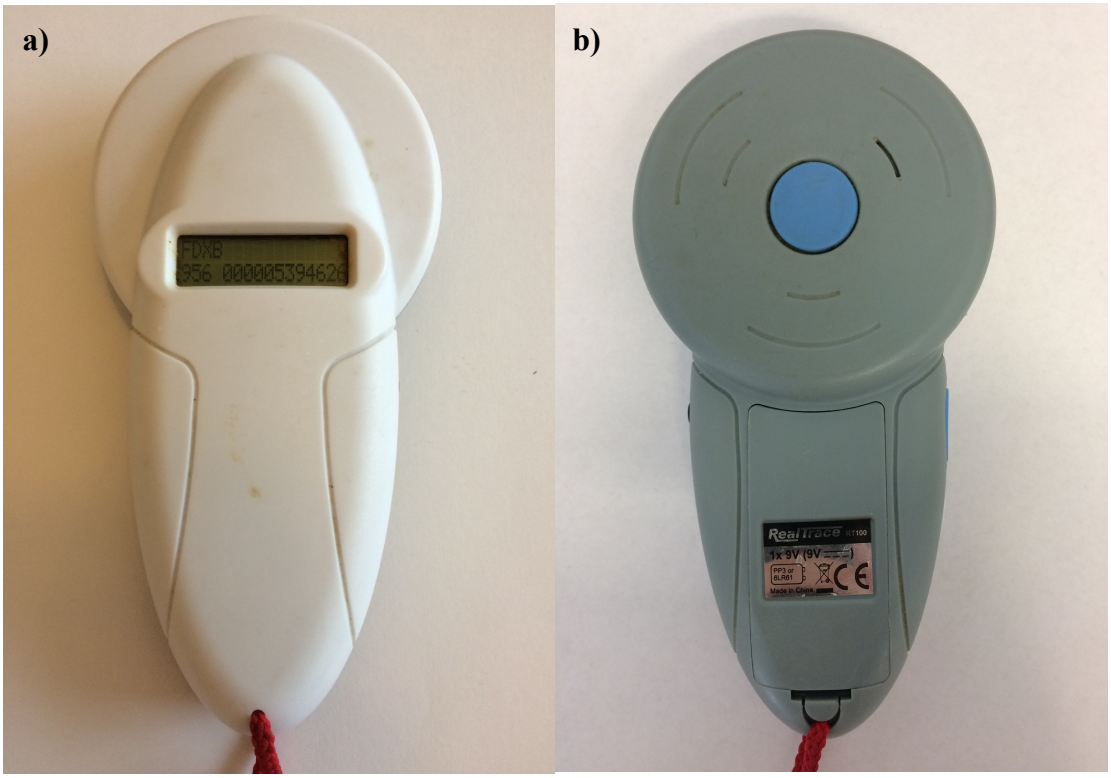


Figure 2: a) Front of RealTrace® microchip reader with screen that shows 12-digit code of scanned PIT tag (numbers to the right of the screen) and b) back of the reader (the blue circle is placed in close proximity to the body of the sea-cucumber when scanning for PIT tag) (*original photo by Gunning*)

Tag retention monitoring

Individuals were scanned with the microchip reader one and six hours after being tagged, and then 1-4 times per week for the duration of each treatment (until the final specimen lost its tag).

Stress and recovery monitoring

Indicators of stress (Table 1) were noted for each individual post injection and for 1 hour (short term recovery monitoring) prior to being returned to each of their respective baskets/tanks. “Short term stress monitoring” took place in individual, aerated 4L aquarium tanks (temperature: 14.36 ± 1.6 °C; DO: 9.4 ± 0.8 mg/L; pH: 8.4 ± 0.16; salinity: 33.5 ± 0.78 ppt). The seawater for these 4 L tanks was from the same source as the acclimatisation and long-term recovery tanks. The presence of skin lesions was monitored each time the specimens were being checked for tag retention for the duration of the trial (long-term recovery monitoring).

Table 1: Morphological, physiological, and behavioural indicators of *H. forskali* stress

Stress Indicators	Description	Severity Level & severity criteria
Morphological		
Skin lesion at point of injection	Tissue damage visible as different colouration than the surrounding tissue	0: No visual indication of skin lesion at point of injection 1: <1 mm diameter skin lesion 2: 1-2 mm diameter skin lesion 3: >2 mm diameter skin lesion
General skin lesion presence		0: No visual indication of skin lesion presence 1: <10% body coverage 2: 10-50% body coverage 3: >50% body coverage
Physiological		
Stress Indicators	**Description**	
Release of cuvierian tubules	Release of defensive threads	
Evisceration	Total or partial extrusion of internal organs	
Swelling	Abnormal enlargement of the body into a balloon shape	

Trial 2: PIT tag effectiveness at two body locations, with two PIT tag sizes, and anaesthetised/not anaesthetised prior to tagging

Animal collection and experimental conditions

H. forskali specimens (n = 216; mean w.w.: 134.09 ± 60.74 g) were wild-caught by a diver from Kenmare Bay, County Kerry, Ireland on the 13th of December 2016 and transported to the Bantry Marine Research Station (BMRS) in polystyrene boxes containing damp *L. digitata*. Upon arrival, each specimen was blotted dry with cloth towels to remove excess water and weighed to the nearest .00 g. This was modified from the paper towels used in trial one, as their use was seen to cause mild skin lesions on some specimens. Specimens were then randomly distributed into plastic mesh baskets (55 cm x 40 cm x 14 cm; see Figure 2) (n=12 specimens per basket) that were divided into four sections with plastic mesh screens (n=3 specimens per section), ensuring as similar a spread of weights (g ± SD) across each basket section as possible. These baskets were then randomly placed in eight 400 L flow through tanks (Figure 3) (n=2-3 baskets per tank). Each section of a basket was randomly assigned a tagging or control treatment, ensuring that no tank had more than one replicate (n=3) from each

tagging or control treatment (temperature: 9.46 ± 1.56 °C; DO: 8.45 ± 0.55 mg/L; salinity: 33.45 ± 0.40 ppt). The seawater was from Bantry Bay, Co. Cork, and was passed through a 60 µm drum filter before entering the tanks.

The sea cucumbers were acclimatised for 7 days prior to trial commencement. No sea cucumber mortalities were recorded during the acclimation period. On 20th December 2016, specimens underwent the experimental or control treatment that was designated to their basket section (see below for details). Following the experimental or control procedure and short-term stress monitoring, specimens were returned to their respective basket/tank. Throughout the acclimatisation and experiment duration, the specimens were fed a powdered seaweed mix (80% *Ascophyllum nodosum* : 20% *Fucus serratus*) *ad libitum*.

Figure 3: 400 L experimental tanks (Trial 2) (*original photo by Gunning*)

Treatments

Trial two consisted of eight tagging (n=12 per treatment) and ten control (n=12 per control) treatments (Table 2). Each tagging treatment assessed a different combination

of tagging location, tag size (small: 1.4 mm x 8 mm; large: 2.12 mm x 11.5 mm) and anaesthetisation. The control treatments consisted of eight injection controls, where the specimens underwent the exact same treatment as its respective tagging treatment (e.g. treatment 1 and injection control 1), however, no tag was released after the specimen was injected. Two further handling controls (no tagging or injection) involved specimens which were handled for 10 seconds (approximate handling time of tagging treatments) with either no anaesthesia (handling treatment 1) or anaesthesia (handling treatment 2) prior to handling.

Table 2: Trial two tagging and control treatments

Tagging treatment (n=12 per treatment)	**Mean weight (g ± SD)**	**Anaesthetised**	**Tag size**	**Injection location**
1	140.39 ± 59.01	No	Small	Mid-dorsal body wall
2	132.02 ± 57.39	Yes		
3	148.84 ± 71.56	No	Large	
4	136.18 ± 75.14	Yes		
5	147.29 ± 73.11	No	Small	Aquapharyngeal bulb
6	153.94 ± 74.68	Yes		
7	131.18 ± 46.65	No	Large	
8	130.07 ± 57.77	Yes		
Injection control (n=12 per control)	**Mean weight (g)**	**Anaesthetised**	**Syringe size**	**Procedure**
1	134.09 ± 59.20	No	Small	Handled and injected as per mid-dorsal tagging; tag not released
2	133.14 ± 69.12	Yes		
3	141.30 ± 68.94	No	Large	
4	130.81 ± 54.75	Yes		
5	133.96 ± 59.81	No	Small	Handled and injected as per aquapharyngeal bulb tagging; tag not released
6	129.02 ± 58.28	Yes		
7	135.33 ± 71	No		
8	132.33 ± 66.33	Yes	Large	
Handling control (n=12 per control)	**Mean weight (g)**	**Anaesthetised**	**Procedure**	
1	113.37 ± 33.53	No	Handled for 10 seconds	
2	110.45 ± 49.51	Yes	Handled for 10 seconds post anaesthesia	

(a) Treatment 1-4: Dorsal body wall (n=12 per treatment)

All individuals tagged mid-dorsally were tagged using the same methods employed in treatment 1 of trial 1. Treatment 1 and 2 were tagged with small PIT tags (1.4 mm x 8 mm) and treatment 3 and 4 with large PIT tags (2.12 mm x 11.5 mm). Specimens from treatment 2 and 4 were anaesthetised prior to being tagged.

(b) Treatment 5-8: Aquapharyngeal bulb (n=12 per treatment)

The method used in treatment 3 of trial 1 was refined, utilising the techniques successfully employed by Gianasi *et al*. (2015). One tentacle was gently held with a flat edge tweezers and the PIT tag implanted at its base. This was done to help maximise the chances of the tag finding its way into the aquapharyngeal bulb via the hydrovascular system, minimising the possibility of implantation into the coelomic cavity or digestive tract. In treatment 5 and 6, specimens were tagged with small PIT tags and in treatment 7 and 8 with large PIT tags. Specimens from treatment 6 and 8 were anaesthetised prior to being tagged.

(c) Injection control 1-4: Injected mid-dorsally – no tag released (n=12 per control)

Specimens were injected mid-dorsally with the small (2 mm x 33 mm) (control 1 & 2) and large (2.7 mm x 40 mm) (control 3 & 4) injector gun without the tag being released. Specimens from injection control 2 and 4 were anaesthetised prior to being injected.

(d) Injection control 5-8: Injected through the aquapharyngeal bulb – no tag released (n=12 per control)

Specimens were injected through the aquapharyngeal bulb with the small (2 mm x 33 mm) (control 5 & 6) and large (2.7 mm x 40 mm) (control 7 & 8) injector gun without the tag being released. Specimens from injection control 6 and 8 were anaesthetised prior to being injected.

(e) Handling control 1 & 2

Specimens (n=12 per control) were handled for 10 seconds without being injected or tagged. Specimens from handling control 2 were anaesthetised prior to being handled.

*****Note: all even numbered treatments and controls were anaesthetised prior to being tagged, injected, or handled***

PIT Tag Methodology

The tagging procedure took approximately 5 seconds for tagging dorsally and approximately 5-10 seconds for tagging through the aquapharyngeal bulb. Each PIT tag was scanned with the microchip reader prior to injection to ensure the tag was

functional and immediately after being implanted to ensure the tag was being read within the injected location. Reading accuracy was corroborated by selecting and dissecting three 'non-signal' tagged individuals from each treatment (total n=24) at the end of the study. For all respective treatments, small PIT tags (1.4 mm x 8 mm) were injected with a 2 mm x 33 mm sterile syringe and large PIT tags (2.12 mm x 11.5 mm) with 2.7 mm x 40 mm sterile syringe (Figure 1).

Sea cucumber anaesthesia methodology

Treatment 2, 4, 6, 8, injection control 2, 4, 6, 8, and handling control 2 were anaesthetised with 2% $MgCl_2$ prior to being tagged, injected, or handled. For each of these treatments twelve individuals were placed in a 20 L aerated tank containing a 2% $MgCl_2$ seawater solution (temp: 12.06 ± 1.13 °C; DO: 8.03 ± 0.45 mg/L; salinity: 33.45 ± 0.35 ppt). All individuals were fully anaesthetised within 15-20 minutes. An individual was deemed fully anaesthetised when each of the following criteria were met; 1) body relaxation (i.e. cessation of crawling movements); 2) failure of tentacles to react to prodding (i.e. the tip of the tentacles were touched with the tip of a forceps); and 3) the inability to anchor firmly (i.e. lack of tube feet attachment to the tank surface). Full recovery from anaesthesia took approximately 20 min and was considered when: 1) crawling movement began; 2) tentacles reacted to prodding; and 3) the majority of tube-feet began to attach.

Tag retention monitoring

One to two times per week, individuals were scanned with the microchip reader (until the final specimen lost its tag).

Stress and recovery monitoring

Indicators of stress (Table 1) were noted for each individual of all control and tagging, treatments, immediately post injection, or handling, and one hour post the aforementioned procedure (short-term recovery monitoring) in individual, aerated 4L aquarium tanks (temperature: 10.12 ± 1.3 °C; DO: 8.34 ± 0.43 mg/L; salinity: 33.04 ± 0.41 ppt). The seawater for these 4L tanks was from the same source as the acclimatisation and long-term recovery tanks. Following short-term monitoring, the specimens were returned to their respective basket/tank and were monitored for morphological signs of stress for 50 days (long-term recovery monitoring).

Statistical analysis

All statistical analysis was compiled using SPSS software (IBM) version 23. Prior to statistical analysis, percentage data was transformed using the arcsine transformation. All data was tested for normal distribution and homogeneity of variance with the Shapiro-Wilk test and Levene's test, respectively ($p>0.05$). Values less than 0.05 were considered statistically significant.

(a) Trial 1

One-way ANOVAs were used to test the significance of differences in tag retention (%) at each monitoring date and the length of time (days) specimens retained tags, between each tagging treatment group. For data that was not normally distributed, individual Kruskal-Wallis tests were used. Individual Kruskal-Walis tests were also used to compare the proportion of specimens from each treatment that experienced different forms of stress post tagging and during short-term stress monitoring.

(b) Trial 2

Kruskal-Wallis tests were used to test the significance of differences in tag retention (%) at each monitoring date and the length of time (days) specimens retained tags, between each tagging treatment group. Individual Kruskal-Wallis tests were also used to compare the proportion of specimens from each tagging treatment that experienced different forms of stress post tagging and during short-term stress monitoring. Independent t-tests were used to compare stress between injection controls and their respective tagging treatment and between handling controls and tagging and injection control treatments. For data that was not normal, individual Kruskal-Wallis tests were used.

5.3 Results

Trial 1: Preliminary assessment of PIT tag effectiveness at three body locations

Tag retention

Retention (%) of PIT tags had dropped below 50% for all tagging locations by 4 days post tagging. Tag retention of specimens tagged ventrally dropped to 40 ± 43.2% after 2 days, while specimens tagged dorsally and through the aquapharyngeal bulb dropped to 40 ± 28.3% and 30 ± 25.8%, respectively, after 4 days (Figure 4). Retention of PIT tags reached 0% by 8, 13, and 41 days post tagging for specimens tagged through the aquapharyngeal bulb, ventrally, and dorsally, respectively. However, it is important to note that from 21 days post tagging, only one specimen (5 ± 10%) tagged dorsally retained its PIT tag (Figure 4). For all days monitored, there was no significant difference in tag retention between treatments ($p>0.05$). There was also no significant difference in the length of time (days) specimens retained tags between all tagging locations ($p>0.05$).

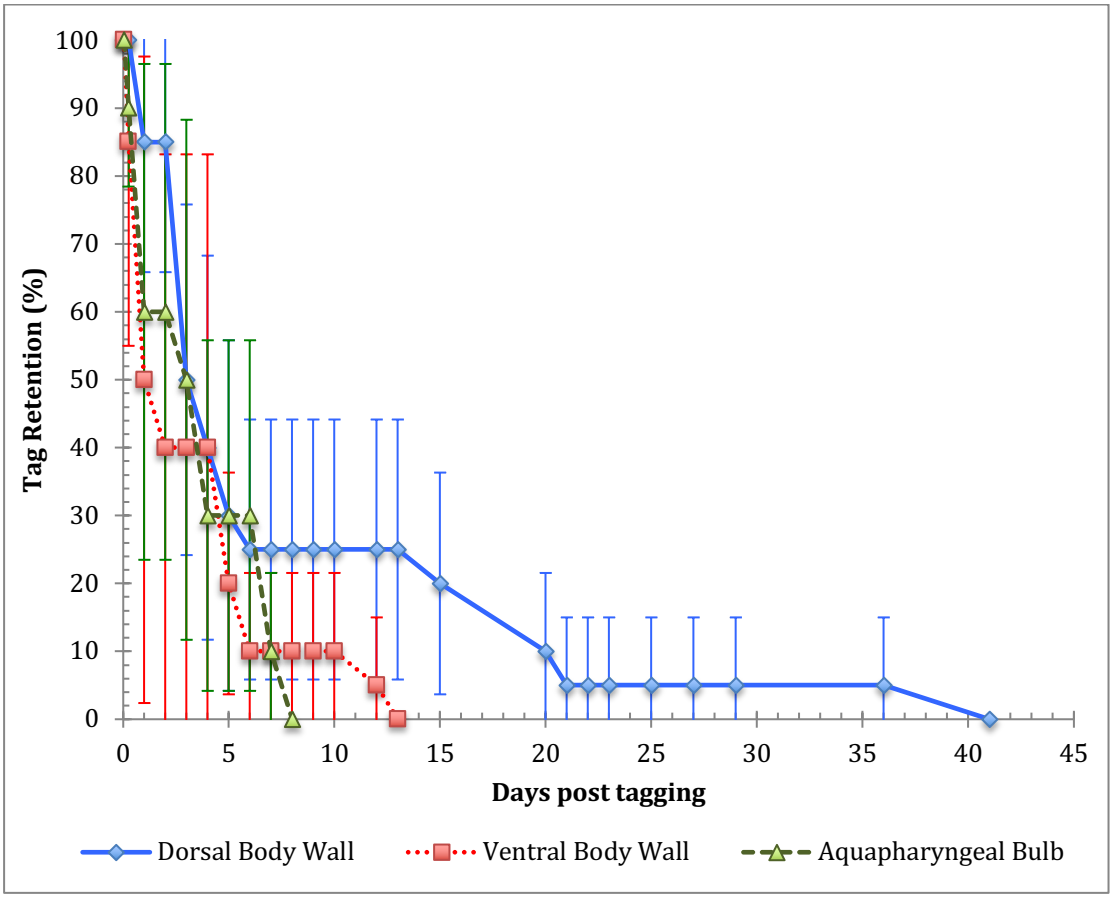


Figure 4: PIT tag retention (%) of *Holothuria forskali* PIT specimens tagged in three different body locations (Trial 1) (mean ± SD)

Stress and mortality monitoring

(a) Post injection/handling (prior to short-term recovery stage)

(i) Morphological stress (i.e. skin lesions):

General body skin lesions: General skin lesion damage (body coverage, site of injection excluded) did not occur for any specimen of any treatment.

Skin lesions at point of injection: Level 1 (<1mm diameter) skin lesion damage at the site of injection post tagging (prior to short-term monitoring) only occurred for 5 ± 10% of specimens tagged ventrally.

(ii) Physiological stress

Cuvierian tubule release: Cuvierian tubule release occurred post tagging (prior to short-term recovery monitoring) for 10 ± 11.55% and 5 ± 10% of specimens tagged dorsally

and ventrally, respectively ($p>0.05$). No specimen released cuvierian tubules post tagging for the aquapharyngeal bulb treatment.

Evisceration & swelling: No evisceration and no swelling occurred post tagging for all tagging treatments.

(b) Short-term recovery monitoring

No cuvierian tubule release occurred for any tagging or control treatment during the short-term recovery period.

(i) Morphological stress (i.e. skin lesions):

General body skin lesions: Although no skin lesions were visible post tagging for those specimens tagged through the aquapharyngeal bulb, level 1 general skin lesion damage became visible for 5 ± 10% of individuals during short-term monitoring.

Skin lesions at point of injection: For specimens tagged ventrally, skin lesion presence at the site of injection increased to 25 ± 10% during short-term monitoring. Specimens tagged dorsally and through the aquapharyngeal bulb had no visible signs of skin lesions at the injection site during short-term monitoring.

(ii) Physiological stress

Swelling: Swelling during short-term monitoring occurred for 15 ± 10%, 30 ± 25.82%, and 5 ± 10% of individuals tagged dorsally, ventrally, and through the aquapharyngeal bulb, respectively ($p>0.05$). . All occurrences of swelling occurred during the first 30 minutes of the 1 hour short-term monitoring phase.

Cuvierian tubule release and evisceration: No evisceration of cuvierian tubule release occurred during the STSM (short term stress monitoring) period for all treatments.

(c) Long-term recovery monitoring

The specimens from the aquapharyngeally tagged treatment fully recovered by day 7 of the long-term recovery period.

By day 5 of long-term recovery, the skin lesion damage at the point of injection had increased to level 3 (>2mm diameter) for 25 ± 10% of ventrally tagged specimens. All specimens with level 3 damage perished by day 10.

Trial 2: PIT tag effectiveness at two body locations, with two PIT tag sizes, and anaesthetised/not anaesthetised prior to tagging

Tag retention

Fifteen days post tagging, specimens from 5 of the 8 tagging treatments had lost all of their tags, while the tag retention of; non anaesthetised specimens tagged dorsally with small PIT tags (Treatment {T} 1), non anaesthetised specimens tagged dorsally with large tags (T3), and anaesthetised specimens tagged through the aquapharyngeal bulb with small tags (T6), was 41 ± 67%, 8.3 ± 16. 7%, and 8.3 ± 16.7%, respectively (Figure 5). By 23 days post tagging, specimens in treatment 3 had 0% retention, while treatment 1 had dropped to 33.3 ± 27.2%, and treatment 6 remained at 8.3 ± 16.7%. By 38 days post tagging, specimens in both treatment 1 and 6 had 8.3 ± 16.7% retention. Specimens from both treatment groups lost their remaining tags by 50 days post tagging (Figure 5).

For all days monitored, there was no significant difference in tag retention between treatments ($p>0.05$). There was also no significant difference in the length of time (days) specimens retained tags between all tagging locations ($p>0.05$).

Reading accuracy was 100%. No PIT tags were found in the 24 dissected specimens (n=3 from each tagging treatment), eliminating the possibility of any potential reading error.

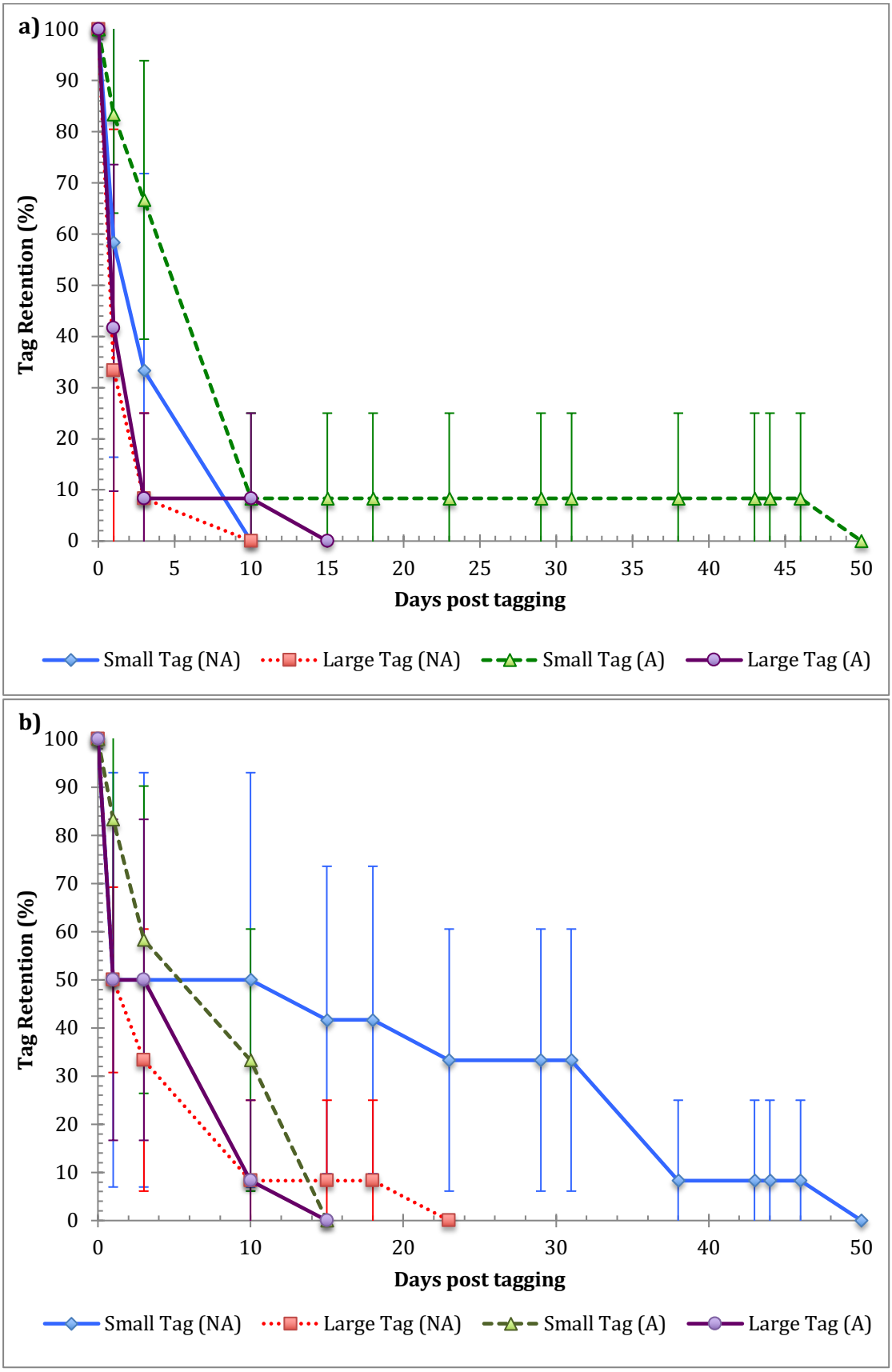


Figure 5: PIT tag retention (%) of *Holothuria forskali* specimens tagged (a) dorsally & (b) through the aquapharyngeal bulb; with small and large tags with (A) or without (NA) an anaesthetic treatment prior to tagging (Trial 2) (mean ± SD)

Stress and recovery monitoring

No specimen from any tagging or control treatment eviscerated at any point during the trial. There were also no mortalities during the trial or recovery stages for any treatment or control. There was no significant difference ($p>0.05$) in the level of stress experienced between all tagging treatments at all stages of the trial, between all tagging treatments and their respective injection treatment, between all tagging treatments and both handling controls, and between all injection controls and both handling controls.

(a) Post injection/handling (prior to short-term recovery stage)

(i) Morphological stress (i.e. skin lesions):

General body skin lesions: Post tagging, level 1 (<10% body coverage) general skin lesion damage (site of injection excluded) occurred for: 16.7 ± 19.4% of anaesthetised specimens tagged dorsally with small tags (T2) (corresponding injection control {IC} incidence - IC 2: 8.3 ± 16.7%); 16.7 ± 33.3% of anaesthetised specimens tagged dorsally with large tags (T4) (IC4: 41.7 ± 16.7%); 8.3 ± 16.7% of anaesthetised specimens tagged through the aquapharyngeal bulb with small tags (T6) (IC6: 8.3 ± 16.7%); 8.3 ± 16.7% of non-anaesthetised specimens tagged through the aquapharyngeal bulb with a large tag (T7) (IC7: 8.33 ± 16.67%); and 8.33 ± 16.67% of anaesthetised specimens tagged through the aquapharyngeal bulb with a large tag (T8) (IC8: 16.7 ± 33.3%). For those specimens that were handled for 10 seconds (no injection) (handling control 1), no general skin lesion damage was observed. Level 1 general skin lesion stress occurred for 50 ± 43.0% of specimens that were anaesthetised prior to being handled for 10 seconds (no injection) (handling control 2).

Skin lesions at point of injection: Post injection, level 1 skin lesion damage (<1 mm diameter) at the site of injection was only noted for 8.3 ± 16.7% (i.e. one individual) of specimens from injection control treatment 2.

(ii) Physiological stress

Cuvierian tubule release: Post tagging, cuvierian tubule release occurred for: 50 ± 43.03% of treatment 1 (CT1: 8.33 ± 16.67%); 50 ± 43.03% of treatment 3 (CT3: 16.67 ± 33.34%); 16.67 ± 33.34% of treatment 4 (CT4: 0%); 33.33 ± 47.14% of treatment 5 (tagged through the aquapharyngeal bulb with small tags) (CT5: 16.67 ± 19.24%);

33.33 ± 27.22% of treatment 7 (CT7: 25 ± 31.92%), and 8.33 ± 16.67% of treatment 8 (CT8: 8.33 ± 16.67%) specimens. Cuvierian tubule release occurred for 8.33 ± 16.67% of specimens from handling control 1 and 2.

Swelling: Post tagging, swelling occurred for 8.33 ± 16.67% of specimens from treatment 7 (CT7: 8.33 ± 16.67%) and 8 (CT8: 8.33 ± 16.67%). Swelling did not occur at this stage for treatment 3, however, it did occur for 16.67 ± 33.34% of specimens from its corresponding control treatment (CT3). No swelling occurred for specimens from handling control 1 and 2.

(b) Short-term recovery monitoring

No cuvierian tubule release occurred for any tagging or control treatment during the short-term recovery period.

(i) Morphological stress (i.e. skin lesions):

General body skin lesions: Level 1 general skin lesion damage increased to 25 ± 16.67%, 16.67 ± 33.34%, and 33.33 ± 27.22% of specimens from tagging treatment 2 (CT2: 8.33 ± 16.67%; no change {NC}), 6 (CT6: 8.33 ± 16.67%; NC), and 8 (CT8: 16.67 ± 33.34%; NC), respectively, and remained at 16.67 ± 33.34% and 8.33 ± 16.67% of specimens from tagging treatment 4 (CT4: 41.67 ± 16.67%; NC) and 7 (CT7: 8.33 ± 16.67%; NC), respectively. There was no change to the incidence of general skin lesion damage for handling control 1 and 2.

Skin lesions at point of injection: The incidence of level 1 skin lesion damage at the point of injection remained the same as in the post injection/handling stage for injection control 2 at 8.33 ± 16.67% of specimens. No other specimens from all other tagging and control treatment had this damage at this stage of the trial.

(ii) Physiological stress

Swelling: During the short-term stress monitoring stage, swelling occurred for: 8.33 ± 16.67% of treatment 1 (CT1: 8.33 ± 16.67%) and 5 (CT5: 0%) specimens, 16.67 ± 19.24% of treatment 3 (CT3: 0%) specimens, and 25 ± 31.92% of treatment 7 (CT7: 16.67 ± 33.34%) specimens. No swelling occurred for handling control 1 or 2 during this stage.

(c) Long-term recovery monitoring

Tagging treatments: Full recovery from level 1 general skin lesion damage occurred 3, 10, 10, 15, and 18 days post tagging for all specimens of treatment 7, 4, 6, 2, and 8, respectively.

Control treatments: 15 days post injection, the specimen from injection control treatment 2 had fully recovered from the skin lesion damage at the injection site. Full recovery from level 1 general skin lesion damage occurred 3,10, and 23 days post tagging for all specimens from injection control treatment 2, 7, 6, 8, and 4, respectively, and 15 days post handling for all specimens from handling control 2.

5.4 Discussion

The capture fishery industry of holothurian species (i.e. Asian and tropical) is declining due to overexploitation and poor management (Gianasi *et al*. 2015; Santos *et al*. 2015; Purcell *et al*. 2014; Anderson *et al*. 2011; Hamel *et al*. 2001; Carpenter and Niem, 1998). Although a number of studies have been conducted on the conservation and management of holothurian fishery grounds and the development of holothurian aquaculture (Mercier and Hamel, 2013; MacTavish *et al*. 2012; Purcell *et al*. 2012; Anderson *et al*. 2011; So *et al*. 2011; So *et al*. 2010; Mercier and Hamel, 2009; Agudo, 2006; Ivy and Giraspy, 2006; Mercier *et al*. 2004; Hamel and Mercier, 1996a; Hamel and Mercier, 1996b), our knowledge of holothurian ecology, biology, and reproduction is still relatively limited. This is, in part, due to the lack of a reliable and easy technique to mark individuals, which has hindered tracking and capture-recapture studies and field and controlled environment studies that require the long-term identification of individuals (Gianasi *et al*. 2015; Santos *et al*. 2015; Navarro *et al*. 2013; Cieciel *et al*. 2009; Shiell, 2006). For tagging procedures to be effective, there must be a high level of tag retention combined with a negligible impact on individual health, behaviour, and survival. Rodríguez-Barreras *et al*. (2016), for example, recommended that a tag retention of 90% was required for effective capture-mark-recapture studies. Most species of holothurian have a life-span of 5 to 10 years (Barnes, 1987).

Due to a lack of hard tissue and the plastic nature of the body wall, it is very difficult for holothurians to retain external physical marks or internal and external tags (Xu *et al*. 2017; Gianasi *et al*. 2015; Shiell, 2006; Conand, 1991). Most of the techniques tested to date (e.g. T-bar tags through the body wall, scratches/brands on the body; coded wires in coelomic cavity and body wall, chemical tags) have yielded limited success and considerable drawbacks in relation to stress, health, mobility, mortality, and growth (Cieciel *et al*. 2009; Purcell and Blockmans, 2009; Purcell *et al*. 2008; Kirshenbaum *et al*. 2006; Shiell, 2006; Purcell *et al*. 2006; Mercier *et al*. 2000; Reichenback, 1999; Ramofafia *et al*. 1997; Lokani, 1992; Conand, 1991). Passive integrated transponder (PIT) tags have been used successfully on a range of taxa (mainly vertebrates) since the mid-1980s, for a variety of studies that require the long-term identification of individuals (Wilson *et al*. 2011; Eymann *et al*. 2006; Mueller *et al*. 2006; Low *et al*. 2005; Skov *et al*. 2005; Gibbons and Andrews, 2004; Galimberti *et al*. 2000; Jehle and

Hödl, 1998). Recently, the number of studies into their suitability for marine invertebrates has increased, however, the success of the PIT transponders has varied considerably (Rodríguez-Barreras and Wangensteen, 2016; Rodríguez-Barreras and Sabat, 2015; Cipriano *et al*. 2014; Lauzon-Guay and Scheibling, 2008; Kurth *et al*. 2007; Woods, 2005; Bubb *et al*. 2002; Caceci *et al*. 1999; Hagen, 1996). To date, only a small number of studies have evaluated the effectiveness of tagging holothurians with PIT tags, with the majority reporting poor tag retention. These studies only trialled a limited number of injection locations and did not assess the impact of anaesthetising the specimens prior to injection/handling (Rodríguez-Barreras *et al*. 2016; Rodríguez-Barreras *et al*. 2014; Purcell *et al*. 2008).

In the first trial of this study, the aim was to assess the efficacy of tagging *Holothuria forskali* specimens at three different locations; dorsal body wall, ventral body wall, and through the aquapharyngeal bulb, with a 1.4 mm x 8 mm PIT tag. As previous studies have reported limited success with releasing PIT tags into the coelomic cavity (Rodríguez-Barreras *et al*. 2016; Rodríguez-Barreras *et al*. 2014; Purcell *et al*. 2008), an effort was made to ensure the tag remained in the body wall when injected dorsally and ventrally. Despite this effort, tag retention reached 0% by 8 and 13 days post tagging for the dorsally and ventrally tagged treatments, respectively. Tagging through the aquapharyngeal bulb also proved to be unsuccessful, with 5 ± 10% retention seen 21 days post tagging, and 0% retention by 41 days. Tagging into the ventral wall proved to be quite stressful for the specimens, resulting in substantial skin lesion damage at the site of injection and the subsequent mortality of 25 ± 10% of specimens. *H. forskali*'s tube-feet are located on the ventral body wall, therefore, this may be a very sensitive region. There was no incidence of morphological (i.e. skin lesions) stress experienced by specimens of the dorsal and aquapharyngeal bulb treatments, and the incidences (5-15% of specimens) and severity of physiological stress (i.e. swelling and cuvierian tubule release) was low.

The PIT tag size utilised differed between previous holothurian PIT tagging studies (e.g. 1.2 mm x 8 mm, 0.05 mm x 8.21 mm, & 2.5 mm x 12 mm) (Rodríguez-Barreras *et al*. 2016; Gianasi *et al*. 2015; Rodríguez-Barreras *et al*. 2014). In trial 2 of this study, two different tag sizes, small (1.4 mm x 8 mm) and large (2.12 mm x 11.5 mm), were utilised. Specimens were tagged dorsally and through the aquapharyngeal bulb again, however, ventral tagging was not attempted due to concerns for the welfare of the sea cucumbers following the outcome of trial 1. In 2015, a study conducted by Gianasi *et*

al. (2015) achieved 92% PIT tag retention for large (11.7 ± 1.5 g immersed weight {IM}) *Cucumaria frondosa* specimens at the end of a 30 day trial, and 68% at the end of a 300 day trial. Tag retention was lower for smaller (2.6 ± 1.1 g IM) individuals, at 84% by the end of the 30 day trial, and 42% by the end of the 300 day trial. The methods employed for tagging through the aquapharyngeal bulb in trial 1 were refined, utilising the techniques successfully implemented by Gianasi *et al*. (2015). Also, the impact of anaesthetising specimens prior to tagging was assessed, an aspect to the PIT tagging of holothurians that has not yet been attempted. Nonetheless, all tagging treatments demonstrated poor tag retention. The most successful treatment consisted of non-anaesthetised specimens tagged dorsally with a small tag (treatment 1), with tag retention of 50 ± 43.0% 10 days post tagging, and 33.3 ± 27.2% 31 days post tagging. However, this treatment reduced to 8.3 ± 16.7% by 38 days post tagging, and 0% by 50 days. Specimens from the other treatments had lost all or the majority (8.3 ± 16.7%) of their tags 15 days post tagging. Despite utilising the same aquapharyngeal bulb tagging technique as Gianasi *et al*. (2015), this study did not achieve the same success rate. However, the tentacles of *C. frondosa* deploy more prominently than those of *H. forskali* (when not under anaesthesia) making them easier to hold with a forceps, which may have contributed to the higher tag retention (Gianasi *et al*. 2015; personal observation 2016, 2014).

Similar to trial 1, injection did not seem to cause damage at the site of injection in trial 2, with only one individual injected dorsally with a small syringe (injection control 2) having level one (<1mm diameter) skin lesion damage post injection. The highest incidence of level 1 (<10% body coverage) general skin lesion damage amongst all treatments (tagged and control) occurred for the anaesthetised handling control specimens (handling control 2) (50 ± 43.03%) and for the anaesthetised specimens injected dorsally with a large syringe (injector control 4) (41.67 ± 16.67%). Also, when tagging treatments with the same tag size and location were compared, those treatments that were anaesthetised prior to tagging experienced a higher incidence of level 1 (<10% body coverage) general skin lesion damage. In fact, only one non-anaesthetised specimen (tagged through the aquapharyngeal bulb with a large tag) experienced level 1 general skin lesion damage (this may have occurred due to the increased difficultly in tagging through the aquapharyngeal bulb when specimens were not anaesethetised, which may have resulted in a more severe handling of specimens. However, this stress was only seen for one specimen). It would appear from these results that anaesthesia is

a significant contributor to general skin lesion stress and does not improve tag retention. It must be noted, however, that all specimens from trial 2 fully recovered from skin lesion damage 3-23 days post tagging, the damage was only minor (<10% body coverage), and the majority only experienced a low incidence (<25%) of this stress. Unsurprising, in the majority of cases, incidences of physiological stress (i.e. cuvierian tubule release & swelling) were higher for specimens form the tagging treatments that were not anaesthetised prior to tagging, indicating that anaesthetising specimens prevented this stress response. Cuvierian tubule release was the only physiological stress response experienced by specimens of the anaesthetised and non-anaesthetised handling controls, with a low incidence of 8.3 ± 16.7%, indicating that handling alone (no injection or tagging) for a short period of time (10 seconds) does not elicit a substantial physiological stress response. Also, in the majority of cases, the incidences of physiological stress was higher for the tagging treatments than the injection control treatments, indicating that the stress was possibly exacerbated by the tag being released into the body. It is important to note, however, that differences in stress levels were not significant ($p>0.05$).

Overall (trial 1 & 2), morphological and physiological stress as a direct result of tagging had a low (1-25%) to medium (25-50%) rate of incidence and low severity. There were also no long-term effects on the health of specimens as a result of tagging (except for ventrally tagged specimens, with 25% experiencing level 3 skin lesion damage at the site of injection, followed by mortality).

Despite assessing three different tagging locations, two different tag sizes, and the impact of anaesthesia, the retention of PIT tags in *H. forskali* was poor. Although tag loss (post tagging) was not observed during the trials, PIT tags were observed passing back through the injection hole during tagging procedure on a total of three occasions (trial 1 and 2) and specimens had to be re-tagged. Although this was a low occurrence, this may have happened on other occasions post-tagging. It is also possible that the body of *H. forskali* specimens (e.g. the immune system) recognised the presence of the tag within a short period of time, despite being encased in a biocompatible polymer, and eliminated it from the body, possibly through the digestive system (Rodríguez-Barreras *et al*. 2016; Rodríguez-Barreras *et al*. 2014). In this study, the use of PIT tags on *Holothuria forskali* did not fulfil the high retention requirements of any short, medium, or long-term studies which require the identification of individual specimens. Although other tagging methods (e.g. T-bars, scarring, chemical tags) have

demonstrated mixed levels of success and various drawbacks when assessed for other species of holothurian, they have not yet been tested on *H. forskali*. Future studies should assess the efficacy of these tagging methods and other novel identification techniques (e.g. pattern recognition technology) (http://www.reijns.com/i3s/) on *H. forskali*.

5.5 Conclusion

Recently, *Holothuria forskali* has emerged as a species with commercial and aquaculture potential. The ability to identify individuals is a requirement for a number of studies that are important for the sustainable development of holothurian fisheries and aquaculture (e.g. reproduction, feeding, demography studies etc.). This study has demonstrated that PIT tagging is not a viable tagging methodology for *H. forskali.* However, it is important to note that other tagging methodologies (e.g. T-bars, chemical tags, pattern recognition photography) have not yet been studied for *H. forskali*.

5.6 Acknowledgements

Benjamin Horgan (UCC) assisted in the setting up and monitoring of trial 1. Prof. Gavin Burnell gave advice on the design of these trials and provided feedback on the chapter. A big thank you to Dean Murphy (Shellfish Ireland, Ltd.) and Declan Sullivan (Marine Harvest Ltd.) who dived for the sea cucumbers used in these trials and to Tony Loveless (Microchipping.biz) who provided the PIT tags for this study.

5.7 References

Agudo, N.S. (2006). Sandfish hatchery techniques. *Australian Centre for International Agricultural Research, Secretariat of the Pacific Community and the WorldFish Centre*, Nouméa, New Caledonia, p. 44.

Anderson, S.C., Flemming, J.M., Watson, R., and Lotze, H.K. (2011). Serial exploitation of global sea cucumber fisheries. *Fish and Fisheries,* 12(3), pp. 317-339.

Barnes, R. (1987). *Invertebrate Zoology*. Dryden Press, Orlando, Florida.

Bordbar, S., Anwar, F., and Saari, N. (2011). High-value components and bioactives from sea cucumbers for functional foods – a review. *Marine Drugs*, 9, pp. 1761-1805.

Bubb, D.H., Lucas, M.C., Thom, T.J., and Rycroft, P. (2002). The potential use of PIT telemetry for identifying and tracking crayfish in their natural environment. *Hydrobiologia*, 483, pp. 225-230.

Caceci, T., Smith, S.A., Toth, T.E., Duncan R.B., and Walker, S.C. (1999). Identification of individual prawns with implanted microchip transponders. *Aquaculture,* 180, pp. 41-51.

Carpenter, K.E., and Niem, V.H. (1998). The living marine resources of the Western Central Pacific – cephalopods, crustaceans, holothurians and sharks. FAO species identification guide for fishery purposes. *United Nations Food and Agriculture Organisation, Rome, Italy,* 2, pp. 687-1396.

Cieciel, K., Pyper, B.J., and Eckert, G.L. (2009). Tag retention and effects of tagging on movement of the giant red sea cucumber *Parastichopus californicus. North American Journal of Fisheries Management*. 29, pp. 288-294.

Cipriano, A., Burnell, G., Culloty, S., and Long, S. Evaluation of 3 tagging methods in marking sea urchin, *Paracentrtus lividus*, populations under both laboratory and field conditions. *Journal of Aquatic Research Development*, 5, DOI: 10.4172/2155-9546.1000276.

Conand, C. (2004). Present status of world sea cucumber resources and utilization: an international overview. In: Lovatelli, A., Conand, C., Purcell, S., Uthicke, Hamel, J-F., and Mercier, A. (eds.). Advances in sea cucumber aquaculture and management, *FAO, Rome, Fisheries Technical Paper, Food and Agriculture Organisation*, 463, pp. 13-24.

Conand, C. (1991). Long-term movement and mortality of some tropical sea cucumbers monitored by tagging and recapture. In: Yanagisawa, T., Yasamasu, I., Oguro, C., Suzuki, N., and Motokawa, T., editors. *Biology of Echinodermata.* A.A. Balkema, Rotterdam, pp. 169-174.

Deudero, S., Tor, A., Alomar, C., Valencia, J.M., Sarriera, P., and Blanco, A. (2011). Integrated multitrophic aquaculture: filter feeders bivalves as efficient reducers of wastes derived from coastal aquaculture assessed with stable isotope analyses. In: Sladonja, B. (ed.). Aquaculture and the Environment – A Shared Destiny, InTech Europe, Eijeka, Croatia, p. 246.

Duggan, R.E., and Miller, R.J. (2001). External and internal tags for the green sea urchin. *Journal of Experimental Marine Biology and Ecology*, 258, pp. 115-122.

Emery, L., and Wydoski, R.S. (1987). Marking and tagging of aquatic animals: an indexed bibliography. *US Fish and Wildlife Service,* 165, p. 57.

Eymann, J., Webley, L.S., Cooper, D.W., and Herbert, C.A. (2006). Performance of Passive Integrated Transponders (PIT tags) as a marking system for common brushtail possums *Trichosurus vulpecula*: a cautionary tale. *Australian Mammalogy*, 28, pp. 209-214.

Galimberti, F., Sanvito, S., and Boitani, L. (2000). Marking of southern elephant seals with Passive Integrated Transponders. *Marine Mammal Science,* 16(2), pp. 500-504.

Gianasi, B. L., Verkaik, K., Hamel, J., and Mercier, A. (2015). Novel use of PIT tags in sea cucumbers: promising results with the commercial species *Cucumaria frondosa*. *PloS one*, 10(5), DOI: 10.1371/journal.pone.0127884.

Gibbons, J.W., and Andrews, K.M. (2004). PIT tagging: simple technology at its best. *Bioscience*, 54, pp. 447-454.

Hagen, N.T. (1996). Tagging sea urchins: a new technique for individual identification. *Aquaculture*, 139, pp. 271-284.

Hamel, J.F., Conand, C., Pawson, D.L., and Mercier, A. (2001). The sea cucumber *Holothuria scabra* (Holothuroidea: Echinodermata): its biology and exploitation as beche-de-mer. *Advances in Marine Biology*, 41, pp. 129-202.

Hamel, J-F., and Mercier, A. (1996a). Early development, settlement, growth, and spatial distribution of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea). *Canadian Journal of Fisheries and Aquatic Sciences*, 53, pp. 253-271.

Hamel, J-F., and Mercier, A. (1996b). Gamete dispersion and fertilisation success of the sea cucumber *Cucumaria frondosa*. *SPC Beche-de-mer Information Bulletin,* 8, pp. 34-40.

Ivy, G., and Giraspy, D.A.B. (2006). Development of large-scale hatchery production techniques for the commercially important sea cucumber *Holothuria scabra* var. *versicolor* (Conand, 1986) in Queensland, Australia. *SPC Beche-de-mer Information Bulletin*, 24, pp. 28-34.

Jehle, R., and Hödl, W. (1998) Pits versus patterns: effects of transponders on recapture rate and body condition of Danube crested newts (*Triturus dobrogicus*) and common spadefoot toads (*Pelobates focus*). *Herpetological Journal*, 8, pp. 181-186.

Kirshenbaum, S., Feindel, S., and Chen, Y. (2006). A study of tagging methods for the sea cucumber *Cucumaria frondosa* in the waters off Maine. *Fishery Bulletin*, 104, pp. 299-302.

Kurth, J., Loftin, C., Zydlewski, J., and Rhymer, J. (2007). PIT tags increase effectiveness of freshwater mussel recaptures. *Journal of the North American Benthological Society*, 26, pp. 253-260.

Lauzon-Guay, J.S., and Scheibling, R.E. Evaluation of passive integrated transponder (PIT) tags in studies of sea urchins: caution advised. *Aquatic Biology*, 2, pp. 105-112.

Lokani, P. (1992). First results of an internal tag retention experiment on sea cucumber. *SPC Beche-de-mer Information Bulletin*, 4, pp. 9-12.

Low, M., Eason, D., and McInnes, K. (2005). Evaluation of passive integrated transponders for identification of kakapo, *Strigops habroptilus. Royal Australasian Ornithologists' Union,* 105, pp. 33-38.

MacDonald, C. L. E., Stead, S. M., and Slater, M. J. (2013). Consumption and remediation of European Seabass (*Dicentrarchus labrax*) waste by the sea cucumber *Holothuria forskali*. *Aquaculture International,* 21(6), pp. 1279-1290.

MacTavish, T., Stenton-Dozey, J., Vopel, K., and Savage, D. (2012). Deposit-feeding sea cucumbers enhance mineralisation in organically-enriched coastal sediments. *PLOS one*, 7(11), e50031, doi:10.1371/journal.pone.0050031.

Mercier, A., and Hamel, J-F. (2013). Sea cucumber aquaculture: Hatchery production, juvenile growth and industry challenges. In: Allan, G., Burnell, G., editors. *Advances in Aquaculture hatchery technology*. Woodhead Publishing, Sawston, Cambridge, pp. 431-454.

Mercier, A., and Hamel, J-F. (2009). Endogenous and exogenous control of gametogenesis and spawning in echinoderms. *Advances in Marine Biology*, 55, pp. 1-302.

Mercier, A., Ycaza, R.H., and Hamel, J-F. (2004). Aquaculture of the Galapagos sea cucumber, *Isostichopus fuscus*. In: Lovatelli, A., Conand, C., Purcell, S., Uthicke, S., Hamel, J-F., and Mercier, A., (eds). *Advances in Sea Cucumber Aquaculture and Management*. FAO Fisheries Technical Paper, $63, FAO, Rome, pp. 347-358.

Mercier, A., Battglene, S.C., and Hamel, J-F. (2000). Periodic movement, recruitment and size-related distribution of the sea cucumber *Holothuria scabra* in Solomon Islands. *Hydrobiologia*, 440, pp. 81-100.

Mowat, G., and Strobeck, C. (2000). Estimating population size of grizzly bears using hair capture, DNA profiling, and mark-recapture analysis. *Journal of Wildlife Management,* 64, pp. 183-194.

Mueller, R.P., Moursund, R.A., and Blleich, M.D. (2006). Tagging juvenile pacific lamprey with Passive Integrated Transponders: methodology, short-term mortality, and influence on swimming performance. *North American Journal of Fisheries Management*, 26, pp. 361-366.

Navarro, P.G., García-Sanz, S., Barrio, J.M., and Tuya, F. (2013). Feeding and movement patterns of the sea cucumber *Holothuria sanctori*. *Marine Biology,* 160(11), pp. 2957-2966.

Nicolaus, M., Bouwman, K.M., and Dingemanse, N.J. (2008). Effect of PIT tags on the survival and recruitment of Great Tits *Parus major. Ardea,* 96, pp. 286-292.

Pradel, R. (1996). Utilization of capture-mark-recapture for the study of recruitment and population growth rate. *Biometrics*, 52, pp. 703-709.

Prentice, E.F., Flagg, T.A., and Mccutcheon, C.S. (1990). Feasibility of using implantable passive integrated transponders (PIT) tags in salmonids. *American Fisheries Society Symposium,* 7, pp. 317-322.

Purcell, S.W., Polidoro, B.A., Hamel, J-F., Gamboa, R.U., and Mercier, A. (2014). The cost of being valuable: Predictors of extinction risk in marine invertebrates exploited as luxury seafood. *Proceedings of the Royal Society B: Biological Sciences,* 281, 20133296, DOI: 10.1371/journal.pone.0095075.

Purcell, S.W., Hair, C.A., and Mills, D.J. (2012). Sea cucumber culture, farming and sea ranching in the tropics: Progress, problems and opportunities. *Aquaculture*, 368-369, pp. 68-81.

Purcell, S.W., and Blockmans, B.F. (2009). Effective flurochrome marking of juvenile sea cucumbers for sea ranching and restocking. *Aquaculture*, 296, pp. 263-270.

Purcell, S.W., Agudo, N.S., and Gossuin, H. (2008). Poor retention rate of passive induced transponder (PIT) tags for mark-recapture studies on tropical sea cucumbers. *SPC Beche-de-mer Information Bulletin*, 28, pp. 341-346.

Purcell, S.W., Blockmans, B.F., and Nash, W.J. (2006). Efficacy of chemical markers and physical tags for large-scale release of an exploited holothurian. *Journal of Experimental Marine Biology and Ecology,* 334, pp. 283-293.

Raj, L. (1998). Photo-identification of *Stichopus mollis*. *SPC Beche-de-mer Information Bulletin*, 10, pp. 29-31.

Ramofafia, C., Foyle, T.P., and Bell, J.D. (1997). Growth of juvenile *Actinopyga mauritiana* (Holothuroidea) in captivity. *Aquaculture*, 152, pp. 119-128.

Reichenbach, N. (1999). Ecology and fishery biology of *Holothuria fuscogilva* in the Maldives, Indian Ocean (Echinodermata: Holothuroidea). *Bulletine of Marine Science*, 64, pp. 103-113.

Rodríguez-Barreras, R., López-Morell, J., and Sabat, A.M. (2016). Effectiveness of two tagging devices in the sea cucumber *Holothuria (Halodeima) grisea. Marine and Freshwater Research,* DOI: http://dx.doi.org/10.1071/MF15473.

Rodríguez-Barreras, R., and Wagensteen, O.S. (2016). Assessing the reliability of two tagging techniques in the echinoid *Echinometra lucunter*. *Regional Studies in Marine Science,* 5, pp. 51-54.

Rodríguez-Barreras, R., and Sabat, A.M. (2015). Evaluation of three tagging methods in the sea urchin *Diadema antillarum*. *Journal of the Marine Biological Association of the United Kingdom*, pp. 1-6, DOI: 10.1017/S002531541000302.

Rodríguez-Barreras, R., Serrano-Torres, S., and Macías-Reyes, D. (2014). A study of two tagging methods in the Caribbean sea cucumber *Holothuria mexicana*. *Marine Biodiversity Records*, 7, pp.1-4.

Rodríguez, E., González, M., Caride, B., Lamas, M. A., and Taboada, M. C. (2000). Nutritional value of *Holothuria forskali* protein and effects on serum lipid profile in rats. *Journal of Physiology and Biochemistry*, 56, pp. 39-44.

Rodríguez, J., Castro, R., and Riguera, R. (1991). Holothurinosides: new antitumour non sulphated triterpenoid glycosides from the sea cucumber *Holothuria forskali*. *Tetrahedron*, 47(26), pp. 4753-4762.

Rogers, L.M., Hounsome, T.D., and Cheeseman, C.L. (2002). An evaluation of passive integrated transponders (PITs) as a means of permanently marking bagers (*Meles meles*). *Mammal Review,* 32, pp. 63-65.

Santos, R., Dias, S., Pinteus, S., Silva, J., Alves, C., Tecelão, Pedrosa, R., and Pombo, A. (2015). Sea cumber *Holothuria forskali*, a new resource for aquaculture? Reproductive biology and nutraceutical approach. *Aquaculture Research*, 47, pp. 2307-2323.

Schooley, R.L., Van Horne, B., and Burnham, K.P. (1993). Passive integrated transponders for marking free-ranging Townsend's ground squirrels. *Journal of Mammalogy*, 74, pp. 480-484.

Shiell, G. (2006). Effect of invasive tagging on the activity of *Holothuria whitmaei* (Echinodermata: Holothuridea): A suitable mark-recapture method for short-term field studies of holothurian behaviour. *Marine and Freshwater Behaviour and Physiology*, 39, pp. 153-162.

Skov, C., Brodersen, J., Brönmark, C., Hansson, L.A., Hertonsson, P., and Nilsson, P.A. (2005). Evaluation of PIT-tagging in cyprinids. *Journal of Fish Biology*, 67, pp. 1195-1201.

Slater, M.J., and Jeffs, A.G. (2010). Do benthic sediment characteristics explain the distribution of juveniles of the deposit-feeding sea cucumber *Australostichopus mollis*? *Journal of Sea Research*, 64, pp. 241-249.

Slater, M.J., Jeffs, A.G., and Carton, A.G. (2009). The use of the waste from green-lipped mussels as a food source for juvenile sea cucumber, *Australostichopus mollis*. *Aquaculture*, 292, pp. 219-224.

Slater, M.J., and Carton, A.G. (2007). Survivorship and growth of the sea cucumber *Australostichopus (Stichopus) mollis* (Hutton 1872) in polyculture trials with green-lipped mussel farms. *Aquaculture*, 272, pp. 389-398.

So, J.J., Uthicke, S., Hamel, J-F., and Mercier, A. (2011). Genetic population structure in a commercial marine invertebrate with long-lived lecithotrophic larvae: *Cucumaria frondosa* (Echinodermata: Holothuridea). *Marine Biology*, 158, pp. 859-870.

So, J.J., Hamel, J-F., Mercier, A. (2010). Habitat utilisation, growth and predation of *Cucumaria frondosa*: implications for an emerging sea cucumber fishery. *Fisheries Management and Ecology*, 17, pp. 473-484.

Steyermark, A.C., Williams, K., Spotila, J.R., Paladino, F.V., Rostal, D.C., Morreale, S.J., Koberg, M.T., and Arauz-Vargas, R. (1996). Nesting leatherback turtles at Las Baulas Nationl Park, Costa Rica. *Chelonian Conservation and Biology,* 2(2), pp. 173-183.

Taboada, M.C., González, M., and Rodríguez, E. (2003). Value and effects on digestive enzymes and serum lipids of the marine invertebrate *Holothuria forskali*. *Nutrition Research*, 23, pp. 1661-1670.

Uthicke, S., Welch, D., and Benzie, J.A.H. (2004). Slow growth and lack of recovery in overfished holothurians on the Great Barrier Reef: Evidence from DNA fingerprints and repeated large-scale surveys. *Conservation Biology*, 18, pp. 1395-1404.

Uthicke, S., and Purcell, S. (2004). Preservation of genetic diversity in restocking of the sea cucumber *Holothuria scabra* investigated by allozyme electrophoresis. *Canadian Journal of of Fisheries and Aquatic Sciences*, 61, pp. 519-528.

Uthicke, S., and Benzie, J.A.H. (2002). A genetic fingerprint recapture technique for measuring growth in 'unmarkable' invertebrates: negative growth in commercially fished holothurians (*Holothuria nobilis*). *Marine Ecology Progress Series*, 241, pp. 221-226.

Van Dyck, S., Gerbaux, P., and Flammang, P. (2009). Elucidation of molecular diversity and body distribution of saponins in the sea cucumber *Holothuria forskali* (Echinodermata) by mass spectrometry. *Comparative Biochemistry and Physiology, Part B,* 152, pp. 124-134.

Wilson, D.W., Arnott, G., Reid, N., Roberts, D. (2011). The pitfall with PIT tags: marking freshwater bivalves for translocation induces short-term behaviour costs. *Animal Behaviour*, 81, pp. 341-346.

Woods, C.M.C. (2005). Evaluation of VI-alpha and PIT-tagging of the seahorse *Hippocampus abdominalis*. *Aquaculture International*, 13, pp. 175-186.

Xu, Q., Gao, F., Zhang, L., Zhang, T., Yang, H. (2017). A new tagging method and its early stress effect on the sea cucumber *Apostichopus japonicus. Aquaculture*, 468, pp. 156-161.

Zamora, L.N., Yuan, X., Carton, A.G., and Slater, M.J. (2016). Role of deposit-feeding sea cucumbers in integrated multitrophic aquaculture: progress, problems, potential, and future challenges. *Reviews in Aquaculture*, pp. 1-18, DOI: 10.1111/raq.12147.

Chapter 6

Fatty acid analysis of organic and inorganic extractive species grown in a zero-exchange maraponic system

Abstract

Maraponics is a land-based marine aquaponic system that combines the aquacultural production of fish with the hydroponic production of halophytes or algae. In this study six species of seaweed (*Pelvetia canaliculata*; *Fucus vesiculosus*; *Fucus serratus*; *Ulva lactuca*; *Laminaria digitata*; and *Ascophyllum nodosum*) were cultivated in small-scale experimental zero water exchange, closed, recirculation maraponic systems (no mechanical water treatment) with blue mussels (*Mytilus edulis*), Japanese abalone (*Haliotis discus hannai*), and the cotton-spinner sea cucumber (*Holothuria forskali*). The presence of *Salmo salar* was simulated by using freeze-dried *S. salar* faeces, feed pellets, and ammonia hydroxide (i.e. ammonia from urea) to simulate waste production. Results indicate that mussels and abalone from the bottom tanks of the maraponic systems grew strongly over the course of the 51 day trial, while sea cucumbers decreased in biomass over the duration of the trial. *A. nodosum, L. digitata*, and *F. vesiculosus* showed positive growth for the first 28 days of the trial, however, after 28 days, all seaweeds decreased in biomass. Fatty acid (FA) analysis was utilised to assess the impact of the trial on the FA composition of all species. There was strong evidence from this analysis that abalone were assimilating salmon waste, however, evidence for assimilation by mussels and sea cucumbers was less clear.

6.1 Introduction

Integrated Multi-Trophic Aquaculture (IMTA) provides a balanced ecosystem-based approach that has the potential to improve upon the resource use efficiency, waste discharge, and economic returns of land-based RAS (Boxman *et al*. 2016; Barrington *et al*. 2009; Troell *et al*. 2009; Naylor and Burke, 2005; Costa-Pierce, 2002; Chopin *et al*. 2001). Aquaponics is a land-based (predominantly closed RAS) IMTA system that combines the aquacultural production of aquatic animals with the hydroponic production of plants. The waste produced by the animals provides nutrients required for plant growth, while the plants remove potentially toxic compounds (e.g. nitrate and phosphorus) resulting from aquaculture production (Shete *et al*. 2016; Love *et al*. 2015; Buzby *et al*. 2014; Love *et al*. 2014; Salam *et al*. 2014; Tyson *et al*. 2011; Endut *et al*. 2009; Rackocy *et al*. 2006; Lennard and Leonard, 2006: Seawright *et al*. 1998).

Currently freshwater aquaponics is the most widely described and practised aquaponic technique and research into saltwater aquaponics is in its infancy. Nevertheless, considering resources of freshwater for land-based food production (aquaculture and aquaculture) are becoming increasingly limited and salinisation of soil and groundwater is progressively increasing in many parts of the world (FAO, 2016; Singh *et al*. 2014; Turcios and Papenbrock, 2014; Ventura and Sagi, 2013), the development of saltwater aquaponics may be invaluable for the production of land-based food products in the future.

Saltwater aquaponics (SA) is a land-based aquaponic system that operates at salinities ranging from brackish to highly saline (when SA systems utilise seawater, they are referred to as marine aquaponics, hereafter referred to as ‘maraponics’). In SA systems, seaweeds, halophytes, and/or salt-tolerant glycophytes are hydroponically cultivated with the wastewater from farmed haline or euryhaline aquatic animals (e.g. fish, molluscs, etc.) (Boxman *et al*. 2016; Fronte, *et al*. 2016; Granada *et al*. 2016; Joesting *et al*. 2016; Nozzi *et al*. 2016; Buhmann *et al*. 2015; Waller *et al*. 2015; Buhmann and Papenbrock, 2013; Lakkireddy *et al*. 2012; Jones, 2005; Wilson, 2005; Neori *et al*. 2004; Troell *et al*. 2003; Dufault *et al*. 2001; Dufault and Korkmaz, 2000; Jensen, 1997).

It is well documented that the marine environment is an important source of bioactive lipids, and in comparison to terrestrial ecosystems, marine ecosystems are characterised

by high levels of n-3 (double bond located on the carbon numbered 3 lower than the highest carbon number) long chain PUFA. Consequently, fish and seafood are the most important source of these vital nutrients in the human diet (Monroig *et al*. 2013; Pereira, *et al.* 2012; Tur *et al*. 2012). A diet with a high ratio of omega (ω) 6 to ω3 PUFA promotes the pathogenesis of many human diseases, such as cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of ω3 PUFA promote suppressive effects. It is recommended that a healthy human diet should consist of a ω6/ω3 ratio of 1:1 - 10:1 and the world health organisation (WHO) recommends a ratio no higher than 10:1 (Stabili *et al*. 2012; Simopoulos, 2008; Ortiz *et al*. 2006; Bergé and Barnathan, 2005).

Physical methods to determine the diet of marine species, for example the analysis of stomach contents or faeces, cannot distinguish assimilated diets, only provide a rough estimate of recent feeding activity (i.e. seconds to hours), and the methods involved tend to be time consuming and labour intensive (Zhao *et al*. 2013; Lehane and Davenport, 2004; Kang *et al*. 1999). Recently, biochemical methods, such as fatty acid (FA) analysis, have been used with considerable success to evaluate nutrient assimilation in animal tissue, providing more accurate and long-term (i.e. weeks to months) dietary information. FA analysis has also been used to determine the FA composition of marine algae and phytoplankton species. Such information is essential for understanding energy and material flows between the various trophic levels of a marine ecosystem (Mæhre *et al*. 2014; Schmid *et al*. 2013; Zhao *et al*. 2013; Kelly and Scheibling, 2012; Guest *et al*. 2010; Dalsgaard *et al*. 2003; Go *et al*. 2002; Fleurence *et al*. 1994).

The development of analytical methods, particularly gas liquid chromatography coupled to mass spectrometry, has been a major contributing factor to the successful identification of FA, even within complex mixtures (Bergé and Barnathan, 2005; Christie, 2003; Ackman, 2002; Rezanka, 1989). FA have been used as tracers (i.e. biomarkers) to determine the source of nutrition of a species, to study trophic relationships among organisms, to assess the impact of diets on farmed species, and to trace the destination of fish farm waste either in wild species located close to cage aquaculture or in co-cultured species of an IMTA system (e.g. molluscs, holothurians). This utilisation of FA analysis relies on the food source and the consumer having distinct FA signatures (Wen *et al*. 2016a; Wen *et al*. 2016b; Irisarri *et al*. 2015; Handå *et al*. 2012; Pleissner *et al*. 2012; Both *et al*. 2011; Redmond *et al*. 2010; Alkanani *et*

al. 2007; Gao *et al*. 2006; Stowasser *et al*. 2006; Su *et al*. 2004; Dalsgaard *et al*. 2003; Mai *et al*. 1996). Understanding how the biochemical composition of co-cultured species may change in an IMTA setting could aid in our understanding of how well they cope with a diet substituted or supplemented with aquacultural waste (Both *et al*. 2011).

In this study, six commercially important (or with commercial potential) species of seaweed (inorganic extractive species) (*Pelvetia canaliculata*; *Fucus vesiculosus*; *Fucus serratus*; *Ulva lactuca*; *Laminaria digitata*; and *Ascophyllum nodosum*) were cultivated in small-scale experimental zero exchange, closed, recirculation maraponic systems with mussels (*Mytilus edulis*), Japanese/disk abalone (*Haliotis discus hannai*), and the cotton-spinner sea cucumber (*Holothuria forskali*) (organic extractive species). These systems had no mechanical forms of water treatment, relying solely on the biofiltering capacity of the organic and inorganic extractive species. Atlantic salmon (*Salmo salar*) are sensitive to rising concentrations of total ammonia nitrogen (TAN) and it is recommended that concentrations in *S. salar* aquaculture should not exceed 2 mg/L (Kolarević, 2012; Knoph and Thorud, 1996; Knoph, 1992). As it was not possible to predict the water quality throughout the duration of this trial, the presence of Atlantic salmon (*Salmo salar*) in the maraponic systems was modelled through the use of the WinFish growth model in accordance with the 3 R's of research principle (i.e. **R**eplace the use of animals with alternative techniques or avoid the use of animals altogether) (Cubillo *et al*. 2016; Ferreira *et al*. 2014; Wolfensohn and Lloyd, 2013; Ferreira *et al*. 2012). The waste (i.e. faeces, urea {N}, uneaten feed) of *S. salar* estimated from this model were added to the systems on a daily basis. This salmon waste, together with naturally accumulating periphyton, were the source of nutrition for the species in the maraponic systems. Periphyton refers to a complex community of phototrophic, multi-species biofilms that develop on surfaces in aquatic environments. These communities harbour a large diversity of organisms, such as: bacteria, viruses, fungi, algae, protozoans, and metazoans (Sanli *et al*. 2015).

The aims of this study were to: (1) evaluate the growth rates of each species within the systems; (2) evaluate water quality throughout the duration of the trial; (3) assess the fatty acid profile of each species in the maraponic systems at the end of the trial and of their wild (or farmed in the case of *H. discus hannai*) counterparts; (4) identify salmon waste and periphyton biomarkers to determine if mussels, abalone, or sea cucumbers were utilising them as a food source; (5) make a comparison of the ω6/ω3 PUFA ratio

of the maraponic species and compare with those of their wild/farmed counterparts.

6.2 Methods and Materials

This trial took place from 8th December 2014 (day 0) to 28th January 2015 (day 51) in a greenhouse at University College Cork (UCC).

Trial preparation

Maraponic system construction

Three maraponic systems were constructed from recycled 1000 L Intermediate Bulk Containers (IBCs) obtained from Folláin, Ballyvourney Industrial Estate, Cork, Ireland. These IBCs contained only grape juice prior to being used in these trials, and therefore were safe for use following a thorough wash.

The top section of each IBC was cut down by 26 cm, inverted, and used as the top tank of each maraponic system, measuring 122 cm x 96 cm x 26 cm (LxWxH). The remainder of the IBC was utilised as the bottom tank and was 122 cm x 96 cm x 70 cm (L x W x H) (Figure 1). The bottom and top tank can hold approximately 600-700 L and 100-200 L of water, respectively. The bottom tank was designed to house finfish and the majority of animals, while the top tank was designed for seaweed. Two wooden boards were place on the top of each bottom tank to support the weight of the top tanks. These top tanks were set back approximately 25 cm from the front of the maraponic systems to ensure the bottom tank was accessible.

Figure 1: a) Intermediate bulk containers (IBCs); b) finished maraponic system (*original photos by Gunning*)

Setup of maraponic trial

Two weeks prior to the commencement of the trial, three replicate maraponic systems were set up in a greenhouse at the Distillery Fields Campus of UCC. The bottom tank of each system contained approximately 600 L of seawater and was pumped to the top tank (approximately 150 L) with a Rio® 2100 submersible pump at a flow rate of approximately 15 L/min. Six 20 L buckets were fitted into the top tank of each system in two rows of three. The water pumped from the bottom tank was directed into each bucket from above at a flow rate of c. 2.5 L/min (Flow rate based on personal communication, Maeve Edwards, 2015). An outflow pipe was inserted into the side of each bucket approximately 10 cm from the top. The water from each bucket flowed back into the top tank, which drained back into the bottom tank through a centrally positioned stand-pipe. Each 20 L bucket was aerated via air-stones powered by a Hailea® Piston-Compressor ACO-009E aerator pump (one aeration pump per system/six 20 L buckets) (Figure 2).

Figure 2: a) Maraponic system prior to trial commencement (seaweed buckets {SB} are numbered from background to foreground; 1-3 on left side of top tank; 4-6 on right side of top tank); b) side view of top trays of the 3 replicate systems *original photos by Gunning*)

Water acclimatisation

Seawater was circulated in each empty system (temperature: 14.4 ± 1.5 °C; dissolved oxygen {DO}: 9.9 ± 0.4 mg/L; pH: 8.0 ± 0.1; salinity: 31.8 ± 0.4 ppt) and dosed with small amounts of ammonia hydroxide for two weeks prior to the commencement of the trial in order to establish a suitable flow-rate and to attract naturally occurring nitrifying bacteria to the tanks (i.e. nitrosomonas bacteria for conversion of ammonia into nitrite and nitrobacter bacteria for conversion of nitrite into nitrate) (Solomon, 2007). This acclimatisation period was considered to be complete when ammonia and nitrite levels were close to 0 mg/L and nitrate levels between 5-10 mg/L.

Modelled salmon waste: collection and addition

Due to the high level of regulation involving the use of vertebrates in Irish university-based experiments and in keeping with the 3 R's of research (Wolfensohn and Lloyd, 2013), it was decided to model the presence of Atlantic salmon (*Salmo salar*) in the systems through the use of an Atlantic salmon growth model. The WinFish growth model (Cubillo *et al*. 2016; Ferreira *et al*. 2014; Ferreira *et al*. 2012) takes a standard net energy balance approach, which simulates fish growth and physiology through mechanistic representation of feeding and feeding regulation; energy transfers (input and loss) through harvestable products, wastes, and biological processes; oxygen consumption through anabolic and catabolic processes; and mass balance equations to account for the inputs and outputs to the production system. Food intake is governed by water temperature and animal size. A component of that food intake is assimilated and converted to energy allocated for growth and metabolism (e.g. basal metabolic rate {BMR}, specific dynamic action {SDA}, anabolism, and swimming). The remainder is excreted as faeces, urea (N) and feed waste (see appendix Chapter 6 for more detail on the WinFish model).

The starting biomass of modelled salmon was ten 100 g *Salmo salar* (Atlantic salmon) per replicate system. This biomass was chosen to ensure it complied with EU regulations for the maximum stocking density of organically produced salmonids (10kg/m^3) (European Union, 2009) and to minimise the potential of poor water quality (e.g. high ammonia) having a negative impact on the health of the species within the systems. Inputs to the model, such as temperature, salinity, water volume, and DO, were based on those parameters that were achieved during the 2 week acclimatisation period (note: DO was lowered slightly to allow for the fact that the presence of animals may

reduce the DO) (see "Water acclimatisation" section for details). The current speed of 0.1 cm s^{-1} was the lowest speed possible for input to the model. Other inputs to the model (chlorophyll-a and ammonia) could not be altered (Figure 3).

The total estimated faeces and ammonia (urea) output and total waste feed (conservative estimate of 5% feed loss) produced by ten 100g (starting biomass) salmon, over the duration of the trial (51 days), was added evenly over each day of the trial. This equated to 2.9 g DW faeces, 1.45 g of uneaten feed, and 0.5 ml ammonia per replicate system per day (Figure 3). No salmon faeces, feed, or ammonia hydroxide were added to the systems from day 27-37 due to ammonia levels going above 1 mg/L. From day 38-51 salmon faeces and feed were added to each system, however, ammonia hydroxide was not added to limit ammonia levels.

Salmo salar faeces were obtained from four 8000 L tanks (c. 40 salmon per tank; 40 kg total biomass) at the Bantry Marine Research Station (BMRS). The faeces were collected from each tank by passing the outflow water through a 60 μm filter. Non-faecal material (e.g. uneaten feed, debris) was removed from the collected waste. Excess water was removed from the faeces by dabbing dry with a paper towel. The faeces were then freeze-dried in a Labconco® shelf freeze-drier to keep them preserved until being added to the systems. Salmon feed pellets were added to each system in conjunction with the faeces to replicate uneaten feed. Ammonia (urea) was replicated by the addition of ammonia hydroxide.

There was no mechanical or artificial waste control methods utilised and the water was continuously recirculated for the duration of the trial (apart from topping up with freshwater periodically to maintain a stable salinity level). However, twice a day (am/pm) the bottom of the tank was agitated with a stick to circulate the waste around the tank.

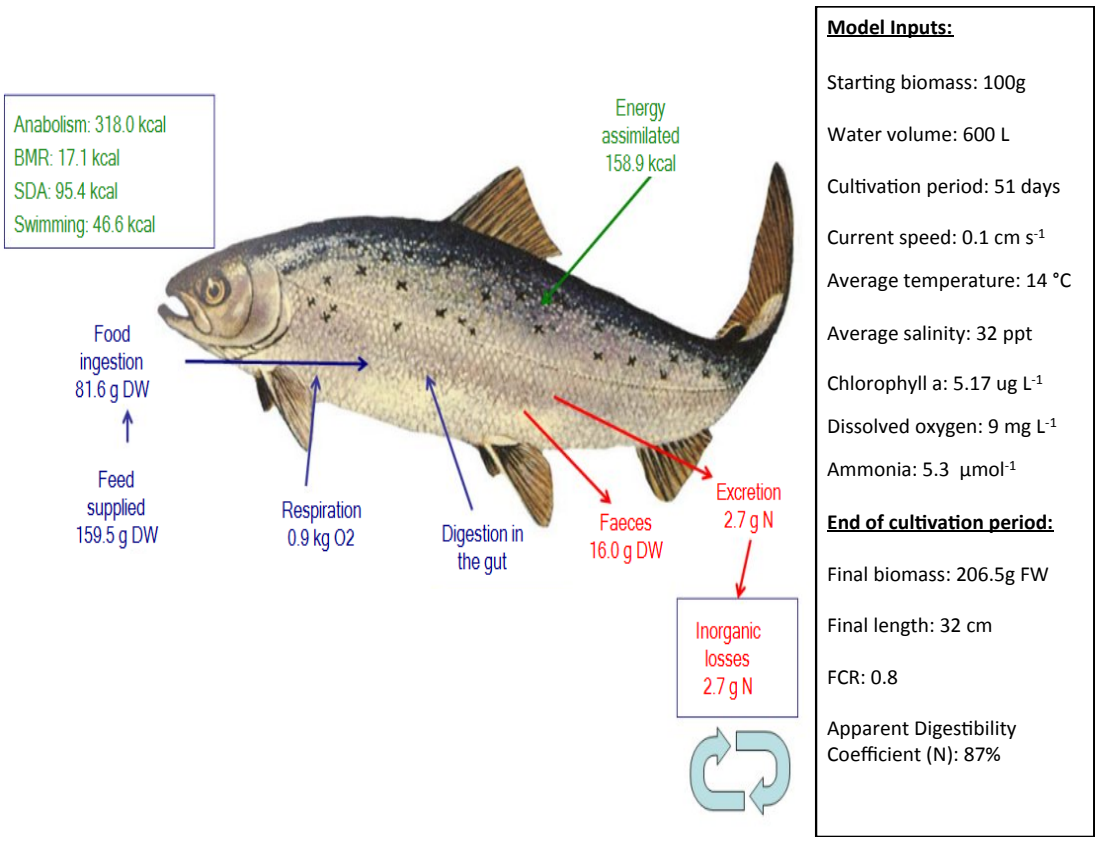


Figure 3: Mass balance for individual *S. salar* growth over a 51 day cultivation cycle (Winfish Model; Cubillo *et al.* 2016; Ferreira *et al.* 2014; Ferreira *et al.* 2012)

Animal and seaweed collection/addition

All reported species weights are wet weight (live) measurements. In the case of mussels and abalone it was live in shell, wet weight measurements. Excess water was removed from all specimens by dabbing them dry with paper towel. The biomass of species added to each replicate system was kept as similar as possible. Due to a limited availability of sea cucumbers, biomass per system was kept as similar as possible, with the same number of species added to each unit.

Mussels

Approximately 4 kg of wild blue mussels (*Mytilus edulis*) were sourced from Bantry Bay, Co. Cork, Ireland and transported to the Aquaculture and Fisheries Development Centre (AFDC), UCC. The mussels were transported in polystyrene boxes with damp *Laminaria digitata* to keep them cool. Twenty mussels were chosen at random and prepared for fatty acid analysis. The remainder were cleaned of any epiphytes and acclimated in an aerated 600 L tank, maintained at the same temperature as the systems

(approximately 14.3 ± 1.27 °C; located next to the systems), for 9 hours prior to being transferred to these systems to reduce the chances of spawning and mortality occurring in the systems. After the acclimation period, as similar a biomass as possible was transferred to the bottom tank of each replicate system and positioned directly under the stand-pipe (i.e. under the water flow from the top tank) to maximise their biofiltration potential (Table 1; Figure 4 and 5). This starting biomass was chosen to ensure that the mussels filtered all of the recirculated water within each maraponic unit at least 2 times per hour and was based on the clearance rate calculations of Petersen *et al*. (2004), which estimated that a 1 g mussel has a clearance rate of approximately 2-11 l/h^{-1}.

Table 1: Quantity and total biomass of mussels added to each replicate system at the beginning of the trial

System 1		System 2		System 3	
Quantity	**Biomass (g)**	**Quantity**	**Biomass (g)**	**Quantity**	**Biomass (g)**
207	1195	244	1195	260	1195

Abalone

Approximately 1 kg (c. 80 individuals) of Japanese abalone (*Haliostis discus hannai*) were sourced from Chonamara Teoranta, Adrigole, Galway Bay, Co. Galway (these abalone had been fed a diet of *Laminaria digitata*). For transport to the AFDC, UCC, the abalone were packed in polyethylene bags (approximately 100 g of abalone per bag) with a small amount of water. The bags were then filled with oxygen and tied. The bags of abalone were placed in styrofoam boxes which had frozen gel blocks to keep the abalone cool while been transported. Each bag contained approximately 8-10 individuals.

Upon arrival, 10 specimens were chosen at random and prepared for fatty acid analysis. The remainder were distributed evenly amongst four 610 cm x 410 cm x 210 cm (c. 52 L) temperature controlled flow through tanks to acclimatise the abalone from the transport temperature (13 °C ± 0.1) to the temperature of the replicate systems (approximately 14 14.4 ± 1.5 °C) by increasing the temperature by 0.5 °C per day (total duration: 3 days; temperature controlled by 2 Teco® 680 units). The abalone were fed *Laminaria digitata* and *Ulva lactuca ab libitum* throughout the acclimatisation period. One abalone mortality occurred during this period.

After the acclimatisation period, as similar a biomass as possible was transferred to the bottom tank of each replicate system. As Japanese abalone are nocturnal creatures, 6 shelters were added to the bottom tank of each system. These were constructed from 20-25 mm sections of PVC piping that were cut in half (Table 2; Figure 4 and 5).

Table 2: Quantity and total biomass of abalone added to each replicate system at the beginning of the trial

System 1		System 2		System 3	
Quantity	**Biomass (g)**	**Quantity**	**Biomass (g)**	**Quantity**	**Biomass (g)**
23	302	23	297	23	297

Sea cucumbers

Cotton spinner sea cucumbers (*Holothuria forskali*) (n=25) were obtained from the Bantry Marine Research Station (BMRS) (Gearhies, Bantry, Co. Cork) and transported to the AFDC, UCC on damp *L. digitata* in a polystyrene box. Upon arrival 10 specimens were chosen at random and prepared for fatty acid analysis. The remainder were acclimatised in an aerated 200 L tank, maintained at the same temperature as the replicate systems (approximately 14.3 ± 1.3 °C; located next to the systems), for 9 hours prior to prior to being transferred to these systems to reduce the chances of spawning, cuvierian tubule release, evisceration, and mortalities. After the acclimatisation period, as similar a biomass as possible was transferred to the bottom tank of each system (Table 3; Table 4 and 5).

Table 3: Quantity and total biomass of sea cucumbers added to each replicate system at the beginning of the trial

System 1		System 2		System 3	
Quantity	**Biomass (g)**	**Quantity**	**Biomass (g)**	**Quantity**	**Biomass (g)**
5	784	5	793	5	737

Seaweeds

Six species of seaweed were collected from the coastline of Gearhies, Bantry Bay, Co. Cork during low tide. Approximately 600 g of channel wrack (*Pelvetia canaliculata*), bladder wrack (*Fucus vesiculosus*), serrated wrack (*Fucus serratus*), sea lettuce (*Ulva lactuca*), Kombu (*Laminaria digitata*), and egg wrack (*Ascophyllum nodosum*) were collected. Care was taken to collect whole clean specimens were possible, as too much cutting can introduce infection and necrosis and seaweed with a high level of epiphyte

coverage would be very slow growing. These seaweeds were transported to the AFDC, UCC in separate polystyrene boxes. Upon arrival, three 50 g of each seaweed was prepared for fatty acid analysis . Approximately 144 g (dabbed dry with paper towel before weighing) of each seaweed was placed in a randomly selected bucket in each replicate system (Maeve Edwards, Irish Seaweed Consultancy, personal communication, 2014) (Table 4; Figure 2, 4, and 5).

Table 4: Weight of seaweed added to each bucket at the beginning of the trial and bucket number assigned

	System 1	**System 2**	**System 3**
	Biomass (g; w.w.) and bucket assigned (n)		
P. canaliculata	144 (1)	144 (2)	144 (1)
F. vesiculosus	144 (2)	144 (1)	144 (3)
F. serratus	145 (4)	144 (5)	145 (4)
U. lactuca	144 (3)	144 (6)	144 (2)
L. digitata	145 (5)	145 (4)	144 (6)
A. nodosum	145 (6)	144 (3)	144 (5)

To improve the growth prospects of the seaweed, artificial lighting over each tank was provided on the 29th day of the trial. These lights were scheduled to run on a light:dark period of 9am to 6pm (mean: 6.37 ± 1.42 x 10 μmol m^{-2} s^{-1}) (note: this trial took place during the winter months of December 2014 and January 2015) (Figure 4).

Light intensity was measured at the water level of the buckets with a Skye® PAR meter (Note: light intensity without the lights was 20.4 ± 4.5 μmol m^{-2} s^{-1} at approximately 9am and 1.3 ± 0.4 μmol m^{-2} s^{-1} between 5 and 6pm).

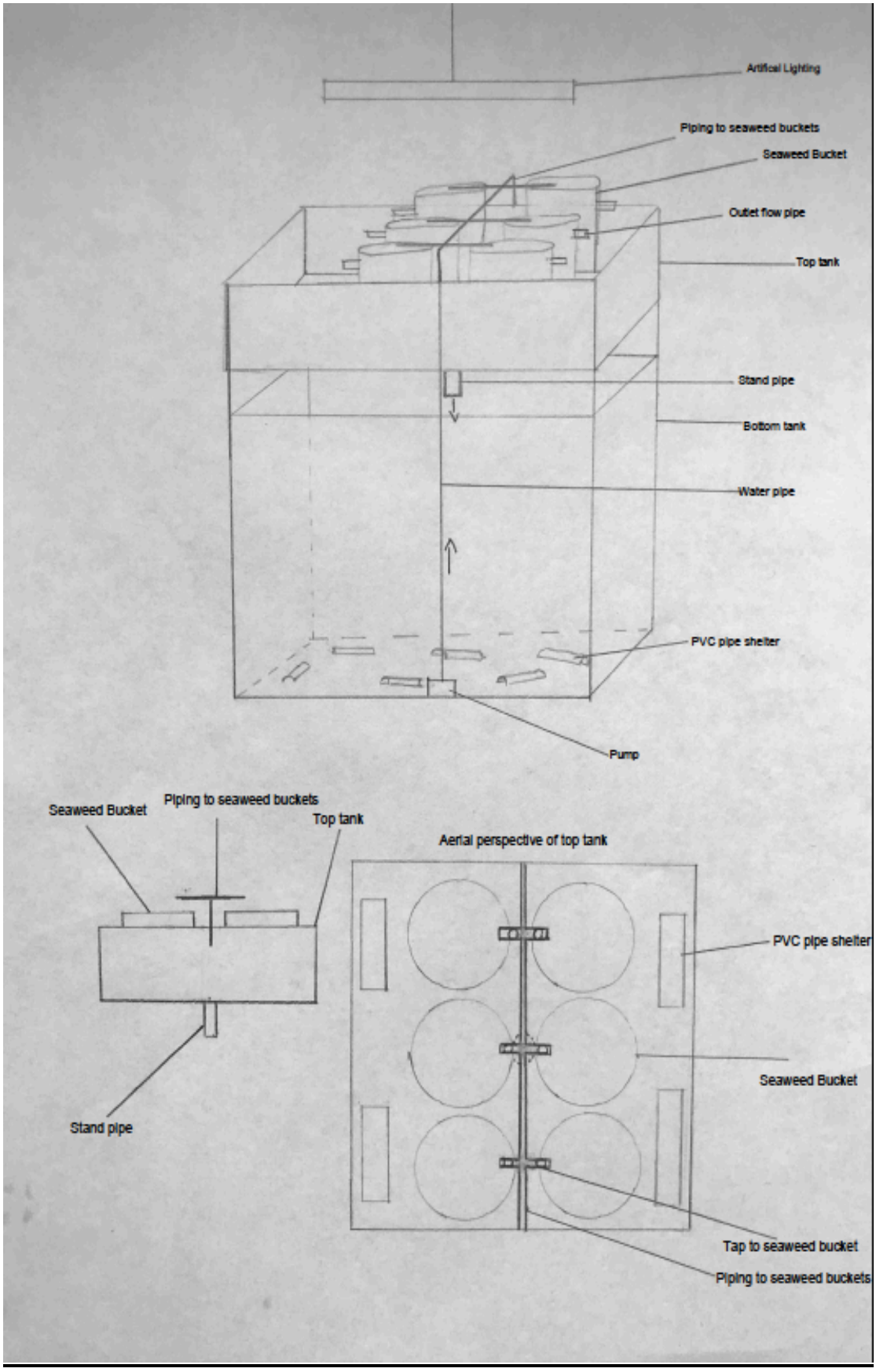


Figure 4: Schematic of Maraponic System

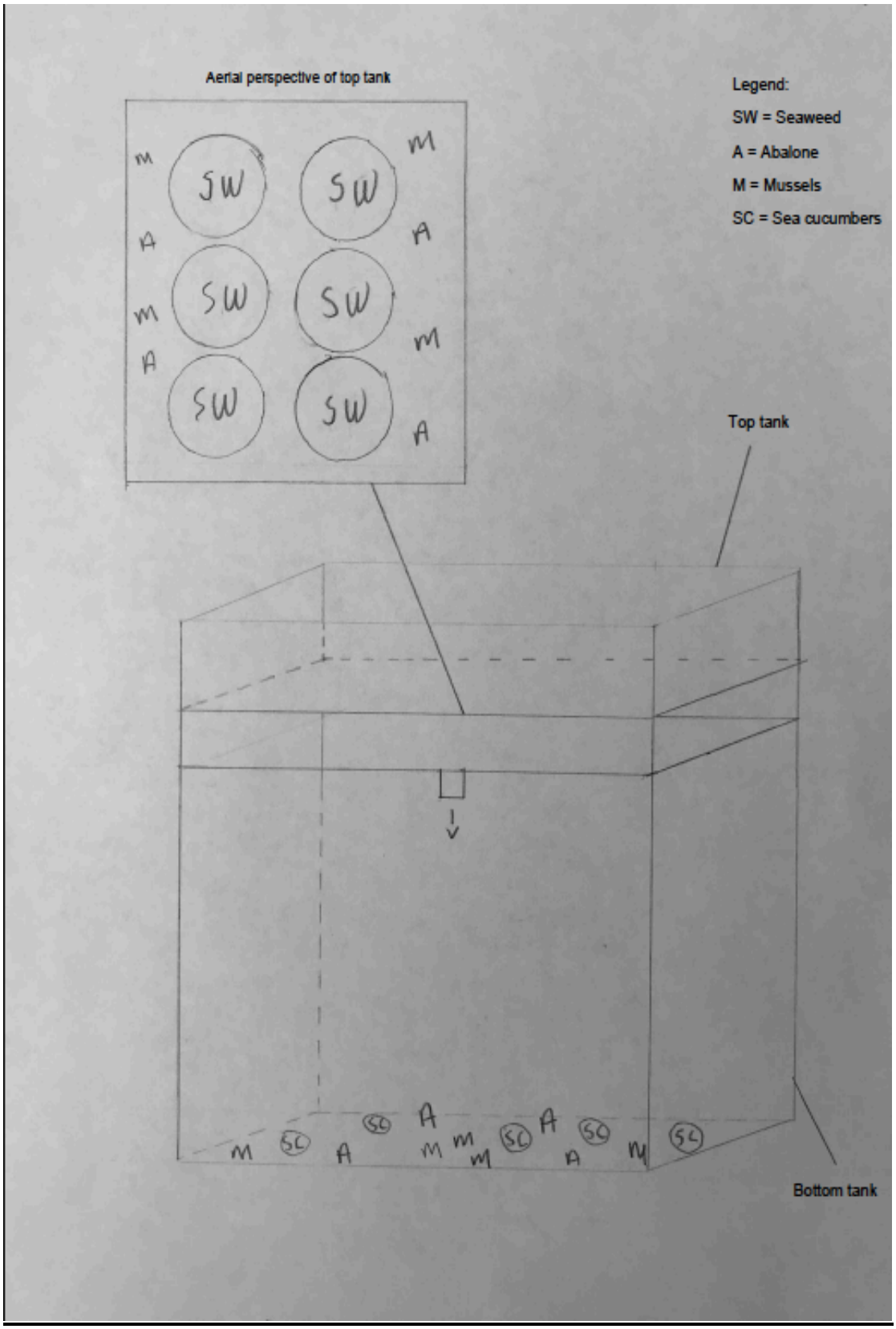


Figure 5: Location of abalone, mussels, sea cucumbers, and seaweed in the maraponic systems.

Growth monitoring

Shellfish tagging and growth monitoring

On the 7th day of the trial, 10 abalone (mean wet weight: 11.72 ± 2.69 g) and 20 mussels (mean wet weight: 4.71 ± 5.02 g) were removed from the bottom tank of each replicate system to apply numbered plastic Dymo® tags and take weight, length, and width measurements. When handled, abalone can grip to a surface very strongly making it difficult to remove individuals and increasing the chances of causing them damage. To prevent this from occurring, a wooden short-handled spatula with a blunt end was used to quickly ease the abalone off the surface before adhesion occurred.

Upon being removed from the systems, the mussels and abalone were dabbed dry with paper towel and left for 15 minutes to air-dry and expel any excess water. Each shell was gently rubbed with acetone to remove any excess water and provided a smooth surface for applying the tag, taking great care to avoid contact with the soft tissue of the mussels and abalone. Quick drying epoxy glue was then applied to each shell and a label applied (Halpin *et al*. 2004; Anthony *et al*. 2001; Shpigel *et al*. 1999; Britz, 1996). The labels were created with a Brother® Label Maker. Abalone were labelled from 1 to 10 and mussels from 1 to 20. The glue was allowed dry for 2 minutes after which each tagged specimen was weighed (g) and length (cm) and width (cm) measured with a vernier calliper. The weight included the weight of the epoxy glue and label. Odd numbered abalone and mussels were placed in the bottom tanks. Even numbered abalone were placed in the top tanks along the left-hand side of the seaweed buckets while even numbered mussels were placed on the right-hand of the seaweed buckets in each maraponic system. Four PVC piping shelters were added to the top tanks, 2 on the left side and 2 on the right-hand side of the seaweed buckets (6 shelters were already present in the bottom tank).

Twenty-four days later (day 31), tagged mussels and abalone were removed from each maraponic system to measure their weights, lengths, and widths as per the techniques described above. Mussels and abalone were replaced in the same location in which they were found prior to measurements. On the last day of the trial (day 51), the final weights and shell lengths and widths of all mussels and abalone were measured in the same manner as the previous two measurement occasions. The growth rates of abalone and mussels were calculated as a mean of the three replicate systems and a comparison was made between individuals that were located in the bottom or top tanks.

Seaweed growth monitoring

Following the introduction of seaweeds to the replicate systems, their weights (g) were measured every 5-13 days over the duration of the project. Prior to being weighed, the seaweeds were dabbed dry with paper towel to remove excess water. The growth rate of each seaweed was calculated as a mean of the three replicate systems.

Sea cucumber growth monitoring

The weight (g) of each sea cucumber was measured on the 28th, 32nd, and 51st (last) day of the trial. As individual sea cucumbers could not be identified, the biomass of sea cucumbers from each replicate system was calculated. The growth rate of sea cucumbers was calculated as the mean biomass of the three replicate systems.

Water parameter monitoring

Water parameters (salinity, temperature, pH, DO, ammonia {TAN}) were measured twice daily, once during the morning (09:00-11:59) and afternoon (12:00-18:00), for the duration of the trial. Measurements were taken from the bottom and top tanks and in one random bucket of each system. TAN levels were measured once a day with the salicylate method (Hach® method #8155; https://www.hach.com/asset-get.download.jsa?id=7639983745) using a Hach® Lange Dr 2800 spectrometer (Hach Co., USA).

A triplicate sample of water (500 ml per sample) was also taken from the bottom tank of each system on day 0 (prior to the addition of waste), 14, 30, 45, and 51 (final day) of the trial, and sent to the Aquatic Services Unit (EPA accredited) of the Environmental Research Institute, UCC for TAN, nitrite, nitrate, and orthophosphate analysis.

Fatty acid analysis

Fatty acid (FA) analysis took place on samples of mussels, abalone, sea cucumbers, and each species of seaweed after 51 days in the maraponic systems and compared with their wild (or farmed in the case of abalone) (control) counterparts. Controls and maraponic specimens were obtained from the same source (see below). The ω6/ω3 ratio of wild/farmed specimens was compared to the maraponic specimens.

FA biomarkers of salmon waste (feed and faeces) and periphyton, which was coating the walls of the systems were compared with wild/farmed (controls) and maraponic abalone, mussel, and sea cucumber specimens to try established if they were feeding upon the salmon waste and/or periphyton.

Sample preparation and transport

The meat of mussels (n=20) and abalone (n=10) obtained from the same source as those used in the maraponics trial (controls) (i.e. wild mussels from Bantry Bay and farmed abalone from Chonamara Teoranta, Adrigole, Co. Galway) were removed from their shells and cleaned of any excess material (e.g. sand, byssal threads etc.), placed in individual 50 ml tubes and stored at -80 °C.

The digestive tract and intestine of 10 sea cucumbers from the same source as those used in the maraponics trial (control) (i.e. Bantry Bay, Co. Cork) were removed, carefully cleaned of any excess material (e.g. cuvierian tubules, gonads etc.), placed in individual 50 ml tubes, and stored at -80 °C. A segment of each sea cucumbers body wall (c. 6 cm x 6 cm) was also removed from each sea cucumber and cleaned of excess material, placed in individual 50 ml tubes, and stored at -80°C.

Three 50 g samples of *P. canaliculata*, *F. vesiculosus*, *F. serratus*, *U. lactuca*, *L. digitata*, and *A. nodosum* from same source as the seaweeds used in the maraponics trial (controls) (i.e. Bantry Bay, Co. Cork) were patted dry with paper towel to remove excess water, placed in individual 50 ml tubes, and stored at -80 °C.

Fifty gram samples of salmon waste from each 8000 L salmon tank and a sample of the salmon feed were placed in individual 50 ml tubes, and stored at -80 °C.

On the last day of the trial (day 51) 5 abalone and 5 mussels from the top tank, 5 abalone and 5 mussels from the bottom tank, all sea cucumbers from the bottom tank, and a 50 g sample of each seaweed from each system, were prepared as described above and stored at -80 °C.

Three approximately 10 cm x 10 cm samples of periphyton were also taken from the inside wall of each system, prepared as described above, and stored at -80 °C.

On 31st January 2015, all samples were transported on dry ice within a sealed polystyrene container to the Institute of Aquaculture, University of Stirling, Stirling, Scotland, United Kingdom, for fatty acid analysis. All samples remained frozen upon arrival and were transferred to a -80 °C freezer until FAME analysis.Due to logistical, financial, and time constraints only 10 wild mussel samples, 3 farmed abalone samples,

and 3 intestine and body wall samples of wild sea cucumbers were assessed. Also, only 3 samples of mussels and abalone from the top and bottom tank of each system and 3 samples of intestine and body wall of sea cucumbers from each system were assessed.

Lipid extraction

For the analysis of the mussels, whole individuals were used to determine the total lipid content with sample weights that varied between 0.5 g and 6 g. The abalone samples were homogenised with a blender (Fisher Scientific®) and approximately 1g of each individual organism was taken for lipid extraction. The body walls of the sea cucumbers were homogenised as above and 1 g taken for lipid extraction. The whole intestine of the sea cucumbers was used for lipid extraction. 0.5 g of salmon feed, 0.75 g of salmon faeces, and 1g of each seaweed sample were used for lipid extraction.

Following the stages described by Folch (1957), the samples were homogenised with an Ultra Turrax TM tissue disruptor (Fisher Scientific®) using 20-36 volumes of chloroform/methanol (C/M, 2:1 v/v). The addition of 0.25 volumes of 0.88% of KCl was necessary to isolate non-lipidic impurities that were subsequently discarded. The remaining solvent was evaporated under a stream of oxygen-free nitrogen and the samples desiccated overnight before total lipid was determined gravimetrically. The samples were re-suspended in 1 ml of C/M (2:1) + 0.01 % (w/v) BHT until determination of the fatty acid composition.

Fatty acid composition

The preparation of the methyl ester derives of fatty acids (FAME) was performed through acid-catalysed esterification and transesterification, using 17:0 fatty acid as internal standard (Christie, 2003). The separation and quantification of the FAME's were realised with gas liquid chromatography TLC. This involved loading 100 µl of each sample on a 20 cm x 2 cm TLC plate (VWR®) and running in a solvent mixture comprising isohexane/diethyl ether/acetic acid (90:10:1, by vol.) Hydrogen was the carrier gas utilised. The thermal gradient varied from 50 to 150 °C at 40 °C min^{-1} to a final temperature of 250 °C at 2 °C min^{-1}. Each different sample was evaluated with mass spectrophotometry to confirm the previous analysis and to determine specific FAs of the different organisms. A comparison with known standards was performed to confirm the methyl esters identification.

Data analysis

All statistical analysis was compiled using SPSS software (IBM) version 23. Prior to statistical analysis, percentage data was transformed using the arcsine transformation. All data was tested for normal distribution and homogeneity of variance with the Shapiro-Wilk test and Levene's test, respectively ($p>0.05$). Values less than 0.05 were considered statistically significant.

(a) Growth analysis

Independent t-tests were used to test the significance of difference in mean length, width, and weight gain of tagged mussels and abalone over the course of the trial. For data that was not normally distributed, Kruskal-Wallis tests were used.

One-way ANOVAs were used to test the significance of difference of the growth rates of the seaweed species ($n=6$). Tukey HSD post-hoc analyses were conducted when ANOVAs gave a statistically significant result ($p<0.05$).

(b) Water parameter analysis

One-way ANOVAs were used to test the significance of difference of ammonia, nitrite, nitrate, and phosphate levels between each replicate maraponic system. Tukey HSD post-hoc analyses were conducted when ANOVAs gave a statistically significant result ($p<0.05$). When equal variance could not be assumed, a Welch test was used; followed by Dunnet's T3 post-hoc analyses test when the Welch test gave a statistically significant result ($p<0.05$). For data that was not normal, Kruskal-Wallis tests were used.

(c) Fatty acid analysis

One-way ANOVAs were used to test the significance of differences in fatty acids between mussels and abalone from the top and bottom tank of the replicate maraponic systems and wild/farmed mussels and abalone. Tukey HSD post-hoc analyses were conducted when ANOVAs gave a statistically significant result ($p<0.05$). When equal variance could not be assumed, a Welch test was used; followed by Dunnet's T3 post-hoc analyses test when the Welch test gave a statistically significant result ($p<0.05$). For data that was not normal, Kruskal-Wallis tests were used.

Independent t-tests were used to test the significance of difference of fatty acids of sea cucumber intestines and body wall from wild sea cucumbers and those that were in the

replicate maraponic systems. For data that was not normally distributed, Kruskal-Wallis tests were used.

Independent t-tests were used to test the significance of differences in fatty acids of each individual seaweed species, and between wild specimens and those that were in the replicate maraponic systems. If the groups' variance were unequal, an adjustment was made to the degrees of freedom using the Welch-Satterthwaite method. For data that was not normally distributed, Kruskal-Wallis tests were used.

6.3 Results

Growth monitoring

Mussels

Over the 44 days that the growth of tagged mussels was monitored; mean length remained at 3.67 ± 1.33 cm (increase of 0 ± 0.06 cm; $p>0.05$) in the top tanks of the replicate systems and increased from 3.57 ± 1.34 cm to 3.59 ± 1.34 cm (increase of 0.02 ± 0.04 cm; $p>0.05$) in the bottom tanks. There was no significant difference in mean length gain between the bottom and top tanks ($p>0.05$) (Figure 6).

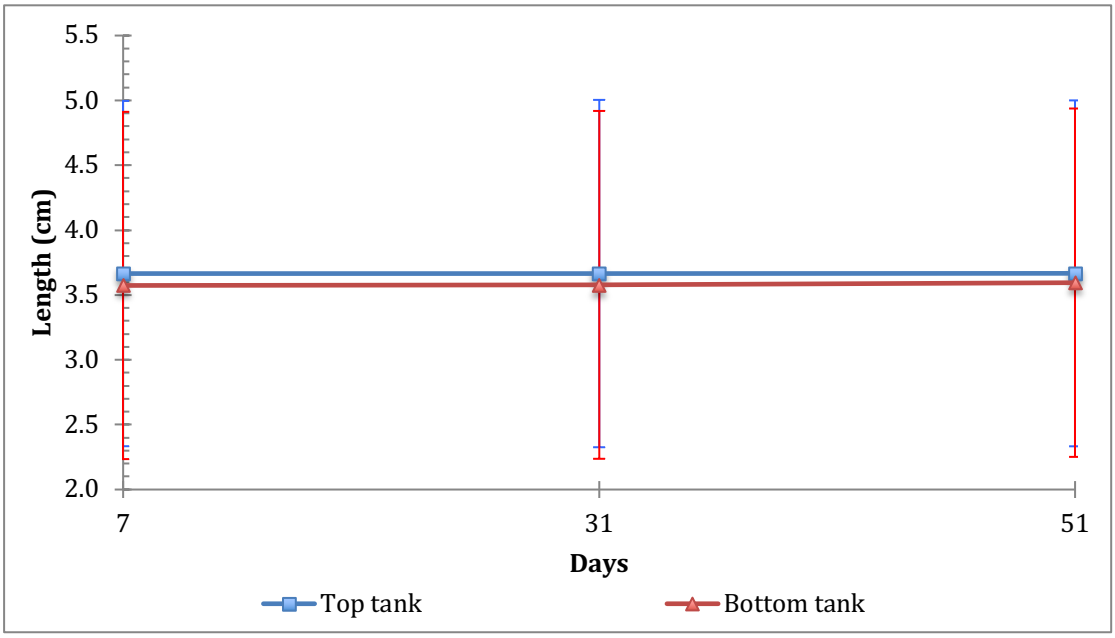


Figure 6: Mean length of tagged mussels in the top and bottom tank of the replicate systems over the course of the trial (mean ± SD)

Mean width increased from 1.77 ± 0.59 cm to 1.78 ± 0.56 cm (increase of 0.01 ± 0.04 cm; $p>0.05$) in the top tanks of the replicate systems and increased from 1.71 ± 0.55 cm to 1.73 ± 0.54 cm (increase of 0.02 ± 0.05 cm; $p>0.05$) in the bottom tanks. There was no significant difference in mean width gain between the bottom and top tanks ($p>0.05$) (Figure 7).

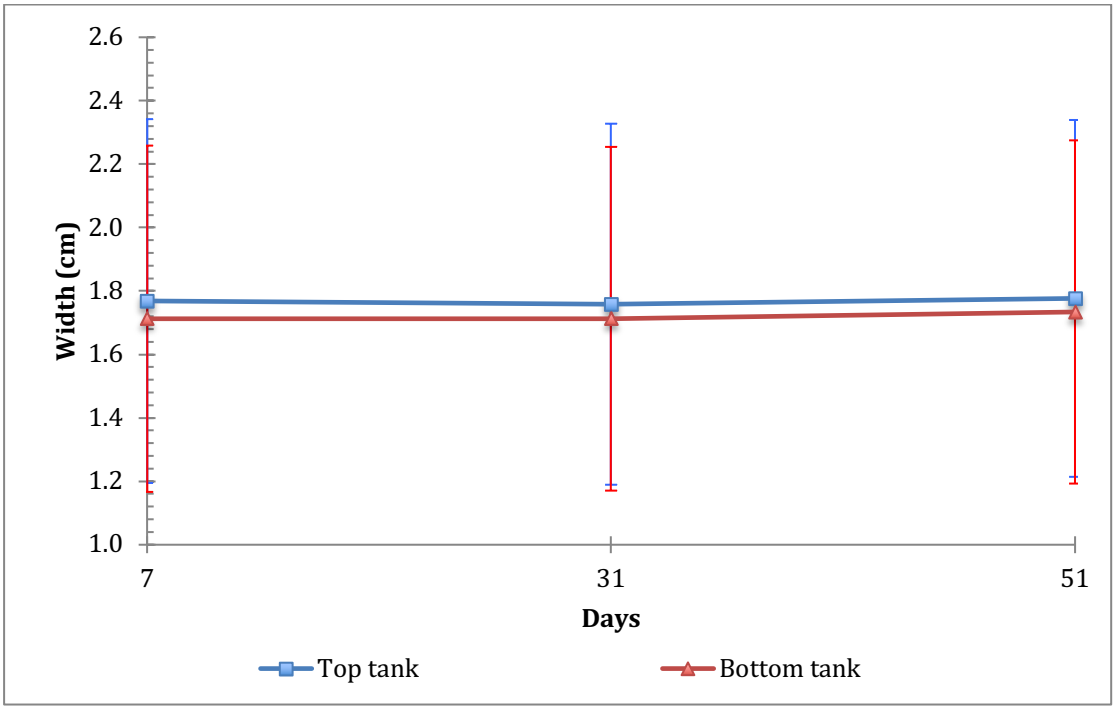


Figure 7: Mean width of tagged mussels in the top and bottom tank of the replicate systems over the course of the trial (mean ± SD)

Mean weight increased from 4.70 ± 4.94 g to 5.09 ± 5.16 g (increase of 0.39 ± 0.53 g; $p>0.05$) in the top tanks of the replicate systems and increased from 4.94 ± 5.28 g to 5.50 ± 5.67 g (increase of 0.57 ± 0.93 g; $p>0.05$) in the bottom tanks. There was no significant difference in mean weight gain between the bottom and top tanks ($p>0.05$) (Figure 8).

Over the course of the trial, the mortality rate of tagged mussels was 0% and 6.67 ± 11.55% for the top and bottom tanks, respectively.

Overall (tagged and non-tagged individuals) biomass increased from 1195 ± 0.41 g to 1345.21 ± 0.79 g and the overall mortality rate was 7.5%.

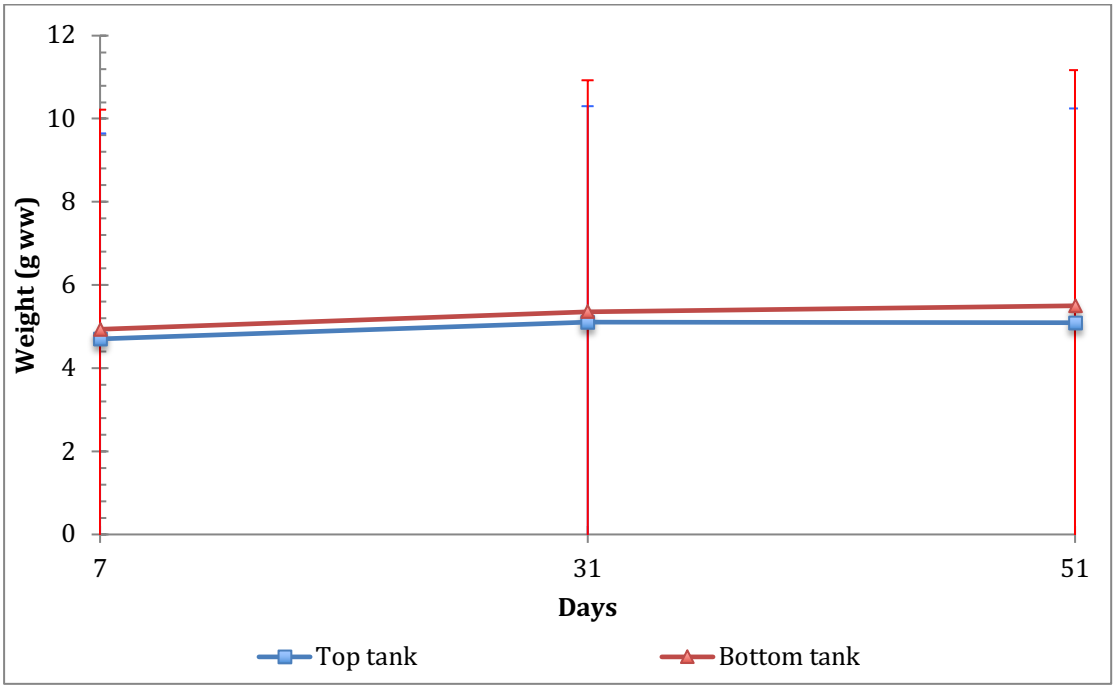


Figure 8: Mean weight of tagged mussels in the top and bottom tank of the replicate systems over the course of the trial (mean ± SD)

Abalone

Over the 44 days that the growth of tagged abalone was monitored; mean length decreased from 4.60 ± 0.38 cm to 4.59 ± 0.35 cm (decrease of 0.01 ± 0.14 cm; $p>0.05$) in the top tanks of the replicate systems and increased from 4.51 ± 0.31 cm to 4.65 ± 0.31 cm (increase of 0.15 ± 0.13 cm; $p>0.05$) in the bottom tanks. There was no significant difference in mean length gain between the bottom and top tanks ($p>0.05$) (Figure 9).

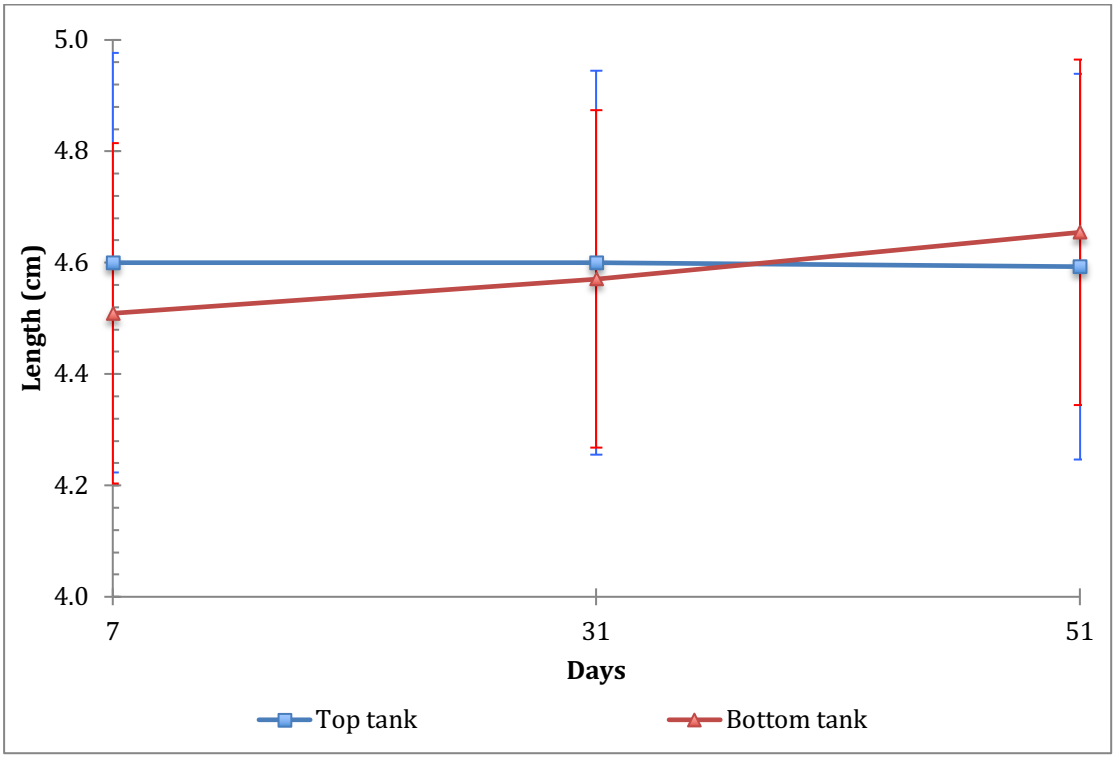


Figure 9: Mean length of tagged abalone in the top and bottom tank of the replicate systems over the course of the trial (mean ± SD)

Mean width increased from 2.97 ± 0.33 cm to 3.07 ± 0.24 cm (increase of 0.10 ± 0.14 cm; $p>0.05$) in the top tanks of the replicate systems and increased from 2.98 ± 0.24 cm to 3.10 ± 0.27 cm (increase of 0.11 ± 0.08 cm; $p>0.05$) in the bottom tanks. There was no significant difference in mean length gain between the bottom and top tanks ($p>0.05$) (Figure 10).

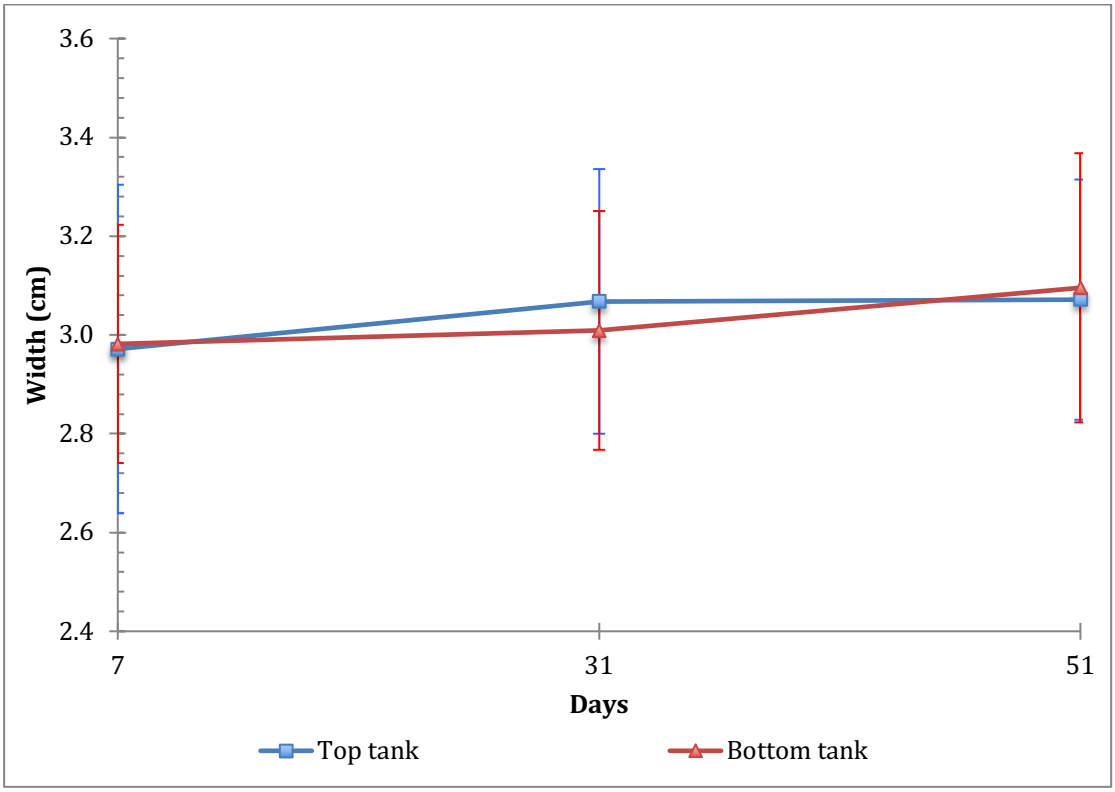


Figure 10: Mean width of tagged abalone in the top and bottom tank of the replicate systems over the course of the trial (mean ± SD)

Mean weight increased from 11.86 ± 2.98 g to 12.21 ± 2.70 g (increase of 0.35 ± 2.81 g; $p>0.05$) in the top tanks of the replicate systems and increased from 11.89 ± 2.54 g to 13.17 ± 2.97 g (increase of 1.28 ± 1.33 g; $p>0.05$) in the bottom tanks. There was no significant difference in mean length gain between the bottom and top tanks ($p>0.05$) (Figure 11).

Over the course of the trial, the mortality rate of tagged abalone was 6.67 ± 11.55% and 26.67 ± 23.09% for the top and bottom tanks, respectively.

Overall (tagged and non-tagged individuals) biomass increased from 298.98 ± 2.84 g to 353.43 ± 2.26 g and the overall mortality was 8.70%.

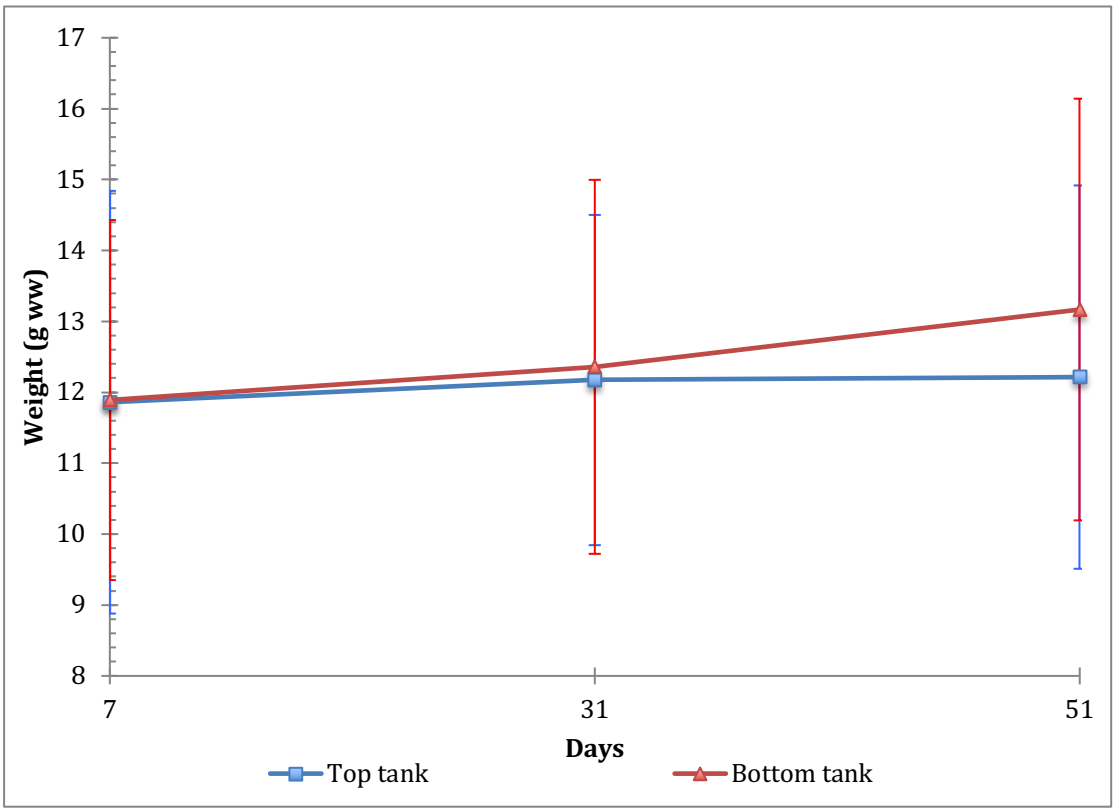


Figure 11: Mean weight of tagged abalone in the top and bottom tank of the replicate systems over the course of the trial (mean ± SD)

Sea cucumbers

The mean weight of sea cucumbers decreased from 140.04 ± 26.46 g to 90.32 ± 35.54 g over the 51 day duration of the trial ($p>0.05$) (Figure 12).

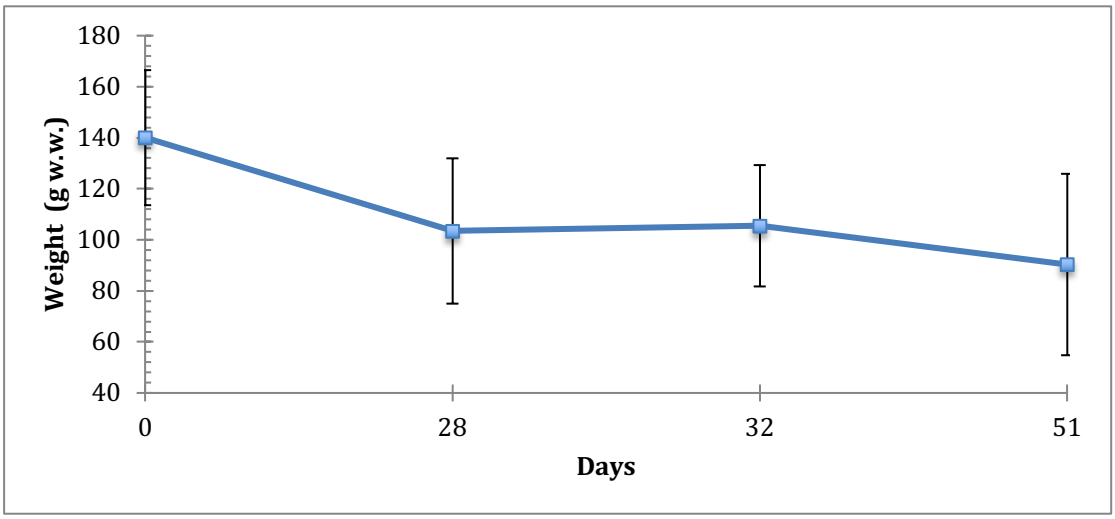


Figure 12: Mean weight of sea cucumbers over the course of the trial (mean ± SD)

By the 28th day of the trial there was a 60% and 20% mortality rate in replicate systems

1 and 2. In order to maintain a balanced ecosystem that was replicated across the three tanks, these mortalities were replaced on the 32nd day of the trial, with a mean weight of 103.03 ± 34.37 g. This mean weight reduced to 85.49 ± 18.24 g by the end of the trial (period of 19 days).

Seaweed

Over the course of the trial, *A. nodosum* showed the greatest level of growth, increasing from 144.30 ± 0.27 g to a peak of 167.27 ± 7.49 g, an increase of 22.97 ± 7.53 g ($p<0.05$), after 28 days. After this point, the biomass of *A. nodosum* began to decrease, reaching 148.77 ± 23.01 g after an additional 23 days, a decrease of 18.50 ± 25.15 g ($p<0.05$) from the 28 day peak. Both *L. digitata* and *F. vesiculosus* also showed growth for the first 28 days of the trial, increasing from 144.64 ± 0.25 g and 143.75 ± 0.02 g to 157.83 ± 16.31 g and 159.18 ± 10.04 g, an increase of 13.19 ± 16.20 g ($p<0.05$) and 15.43 ± 10.02 g ($p<0.05$), respectively. But as was the case with *A. nodosum*, both *L. digitata*'s and *F. vesiculosus'* biomass decreased after 28 days, reaching 143.16 ± 11.16 g and 127.98 ± 30.84 g after an additional 23 days, a decrease of 14.67 ± 27.38 g ($p<0.05$) and 31.20 ± 27.66 g ($p<0.05$), respectively. Both *P. canaliculata* and *F. serratus* had an increase in biomass for the first 9 days of the trial, increasing from 144.11 ± 0.16 g and 144.63 ± 0.28 g to 150.33 ± 1.33 g and 150.90 ± 0.63 g, an increase of 6.22 ± 1.37 g ($p<0.05$) and 6.28 ± 0.62 g ($p<0.01$), respectively. However, for the remainder of the trial, both seaweeds had a decrease in biomass. After a further 19 days, *P. canaliculata* biomass decreased by 91.01 ± 13.25 g to 59.32 ± 12.70 g ($p<0.05$) and specimens were very badly degraded. At this stage, specimens were removed from the each system and replaced with fresh biomass. Despite this, the newly added *P. canaliculata* degraded even quicker, reducing from 144.30 ± 0.27 g to 0 g over the remaining period of the trial. After a further 42 days *F. serratus* decreased by 56.17 ± 26.59 g to 94.74 ± 26.95 g ($p<0.01$). *U. lactuca* decreased in biomass over the duration of the 51 day trial, decreasing from 144.04 ± 0.02 g to 29.51 ± 9.62 g, a decrease of 114.53 ± 9.62 g ($p<0.001$) (Figure 13). However, it is important to note that *Ulva* species are very difficult to keep alive in cultivation (Oliveira *et al*. 2000; Santelices and Doty, 1989).

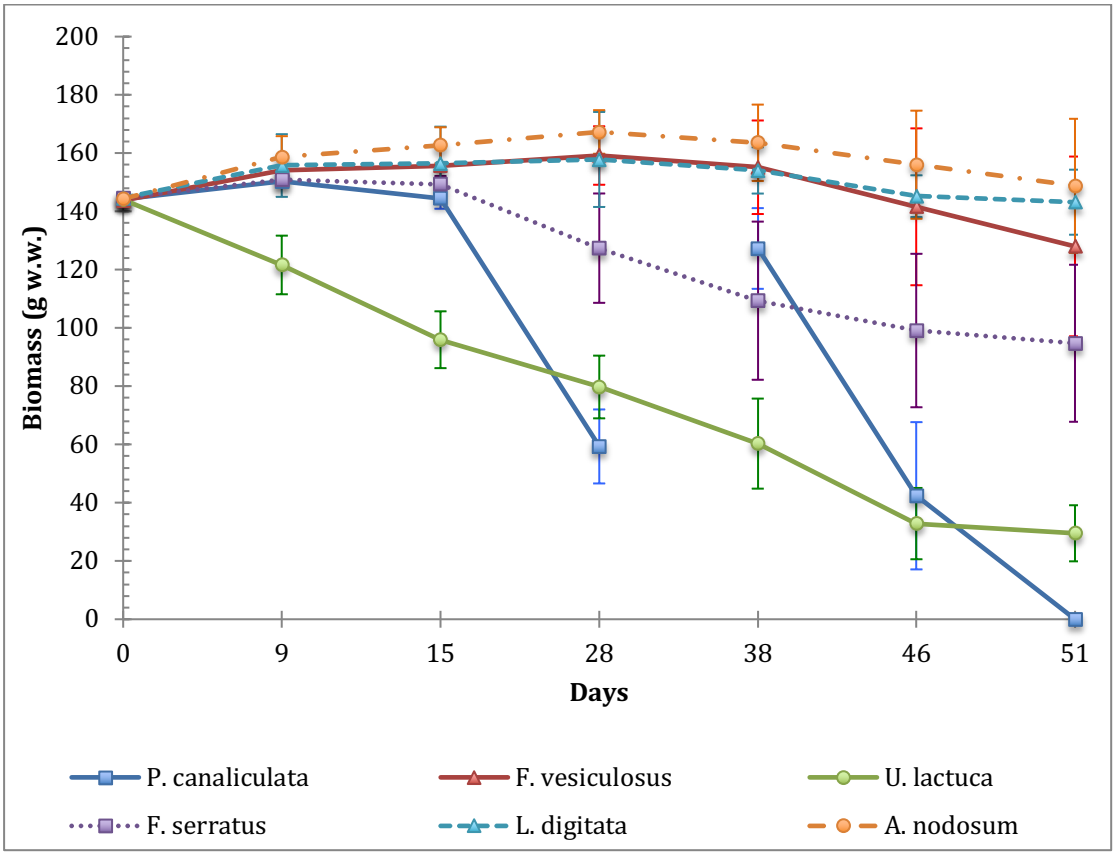


Figure 13: Mean biomass of each seaweed species over the course of the trial (mean ± SD)

Water parameters

Over the 51 days of the trial, salinity, pH, temperature, and dissolved oxygen (DO) remained relatively consistent (Table 5) (see appendix, Table 11).

Table 5: Mean water parameters of the bottom tanks, top tanks, and seaweed buckets of replicate system 1, 2, and 3 measured in the morning (09:00-11:59) and afternoon (12:00-18:00) (mean ± SD)

Parameter	Bottom Tank		Top Tank		Seaweed Buckets	
	Morning	*Afternoon*	*Morning*	*Afternoon*	*Morning*	*Afternoon*
Salinity (ppt)	31.79±0.48	31.72±0.38	31.82±0.48	31.77±0.37	31.84±0.49	31.79±0.38
pH	7.97±0.06	8.07±0.10	8.01±0.09	8.10±0.10	7.99±0.06	8.08±0.08
Temp (°C)	14.0±1.69	14.66±1.39	14.05±1.70	14.74±1.37	13.99±1.68	14.67±1.38
DO (mg/L)	9.71±0.42	9.75±0.43	10.11±0.47	10.06±0.48	10.01±0.38	9.90±0.35
DO (% Sat)	96.84±3.15	99.06±2.69	100.76±4.22	101.98±4.27	99.05±1.73	99.69±1.68
TAN (Hach; mg/L)	0.81±0.49		-		-	

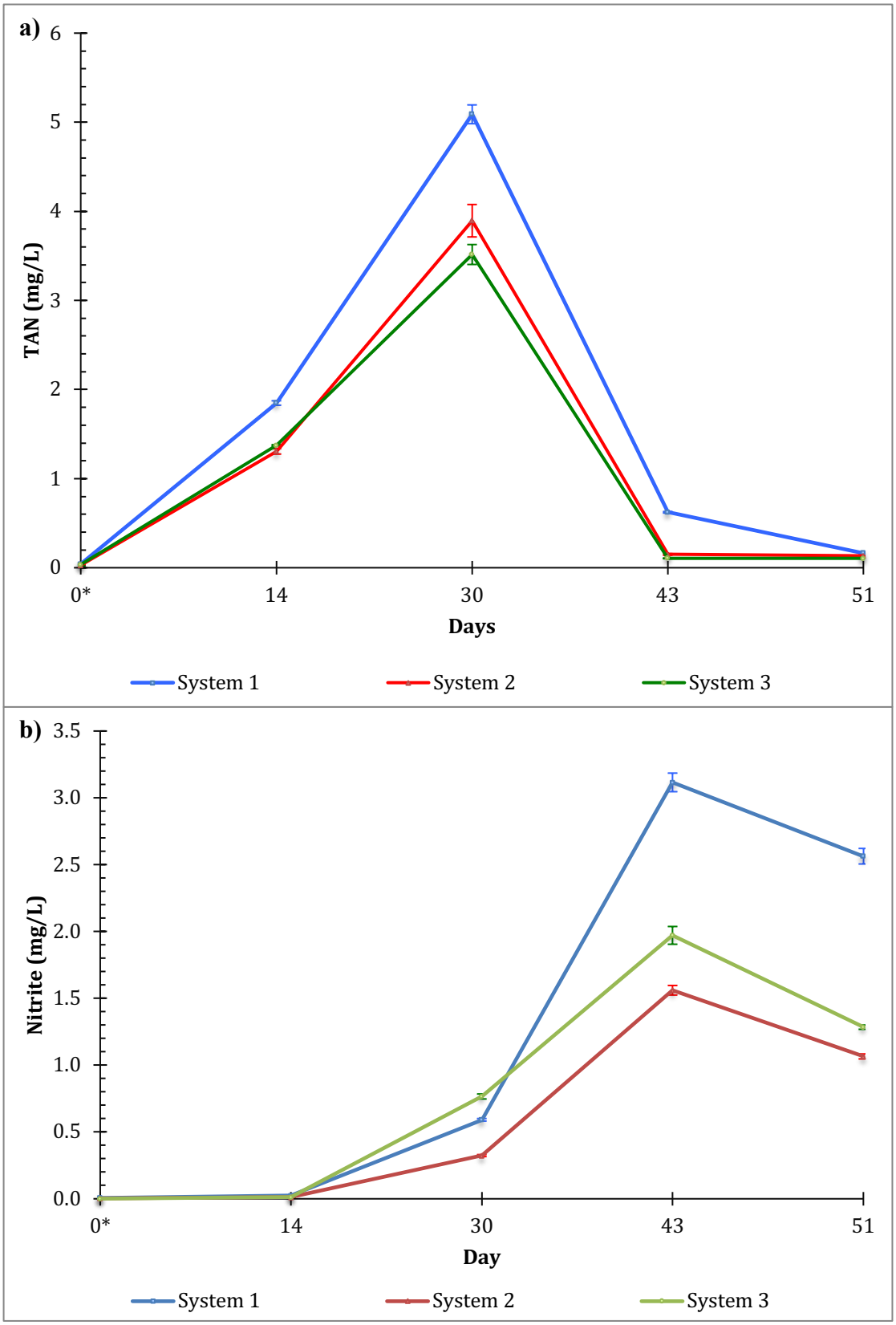
TAN steadily increased in all systems over the first 30 days of the trial, peaking on day 30 at 5.09 ± 0.11, 3.89 ± 0.18, and 3.52 ± 0.11 mg/L for system 1, 2, and 3, respectively

($p<0.001/p<0.05$) (Figure 13a). These levels reduced substantially on the remaining 2 sampling dates as a result of waste reduction measures (see methods), reaching 0.62 ± 0.01, 0.15 ± 0.01, and 0.11 ± 0.004 mg/L for system 1, 2, and 3, respectively, by day 43 ($p<0.001/p<0.01$), and 0.17 ± 0.01, 0.13 ± 0.004, and 0.11 ± 0.002 mg/L for system 1, 2, and 3, respectively, by day 51 ($p<0.05/p<0.01$) (Figure 14a).

Nitrite levels remained low for the first 14 days of the trial, increasing to 0.59 ± 0.01, 0.32 ± 0.01, and 0.77 ± 0.02 mg/L for system 1, 2, and 3, respectively, by day 30 ($p<0.001$), peaking at 3.12 ± 0.07, 1.56 ± 0.04, and 1.97 ± 0.07 for system 1, 2, and 3, respectively, by day 43. These levels decreased to 2.56 ± 0.06, 1.07 ± 0.02, and 1.28 ± 0.02 mg/L, for system 1, 2, and 3, respectively, by day 51 of the trial ($p<0.001/p<0.01$) (Figure 14b).

Nitrate levels were below 0.31 mg/L for all systems when monitored on day 0, 14, and 43. However, levels were 0.72 ± 0.03, 0.48 ± 0.14, and 0.81 ± 0.02 mg/L for system 1, 2, and 3, respectively, on day 30 ($p< 0.05$; 1 vs. 3), and 3.15 ± 0.14, 1.12 ± 0.07, and 1.31 ± 0.02 for system 1, 2, and 3, respectively, on day 51 ($p<0.001$; 1 vs. 2 & 1 vs. 3) (Figure 14c).

Phosphate levels increased steadily over the duration of the trial, dropping slightly between day 30 and 43, but peaking on day 51 at 1.93 ± 0.15, 1.60 ± 0.04, and 1.40 ± 0.02 mg/L for system 1, 2, and 3, respectively ($p<0.05$) (Figure 14d).


a)
TAN (mg/L)
0
1
2
3
4
5
6
0*
14
30
43
51
Days
System 1
System 2
System 3
b)
Nitrite (mg/L)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
0*
14
30
43
51
Day
System 1
System 2
System 3

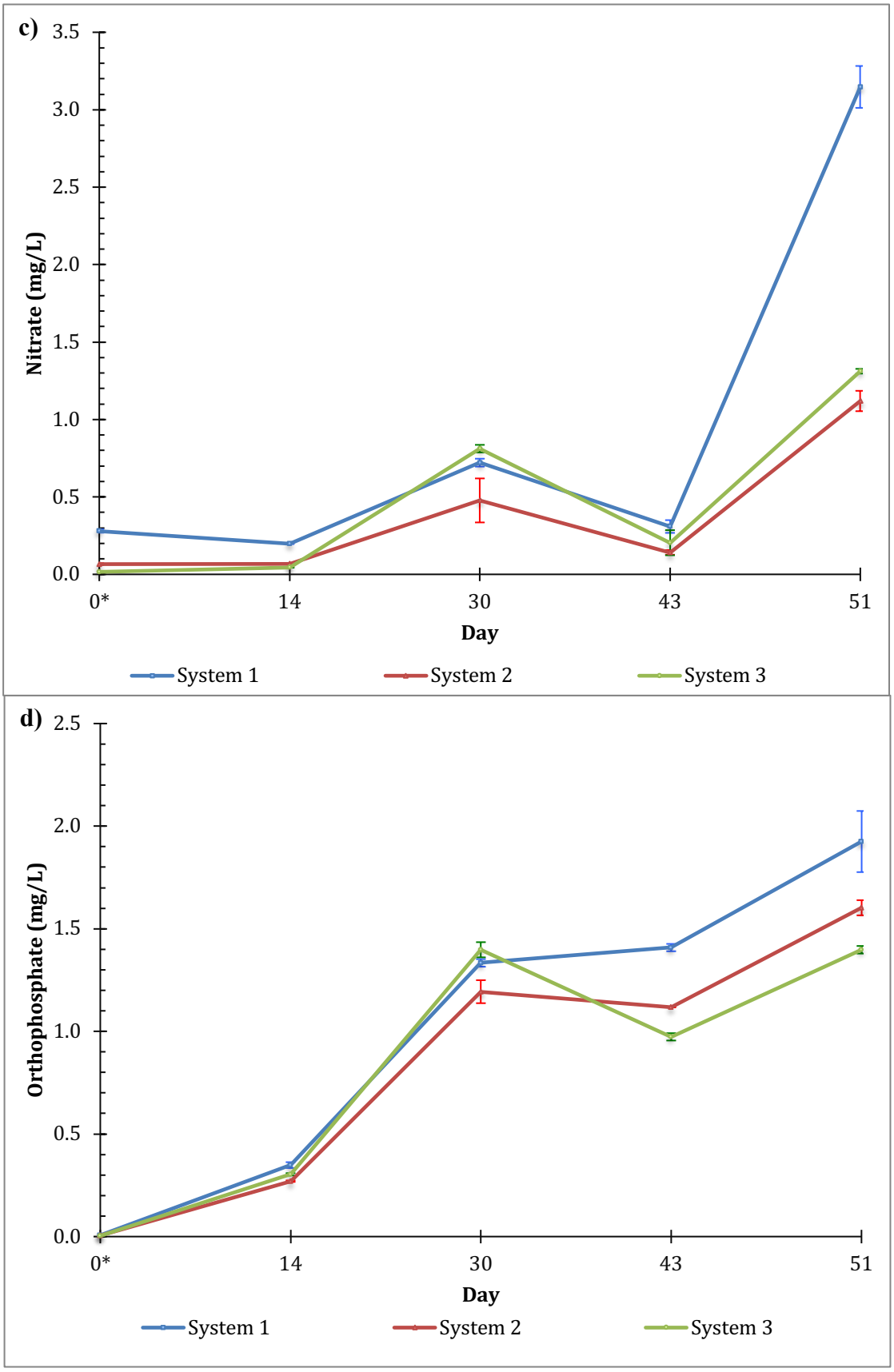


Figure 14: a) TAN; b) nitrite; c) nitrate; & d) orthophosphate (mg/L) levels in each replicate system pre waste addition (day 0) and over the course of the trial (mean ± SD; * first day of trial, prior to waste addition)

Fatty acid analysis

Salmon waste

The most abundant group of fatty acids (FA) in salmon feed (25.94 ± 0.75% lipid content) were monounsaturated fatty acids (MUFA) (44.71 ± 0.23% of total FA), followed by polyunsaturated fatty acids (PUFA) (32.50 ± 0.13%) and saturated fatty acids (SFA) (22.79 ± 0.22%) (Table 6). Salmon faeces had a lower lipid content than the salmon feed, at 2.90 ± 0.45%. The most abundant FA groups in the salmon faeces were MUFA (45.22 ± 1.66%), followed by SFA (32.63 ± 2.77%) and PUFA (22.15 ± 2.68%) (Table 6).

In the salmon feed and faeces palmitic acid (16:0), oleic acid (18:1n-9), gondoic acid (20:1n-9), cetoleic acid (22:1n-11), and docosahexaenoic acid (22:6n-3; DHA) were identified as the most common FAs. Due to their high levels, they were identified as salmon waste biomarkers. A significant increase in these biomarkers in the tissue of the specimens present in this study (i.e. abalone, mussels, or seaweed) at the end of the trial may be an indication that the specimen is consuming the salmon waste. Some of these FAs have also been identified as salmon waste (feed and faeces) biomarkers in the literature. Although linoleic acid (18:2n-6) and 20:4n-6 (arachidonic acid; ARA) have been identified as salmon waste biomarkers in the literature, their presence in the feed and faeces of this study was relatively low and lower than the other identified biomarkers (Irisarri *et al*. 2015; Handå *et al*. 2012). Subsequently, they were not included as salmon waste biomarkers in this study. Palmitic acid (16:0) was also identified as a strong biomarker of periphyton, sharing a similarly high concentration as the salmon waste. Subsequently it would be difficult to distinguish if any potential increase of 16:0 in mussels, abalone, or mussels was due to the assimilation of salmon waste or periphyton. Therefore, it was not considered a suitable biomarker of salmon waste (or periphyton) in this study (Table 6) . Unfortunately, a limitation of this study was the fact that salmon feed and faeces were added together to the systems and had a similar FA profile. It was therefore difficult to infer if an increase in salmon waste biomarker was as a result of assimilation of salmon feed or faeces. Subsequently, for this study, salmon feed and faeces were referred to under the umbrella-term; salmon waste. It is important to keep in mind, however, that the utilisation of FAs as biomarkers relies on the food source and the consumer having distinct FA signatures (Wen *et al*. 2016a; Irisarri *et al*. 2015; Handå *et al*. 2012; Pleissner *et al*. 2012). The ω6/ω3 ratio of

salmon feed and waste was 0.19 ± 0.01 and 0.29 ± 0.02, respectively.

Table 6: Fatty acid composition (%) of salmon waste (feed and faeces) (mean ± SD; *=biomarker)

	Feed	Faeces
Lipid	**25.94 ± 0.75**	**2.90 ± 0.45**
SFA		
14:0	6.01 ± 0.17	6.80 ± 0.46
15:0	0.48 ± 0.01	0.64 ± 0.05
16:0	14.21 ± 0.16	21.39 ± 1.89
18:0	1.89 ± 0.02	3.32 ± 0.44
20:0	0.16 ± 0.01	0.35 ± 0.05
22:0	0.04 ± 0.02	0.13 ± 0.02
Total SFA	**22.79 ± 0.22**	**32.63 ± 2.77**
MUFA		
16:1n-9	0.16 ± 0.06	0.06 ± 0.03
16:1n-7	6.19 ± 0.10	4.11 ± 0.40
18:1n-9*	**9.57 ± 0.16**	**8.16 ± 0.65**
18:1n-7	1.97 ± 0.01	1.84 ± 0.06
20:1n-9*	**10.76 ± 0.04**	**10.87 ± 0.47**
20:1n-7	0.25 ± 0.01	0.32 ± 0.03
22:1n-11*	**14.16 ± 0.06**	**16.93 ± 1.18**
22:1n-9	0.94 ± 0.02	1.38 ± 0.12
24:1n-9	0.72 ± 0.02	1.55 ± 0.22
Total MUFA	**44.71 ± 0.23**	**45.22 ± 1.66**
n-6 PUFA		
18:2n-6	3.72 ± 0.10	3.88 ± 0.40
18:3n-6	0.18 ± 0	0.10 ± 0.02
20:2n-6	0.25 ± 0.01	0.24 ± 0.02
20:3n-6	0.11 ± 0.07	0.15 ± 0.06
20:4n-6 (ARA)	0.43 ± 0.01	0.33 ± 0.04
22:4n-6	0	0.02 ± 0.08
22:5n-6	0.14 ± 0	0.10 ± 0.01
Total n-6 PUFA	**4.83 ± 0.12**	**4.82 ± 0.44**
n-3 PUFA		
18:3n-3	1.37 ± 0.02	0.96 ± 0.11
18:4n-3	3.65 ± 0.05	1.85 ± 0.34
20:3n-3	0.13 ± 0	0.13 ± 0.01
20:4n-3	0.62 ± 0.02	0.41 ± 0.08
20:5n-3 (EPA)	9.08 ± 0.08	5.05 ± 0.86
22:5n-3	0.89 ± 0.03	0.66 ± 0.11
22:6n-3 (DHA)*	**10.27 ± 0.14**	**7.47 ± 0.78**
Total n-3 PUFA	**26.00 ± 0.10**	**16.52 ± 2.17**
Other PUFAs		
16:2	0.56 ± 0.01	0.30 ± 0.06
16:3	0.40 ± 0.08	0.18 ± 0.05
16:4	0.70 ± 0.02	0.32 ± 0.07
Total other PUFAs	**1.66 ± 0.09**	**0.80 ± 0.17**
Total PUFA	**32.50 ± 0.13**	**22.15 ± 2.68**

Periphyton

The most abundant group of FAs in periphyton (1.75 ± 0.65% lipid content) were PUFA (43.10 ± 3.74%), followed by MUFA (26.45 ± 2.95%) and SFA (21.55 ± 1.78%). Furan FAs were also identified in the periphyton (8.89 ± 0.49%) (Table 7).

Palmitic acid (16:0), palmitoleic acid (16:1n-7), and eicosapentaenoic acid (EPA; 20:5n-3) were identified as the most common FAs. As noted above, 16:0 was also identified as a biomarker of salmon waste and was therefore not suitable as a periphyton biomarker in this study (Table 7). As a result, 16:1n-7 and EPA were chosen as periphyton biomarkers. The ω6/ω3 ratio of periphyton was 0.26 ± 0.04.

Table 7: Fatty acid composition (%) of periphyton (mean ± SD; *=biomarker)

Lipid	**1.75 ± 0.65**
SFA	
14:0	4.13 ± 0.99
Anteiso 15:0	0.90 ± 0.28
15:0	0.45 ± 0.09
16:0	15.02 ± 1.77
18:0	0.61 ± 0.10
20:0	0.02 ± 0.07
22:0	0.02 ± 0.05
24:0	0.40 ± 0.18
Total SFA	**21.55 ± 1.78**
MUFA	
14:1	0.76 ± 0.42
16:1n-9	0.31 ± 0.10
16:1n-7*	**15.51 ± 2.26**
16:1	0.69 ± 0.37
17:1	0.04 ± 0.08
18:1n-9	2.55 ± 0.49
18:1n-7	5.71 ± 0.56
18:1	0.76 ± 0.17
20:1n-11	0.02 ± 0.06
20:1n-7	0.05 ± 0.11
22:1n-11	0.02 ± 0.06
24:1	0.02 ± 0.06
Total MUFA	**26.45 ± 2.95**
n-6 PUFA	
18:2n-6	3.32 ± 0.70
18:3n-6	0.53 ± 0.07
20:2n-6	0.05 ± 0.10
20:3n-6	0.24 ± 0.13
20:4n-6 (ARA)	2.10 ± 0.45
22:5n-6	0.90 ± 0.42
Total n-6 PUFA	**7.15 ± 1.12**
n-3 PUFA	
18:3n-3	5.13 ± 1.85
18:4n-3	4.31 ± 1.76
20:4n-3	0.26 ± 0.06
20:5n-3 (EPA)*	**16.42 ± 4.20**
22:6n-3 (DHA)	1.86 ± 0.35
Total n-3 PUFA	**27.98 ± 3.69**
Other PUFA	
14:2	1.18 ± 0.69
16:2	1.91 ± 0.35
16:3	3.90 ± 1.13
16:4	0.32 ± 0.04
18:2	0.66 ± 0.10
Total other PUFA	**7.98 ± 1.19**
Total PUFA	**43.10 ± 3.74**
Furan Fatty Acids	**8.89 ± 0.49**

Mussels

The lipid content of wild (control) mussels (2.43 ± 0.50% lipid comtent) and those contained in the top (2.45 ± 0.61%) and bottom tank (2.14 ± 0.60%) were not significantly different ($p>0.05$). The most abundant FAs were PUFA followed by SFA and MUFA for the wild mussels and the mussels contained in the top and bottom tank of the systems (Table 8).

SFA was significantly higher ($p<0.05$) in the bottom tank mussels than the top. PUFA was higher in the wild than maraponic (both the top and bottom tank) mussels ($p<0.05$; wild vs. bottom tank). Dimethylacetal (DMA) FAs and non-methylene-interrupted dienoic (NMID) FAs were present in all mussel samples; however, there was no significant difference between the tank and wild mussels ($p>0.05$) (Table 8). The PUFA ω6/ω3 ratio of wild (0.16 ± 0.32) and top (0.20 ± 0.37) and bottom (0.18 ± 0.13) tank mussels were within the recommend levels for a healthy diet (Stabili *et al*. 2012; Bergé and Barnathan, 2005) (there was no significant difference in the ω6/ω3 ratio between all mussel types; $p>0.05$).

The salmon waste biomarker 20:1n-9 was significantly higher in the maraponic mussels (top and bottom tanks) than wild mussels ($p<0.01$) 22:1n-11 was also significantly higher in bottom tank thank wild mussels ($p<0.05$). There was no significant difference in the periphyton biomarkers, 16:1n-7 and EPA, between the top and bottom tank mussels and between the maraponic (bottom and top tank) and wild mussels ($p>0.05$) (Table 8; see appendix, Table 12).

Table 8: Overall fatty acid composition (%) of mussels and identified salmon waste & periphyton biomarkers (mean ± SD; non-matching sub-script letters = significant difference; NS = non significant difference)

	Wild	Top tanks	Bottom tanks	P-value
Lipid Content (%)				
	2.43 ± 0.50	2.45 ± 0.61	2.14 ± 0.60	NS
Overall Fatty Acid Composition				
SFA	23.98 ± 1.13$^{a/b}$	22.67 ± 1.63^{a}	24.23 ± 0.85^{b}	<0.05
MUFA	15.30 ± 1.62	16.70 ± 2.25	17.15 ± 3.94	NS
PUFA	45.51 ± 1.70^{a}	41.34 ± 2.05$^{a/b}$	41.89 ± 3.15^{b}	<0.05
DMA	8.07 ± 1.84	10.41 ± 2.90	9.11 ± 1.72	NS
NMID	7.14 ± 1.32	8.88 ± 2.48	7.62 ± 1.98	NS
Salmon waste biomarkers				
18:1n-9	2.15 ± 0.58	2.56 ± 0.48	2.89 ± 1.01	NS
20:1n-9	2.69 ± 0.47^{a}	3.61 ± 0.77^{b}	3.63 ± 0.42^{b}	<0.01
22:1n-11	0.22 ± 0.45^{a}	0.24 ± 0.18$^{a/b}$	0.47 ± 0.22^{b}	<0.05
22:6n-3 (DHA)	19.18 ± 1.76	17.52 ± 2.17	18.14 ± 3.26	NS
Periphyton biomarkers				
16:1n-7	5.35 ± 1.75	4.20 ± 2.50	4.79 ± 2.26	NS
20:5n-3 (EPA)	13.53 ± 1.16	11.93 ± 1.66	12.33 ± 2.25	NS

Abalone

The lipid content of farmed abalone (control) (1.14 ± 0.22% lipid content) and those contained in the top (1.12 ± 0.18%) tank were not significantly different ($p>0.05$), however, there was a significant difference between abalone from the bottom (1.47 ± 0.35%) and top tank and between farmed and bottom tank abalone ($p<0.05$). The most abundant FAs were PUFA followed by MUFA and SFA for the farmed abalone and the abalone contained in the top and bottom tank of the systems (Table 9).

MUFA was lower in both the top and bottom tank abalone than the farmed abalone ($p<0.001$; farmed vs. top). MUFA was significant higher ($p<0.05$) in bottom tank abalone than top tank. PUFA was higher in the top tank abalone than the farmed abalone ($p<0.01$). There was a significant difference in PUFA between top and bottom tank abalone (<0.01). DMA and NMID FAs were also present in all abalone samples with DMA being higher in the top tank abalone than the farmed abalone ($p<0.01$). DMA was significantly higher in the top tank abalone than the bottom tank (<0.05). NMID was lower in both the top and bottom tank abalone than the farmed abalone ($p<0.01$; farmed vs. bottom) (Table 9). The PUFA ω6/ω3 ratio of farmed (0.41 ± 0.02) and top (0.36 ± 0.04) and bottom (0.33 ± 0.01) tank abalone were within the recommend levels for a

healthy diet, with the ratio of top and bottom abalone significantly lower (<0.05/<0.01) than farmed abalone. There was no significant difference in the ω6/ω3 ratio between the top and bottom tank abalone.

The salmon waste biomarkers, 22:1n-11 and DHA, were higher in both the top and bottom tank abalone when compared to farmed abalone (22:1n-11 – farmed vs. bottom, $p<0.01$; DHA – $p<0.01$), whereas 18:1n-9 was lower in both the top and bottom tanks (farmed vs. top; $p<0.001$) and 20:1n-9 was lower in the top tank abalone and higher in the bottom tank abalone than the farmed abalone (farmed vs. bottom; $p<0.01$). The periphyton biomarker, 16:1n-7, was lower in both the top and bottom tank abalone than the farmed abalone (farmed vs. top; $p<0.001$). 16:1n-7 was also significantly higher ($p<0.001$) in the bottom than the top tank abalone. EPA was significantly higher ($p<0.05$) in top tank abalone than farmed abalone and lower in bottom tank than farmed abalone ($p>0.05$). EPA was significantly higher ($p<0.001$) in the top tank than bottom tank abalone (Table 9; see appendix, Table 13).

Table 9: Overall fatty acid composition (%) of abalone and identified salmon waste & periphyton biomarkers (mean ± SD; non-matching sub-script letters = significant difference; NS = non significant difference)

	Farmed	**Top tanks**	**Bottom tanks**	**P-value**
Lipid Content (%)				
	1.14 ± 0.22[a]	1.12 ± 0.18[a]	1.47 ± 0.35[b]	<0.05
Overall Fatty Acid Composition				
SFA	23.39 ± 9.37	27.31 ± 1.53	27.41 ± 0.54	NS
MUFA	32.63 ± 1.34[a]	24.70 ± 2.17[b]	32.27 ± 2.54[a]	<0.001/<0.05
PUFA	28.03 ± 7.91[a]	31.10 ± 1.37[b]	28.83 ± 0.91[a]	<0.01
DMA	9.14 ± 2.12[a]	10.98 ± 0.91[b]	6.63 ± 1.61[a]	<0.01/<0.05
NMID	6.81 ± 0.93[a]	5.90 ± 0.60[a/b]	4.87 ± 0.79[b]	<0.01
Salmon waste biomarkers				
18:1n-9	8.06 ± 0.70[a]	5.83 ± 0.72[b]	7.62 ± 0.78[a]	<0.001
20:1n-9	1.05 ± 0.91[a]	0.87 ± 0.12[a]	4.88 ± 1.26[b]	<0.01
22:1n-11	0.26 ± 0.11[a]	0.32 ± 0.10[a]	3.28 ± 0.94[b]	<0.01/<0.05
22:6n-3 (DHA)	0.09 ± 0.08[a]	0.88 ± 0.35[b]	4.08 ± 1.16[c]	<0.01
Periphyton biomarkers				
16:1n-7	3.62 ± 0.75[a]	2.09 ± 0.45[b]	3.36 ± 0.62[a]	<0.001
20:5n-3 (EPA)	10.09 ± 2.73[a]	11.18 ± 1.03[b]	9.54 ± 0.44[a]	<0.05/<0.001

Sea cucumbers

Intestines

The lipid content of sea cucumber intestines from the maraponic system (1.86 ± 0.75% lipid content) was higher than the wild (control) sea cucumbers (1.43 ± 0.45%) ($p<0.05$). The most abundant FAs were PUFA followed by MUFA and SFA for both the wild and maraponic sea cucumbers (Table 10).

There was no significant difference in the SFA, MUFA, PUFA, and DMA content of sea cucumber intestine between maraponic and wild specimens ($p>0.05$). Subsequently, there was no difference in the salmon waste and periphyton biomarkers (Table 10). The PUFA ω6/ω3 ratio of intestines of wild (0.82 ± 0.11) and maraponic (0.76 ± 0.23) sea cucumbers were within the recommend levels for a healthy diet (maraponic vs. wild; $p>0.05$) (see appendix, Table 14).

Table 10: Overall fatty acid composition (%) of sea cucumber intestine and identified salmon waste & periphyton biomarkers (mean ± SD; non-matching sub-script letters = significant difference; NS = non significant difference)

	Wild	**Maraponic System**	**P-value**
Lipid Content			
	1.43 ± 0.45	1.86 ± 0.75	NS
Overall Fatty Acid Composition			
SFA	19.67 ± 0.87	20.03 ± 4.28	NS
MUFA	31.71 ± 6.85	30.19 ± 3.94	NS
PUFA	40.84 ± 6.42	41.70 ± 6.37	NS
DMA	7.78 ± 1.35	8.07 ± 1.96	NS
ω6/ω3 ratio			
n-6 PUFA	18.12 ± 2.29	17.50 ± 4.16	NS
n-3 PUFA	22.30 ± 4.40	23.78 ± 4.83	NS
Salmon waste biomarkers			
18:1n-9	3.81 ± 2.25	3.40 ± 1.54	NS
20:1n-9	1.89 ± 0.80	2.05 ± 1.13	NS
22:1n-11	0.91 ± 0.25	1.05 ± 0.36	NS
22:6n-3 (DHA)	2.30 ± 0.26	2.52 ± 0.71	NS
Periphyton biomarkers			
16:1n-7	3.31 ± 0.59	3.20 ± 2.11	NS
20:5n-3 (EPA)	16.37 ± 3.46	17.43 ± 4.94	NS

Body wall

The lipid content of the body wall from the maraponic sea cucumbers (0.32 ± 0.08%) was lower than the wild (control) sea cucumbers (0.28 ± 0.12%) ($p<0.05$). The most abundant FAs were PUFA followed by MUFA and SFA for both the wild and maraponic sea cucumbers (Table 11).

There was no significant difference in the SFA, MUFA, PUFA, and DMA content of sea cucumber body wall between maraponic and wild specimens ($p>0.05$). Subsequently, there was no difference in the salmon waste and periphyton biomarkers (Table 11). The PUFA ω6/ω3 ratio of intestines of wild (1.65 ± 0.12) and maraponic (1.61 ± 0.38) sea cucumbers were within the recommend levels for a healthy diet (maraponic vs. wild; $p>0.05$) (see appendix, Table 15).

Table 11: Overall fatty acid composition (%) of sea cucumber body wall and identified salmon waste & periphyton biomarkers (mean ± SD; NS = non significant difference)

	Wild	**Maraponic System**	**P-value**
Lipid Content (%)			
	0.28 ± 0.12	0.32 ± 0.08	NS
Overall Fatty Acid Composition			
SFA	17.46 ± 2.81	19.78 ± 8.11	NS
MUFA	31.93 ± 1.27	32.87 ± 1.78	NS
PUFA	40.08 ± 3.19	36.15 ± 9.96	NS
DMA	10.52 ± 0.72	11.19 ± 0.83	NS
Salmon waste biomarkers			
18:1n-9	3.35 ± 0.53	3.96 ± 1.30	NS
20:1n-9	0.55 ± 0.84	0.89 ± 0.52	NS
22:1n-11	0.74 ± 0.05	0.61 ± 0.09	NS
22:6n-3 (DHA)	1.11 ± 0.27	0.92 ± 0.36	NS
Periphyton biomarkers			
16:1n-7	1.20 ± 0.77	1.15 ± 0.40	NS
20:5n-3 (EPA)	12.10 ± 1.26	11.46 ± 4.12	NS

Seaweeds

Ascophyllum nodosum

The lipid content of *A. nodosum* from the maraponic systems (1.75 ± 0.36% lipid content) was significantly lower than the wild (control) specimens (2.29 ± 0.16%) ($p<0.05$). The most abundant FAs were MUFA followed by PUFA and SFA for both the maraponic and wild *A. nodosum.* SFA, PUFA, and furan FA content was higher in the maraponic *A. nodosum* than wild *A. nodosum* (MUFA; $p<0.05$ & Furan FAs; $p<0.01$), while MUFA was lower ($p>0.05$) (Table 12). The PUFA ω6/ω3 ratio of maraponic (2.11 ± 0.34) and wild (2.04 ± 0.12) *A. nodosum* were within the recommend levels for a healthy diet (maraponic vs. wild; $p>0.05$) (see appendix, Table 16).

Table 12: Overall fatty acid composition (%) of *A. nodosum* (mean ± SD)

	Wild	**Maraponic Systems**	**P-value**
Lipid Content (%)			
	2.29 ± 0.16	1.75 ± 0.36	<0.05
Overall Fatty Acid Composition			
SFA	17.47 ± 1.03	21.35 ± 3.65	NS
MUFA	45.54 ± 1.29	38.33 ± 7.11	<0.05
PUFA	36.02 ± 0.64	38.25 ± 3.13	NS
Furan FA	0.97 ± 0.10	2.07 ± 0.74	<0.01

Laminaria digitata

The lipid content of *L. digitata* from the maraponic systems (0.78 ± 0.22%) was significantly lower than the wild (control) specimens (1.21 ± 0.36%) ($p<0.05$). The most abundant FAs were PUFA followed by SFA and MUFA for both the maraponic and wild *L. digitata.* PUFA was significantly lower in the maraponic than wild *L. digitata* ($p<0.05$) (Table 13). The PUFA ω6/ω3 ratio of maraponic (0.89 ± 0.38) and wild (0.39 ± 0.04) *L. digitata* were within the recommend levels for a healthy diet (maraponic vs. wild; $p<0.05$) (see appendix, Table 17).

Table 13: Overall fatty acid composition (%) of *L. digitata* (mean ± SD)

	Wild	Maraponic Systems	P-value
Lipid Content (%)			
	1.21 ± 0.36	0.78 ± 0.22	<0.05
Overall Fatty Acid Composition			
SFA	29.55 ± 1.03	31.67 ± 2.44	NS
MUFA	25.48 ± 0.44	26.39 ± 3.10	NS
PUFA	40.70 ± 0.64	34.41 ± 7.62	<0.05
Furan FA	4.29 ± 0.80	7.53 ± 5.32	NS

Fucus vesiculosus

There was no significant difference in the lipid content of the maraponic (1.36 ± 0.14%) and wild (2.97 ± 1.14%) *F. vesiculosus* ($p>0.05$). The most abundant FAs were PUFA, followed by SFA and MUFA for maraponic *F. vesiculosus*, whereas, the most abundant FAs were MUFA, followed by PUFA and SFA for wild *F. vesiculosus*. SFA ($p<0.01$), PUFA ($p<0.01$), and furan FA ($p<0.001$) content was significantly higher in the maraponic *F. vesiculosus* than wild *F. vesiculosus*, while MUFA was significantly lower ($p<0.001$) (Table 14). The PUFA ω6/ω3 ratio of maraponic (0.96 ± 0.13) and wild (1.90 ± 0.60) *F. vesiculosus* were within the recommend levels for a healthy diet (maraponic vs. wild; $p>0.05$) (see appendix, Table 18).

Table 14: Overall fatty acid composition (%) of *F. vesiculosus* (mean ± SD)

	Wild	Maraponic Systems	P-value
Lipid Content (%)			
	2.97 ± 1.14	1.36 ± 0.14	NS
Overall Fatty Acid Composition			
SFA	24.42 ± 2.80	29.42 ± 1.40	<0.01
MUFA	40.29 ± 10.92	20.39 ± 3.48	<0.001
PUFA	33.91 ± 7.71	46.07 ± 3.84	<0.01
Furan FA	1.39 ± 0.86	4.12 ± 0.51	<0.001
ω6/ω3 ratio			
n-6 PUFA	21.31 ± 2.78	22.26 ± 1.79	NS
n-3 PUFA	12.22 ± 5.13	23.44 ± 2.93	<0.01
ω6/ω3	1.90 ± 0.60	0.96 ± 0.13	NS

Fucus serratus

The lipid content of *F. serratus* from the maraponic systems (1.44 ± 0.19%) was significantly lower than the wild (control) specimens (1.96 ± 0.12%) ($p<0.01$). The most abundant FA was PUFA in both the maraponic and wild *F. serratus*, followed by SFA and MUFA for maraponic *F. serratus* and MUFA and SFA for wild *F. serratus.*

SFA and furan FA content was higher in the maraponic *F. serratus* than wild *F. serratus* (Furan FA; $p<0.05$) (Table 15). The PUFA ω6/ω3 ratio of maraponic (1.30 ± 0.34) and wild (1.33 ± 0.11) *F. serratus* were within the recommend levels for a healthy diet (maraponic vs. wild; $p>0.05$) (see appendix, Table 19).

Table 15: Overall fatty acid composition (%) of *F. serratus* (mean ± SD)

	Wild	**Maraponic Systems**	**P-value**
Lipid Content (%)			
	1.96 ± 0.12	1.44 ± 0.19	<0.01
Overall Fatty Acid Composition			
SFA	26.86 ± 2.11	29.78 ± 1.88	NS
MUFA	30.52 ± 1.58	26.71 ± 6.06	NS
PUFA	40.95 ± 3	39.91 ± 4.88	NS
Furan FA	1.67 ± 0.23	3.61 ± 1.11	<0.05

Pelvetia canaliculata

The lipid content of *P. canaliculata* from the maraponic systems (1.53 ± 0.32%) was significantly lower than the wild (control) specimens (3 ± 0.16%) ($p<0.001$). The most abundant FA was MUFA, followed by PUFA and SFA in the maraponic *P. canaliculata*, whereas in the wild *P. canaliculata* it was PUFA, followed by MUFA and SFA. SFA, MUFA. PUFA content was significantly lower in the maraponic than wild *P. canaliculata* ($p<0.001$) (Table 16). The PUFA ω6/ω3 ratio of maraponic (3.31 ± 1.32) and wild (2.15 ± 0.07) *P. canaliculata* were within the recommend levels for a healthy diet (maraponic vs. wild; $p>0.05$) (see appendix, Table 20).

Table 16: Overall fatty acid composition (%) of *P. canaliculata* (mean ± SD)

	Wild	**Maraponic Systems**	**P-value**
Lipid Content (%)			
	3 ± 0.16	1.53 ± 0.32	<0.001
Overall Fatty Acid Composition			
SFA	20.18 ± 1.01	24.93 ± 5.50	NS
MUFA	35.12 ± 0.85	45.49 ± 11.59	NS
PUFA	42.88 ± 1.05	26.05 ± 4.76	<0.001
Furan FA	1.82 ± 0.18	3.54 ± 3.44	NS
ω6/ω3 ratio			
n-6 PUFA	28.82 ± 1.02	17.36 ± 4.77	<0.001
n-3 PUFA	13.40 ± 0.23	7.46 ± 6.99	NS
ω6/ω3	2.15 ± 0.07	3.31 ± 1.32	NS

Ulva lactuca

There was no significant difference in the lipid content of the maraponic (1.43 ± 0.13%) and wild (1.35 ± 0.31%) *U. lactuca* ($p>0.05$). The most abundant FA was PUFA, followed by SFA and MUFA in the maraponic *U. lactuca*, whereas in the wild *U. lactuca* it was SFA, followed by PUFA and MUFA. PUFA, SFA was significantly lower in the maraponic than wild *U. lactuca* ($p<0.05$) (Table 17). The PUFA ω6/ω3 ratio of maraponic (0.27 ± 0.08) and wild (0.24 ± 0.08) *U. lactuca* were within the recommend levels for a healthy diet (maraponic vs. wild; $p>0.05$) (see appendix Table 21).

Table 17: Overall fatty acid composition (%) of *U. lactuca* (mean ± SD)

	Wild	**Maraponic Systems**	**P-value**
Lipid Content (%)			
	1.35 ± 0.31	1.43 ± 0.13	NS
Overall Fatty Acid Composition			
SFA	40.53 ± 4.48	31.77 ± 5.07	<0.05
MUFA	21.54 ± 6.26	19.29 ± 2.35	NS
PUFA	26.72 ± 5.28	35.44 ± 6.88	NS
Furan FA	11.22 ± 6.91	13.50 ± 0.72	NS
ω6/ω3 ratio			
n-6 PUFA	4.50 ± 0.91	6.56 ± 2.22	NS
n-3 PUFA	19.44 ± 4.32	24.13 ± 2.31	<0.05
ω6/ω3	0.24 ± 0.08	0.27 ± 0.08	NS

6.4 Discussion

Growth rate of species

Seaweeds

Although *A. nodosum, L. digitata,* and *F. vesiculosus* all showed growth over the first 28 days of the trial, the biomass of each of these species decreased over the remaining duration of the trial (23 days). Unfortunately, due to logistical constraints artificial lighting above the seaweed buckets were not installed until the 29th day of the trial. As this trial took place during winter (December/January), daylight hours were approximately 8-9 and the light intensity was only 20.4 ± 4.5 μmol m^{-2} s^{-1} at approximately 09:00 and 1.3 ± 0.4 μmol m^{-2} s^{-1} between 17:00 and 18:00 over the buckets, in comparison to the intensity an intensity of 63.7 ± 14.2 μmol m^{-2} s^{-1} over the buckets under artificial lighting (artificial lights were on from 09:00 to 18:00). This lack of sufficient light may have contributed considerably to the poor level of seaweed growth achieved, particularly for *F. serratus* and *P. canaliculata*, which only showed growth for the first nine days of the trial, and for *U. lactuca* which did not increase in biomass throughout the trial duration. Potential design flaws of these prototype maraponic systems may also have contributed to poor seaweed growth. The 20 L seaweed buckets used were quite tall and had a relatively small top diameter in relation to size of the bucket. Despite each bucket being aerated, it may have been the case that a sufficient tumble culture in each bucket was not achieved at that only a proportion of the seaweed was exposed to the light at the surface of the bucket at any one time. The seaweed from each bucket was manually tumbled twice a day, however, this may not have been sufficient. Utilising transparent buckets may also have improved the level of light reaching the seaweeds (note: it was attempted to obtain transparent buckets for this trial, however, it proved very difficult to do so). For future studies using these systems, it would be recommended to conduct trials during summer months and/or with artificial lighting for the whole duration of the trial. The bucket design was chosen for this study to maximise the number of species trialled and to allow for the effective assessment of each seaweed's growth. However, to maximise the surface area of the seaweed exposed to light, future studies could remove the seaweed buckets and cultivate in the entire top tank of the maraponic system. A number of different species

could be assessed at once by dividing the tank into different sections (e.g. with mesh-screens).

However, it is important to note that poor seaweed growth is a very complex problem and that the cultivation of seaweed, especially from wild, collected specimens, is a very difficult process. The systems used for this trial may not have replicated a natural environment effectively enough and a dump-bucket system, as described in Adey and Loveland, (2011). In such systems, the dump rate of water can be set to a rate that closely resembles natural wave action that the seaweeds would experience in their natural environment (Adey and Loveland, 2011). Also, throughout the trial, it was noted that for a number of the seaweed species (especially the *U. lactuca*) the seaweed was breaking apart into smaller pieces. This may have been the natural algal fragmentation process, whereby coenobic colonies or filaments break into fragments having the capacity to development into new individuals. The study of this process and how it impacts upon the growth of the selected seaweed species was beyond the scope of this trial (Barsanti and Gualtieri, 2014).

Mussels

Tagged *M. edulis* from the top and bottom tanks increased in length and weight by 0.002 ± 0.06 cm and 0.02 ± 0.04 cm, and 0.39 ± 0.53 g and 0.57 ± 0.93 g, respectively, over the 44 day monitored growth period. By comparison, a study by Garcia and Kamermans, (2013) found that mussels cultivated for 4 weeks in a recirculating aquaculture system (RAS) and a flow-through system (FTS) had an increase in length of 1.004 cm and 0.84 cm, respectively. A study by Stirling and Okumuş (1995) found that *M. edulis* cultivated in two Scottish lochs increased in length by a mean of 0.2 cm and 0.2 cm and weight increased by a mean of 0.82 g and 0.91 g per month when cultivated at a mussel farm and a *S. salar* farm, respectively.

Although total ammonia nitrogen (TAN) reached high concentrations during the trial, ranging from 0.11 to 5.9 mg/L (min/max levels of all systems), it was not certain if these levels negatively impacted the growth of *M. edulis*. Literature on the tolerance of *M. edulis* to TAN is limited, however, a study by Eggermont *et al*. (2014) found that their tolerance is high, with 90% of specimens surviving a TAN concentration of 25 mg/L over a 5 day period, consistent with the tolerance level of other bivalves (Eggermont *et al*. 2014; Epifanio and Srna, 1975). Unfortunately this study did not assess the impact of TAN concentrations on growth (or survival) over a longer period

of time, and the greater growth seen in the Garcia and Kamermans, (2013) study may have been due to the low TAN concentrations in their study of 0.05 ± 0.01 mg/L and 0.06 ± 0.02 mg/L for RAS and FTS, respectively. It may also have been due to the fact that the mussels were being fed a natural diet of microalgae, and the salmon waste provided in this trial may not have been sufficient for optimal growth (Garcia and Kamermans, 2013).

Abalone

Tagged *H. discus hannai* from the bottom tanks increased in length and weight by 0.15 ± 0.13 cm (c.0.003 cm per day) and 1.28 ± 1.33 g (c. 0.03 g), respectively, and tagged abalone from the top tanks decreased by 0.01 ± 0.14 cm (c. 0.0002 cm per day) and increased in weight by 0.35 ± 2.81 g (c. 0.008 g per day), respectively, over the 44 day monitored growth period. By comparison, a study by Park *et al*. (2008) found that *H. discus hannai* cultivated in a experimental-scale RAS over a 180 day period and fed a diet of *Undaria pinnatifada* and *Laminaria japonica*, experienced a mean shell length increase of 0.3 cm per month (c.0.00017 cm per day) and a mean weight increase of 5.35 g (c. 0.03 g per day). The higher/equal level of length/weight increase of the bottom tank abalone, for this study, was despite exposure to peaks of high ammonia and a lack of natural diet (i.e. macroalgae) for the duration of the trial (Bansemer *et al*. 2014; Garcia-Carreno *et al*. 2003; Mai *et al*. 1996; Hahn, 1989). Despite a lack of literature of *H. dicus hannai*'s tolerance to TAN, studies have found a variation in TAN tolerance levels for different species of abalone. For example, Reddy-Lopata *et al*. (2006) found that *H. midae*'s tolerance to ammonia increases with body size and that juveniles (most vulnerable size class) should be cultured at a TAN concentration of below 0.16 mg/L. Basuyaux and Mathieu (1999) found that a TAN concentration of 1 mg/L was safe for *H. tuberculata*, while a concentration of 5mg/L had a low level of toxicity. Harris *et al*. (1998) found significant reductions in length and weight of *H. laevigata* at TAN concentrations of 0.001 mg/L and 0.002 mg/L, respectively. These variations in results would suggest that TAN tolerance amongst abalone is species specific. Future studies should assess the TAN tolerance of *H. discus hannai*, however, the successful growth seen in this study would suggest that it has a relatively high level of tolerance.

Sea cucumber

H. forskali did not grow well in the maraponic systems, decreasing in weight over the duration of the trial. They also showed signs of stress throughout the trial, releasing cuvierian tubules and eviscerating on a number of occasions. The total mortality rate of sea cucumbers in this study was 26.67%. These observations would indicate that *H. forskali* were stressed by the conditions of the systems. Although there are no published studies on the TAN tolerance level of holothurians, studies have shown that the growth of Echinodermata species is negatively impacted by TAN concentrations above 1.55mg/L (Siikavuopio *et al*. 2004; Basuyaux and Mathieu, 1999).

Water parameters

At various points throughout the trial, concentrations of TAN, nitrite, nitrate, and phosphate peaked at the relatively high levels of 4.17, 2.22, 1.86, and 1.64 mg/L (mean of systems), respectively. These high levels may have been a result of a number/combination of the following reasons. (1) Insufficient biomass/ratio of inorganic and/or organic extractive species. (2) Poor design of seaweed buckets . (3) An unsuitable salmon waste model. The WinFish model used was designed for cage aquaculture and the calculated waste outputs may have been too high for small-scale maraponics systems despite the model input being as close to scale as possible. (4) Periodic water exchange and additional treatment of the water (e.g. mechanical filtration, sump tank) may be necessary.

Fatty acid analysis

Salmon waste

The origin of lipids in salmon feeds have traditionally been marine oils from pelagic fish (e.g. capelin and herring). Long chain MUFA, such as 20:1n-9 and 22:1n-11, originally from these marine sources, are documented to be typical of salmon farm waste (i.e. uneaten feed and/or faeces) (Johnsen *et al*. 2000; Henderson *et al*. 1997). In recent years, however, a higher level of plant oils have been incorporated into salmon

feeds and terrestrial sources naturally contain a higher concentration of the FAs 18:1n-9, 18:2n-6, and 18:3n-6 (Narváez *et al*. 2008; Dalsgaard *et al*. 2003; Skog *et al*. 2003). The FAs 20:1n-9, 22:1n-11, and 18:1n-9 were present in high amounts in the salmon waste from this trial, indicating that the pellets used to feed the salmon utilised for this trial had a high content of marine oils from pelagic fish, however, some plant oils may also present in the feed. Unfortunately, the FA contents of the feed utilised by the salmon for this trial was not known.

Periphyton

Published literature on the FA composition of marine periphyton are very limited, however, a study by Hanson *et al*. (2010), found that the periphyton from the leaves of *Posidonia sinuosa* obtained from the coastal waters of Jurien Bay Marine Park, Australia, was highest in SFA (49.45 ± 4.23%), followed by PUFA (26.17 ± 6.03%) and MUFA (20.88 ± 5.36%). By comparison, the periphyton from our study was highest in PUFA (43.10 ± 3.74%), followed by MUFA (26.45 ± 2.95%) and SFA (21.55 ± 1.78%). This is not surprising however, as periphyton consists of a diverse community of bacteria, viruses, fungi, algae, protozoans, and metazoans (Sanli *et al*. 2015) and the FA composition of periphyton will most likely be site and/or sample specific.

Mussels

The fatty acid (FA) composition of wild mussels (control) from this study was comparable with that reported in the published literature (Redmond *et al*. 2010; Alkanani *et al*. 2007; Murphy *et al*. 2002). There is a general agreement in the literature that phytoplankton (or microalgae) are the major source of essential fatty acids in the marine environment and can provide up to c. 40% of the FAs of *M. edulis* and other marine bivalves (Budge *et al*. 2000; Parrish *et al*. 1998; Pazos *et al*. 1997; Napolitano *et al*. 1992; Fernandez-Reiriz *et al*. 1989).

The lipid content of mussels from the top and bottom tanks were not significantly different from that of wild mussels ($p>0.05$). The most abundant FAs in both the maraponic (top and bottom tanks) and wild mussels were DHA, 16:0,, and EPA (all differences were non-significant, apart from 16:0; wild > bottom tank; $p<0.01$; appendix Table 12). Overall, PUFA was the most abundant of FAs present in wild and top and bottom tank mussels, followed by SFA, MUFA, DMA, and NMID, an indication that the overall FA composition of mussels were not substantially impacted

by being in the maraponic systems.

The salmon waste biomarker 22:1n-9 was significantly higher in the maraponic (top and bottom tank) than in wild mussels ($p<0.01$), while 22:1n-11 was significantly higher in the bottom tank than in wild mussels ($p<0.05$). This may be an indication that the mussels were assimilating salmon waste (note: DHA was quite high in wild mussels, subsequently, it is not a suitable salmon waste biomarker for *M. edulis*. It was also lower in the maraponic mussels). It must be noted, however, that there was no significant difference in all other salmon biomarkers between maraponic and wild mussels. Subsequently, evidence of mussels assimilating salmon waste in this study is far from conclusive. From this study, it is unclear to what extent *M. edulis* was utilising periphyton as a food source.

M. edulis specimens from this trial had a lower level of growth when compared with *M. edulis* cultivated on the cages of a salmon farm, and fed a natural microalgal diet (Garcia and Kamermans, 2013; Reid *et al*. 2008; Stirling and Okumuş, 1995). Zhukova *et al*. (1992) suggested that bivalves synthesise NMID when there is a deficiency in dietary unsaturated FAs. The 22:2 NMID FA was significantly higher ($p<0.05$) in top tank mussels (4.23 ± 1.28%) than wild mussels (3.07 ± 0.64%). The evidence from this study suggests that the maraponic mussels may have been assimilating the salmon waste, however, they may not have been receiving an adequate diet for optimal growth.

Abalone

The farmed *H. discus hannai* (control) from this study consumed a diet of *L. digitata*. Although we did not conduct FA analysis on the specific *L. digitata* that they were feeding upon (source: Atlantic; Galway coast), the wild *L. digitata* from this study (source: Atlantic; West Cork coast) was highest in 16:0 (19.88 ± 0.10%), 18:1n-9 (17.40 ± 0.19%), and EPA (17.12 ± 0.75%), which is consistent with that of other published studies (Mæhre *et al*. 2014; Peinado *et al*. 2014; Schmid *et al*. 2013; Chuecas and Riley, 1966). The FA profile of the farmed *H. discus hannai* showed that they were highest in 16:0 (12.16 ± 10.54%), 18:1n-7 (11.91 ± 0.63%), EPA (10.09 ± 2.73%), and 18:1n-9 (8.06 ± 0.70%), and was consistent with another study that fed *H. discus hannai* a diet of *L. digitata* (Mai *et al*. 1996). The evidence from this study and published literature confirm that the farmed abalone from this study consumed a diet of *L. digitata*.

The lipid content of abalone from the bottom tank was 0.39% ($p<0.05$) higher than the farmed abalone. The most abundant FAs in both the maraponic (top and bottom tanks)

and farmed abalone were 16:0, 18:1n-7, and EPA. The content of 18:1n-7 was significantly higher in farmed than maraponic (top and bottom tank) abalone ($p<0.001$). Overall, both farmed and bottom tank maraponic abalone was highest in PUFA, followed by MUFA, SFA, DMA, and NMID. Top tank abalone only varied slightly in the overall fatty acid composition, being highest in PUFA, followed by SFA, MUFA, DMA, and NMID. This would indicate that the FA composition of abalone was not substantially impacted by being in the maraponic systems.

There is strong evidence from this study that abalone were feeding upon salmon waste, with the biomarkers 20:1n-9, 22:1n-11, and DHA being significantly ($p<0.01$) higher in the bottom tank abalone than farmed abalone. DHA ($p<0.01$) was also higher in top tank than farmed abalone. The higher level of evidence from the bottom tank is not surprising, as this is the tank to which the salmon waste was added and most ended up accumulating here. DHA provides very strong evidence of salmon waste assimilation by maraponic abalone, as DHA was present at very low concentrations in farmed abalone ($0.09 \pm 0.08\%$), increasing to $0.88 \pm 0.35\%$ and $4.08 \pm 1.16\%$ in top and bottom tank abalone, respectively. The low level of DHA in abalone fed a natural diet is consistent with a study by Nelson *et al*. (2002).

It would also seem that maraponic abalone were feeding upon salmon waste as opposed to periphyton. The periphyton biomarker, 16:1n-7 was significantly lower in top ($p<0.001$) tank than farmed abalone. EPA was significantly higher ($p<0.05$) in the top tank than farmed abalone, which may be evidence of these abalone feeding upon periphyton. This may be as a result of less salmon waste accessing the top tank, and abalone feeding more on periphyton instead. However, it is important to note that EPA concentrations in farmed abalone were relatively high ($10.09 \pm 2.73\%$), so the use of EPA as a periphyton biomarker should be taken with caution.

From this study, it was unclear whether a diet of salmon waste was a sufficient source of food for the growth of abalone in the bottom tank. The growth rate of (surviving) abalone in the bottom tank was strong and they had a higher lipid content in comparison to farmed abalone. Bivalves synthesise NMID when there is a deficiency of unsaturated FAs in the diet (Zhukova *et al*. 1992) and NMID FA concentrations were significantly lower ($p<0.01$) in bottom tank than farmed abalone. Although the mortality rate of tagged abalone in the bottom tank of the systems was relatively high, at $26.67 \pm 23.09\%$ (i.e. 4 out of 15 specimens), overall mortality (tagged and non-tagged abalone from the bottom and top tank) was only 8.70%. It was not clear what proportion of the mortalities

was as a result of natural causes, assimilation of salmon waste or the high level of ammonia. Abalone are quite sensitive to handling stress, and the repeated handling for growth measurements may have contributed to the mortality rate of tagged abalone (Gavin Burnell, personal communication, 2015). Nevertheless, the fact that *H. discus hannai* displayed growth on a diet of salmon waste was surprising, as various studies have determined that algae are the predominant diet of abalone (Bansemer *et al*. 2014; Garcia-Carreno *et al*. 2003; Mai *et al*. 1996; Hahn, 1989).

Sea cucumbers

The most common FAs in both the intestine and body wall of wild *H. forskali* (control) from this study were PUFA (40.84 ± 6.42% and 40.08 ± 3.19%, respectively), followed by MUFA (31.71 ± 6.85% and 31.93 ± 1.27%) and SFA (19.67 ± 0.87% and 17.46 ± 2.81%). By comparison, a study by Allen (1968), found that *H. forskali* collected from the coast of Plymouth, England was highest in PUFA content, at 64.3% for the gut and 52.2% for the body wall. This was followed by SFA, at 16.7% and 25.2%, respectively, and MUFA, at 12.7% and 16.9%, respectively (Allen, 1968).

There was no significant difference in the lipid content of the intestine and body wall of maraponic compared to wild sea cucumbers ($p>0.05$). There was very little difference in the FA composition of the intestine and body wall of wild and maraponic sea cucumbers, with only the concentration of 16:3 showing a significant difference in the body wall of wild (0.57 ± 0.11%) and maraponic sea cucumbers (0.29 ± 0.29%) ($p<0.05$). Overall, the fatty acid composition of the intestine and body wall of both wild and maraponic sea cucumbers were highest in PUFA, followed by MUFA, SFA, and DMA, an indication that the FA composition of sea cucumbers was not substantially impacted by being in the maraponic systems.

There was no significant difference in salmon waste or periphyton biomarkers between maraponic and wild sea cucumbers ($p>0.05$) (note: the periphyton biomarker, EPA, was quite high in the intestine and body wall of wild sea cucumbers, subsequently, it is not suitable periphyton biomarker for sea cucumbers). There was no significant difference in the concentration of any biomarker in the intestine or body wall of wild and FA sea cucumbers. The sea cucumbers showed signs of stress and had poor growth rates over the duration of the trial. Combined with the FA evidence of this study, it would appear that sea cucumbers were not receiving an adequate diet and/or were too stressed to feed effectively.

Seaweeds

The FA composition of all wild seaweed species (controls) from this study was comparable with that reported in the published literature, being particularly high in levels of PUFA for the majority of species (Mæhre *et al*. 2014; Peinado *et al*. 2014; Monroig *et al*. 2013; Van Ginneken *et al*. 2011; Kumari *et al*. 2010; Ortiz *et al*. 2006: Herbreteau, 1997; Fleurence *et al*. 1994; Jones and Harwood, 1992; Smith and Harwood, 1984; Munda, 1977; Chuecas and Riley, 1966).

With the exception of maraponic *U. lactuca*, which had no significant difference ($p>0.05$) in lipid content compared to its wild counterpart, all other seaweeds from the maraponic systems had a lower lipid content than their wild counterpart, with *A nodosum*, *L. digitata*, *F. vesiculosus*, *F. serratus*, and *P. canaliculata* being 0.54% ($p<0.05$), 0.43% ($p<0.05$), 1.61% ($p>0.05$), 0.52% (<0.01), and 1.47% ($p<0.001$) lower, respectively. The order of FA categories (i.e. SFA, MUFA, PUFA, and Furan FA) from highest to lowest concentration, of maraponic *A. nodosum* and *L. digitata* were the same as their wild counterparts. The FA composition of all other maraponic seaweed species differed from their wild counterparts, mainly as a result of relatively large changes (reductions and/or increases) to concentrations of MUFA and PUFA. It is clear from the reduction in lipid content (exception – *U. lactuca*), alterations to the FA composition, and poor growth rates, that the conditions of the maraponic systems in this study were not suitable for the growth of these species of seaweed.

ω6/ω3 ratio

The ω6/ω3 ratios of all species from the maraponic systems, by the end of the trial, were well within the limits recommended by the WHO for a healthy diet (i.e. <10:1) (Stabili *et al*. 2012; Simopoulos, 2008; Ortiz *et al*. 2006).

6.5 Conclusion

Further work is required to identify a suitable ratio/biomass of extractive and inorganic species for the efficient biofiltration of the water. The need for additional filtration and water exchange should also be assessed. In Ireland, the regulations surrounding experimentation with vertebrates are very strict (and getting more restrictive). Therefore, the need to model the presence of vertebrate species in IMTA trials may

become increasingly necessary. This study demonstrates the potential of FA analysis to investigate the performance of Aquaponic systems. Yet, due to the similarity of their FA composition and resulting biomarkers, future studies should consider the addition of salmon feed and faeces in different systems in order to determine the effects of them separately.

6.6 Acknowledgements

Enya O' Reilly tagged and monitored the growth of abalone and mussels. She also assisted in monitoring water parameters. Teresa Fernández (University of Stirling) performed the fatty acid analysis of salmon waste, mussels, abalone, and sea cucumbers. Prof. Burnell gave advise on the design of this study and provided feedback on the chapter. A big thanks to all the staff at the Institute of Aquaculture, University of Stirling, for their assistance in performing FA analysis of the seaweed species and periphyton. A particular thank you to Matthew Sprague, James Dick, Douglas Tocher, Mónica Betancor, and all the laboratory staff at the Institute of Aquaculture, without your assistance, guidance, and advice, the FA component of this chapter would not have been possible. Finally, thank you to Luke Harman (UCC) for constructing the maraponic systems and providing technical assistance throughout this trial. Cindy O' Brien (Abalone Chonamara Ltd.) provided the abalone for this trial and Colin Hannon (Abalone Chonamara Ltd.) transported them from Galway to UCC.

6.7 References

Ackman, R.G. (2002). The gas chromatography in practical analysis of common and uncommon fatty acids for the 21st century. *Analytica Chimica Acta*, 465, pp. 175-192.

Adey, W.H., and Loveland, K. (2011). *Dynamic Aquaria: Building Living Ecosystems* (3rd Ed.), Academic Press, London, UK, p. 528.

Alkanani, T., Parrish, C.C., Thompson, R.J., and McKenzie, C.H. (2007). Role of fatty acids in cultured mussels, *Mytilus edulis*, grown in Notre Dame Bay, Newfoundland. *Journal of Experimental Marine Biology and Ecology*, 348, pp. 33-45.

Allen, W.V. (1968). Fatty-acid synthesis in echinoderms: *Asterias rubens*, *Echinus esculentus* and *Holothuria forskali*. *Journal of the Marine Biological Association of the United Kingdom*, 48, pp. 521-533.

Anthony, J.L., Kesler, D.H., Downing, W.L., and Downing, J.A. (2001). Length-specific growth rates in freshwater mussels (Bivalvia: Unionidae): extreme longevity or generalized growth cessation? *Freshwater Biology*, 46, pp. 1349-1359.

Bansemer, M.S., Qin, J.G., Harris, J.O., Howarth, G.S., and Stone, D.A. (2014). Nutritional requirements and use of macroalgae as ingredient in abalone feed. *Reviews in Aquaculture*, 8(2), pp. 121-135.

Barrington, K., Chopin, T., and Robinson, S., (2009). Integrated multi-trophic aquaculture (IMTA) in marine temperate waters. In D., Soto. (eds). *Integrated mariculture: a global review*. FAO Fisheries and Aquaculture Technical Paper, 529, FAO Rome, pp. 7–46.

Barsanti, L., and Gualtieri, P. (2014). *Algae: Anatomy, Biochemistry, and Biotechnology,* (2nd Ed.). CRC Press, Boca Raton, Florida, USA, p. 361.

Basuyaux, O., and Mathieu, M. (1999). Inorganic nitrogen and its effect on the growth of the abalone *Haliotis tuberculata* Linnaeus and the sea urchin *Paracentrotus lividus* Lamarck. *Aquaculture*, 174, pp. 95-107.

Bergé, J., and Barnathan, G. (2005). Fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. *Marine Biotechnology 1 – Advances in Biochemical Engineering/Biotechnology*, 96, pp. 49-125.

Both, A., Parrish, C., Penney, R.W., and Thompson, R.J. (2011). Lipid composition of *Mytilus edulis* reared on organic waste from a *Gadus morhua* aquaculture facility. *Aquatic Living Resources*, 24, pp. 295-301.

Boxman, S.E., Nystrom, M., Capodice, J.C., Ergas, S.J., Main, K.L., and Trotz, M.A. (2016). Effect of support medium, hydraulic loading rate and plant density on water quality and growth of halophytes in marine aquaponic systems. *Aquaculture Research*, 48(5), pp. 2463-2477.

Britz, P.J. (1996). The suitability of selected protein sources for inclusion in formulated diets for the South African abalone, *Haliotis midae*. *Aquaculture*, 140, pp. 63-73.

Budge, S.M., Parrish, C.C., Thompson, R.J., and McKenzie, C.H. (2000). Fatty acids in phytoplankton in relation to bivalve dietary requirements. In: *Seafood in Health and Nutrition*; Shahidi, F., Ed.; ScienceTech Publishing Company, St. John's, Canada, pp. 495-520.

Buhmann, A.K., Waller, U., Wecker, B., and Papenbrock, J. (2015). Optimisation of culturing conditions and selection of species for the use of halophytes as biofilter for nutrient-rich saline water. *Agricultural Water Management*, 149, pp. 102–114.

Buhmann, A., and Papenbrock, J. (2013). An economic point of view of secondary compounds in halophytes. *Functional Plant Biology*, 40, pp. 952–967.

Burridge, L., Weis, J.S., Cabello, F., Pizarro, J., and Bostick, K. (2010). Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. *Aquaculture*, 306, pp 7–23.

Chopin, T., Buschmann, A.H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G.P., Zertuche-González, J.A., Yarish, C., and Neefus, C. (2001). Integrating seaweeds into marine aquaculture systems: A key toward sustainability. *Journal of Phycology*, 37, pp. 975–986.

Christie, W.W. (2003). *Lipid analysis: isolation, separation, identification, and structural analysis of lipids* (3rd ed.) Oily Press, Bridgwater, Somerset, p. 416.

Chuecas, L., and Riley, J.P. (1966). The component fatty acids of some sea-weed fats. *Journal of the Marine Biological Association of the United Kingdom*, 46, pp. 153-159.

Costa-Pierce, B.A. (2002). Ecology as the paradigm for the future of aquaculture. In *Ecological Aquaculture: The Evolution of the Blue Revolution*; Costa-Pierce, B.A., Ed.; Blackwell Science: Malden, MA, USA, pp. 339–372.

Cubillo, A.M., Ferreira, J.G., Robinson, S.M.C., Pearce, C.M., Corner, R.A., and Johansen. (2016). Role of deposit feeders in integrated mulit-trophic aquaculture – A model analysis. *Aquaculture*, 453, pp. 54-66.

Dalsgaard, J., St. John, M., Kattner, G., Müller-Navarra, D., and Hagen, W. (2003). Fatty acid trophic markers in the pelagic marine environment. *Advances in Marine Biology*, 46, pp. 225-340.

DeBusk, T.A., Blakeslee, M., and Ryther, J.H. (1986). Studies on the outdoor cultivation of *Ulva lactuca* L. *Botanica Marina*, 29, pp. 381-386.

Dufault, R.J., Korkmaz, A., and Ward, B. (2001). Potential of biosolids from shrimp aquaculture as a fertiliser for broccoli production. *Compost Science and Utilization*, 9, pp. 107–114.

Dufault, R.J., and Korkmaz, A. (2000). Potential of biosolids from shrimp aquaculture as a fertiliser in bell pepper production. *Compost Science and Utilisation*, 3, pp. 310–319.

Eggermont, M., Tamanji, A., Nevejan, N., Bossier, P., Sorgeloos, P., and Defoirdt, T. (2014). Stimulation of heterotrophic bacteria associated with wild-caught blue mussel (*Mytilus edulis*) adults results in mass mortality. *Aquaculture*, 431, pp. 136-138.

Epifanio, C.E., and Sma, R.F. (1975). Toxicity of ammonia, nitrite ion, nitrate ion, and orthophosphate to *Mercenaria mercenaria* and *Crassostrea virginica*. *Marine Biology*, 33, pp. 241-246.

European Union. (2009). Commission Regulation (EC) No 710/2009. Available online: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32009R0710 (Accessed on: 23/01/14).

Fernandez-Reiriz, M.J., Perez-Camacho, A., Ferreiro, M.J., Blanco, J., Planas, M., Campos, M.J., and Labarta, U. (1989). Biomass production and variation in the biochemical profile (total protein, carbohydrates, RNA, lipids and fatty acids) of seven species of marine microalgae. *Aquaculture*, 83, pp. 17-37.

Ferreira, J.G., Falconer, L., Kittiwanich, J., Ross, L., Saurel, C., Wellman, K., Zhu, C.B., and Suvanachai, P. (2014). Analysis of production and environmental effects of Nile tilapia and white shrimp culture in Thailand. *Aquaculture,* 447, pp. 23-36.

Ferreira, J.G., Saurel, C., and Ferreira, J.M. (2012). Cultivation of gilthead bream in monoculture and integrated multi-trophic aquaculture. Analysis of production and environmental effects by means of the FARM model. *Aquaculture*, 358-359, pp. 23-34.

Fleurence, J., Gutbier, G., Mabeau, S., and Leray, C. (1994). Fatty acids from 11 marine macroalgae of the French Brittany coast. *Journal of Applied Phycology*, 6, pp. 527-532.

Folch, J., Lees, M. and Sloane-Stanley, G. (1957), A simple method for the isolation and purification of total lipids from animal tissues. *The Journal of Biological Chemistry*, 226(1), pp. 497-509.

Food and Agriculture Organisation of the United Nations. (2016). The State of World Fisheries and Aquaculture 2016: *Contributing to Food Security and Nutrition for All*; FAO, Rome, Italy. p. 200.

Fronte, B., Galliano, G., and Bibbiani, C. (2016). From freshwater to marine aquaponic: New opportunities for marine fish species production. In: Proceedings of the 4th VIVUS Conference on Agriculture, Environmentalism, Horticulture and Floristics, *Food Production and Processing and Nutrition*, Naklo, Slovenia, 20–21st April 2016.

Gao, Q-F., Shin, P.K.S., Lin, G-H., Chen, S-H., and Cheung, S.G. (2006). Stable isotope and fatty acid evidence for the uptake of organic waste by green-lipped mussels *Perna viridis* in a polyculture fish farm system. *Marine Ecology Progress Series*, 317, pp. 273-283.

Garcia, A.B., and Kamermans, P. (2015). Optimization of blue mussel (*Mytilus edulis*) seed culture using recirculation aquaculture systems. *Aquaculture Research*, 46(4), pp. 977-986.

Garcia-Carreno, F., Del Toro, M.N., and Serviere-Zaragoza, E. (2003). Digestive enzymes in juvenile green abalone, *Haliotis fulgens*, fed natural food. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 134(1), pp. 143-150.

Go, J.V., Řezanka, T., Srebnik, M., and Dembitsky, V.M. (2002). Variability of fatty acid components of marine and freshwater gastropod species from the littoral zone of the Red Sea, Mediterranean Sea, and Sea of Galilee. *Biochemical Systematics and Ecology*, 30, pp. 819-835.

Granada, L., Sousa, N., Lopes, S., and Lemos, M.F.L. (2016). Is integrated multitrophic aquaculture the solution to the sectors' major challenges? - A review. *Reviews in Aquaculture*, 8, pp. 283–300.

Guest, M., Hirst, A., Nichols, P., and Frusher, S. (2010). Multi-scale spatial variation in stable isotope and fatty acid profiles amongst temperate reef species: implications for design and interpretation of trophic studies. *Marine Ecology Progress Series*, 410, pp. 25-41.

Hahn, K.O. (1989). Nutrition and growth of abalone. In: *Handbook of culture of abalone and other marine gastropods*; Hahn, K.O., Ed.; CRC Press, Boca Raton, Florida, USA, pp. 135-156.

Halpin, P.M., Menge, B.A., and Hofmann, G.E. (2004). Experimental demonstration of plasticity in the heat shock response of the intertidal mussel *Mytilus californianus*. *Marine Ecology Progress Series*, 276, pp. 137-145.

Handå, A., Ranheim, A., Olsen, A.J., Altin, D., Reitan, K.I., Olsen, Y., and Reinertsen, H. (2012). Incorporation of salmon fish feed and faeces components in mussels (*Mytilus edulis*): implications for integrated multi-trophic aquaculture in cool-temperate North Atlantic waters. *Aquaculture*, 370-371, pp. 40-53.

Hanson, C.E., Hyndes, G.A., and Wang, S.F. (2010). Differentiation of benthic marine primary producers using stable isotopes and fatty acids: implications to food web studies. *Aquatic Botany*, 93, pp. 114-122.

Harris, J.O., Maguire, G.B., Edwards, S., and Hindrum, S.M. (1998). Effect of ammonia on the growth rate and oxygen consumption of juvenile greenlip abalone *Haliotis laevigata* Donovan. *Aquaculture*, 160, pp. 259-272.

Henderson, R.J., Forrest, D.A.M., Black, K.D., and Park, M.T. (1997). The lipid compostion of sealoch sediments underlying salmon cages. *Aquaculture*, 158, pp. 69-83.

Herbreteau, F., Coiffard, L.J.M., Derrien, A., and De Roeck-Holtzhauer, Y. (1997). The fatty acid composition of five species of macroalgae. *Botanica Marina*, 40, pp. 25-27.

Irisarri, J., Fernández-Reiriz, M.J., Labarta, U., Cranford, P.J., and Robinson, S.M.C (2015). Availability and utilisation of waste fish feed by mussels, *Mytilus edulis*, in a commercial integrated multi-trophic aquaculture (IMTA) system: A multi-indicator assessment approach. *Ecological Indicators*, 48, pp. 673-686.

Jensen, M.H. (1997). Hydroponics worldwide—A technical overview. *International Symposium on Growing Media and Hydroponics*, 481, pp. 719–730.

Joesting, H.M., Blaylock, R., Biber, P., and Ray, A. (2016). The use of marine aquaculture solid waste for nursery of salt marsh plants *Spartina alterniflora* and *Juncus roemerianus*. *Aquaculture Reports*, 3, pp. 108–114.

Johnson, R.I., Grahl-Nielsen, O., and Roem, A. (2000). Relative absorption of fatty acids by Atlantic salmon *Salmo salar* from different diets, as evaluated by multivariate statistics. *Aquaculture Nutrition*, 6, pp. 255-261.

Jones, J.B., Jr. (2005). Hydroponics—A Practical Guide for the Soilless Grower, 2nd ed.; CRC Press: Boca Raton, FL, USA, p. 440.

Jones, A.L., and Harwood, J.L. (1992). Lipid composition of the brown algae *Fucus vesiculosus* and *Ascophyllum nodosum*. *Phytochemistry*, 31(10), pp. 3397-3403.

Kang, C.K., Sauriau, P.G., Richard, P., and Blanchard, G.F. (1999). Food sources of infaunal suspension-feeding bivalve *Cerastoderma edule* in a muddy sandflat of Marennes-Oléron Bay, as determined by analyses of carbon and nitrogen stable isotopes. *Marine Ecology Progress Series*, 145, pp. 147-158.

Kelly, J.R., and Scheibling, R.E. (2011). Fatty acids as dietary tracers in benthic food webs. *Marine Ecology Progress Series*, 446, pp. 1-22.

Knoph, M.B., and Thorud, K. Toxicity of ammonia to Atlantic (*Salmo salar* L.) in seawater – effects on plasma osmolality, ion, ammonia, urea, and glucose levels and hematologic parameters. *Comparative Biochemistry and Physiology Part A: Physiology*, 113(4), pp. 375-381.

Knoph, M.B. (1992). Acute toxicity of ammonia to Atlantic salmon (*Salmo salar*) parr. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*, 101(2), pp. 275-282.

Kolarević, J. (2012). Water quality requirements for optimal growth of Atlantic salmon smolt in recirculating aquaculture systems (RAS). *Fremtidens Smoltproduksjon, Andre Konferanse om Resirkulering av vann i Akvakultur*, Sunndalsøra, 23rd – 24th October, 2012.

Kumari, P., Kumar, M., Gupta, V., Reddy, C.R.K., and Jha, B. (2010). Tropical marine macroalgae as potential sources of nutritionally important PUFAs. *Food Chemistry*, 120, pp. 749-757.

Lakkireddy, K.K.R., Kasturi, K., and Sambasiva Rao, K.R.S. (2012). Role of hydroponics and aeroponics in soilless culture in commercial food production. *Research and Reviews: Journal of Agricultural Science and Technology*, 1, pp. 26–35.

Lehane, C., and Davenport, J. (2004). Ingestion of bivalve larvae by *Mytilus edulis*: experimental and field demonstrations of larviphagy in farmed blue mussels. *Marine Biology*, 145, pp. 101-107.

Mai, K., Mercer, J.P., and Donlon, J. (1996). Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. V. The role of polyunsaturated fatty acids of macroalgae in abalone nutrition. *Aquaculture*, 139, pp. 77-89.

Mæhre, H.K., Malde, M.K., Eilertsen, K-E., and Elvevoll, E.O. (2014). Characterization of protein, lipid, and mineral contents in common Norwegian seaweeds and evaluation of their potential as food. *Journal of the Science of the Science of Food and Agriculture*, 94, pp. 3281-3290.

Monroig, Ó., Tocher, D.R., and Navarro, J.C. (2013). Biosynthesis of polyunsaturated fatty acids in marine invertebrates: recent advances in molecular mechanisms. *Marine Drugs*, 11, pp. 3998-4018.

Munda, I.M. (1977). Differences in amino acid composition of estuarine and marine fucoids. *Aquatic Botany*, 3, pp. 273-280.

Murphy, K.J., Mooney, B.D., Mann, N.J., Nichols, P.D., and Sinclair, A.J. (2002). Lipid, FA, and sterol composition of New Zealand green lipped mussel (*Perna canaliculus*) and Tasmanian blue mussel (*Mytilus edulis*). *Lipids*, 37, pp. 587-595.

Napolitano, G.E., MacDonald, B.A., and Thompson, R.J. (1992). Lipid composition of eggs and adductor muscle in the giant scallops (*Placopecten magellanicus*) from different habitats. *Marine Biology*, 113, pp. 71-76.

Narváez, M., Freites, L., Guevara, M., Mendoza, J., Guderley, H., Lodeiros, C.J., and Salazar, G. (2008). Food availability and reproduction affects lipids and fatty acid composition of the brown mussel, *Perna perna*, raised in suspension culture. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 149(2), pp. 293-302.

Naylor, R., and Burke, M. (2005). Aquaculture and ocean resources: Raising tigers of the sea. *Annual Review of Environment and Resources*, 30, pp. 185–218.

Nelson, M.M., Leighton, D.L., Phleger, C.F., and Nichols, P.D. (2002).Comparison of growth and lipid composition in the green abalone, *Haliotis fulgens*, provided specific macroalgae diets. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 131(4), pp. 695-712.

Neori, A., Chopin, T., and Troell, M. (2004). Integrated aquaculture: Rationale, evolution, and state of the art emphasizing seaweed biofiltration in modern Mariculture. *Aquaculture*, 231, pp. 361–391.

Nozzi, V., Parisi, G., Di Crescenzo, D., Giordano, M., and Carnevali, O. (2016). Evaluation of *Dicentrarchus labrax* meats and the vegetable quality of *Beta vulgaris* var. *cicla* farmed in freshwater and saltwater aquaponic systems. *Water*, 8(423), pp. 1-14.

Ortiz, J., Romero, N., Robert, P., Araya, J., Lopez-Hernández, J., Bozzo, C., Navarrete, E., Osorio, A., and Rios, A. (2006). Dietary fiber, amino acid, fatty acid, and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea Antarctica*. *Food Chemistry,* 99(1), pp. 98-104.

Park, J., Kim, P-K., and Jo, J-Y. (2008). Growth performance of disk abalone *Haliotis discus hannai* in pilot- and commercial-scale recirculating aquaculture systems. *Aquaculture International*, 16, pp. 191-202.

Parrish, C.C., Wells, J.S., Yang, Z., and Dabinett, P. (1998). Growth and lipid composition of scallop juveniles *Placopecten magellanicus* fed the flagellates *Isochrysis galbana* with varying lipid compositions and the diatom *Chaetoceros muelleri*. *Marine Biology*, 133, pp. 461-471.

Pazos, A.J., Roman, G., Acosta, C.P., Sanchez, J.L., and Abad, M. (1997). Lipid classes and fatty acid composition of *Pecten maximus* in relation to reproductive cycle and environmental variables. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 117(3), pp. 393-402.

Peinado, I., Girón, J., Koutsidis, G., and Ames, J.M. (2014). Chemical composition, antioxidant activity and sensory evaluation of five different species of brown edible seaweed. *Food Research International*, 66, pp. 36-44.

Pereira, H., Barreira, L., Figueiredo, F., Custódio, L., Vizetto-Duarte, C., Polo, C., Rešek, E., Engelen, A., and Varela, J. (2012). Polyunsaturated fatty acids of marine macroalgae: potential for nutritional and pharmaceutical applications. *Marine Drugs*, 10, pp. 1920-1935.

Petersen J.K., Bougrier, S., Smaal, A.C., Garen, P., Robert, S., Larsen, J.E.N, and Brummelhuis, E. (2004). Intercalibration of mussel *Mytilus edulis* clearance rate measurements. *Marine Ecology Progress Series*., 267, pp. 187-194.

Pleissner, D., Eriksen, N.T., Lundgreen, K., and Riisgård, H.U. (2012). Biomass compostion of blue mussels, *Mytilus edulis*, is affected by living site and species of ingest microalgae. *International Scholarly Research Notices – Zoology*, 2012, pp. 1-12.

Reddy-Lopata, K., Auerswald, L., and Cook, P. (2006). Ammonia toxicity and its effect on the growth of the South African abalone *Haliotis midae* Linnaeus. *Aquaculture*, 261, pp. 678-687.

Redmond, K.J., Magnesen, T., Hansen, P.K., Strand, Ø., and Meier, S. (2010). Stable isotopes and fatty acids as tracers of the assimilation of salmon fish feed in blue mussels (*Mytilus edulis*). *Aquaculture*, 298, pp. 202-210.

Reid, G.K., Robinson, S.M.C., Chopin, T.R., Mullen, J., Lander, T., Sawhney, M., MacDonald, B.A., Haya, K., Burridge, L., Page, F., Ridler, N., Boyne-Travis, S., Sewester, J., Marvin, R., Szmerda, M., and Powell, E. (2008). Recent developments and challenges for open water, Integrated Multi-trophic Aquaculture (IMTA) in the Bay of Fundy, Canada. *Proceedings of Canadian Freshwater Symposium, Aquaculture Canada,* pp. 1-12.

Rezanka, T. (1989). Very long-chain fatty acids from the animal and plant kingdoms. *Progress in Lipid Research*, 28, pp. 147-187.

Sanli, K., Bengtsson-Palme, J., Nilsson, R.H., Kristiansson, E., Rosenblad, M.A., Blank, H., and Eriksson, K.M. (2015). Metagenomic sequencing of marine periphyton: taxonomic and functional insight into biofilm communities. *Frontiers in Microbiology*, 6. DOI: 10.3389/fmicb/2015.01192.

Schmid, M., Guihéneuf, F., and Stengel, D.B. (2013). Fatty acid contents and profiles of 16 macroalgae collected form the Irish coast at two seasons. *Journal of Applied Phycology*, 26(1), pp. 451-463.

Shpigel, M., Ragg, N.L., Lupatsch, I., and Neori, A. (1999). Protein content determines the nutrional value of the seaweed *Ulva lactuca* L for the abalone *Haliotis tuberculata* L. and *H, discus hanni* INO. *Journal of Shellfish Research*, 18(1), pp. 227-233.

Siikavuopio, S.I., Dale, T., Foss, A., and Mortensen, A. (2004). Effects of chronic ammonia exposure on gonad growth and survival in green sea urchin *Strongylocentrotus droeachiensis*. *Aquaculture*, 242, pp. 313-320.

Simopoulos, A.P. (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Experimental biology and medicine*, 233(6), pp. 674-688.

Singh, D., Buhmann, A. K., Flowers, T. J., Seal, C. E., and Papenbrock, J. (2014). *Salicornia* as a crop plant in temperate regions: selection of genetically characterised ecotypes and optimization of their cultivation conditions. *AoB PLANTS*, 6(plus071); doi:10.1093/aobpla/plu071.

Skog, T-E., Hylland, K., Torstensen, B.E., Bernstssen, M.H.G. (2003). Salmon farming affects the fatty acid composition and taste of wild saithe *Pollachius virens* L. *Aquaculture Research*, 34, pp. 999-1007.

Smith, K.L., and Harwood, J.L. (1984). Lipids and lipid metabolism in the brown alga, *Fucus serratus*. *Phytochemistry*, 23(11), pp. 2469-2473.

Solomon, M. (2007). Fishless cycling – ammonia required. Available online: http://www.fishforums.net/aquarium-calculator.htm (accessed on 29/04/15).

Stabili, L., Acquaviva, M.I., Biandolino, F., Cavillo, R.A., De Pascali, S.A., Fanizzi, F.P., Narracci, M., Petrocelli, A., and Cecere, E. (2012). The lipidic extract of the seaweed *Gracilariopsis longissima* (Rhodophyta, Gracilariales): a potential resource for biotechnological purposes? *New Biotechnology*, 29(3), pp. 443-450.

Stirling, H.P., and Okumuş, İ. (1995). Growth and production of mussels (*Mytilus edulis* L.) suspended at salmon cages and shellfish farms in two Scottish sea lochs. *Aquaculture*, 134, pp. 193-210.

Stowasser, G., Pierce, G.J., Moffat, C.F., Collins, M.A., and Forsythe, J.W. (2006). Experimental study on the effect of diet on fatty acid and stable isotope profiles of the squid *Lolliguncula brevis*. *Journal of Experimental Marine Biology and Ecology*, 333, pp. 97-114.

Su, X.Q., Antonas, K.N., and Li, D. (2004). Comparison of n-3 polyunsaturated fatty acid contents of wild and cultured Australian abalone. *International Journal of Food Sciences and Nutrition.* 55(2), pp. 149-154.

Subbaramaiah, K. (1970). Growth and reproduction of *Ulva fasciata* Debile in nature and in culture. *Botanica Marina*, 13, pp. 25-27.

Troell, M., Joyce, A., Chopin, T., Neori, A., Buschmann, A.H., and Fang, J.G. (2009). Ecological engineering in aquaculture—Potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. *Aquaculture*, 297, pp. 1–9.

Troell, M., Halling, C., Neori, A., Chopin, T., Buschmann, A.H., Kautsky, N., and Yarish, C. (2003). Integrated mariculture: Asking the right questions. *Aquaculture*, 226, pp. 69–90.

Tur, J.A., Bibiloni, M.M., Sureda, A., and Pons, A. (2012). Dietary sources of omega 3 fatty acids: public health risks and benefits. *British Journal of Nutrition,* 107, pp. S23-S52.

Turcios, A. E., and Papenbrock, J. (2014). Sustainable treatment of aquaculture effluents – what can we learn from the past for the future? *Sustainability*, 6, pp. 836-856.

Van Ginneken, V.J.T., Helsper, J.P.F.G., de Visser, W., van Keulen, H., and Brandenburg, W.A. (2011). Polyunsaturated fatty acids in various macroalgal species from north Atlantic and tropical seas. *Lipids in Health and Disease*, 10(104), pp. 1-8.

Ventura, Y., and Sagi, M. (2013). Halophyte crop cultivation: The case for *Salicornia* and *Sarcocornia*. *Environmental and experimental botany*, 92, pp. 144-153.

Waller, U., Buhmann, A.K., Ernst, A., Hanke, V., Kulakowski, A., Wecker, B., Orellana, J., and Papenbrock, J. (2015). Integrated multi-trophic aquaculture in a zero-exchange recirculation aquaculture system for marine fish and hydroponic halophyte production. *Aquaculture International,* 23, pp. 1473–1489.

Wen, B., Gao, Q-F., Dong, S-L., Hou, Y-R., Yu, H-B., and Li, W-D. (2016a). Effects of dietary inclusion of benthic matter on feed utilisation, digestive and immune enzyme activities of sea cucumber *Apostichopus japonicus* (Selenka). *Aquaculture*, 458, pp. 1-7.

Wen, B., Gao, Q-F., Dong, S-L., Hou, Y-R., Yu, H-B., and Li, W-D. (2016b). Uptake of benthic matter by sea cucumber *Apostichopus japonicus* (Selenka): insights from carbon stable isotopes and fatty acid profiles. *Journal of Experimental Marine Biology and Ecology*, 474, pp. 46-53.

Wilson, G. (2005). Seaweed is the common denominator in exciting saltwater aquaponics. *Aquaponics Journal*, 36, pp. 12–16.

Wolfensohn, S., and Lloyd, M. (2013). Ethical considerations around using animals in research. In: Handbook of Laboratory Management and Animal Welfare, 4th Edition. *Wiley-Blackwell, Chichester, West Sussex*, pp. 7-18.

Zhao, L., Yang, F., and Yan, X. (2013). Stable isotopes and fatty acids as dietary tracers of intertidal bivalves. *Fisheries Science*, 79, pp. 749-756.

Zhukova, N.V., Kharlamenko, V.I., Svestashev, V.I., and Rodionov, I.A. (1992). Fatty acids as markers of bacterial symbionts of marine bivalve molluscs. *Journal of Experimental Marine Biology and Ecology*, 162, pp. 253-263.

Chapter 7

Integrated multitrophic aquaculture and resource efficiency in European mariculture: a case study from an Irish organic salmon farm

Abstract

Integrated Multi-Trophic Aquaculture (IMTA) has the potential to help aquaculture achieve environmental sustainability through bio-mitigation of aquaculture wastes or by providing the farmer with higher levels of economic stability through product diversification, risk reduction, or by generating revenue from nutrients that would have otherwise been lost. In 2012, a European FP7 funded project (IDREEM: Increasing Industrial Resource Efficiency in European Mariculture) was launched to investigate the feasibility of developing commercial-scale IMTA systems to assist the European aquaculture industry in adopting more environmentally and economically efficient practises. This chapter details practical experiences and theoretical considerations related to trials of cultivating co-cultured species (*Alaria esculenta* and *Saccharina latissima*) next to an operational organic salmon farm as part of the IDREEM project. Due to the small-scale nature of these trials, it was not possible to ascertain the extent to which the seaweed was uptaking excess nutrients. However, this study showed that both seaweed species could achieve a high level of biomass generation in both the IMTA and control (1 km upstream of salmon farm) locations and that locating seaweed longlines in close-proximity to the cages did not negatively impact upon growth. From conducting this trial, BMRS identified five main steps that would need to be taken to produce seaweed as a co-cultured crop on a commercial scale: (1) positioning of the seaweed longlines next to the salmon cages in a location that receives the optimal flow of nutrients from the farm; (2) Acquisition of a dedicated boat for maintaining and harvesting the seaweed longlines; (3) Development of on-shore seaweed processing facilities; (4) Identification of a market for the co-cultured seaweed and (5) Hiring of dedicated staff.

7.1 Introduction

Marine aquaculture farms can discharge large volumes of wastewater containing excreta, food waste, and dissolved metabolites such as organic matter, inorganic nitrogen, and phosphorous into surrounding waters, which has the potential to damage ecosystems and also negatively impact upon other stakeholders of these water-bodies, such as tourism and fisheries. High levels of suspended organic solids can damage the gills of cultured and wild organisms. Also, it is estimated that 52-95% of the nitrogen and 85% of the phosphorous input to marine aquaculture systems may be lost to the environment through feed wastage, faeces production, and fish excretion (Granada *et al*. 2016; Buhmann and Papenbrock, 2013; Webb *et al*. 2012; Grigorakis and Rigos, 2011; Marinho-Soriano *et al*. 2011; Primavera, 2006; Zhou *et al*. 2006; Brown *et al*. 1999). Despite this, the most commonly practiced waste management solution for cage aquaculture is to release untreated effluent into surrounding waters, having a potential negative impact on the ocean floor extending 30-150m in diameter from the farm (Naylor and Burke, 2005; Naylor *et al*. 2003; Bridger and Garber, 2002; Brown, 2002). IMTA allows for the fed aquaculture species' uneaten feed, waste, nutrients and by-products to be recaptured and converted into feed, fertiliser and energy for the other crops (Hannah *et al*. 2013; Chopin *et al*. 2012). IMTA has the potential to help aquaculture farm operators achieve environmental sustainability through bio-mitigation of aquaculture wastes and can also provide the farmer with economic stability through product diversification, risk reduction, eco-tourism, eco-labelling, and by generating revenue from nutrients that would have otherwise been lost (Ma *et al*. 2013; Klinger and Naylor, 2012; Roheim *et al*. 2011; Troell *et al*. 2009; Culver and Castle, 2008). The co-culturing of various species from different trophic levels is a complex, often site specific, process. Subsequently the development of a successful IMTA system that produces marketable and profitable biomass of additional crops can be a lengthy process, resulting in economic risk and uncertainty of production. A lack of dedicated EU policy and legislation for IMTA and a mixed level of awareness and understanding of IMTA amongst the public and relevant stakeholders could be an additional hindrance to its development in the EU (Alexander and Hughes, 2017; Alexander *et al*. 2016a; Alexander *et al*. 2016b; Hughes and Black, 2016; Alexander *et al*. 2015; Landers *et al*. 2013; Klinger and Naylor, 2012; Chopin, 2011; Troell *et al*. 2009). Although some

marine IMTA systems have been successfully trialled at an industrial scale in Asia (mainly China) and experimental projects are scaling up towards commercialisation in the USA, Canada, Chile, and some European countries, the majority of recent research on marine IMTA systems in industrialised nations has consisted of small-scale experimental operations, which are difficult to extrapolate to larger industrial scale farms (Granada *et al*. 2016; Troell *et al*. 2009; Troell *et al*. 2003). Also, published information on the European experience of developing IMTA systems (i.e. difficulties encountered, economic feasibility, suitable species etc.) is limited (Alexander and Hughes, 2017; Alexander *et al*. 2016a; Alexander *et al*. 2016b; Hughes and Black, 2016; Alexander *et al*. 2015).

Seaweeds are very effective and efficient at taking up nutrients (i.e., nitrogen and phosphorus), making them an ideal bioremediation tool for aquaculture. Studies have shown that seaweeds can remove up to 60% of dissolved inorganic nitrogen and phosphorus over their cultivation period (dependent of species and farm size) (Huo *et al*. 2011; Marinho-Soriano *et al*. 2011; Abreu *et al*. 2009). Intensive seaweed production requires a constant nutrient supply and integrating seaweed into fish aquaculture in coastal waters can alleviate potential seasonal nutrient depletions by having a constant nutrient supply from fish farms (Zhou *et al*. 2006; Chopin *et al*. 2001). Seaweeds have a high market value and are sold worldwide for human consumption, phycocolloids, feed supplements, agrichemicals, neutrachemicals, and pharmaceuticals. In 2014 alone, the global culture of algae reached approximately 27-28 million tons at an estimated value of US$ 5-6 billion (FAO, 2016; Granada *et al*. 2016; Neori *et al*. 2004).

This chapter details the IMTA trials that took place next to an organic salmon farm (Murphy's Irish Seafood Ltd.) in Bantry Bay, Bantry, Cork, Ireland; conducted by the Bantry Marine Research Station (BMRS) as part of the IDREEM project. *Alaria esculenta* (Atlantic wakame/dabberlocks) (trial 1 and 2) and *Sacharina latissima* (Sugar or sweet kelp/kombu) (trial 3), two Phaeophyceae species that are native to Irish coasts (Bunker *et al*. 2010), were utilised as the co-cultured species for these trials. Seaweeds were chosen as it was believed they would have a minimal impact on the daily operations of the existing salmon farm and staff with hatchery experience of these particular species was available on-site (Freddie O'Mahony, Carton Point Shellfish Ltd). *A. esculenta* and *S. latissima* can be used for a variety of purposes ranging from human consumption and alginate production to fodder and use in cosmetics. *A.*

esculenta is high in sugars, proteins, calcium, iodine, bromine, trace elements, and vitamins A, B2, B6, B12, and K. It also contains vitamin C, nitrogen, boron, radium, rubidium, cobalt, and nickel. As a result, it is gaining popularity in the natural food market. *S. latissima* is high in protein, and calcium and also contains significant amounts of vitamin C. It also contains a high amount of mineral elements such as sodium, magnesium, potassium, chlorine, sulphur, phosphorus, and micronutrients, such as iodine, zinc, copper, selenium, and molybdenum. Historically it has been used in Chinese medicine for treatment of various aliments, including cancer (Guiry, 2017; Barsanti and Gualtieri, 2014; University of Coimbra, 2008a; University of Coimbra, 2008b; Irish Seaweeds 2016a; Irish Seaweeds 2016b).

The aims of this case study were to: 1) assess the feasibility of operating integrating IMTA into an operational salmon farm; 2) identify the biomass potential of growing *A. esculenta* and *S. latissima* next to the organic salmon farm; 3) assess the bioremediation potential of *A. esculenta* and *S. latissima* for this farm; and 4) assess the level of contaminants (e.g. heavy metals, polychlorinated biphenyls (PCBs), and *Escherichia coli*) present in the seaweeds.

7.2 Methods and Materials

Location of trials

Bantry Bay is a major inlet on the southwest coast of Ireland, approximately 40 km in length, varying in width from 8 km at the entrance to 5 km at the landward end. At its deepest point, the bay is approximately 35 m deep. At the salmon farm (IMTA site for this study) and control site (1 km northeast of the IMTA site) the depth was approximately 20-25 m. In Bantry Bay the prevailing southwest winds push warmer surface water towards the inner harbour area and causes a thermocline further down the bay. When the wind calms or changes direction, the thermocline moves towards a horizontal position and this causes a vast exchange of water, with up to 70% of the water in the bay being exchanged over a 2 to 3 day period. Tides are the main feature providing water movement within Bantry Bay, which runs relatively uniformly parallel to the shore (northeast/southwest direction) (AquaFact, 2012; Maguire and Burnell, 2001; Elliott *et al*. 1997; Edwards *et al*. 1996; Raines, 1996).

Monoculture site

The existing monocultural aquaculture site used for this IMTA study was an organic salmon (*Salmo salar*) farm located in Bantry Bay, approximately 500m north of the BMRS onshore facility at the port of Gearhies, Bantry, Co. Cork (51° 38' N; 09° 36' E). It consisted of three on-growing cages and two smolt cages (Figure 1).

Salmon cage biomass and cultivation

From November 2012 to October 2013, there was a mean total biomass of 168 tonnes in the smolt cages. There were no smolts present in these cages after October 2013. From November 2012 to November 2013, there was a mean total biomass of 237 tonnes in the on-growing (adult) cages. Harvested biomass over this period was approximately 800 tonnes (trial 1 took place within this time period).

Following winter storms in 2013/2014, the total biomass in the on-growing cages reduced to approximately 90,000 kg for 2014 and 2015 and the harvested biomass over this period was approximately 200 tonnes per annum (trial 2 and 3 took place during this time period) (David O'Neill, Murphy's Irish Seafood, personal communication, 2015).

Overview of trials

Trial 1 and 2 consisted of a 200 m *Alaria esculenta* longline 50 m adjacent to the three on-growing cages (IMTA longline/site) and a 100 m *A. esculenta* longline approximately 1 km northeast of the IMTA site (control longline/site). Water parameters were monitored at both the IMTA and control site for trial 1 and 2. Due to logistical and financial constraints, water parameter analysis was not carried out for trial 3. Over the course of trial 1 and 2 (2013/2014) temperatures ranged from 8.1 °C (March 2013) at the surface (0 m) and near the seabed (20 m) to 17.1°C (July 2014) and 15.5 °C (September 2013) at 0 m and 20 m, respectively, at the IMTA site. At the control site, temperatures ranged from 8 °C (January 2014) and 8.1 °C (March 2013) at 0 m and 20 m, respectively, to 17.7 °C (July 2014) and 16.3 °C (September 2013) at 0m and 20 m, respectively. Salinity ranged from 31.8 ppt (0 m; March 2014) and 34.2 (20 m; January 2014) to 34.8 ppt (0 m; June 2013) and 35 ppt (20 m; June 2013) at the IMTA site, and from 30.7 ppt (0 m; May 2014) and 34 ppt (20 m; January 2014) to 34.7 ppt (0 m; November 2013) and 34.8 ppt (20 m; May, June, & November 2013; April 2014) at the control site (see appendix, Figure 1 and 2, pg. 391-392). Growth and

Carbon, Hydrogen, Nitrogen (CHN) analysis of *A. esculenta* from both the IMTA and control longline was planned for trial 1 and 2, however, this analysis was only conducted for trial 1 due to weather difficulties experienced during trial 2. The third trial consisted of a 300 m longline consisting of 220 m of *Saccharina latissima* and 80 m of *A. esculenta* 150 m from the salmon cages (IMTA longline/site) and a 100 m longline consisting of 80 m of *S. latissima* and 20 m of *A. esculenta* 1km northeast of the IMTA site (control longline/site). Growth and contaminant analysis of both species of seaweed on both the IMTA and control longline was conducted for trial 3. Details of BMRS' experience with harvesting the co-cultured crops and with running the pilot IMTA system, is also detailed.

Figure 1: IMTA setup in Bantry Bay, off the coast of Gearhies Pier, Bantry, Co. Cork (51° 38' N; 09° 36' E)

Set-up of IMTA Trials

Longline and seeded string deployment

Seeded-string of *A. esculenta* and *S. latissima* for this study was obtained from Freddie O'Mahony, Cartron Point Shellfish Ltd (seaweed hatchery), Gearhies, Bantry, Co. Cork. They are prepared for deployment as seeded-string wrapped around collectors (Figure 2). Seaweed longlines and seeded string were deployed utilising the methods described in Edwards and Watson, (2011) and Arbona and Molla, (2006).

Figure 2: *A. esculenta* seeded string collector prior to deployment on a seaweed longline (*original photo by Gunning*)

(a) Trial 1 (2012/2013)

On 5th November 2012, a 200 m longline (IMTA line) was deployed at a depth of approximately 0.5-0.75 m below the water surface, with floatation buoys installed at 15-20 m intervals and anchor buoys at either end of the lines, 50 m adjacent (north) to three organic Atlantic salmon (*Salmo salar*) cages (Figure 3). A 100 m control longline was also deployed northeast of the salmon cages, at the same depth and with the same frequency of buoys.

On 18th of January 2013, Kuralon™ string seeded with *A. esculenta* culture was deployed on both the IMTA and control longlines. The longline was lifted out of the water with a boat-operated crane and, starting at the west end of the line, the longline was placed through the centre of the 1st seeded-string collector. The seeded string was attached securely to the longline and the collector was then pulled carefully along the longline, causing the seeded string to be pulled from the collector and wrapped around the line. It is important that the seeded string is tight against the longline to ensure that the growing seaweed successfully anchors to the longline (Figure 5). Each collector has approximately 40 m of seeded string and approximately 1.33 m of seeded string is required for each metre of longline. Therefore, approximately 266 m of seeded string (c. 6.65 collectors) was deployed on the 200 m IMTA line and 133 m (c. 3.33 collectors) on the 100 m control line. When the whole longline was wrapped with the seeded string, the longline was lowered back to a depth of 0.75-1 m.

(b) Trial 2 (2013/2014)

On 21st October 2013, both the 200 m IMTA and 100 m control longlines were deployed as described for trial 1 (Figure 3). On 6th of November 2013, approximately 120 m (c. 3 collectors) of *A. esculenta* seeded string was deployed on the IMTA line and approximately 40 m (c. 1 collectors) on the control line. On 4th of December 2013, the remaining seeded string was deployed on both the IMTA and control line (Figure 5). Therefore, as with trial 1, a total of c.266 m of seeded string was deployed on the 200 m IMTA line and c.133 m deployed on the 100 m control line. The seeded sting was deployed as per the methods described for trial 1, however, the seeded string was deployed on two separate occasions due to an unexpected delay in production. Although enough seeded string was not ready for full deployment on the first deployment date (6th November 2013), the quantity that was ready had to be deployed at this time to

remain viable. The remaining *A. esculenta* seeded string was ready for deployment by the 4th December 2013.

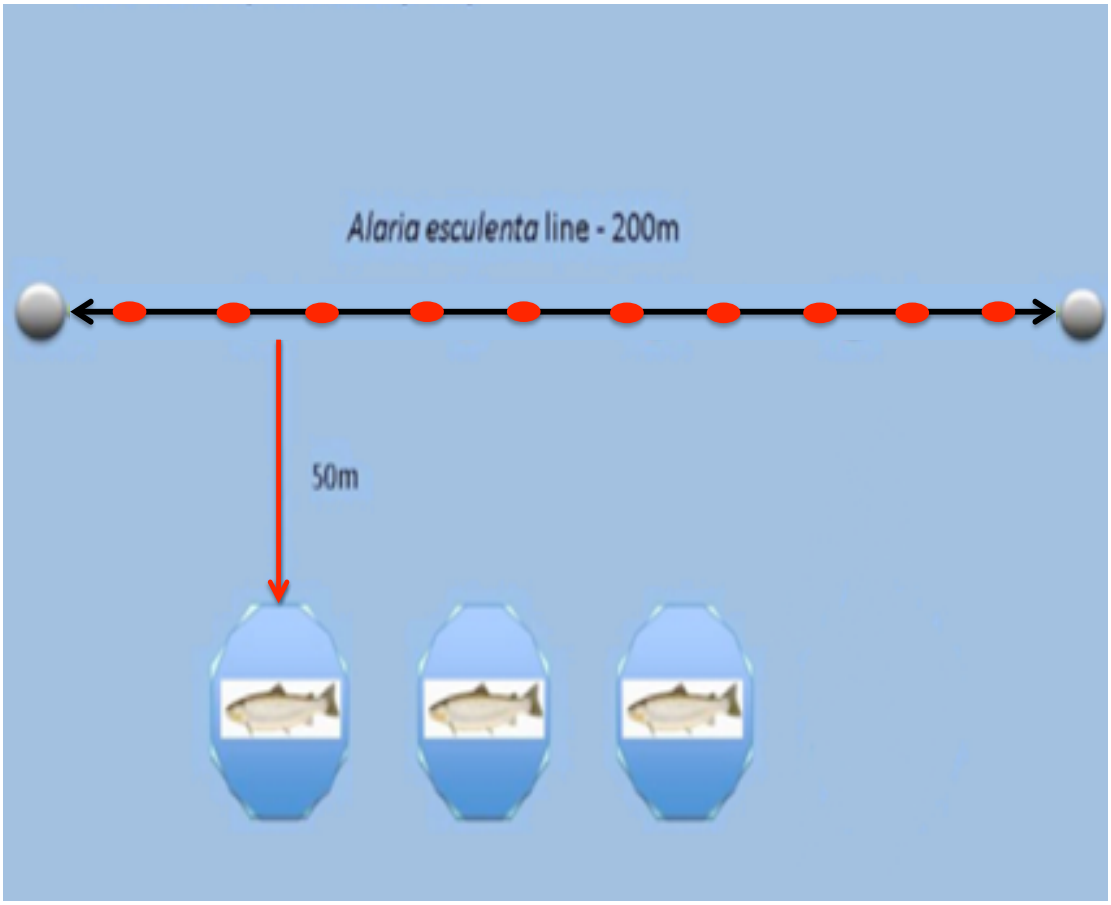


Figure 3: IMTA setup for trial 1 and 2

(c) Trial 3 (2015)

On 28th January 2015, a 300 m longline (IMTA line) was deployed at a depth of approximately 0.5-0.75 m below the water surface, with floatation buoys installed at 10-14 m intervals, 150 m adjacent (north) to the same three organic *S. salar* cages from trial 1 and 2 (Figure 4). Violent storms during the winter of 2013/2014 caused substantial damage to the salmon cages. Subsequently, the distance of the IMTA longline from the cages had to be increased to 150 m (50 m in trial 1 and 2) to allow space for the newly installed salmon cage reinforcement anchorage lines. The frequency of floatation buoys was also increased over that used in trial 1 and 2. This was done to help alleviate the longline sinkage issues experienced in trial 1, which resulted from the weight of the seaweeds at the end of the growth cycle. A 100 m control

line was also deployed 1 km northeast of the IMTA site, at the same depth and with the same frequency of buoys as the IMTA line.

On the 3rd of February 2015, approximately 106.4 m (c.2.66 collectors) of *A. esculenta* seeded string was deployed on the first 80 m of the west end of the 300 m IMTA longline and 26.6 m (c.1.5 collectors) on the first 20 m of the west end of the 100 m control longline. On 6th of February 2015, approximately 292.6 m (c.7.32 collectors) of *Saccharina latissima* seeded string was deployed on the remaining 220 m of the IMTA longline and approximately 106.4 m (c. 2.66 collectors) to the remaining 80 m of the control longline (Figure 5). The method for deploying the seeded string was the same as in trial 1 and 2.

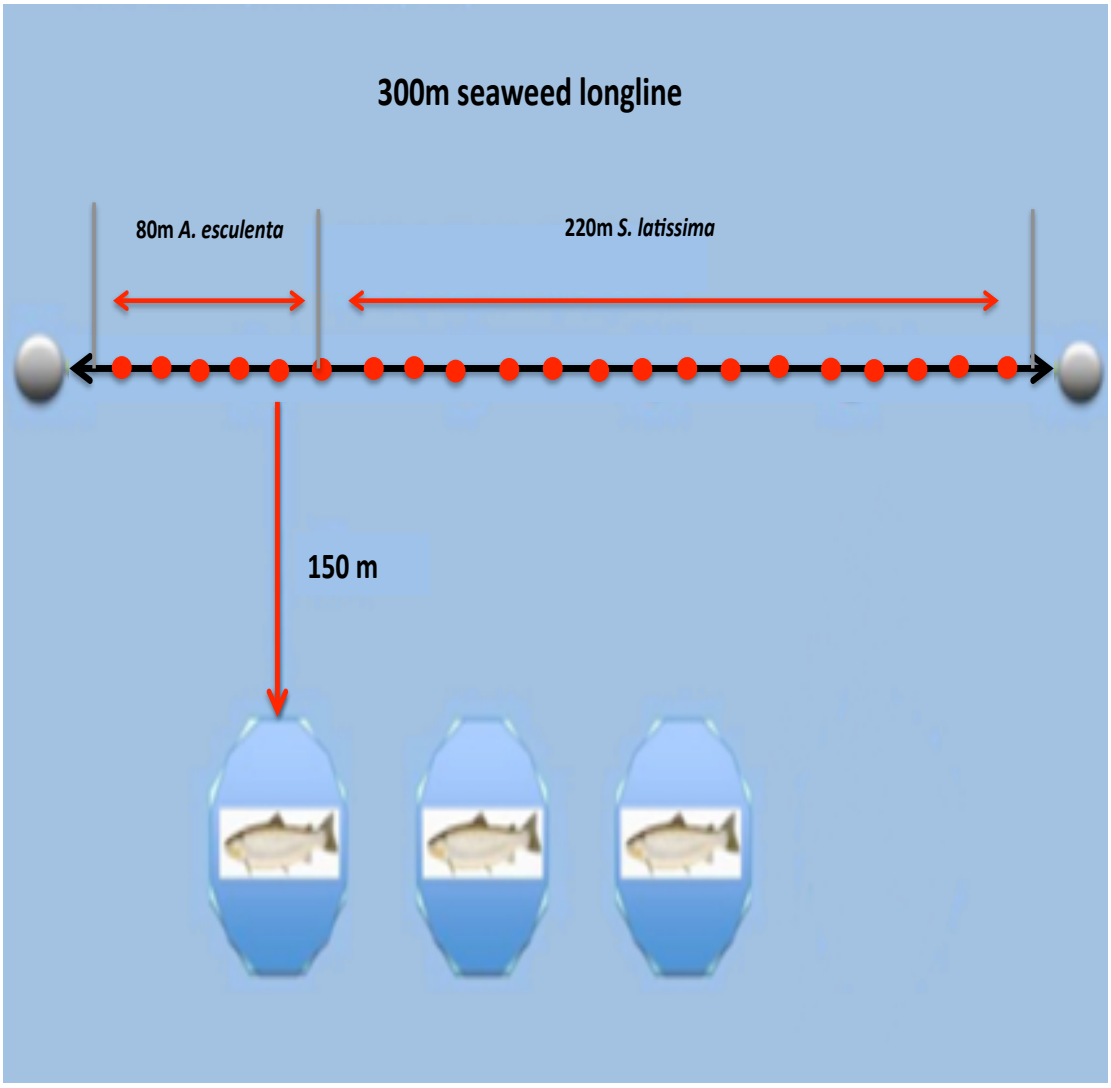


Figure 4: IMTA setup for trial 3

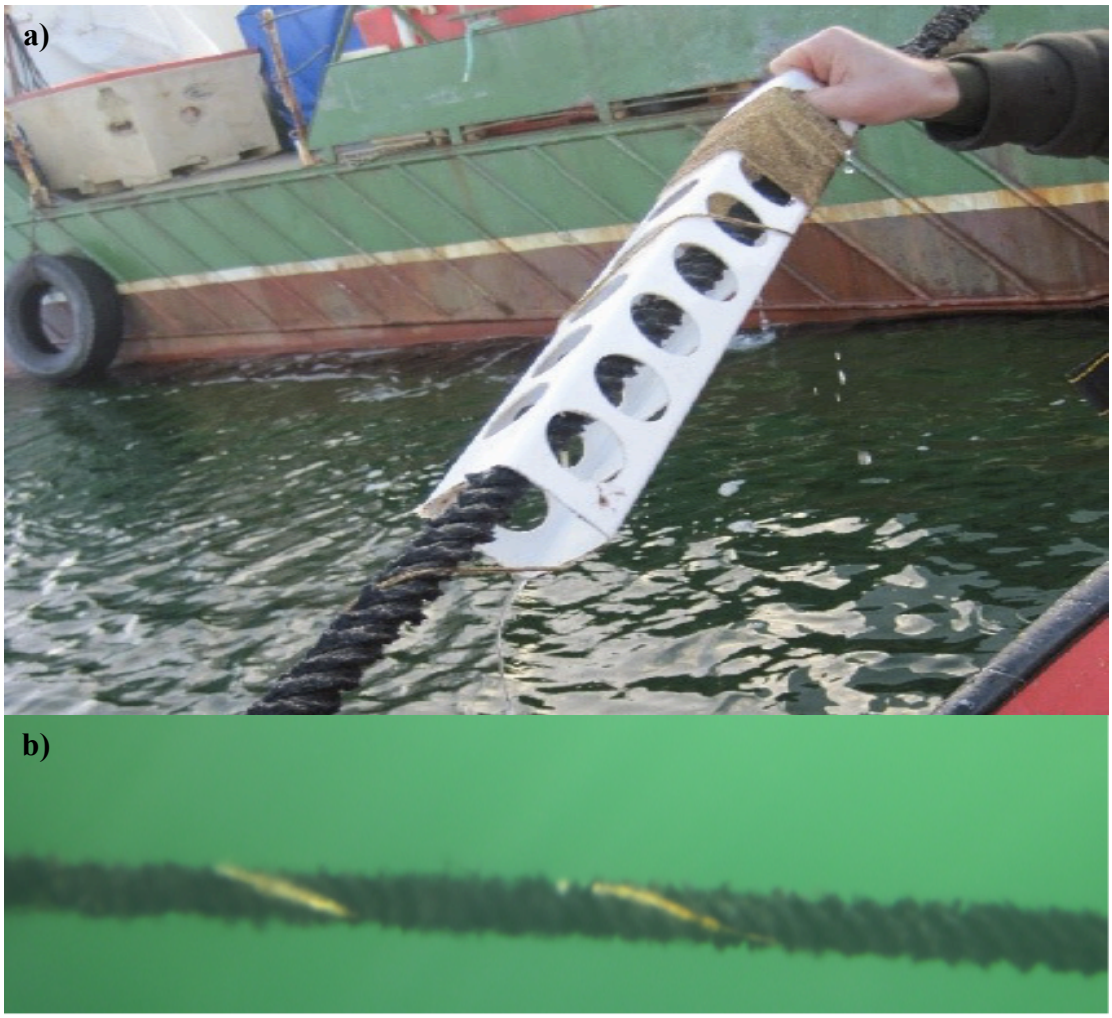


Figure 5: a) Deployment of seeded string; b) underwater image of seeded string on longline following deployment (*original photo by Gunning*)

Trial monitoring

Seaweed biomass monitoring

(a) Trial 1

Samples (n=3) of *A. esculenta* were taken from a 10 cm long section of the IMTA longline at a random location along the first (west end), middle, and final (east end) 66 m of the line. The same sampling regime was also conducted on the control longline at a random location along the first (west end), middle, and final 33 m (east end) of the line. An average wet weight biomass (kg/m) was calculated from these samples. Seaweed sampling for biomass calculations took place at the IMTA longline on 11th April, 31st May, and 26th of June 2013. Seaweed sampling of the control longline took

place on the 11th April and 31st of May 2013. It was noted on the 31st May 2013 that the east end of the control line was beginning to sink and no sampling of the control line took place on the last date of sampling (26/6/13). By this date the whole line had sunk to a depth that made sampling impossible. Unfortunately only a small RIB boat was available for sampling and did not have the capacity to lift the line out of the water. Samples collected from each biomass sampling date were freeze-dried in a Labconco® shelf freeze-drier prior to being transported to University College Cork for CHN analysis.

(b) Trial 2

It was planned to sample the IMTA and control longlines via the same methodology employed in trial 1, however, a large number of violent storms occurred from mid December 2013 to February 2014. During this period of bad weather, it was not safe to access either longline by boat. By March 2014 both the IMTA and control site were accessed. Unfortunately, both longlines and the salmon cages were significantly damaged. Subsequently, both sites were no longer operational for the remainder of trial 2. On April 2nd 2014, a boat-operated crane removed the IMTA and control line from the water and returned them to shore.

(c) Trial 3

For trial 3, the IMTA longline was 300 m in length, with 80 m (starting from the west end of the line) containing *A. esculenta* and the remainder of the line (220 m; finishing at the east end) containing *S. latissima*. The same sampling regime employed in trial 1 and 2 was conducted for trial 3, with 10 cm long sections of *A. esculenta* taken from a random location along the first (west end), middle, and final (east end) 26 m and 6 m of the IMTA and control line, respectively (n=3 for both IMTA and control line) and 10 cm long sections of *S. latissima* taken from a random location along the first (west end), middle, and final (east end) 73 m and 26 m of the IMTA and control line, respectively (n=3 for both IMTA and control line). Biomass sampling of both the IMTA and control longline took place on the 13th March, 7th April, 14th May, and 8th June 2015. Samples from the final biomass sampling date (8/6/15) were freeze-dried prior to being sent to Aqua (the Italian partner of the IDREEM project) for contaminant analysis.

Water parameter monitoring (trial 1 & 2)

Water sampling took place at two locations; (1) approximately 3m from the western end of the IMTA seaweed longline (or approximately 50 m from the salmon cages) and (2) 3m from the western end of the control seaweed longline, once per month, for trial 1 and 2. Due to logistical and/or weather constraints, sampling did not take place at these locations in July, August, and December of 2013, and in February, October, November, and December of 2014. A water sample was taken from the surface (0m) and near the seabed (c. 20 m) at both locations. Water sampling and analysis was conducted by Hensey Glan-Uisce Teo, Coismeigmore, Furbo, Co. Galway, Ireland (ISO 17025 Irish National Accreditation Board accredited).

Carbon and Nitrogen content in A. esculenta - sample preparation for CHN analysis (trial 1)

On each biomass sampling date in trial 1, 100 g from each of the 3 samples from the IMTA longline were mixed together (total – 300 g) and freeze-dried. The same procedure was applied to the control samples. As the control line could not be sampled on the final sampling date (26/6/13), when the longlines were brought ashore for harvesting on the 2nd of July 2013, three samples were taken for CHN analysis from random locations along the line where the seaweed was not degraded or had a heavy covering of epiphytes. These samples were also mixed as described above and transferred to the freeze dryer. All freeze-drying took place at BMRS using a Labconco® shelf freeze-dryer (serial number: 100830101D) and all samples were freeze-dried for 24-48 hours.

When the samples were removed from the freeze drier they were ground to a fine powder using a DeLonghi® KG49 desktop grinder. Ten mg of powdered seaweed per sample was sent to the micro-analytical laboratory, Chemistry Department, University College Cork, Co. Cork, Ireland for CHN analysis (this laboratory utilises standardised methods according to industry standards). Note: it was planned to conduct CHN analysis for trial 2, however, due to the storm damage, samples were not collected during this trial.

Contaminants analysis – sample preparation (trial 3)

On the final day of biomass sampling for trial 3 (8/06/15) approximately 700-705 g wet weight (WW) of *A. esculenta* and *S. latissima* from the IMTA longline were freeze-dried (approximately 230-235 g WW from each sampling location for each seaweed were mixed together). The same was also applied to the samples of *A. esculenta* and *S. latissima* from the control longline. All freeze-drying took place at BMRS using a Labconco® shelf freeze-dryer (serial number: 100830101D) and all samples were freeze-dried for 24-48 hours. Following removal from the freeze-dryer, 100 g dry weight (DW) of each freeze-dried seaweed sample was vacuum-packed and sent by express courier to the Italian partners of the IDREEM project, who had the samples tested for heavy metals, polychlorinated biphenyls (PCBs), and *Escherichia coli* at the Chemical Applied Water Purification Laboratory, Menfi, Sicily (accreditation: *Accredia L'ente Italiano di Accreditamento*; *Certiquality – Sistemi di Gestione Certificati*; UNI EN ISO 9001:2008; UNI EN ISO 1400:2004; http://www.cadaonline.it/en/home/).

Although a number of heavy metals and PCBs were tested for, this study highlights those that have EU regulatory thresholds for human consumption (Directive 2006/1881/EC) and use as or in animal feeds (Directive 2002/32/EC) (i.e. arsenic, cadmium, mercury, lead, ICES-6 PCBs) (European Commission 2006; European Commission, 2002). [Note: ICES-6 refers to the International Council for Exploration of the Seas - 6 indicator PCBs, an EU-uniform group of non-dioxin like PCBs that are of concern for human health above a certain threshold (European Commission 2006)].

The samples were sent as dried weight to ensure they did not degrade during transport. Directive 2006/1881/EC presents thresholds in mg/kg WW for heavy metals and ng/g WW for PCBs (European Commission, 2006), therefore, the mg/kg DW results obtained in the laboratory were converted to mg/kg WW or ng/g WW based on the percentage moisture content of the samples prior to freeze-drying (85.1 ± 1.43 %). Directive 2002/32/EC presents thresholds in mg/kg relative to a feeding-stuff with a 12% moisture content (European Commission, 2002). Therefore, the mg/kg DW results obtained in the laboratory were converted to mg/kg WW if the seaweeds were reduced to a 12% moisture content.

Final harvesting of seaweed (trial 1 and 3)

Both the IMTA and control longlines from trial 1 were brought ashore for harvesting on the 2nd July 2013. Only the IMTA line was brought ashore for harvesting in trial 3, which took place on the 12th June 2015. As there was no demand for further harvested seaweed, the control longline from trial 3 was left at sea to degrade naturally.

For both trials, the seaweed longline was removed from the sea via the use of a boat-operated crane. Firstly, the longline was detached manually from both the anchor-buoys (accessed via a RIB). The crane-hook was then attached to the end of the line and the first section of the longline was pulled on-board. The crane-hook was then detached and reattached to the section of the longline that was still in the water, which was pulled on-board. These steps were repeated until the whole longline was on-board (Figure 6 a & b). Once on-land, the longline was suspended from the roof of the polytunnel at BMRS and manually stripped with a knife (Figure 6 c - e).

As part of the harvesting deliverable for IDREEM, each partner of the project was asked about: (1) difficulties encountered while harvesting; (2) steps required to achieve harvesting of co-cultured crops on a commercial scale; and (3) market and harvesting potential of chosen co-cultured crops. A meeting was held after the harvesting of seaweed from trial 3, where all staff from BMRS who were involved in harvesting contributed to the answering of these questions. Daryl Gunning and Marc Shorten (BMRS), as part of a deliverable for the IDREEM project, developed the questions.

Figure 6: a & b) Removal of *S. latissima* from Bantry Bay (Trial 3) via boat-operated crane; c-e) Manual harvesting of *S. latissima* (Trial 3) (*original photos by Gunning*)

Statistical analysis

All statistical analysis was done using SPSS software (IBM) version 23. Prior to statistical analysis, percentage data was transformed using the arcsine transformation. All data was tested for normal distribution and homogeneity of variance with the Shapiro-Wilk test and Levene's test, respectively ($p>0.05$). Values less than 0.05 were considered statistically significant.

Independent t-tests were used to test the significance of difference of biomass (kg/m) and carbon and nitrogen content (%) between *A. esculenta* and/or *S. latissima* from the IMTA and control longline. If the groups' variance were unequal, an adjustment was made to the degrees of freedom using the Welch-Satterthwaite method.

7.3 Results

Seaweed biomass (trial 1 & 3)

Trial 1

The mean biomass of the *A. esculenta* raised on either an IMTA or a control longline reached 14.67 ± 3.44 kg/m WW and 18.13 ± 1.09 kg/m, respectively, after 133 days of growth ($p>0.05$). The *A. esculenta* biomass on the IMTA longline increased to 17.22 ± 1.69 kg/m WW after a further 26 days of growth (Figure 7). The biomass on the control longline had sunk after the 2nd sampling date (31/5/13; 133 days of growth), and biomass sampling did not take place. There was no significant difference ($p>0.05$) between biomass on the IMTA and control longline on any of the sampling dates.

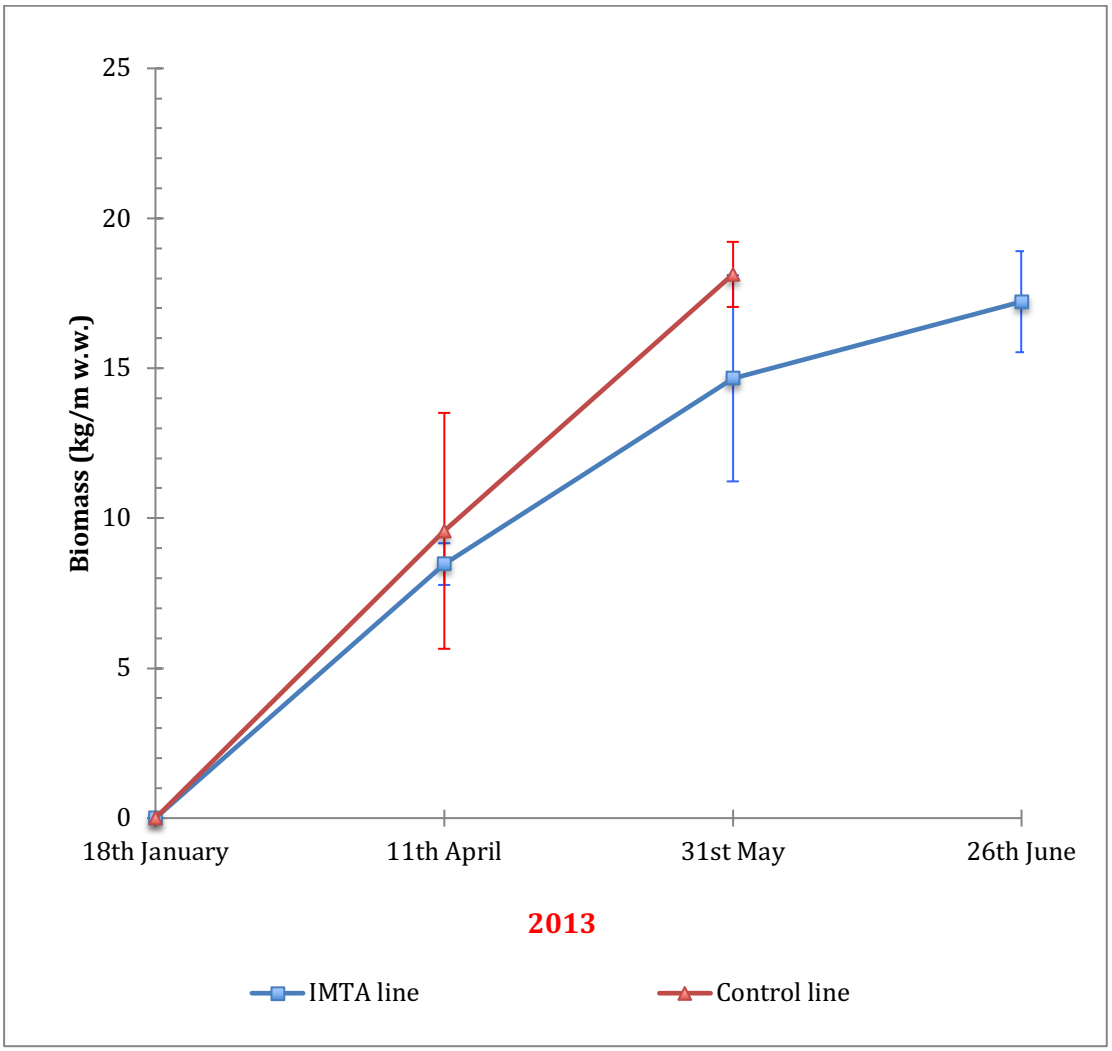


Figure 7: Biomass of *A. esculenta* from the IMTA and control longline (Trial 1; mean ± SD)

Trial 3:

The mean biomass of the *A. esculenta* on the IMTA and control longlines reached 11.90 ± 3.47 kg/m WW and 11.73 ± 0.66 kg/m WW, respectively, after 122 days of growth ($p>0.05$) (Figure 8). There was only a significant difference between the mean biomass of *A. esculenta* grown on the IMTA and control longline on the second sampling date (07/04/15; $p<0.05$).

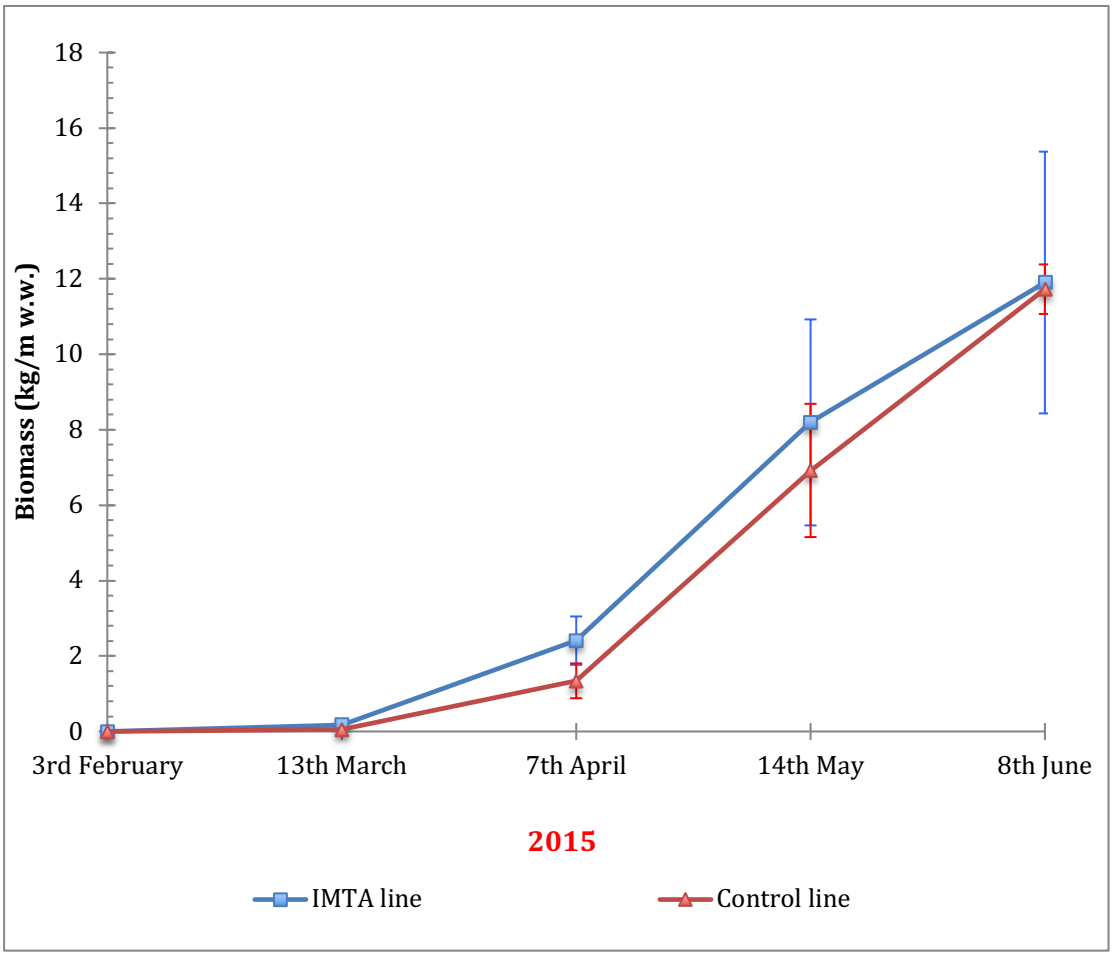


Figure 8: Biomass of *A. esculenta* from the IMTA and control longline (Trial 3; mean ± SD)

The mean biomass of the *S. latissima* grown on the IMTA and control longlines reached 6.02 ± 2.39 kg/m WW and 7.44 ± 0.84 kg/m WW, respectively, after 125 days of growth ($p>0.05$) (Figure 9). There was only a significant difference between the mean biomass of *A. esculenta* grown on the IMTA and control longline on the first sampling date (13/03/15; $p<0.01$).

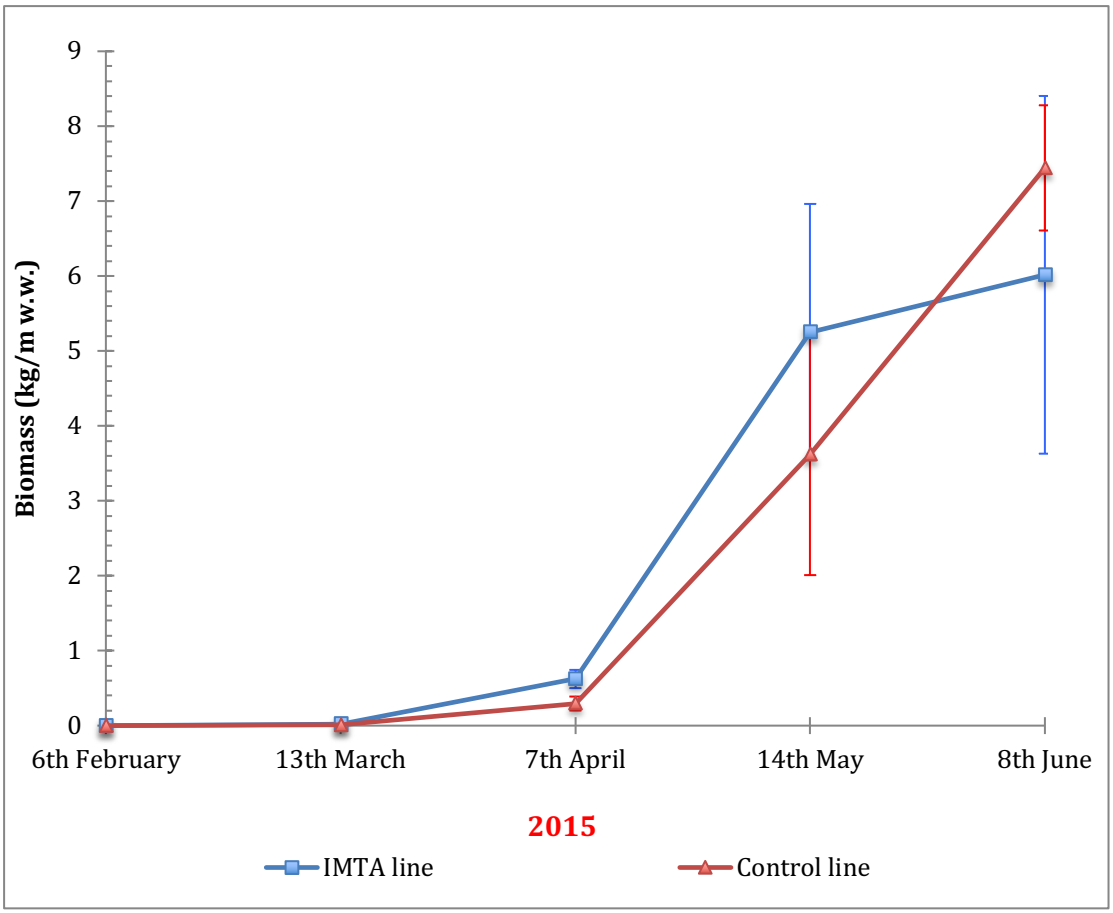


Figure 9: Biomass of *S. latissima* from the IMTA and control longline (Trial 3; mean ± SD)

Water parameters (trial 1 and 2)

Apart from a few exceptions, water parameters (i.e. ammonia, nitrite, nitrate, phosphate, total particulate matter {TPM}, and chlorophyll-a) were very similar at both the IMTA and control site (Figure 10-15).

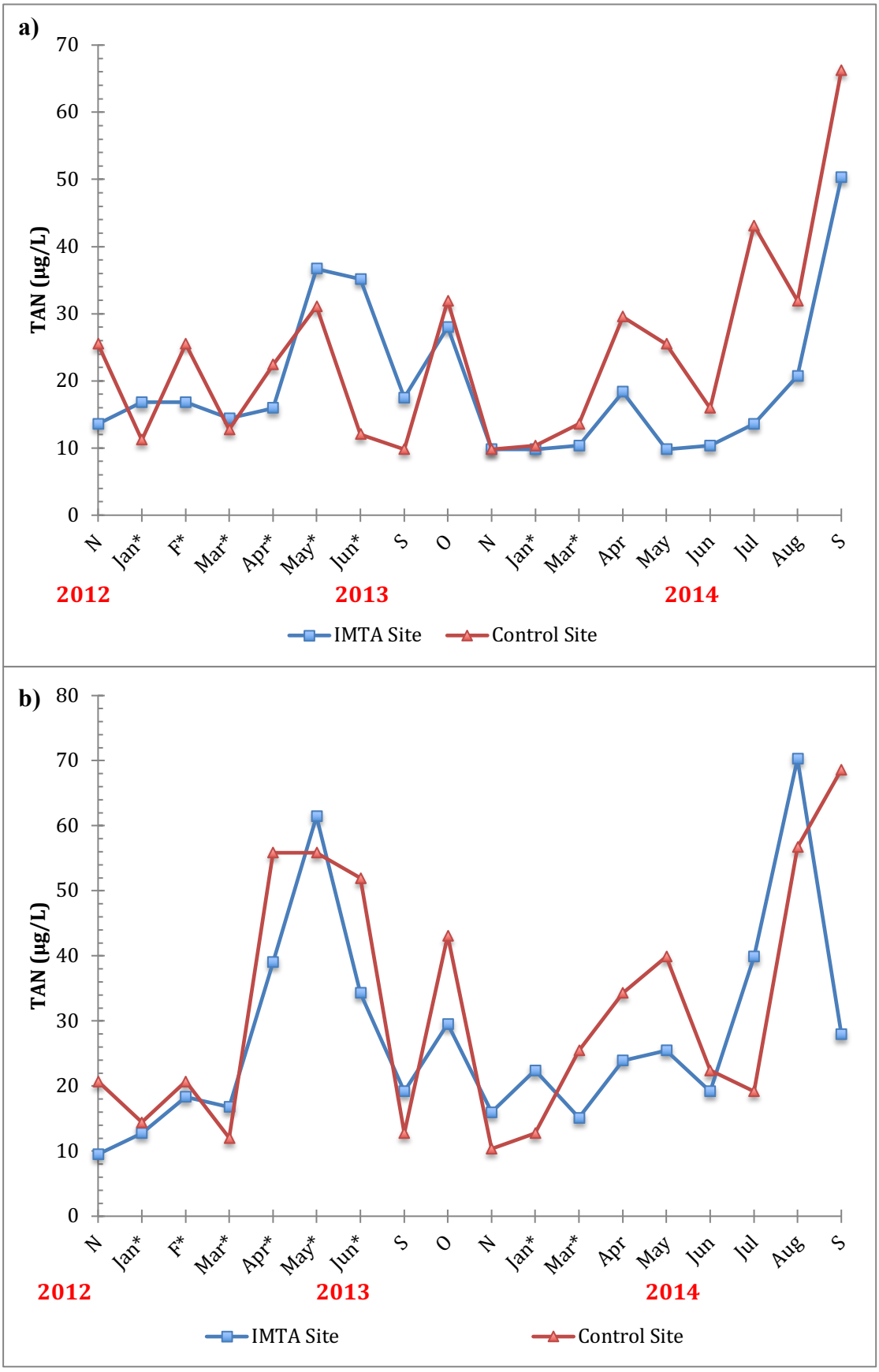


Figure 10: TAN levels at a) 0 m & b) 20 m depths at the IMTA and control site over the course of trial 1 & 2 (* = seaweed longlines present)

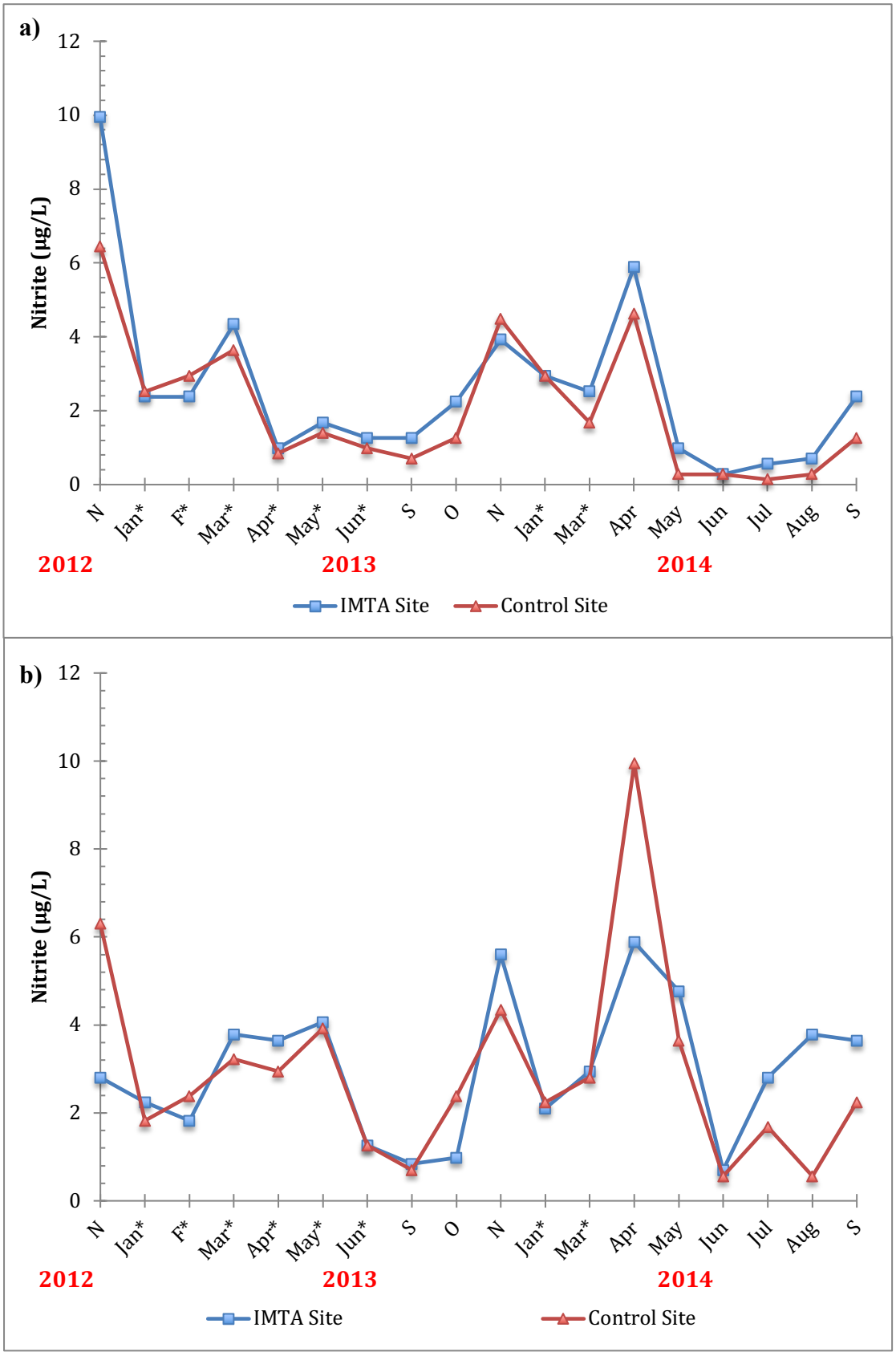


Figure 11: Nitrite levels at a) 0 m & b) 20 m depths at the IMTA and control site over the course of trial 1 & 2 (* = seaweed longlines present)

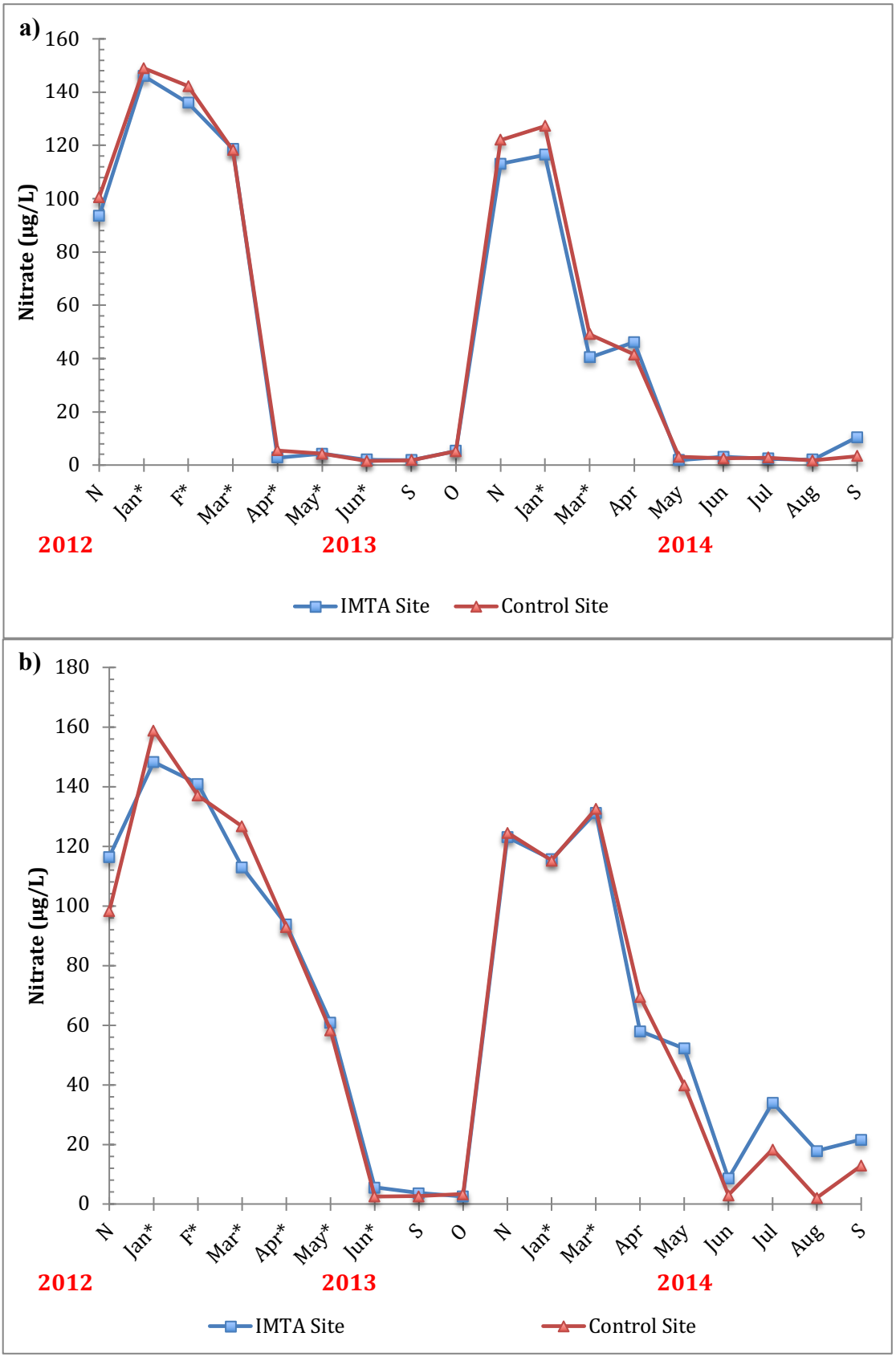


Figure 12: Nitrate levels at a) 0 m & b) 20 m depths at the IMTA and control site over the course of trial 1 & 2 (* = seaweed longlines present)

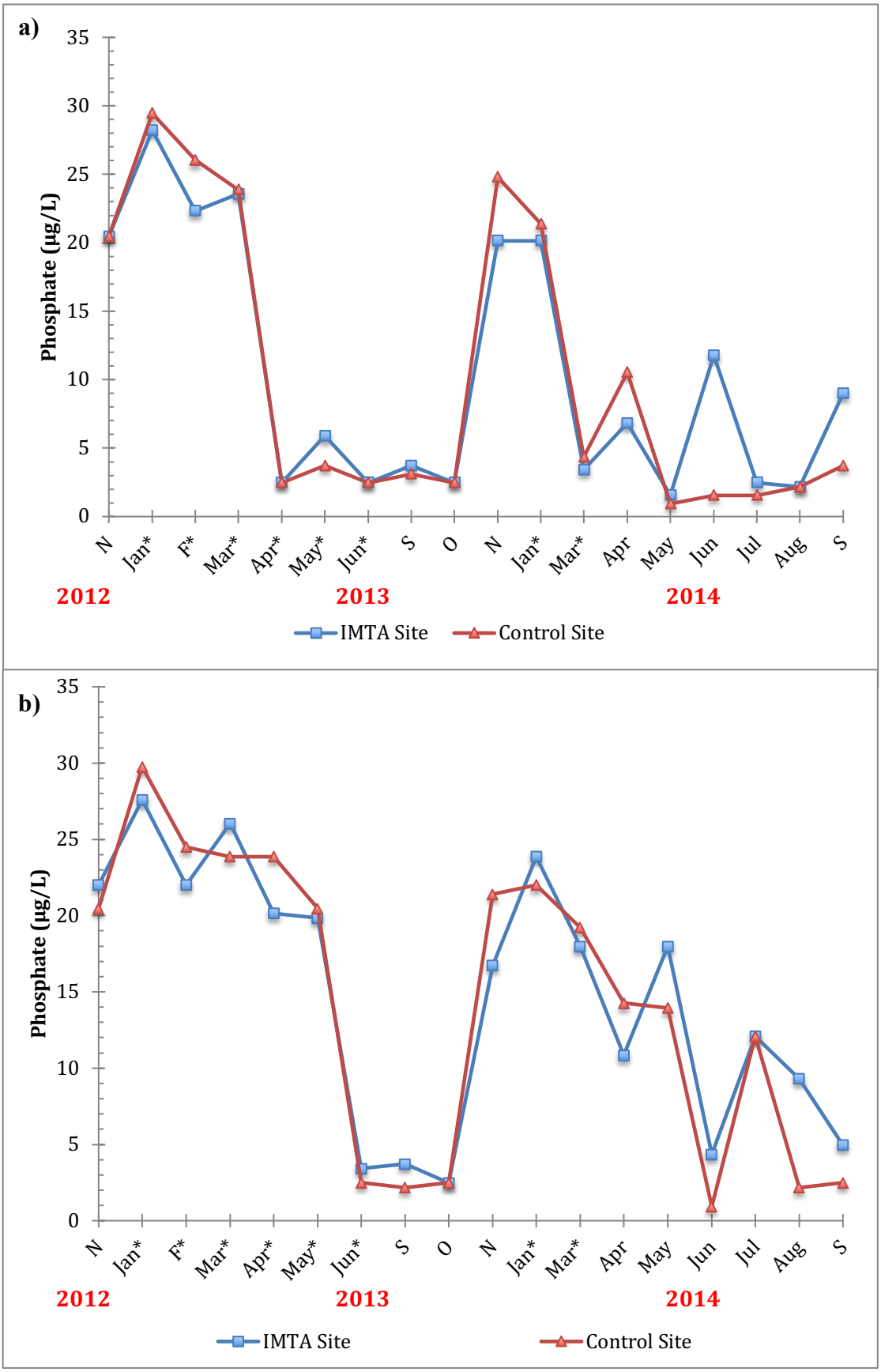


Figure 13: Phosphate levels at a) 0 m & b) 20 m depths at the IMTA and control site over the course of trial 1 & 2 (* = seaweed longlines present)

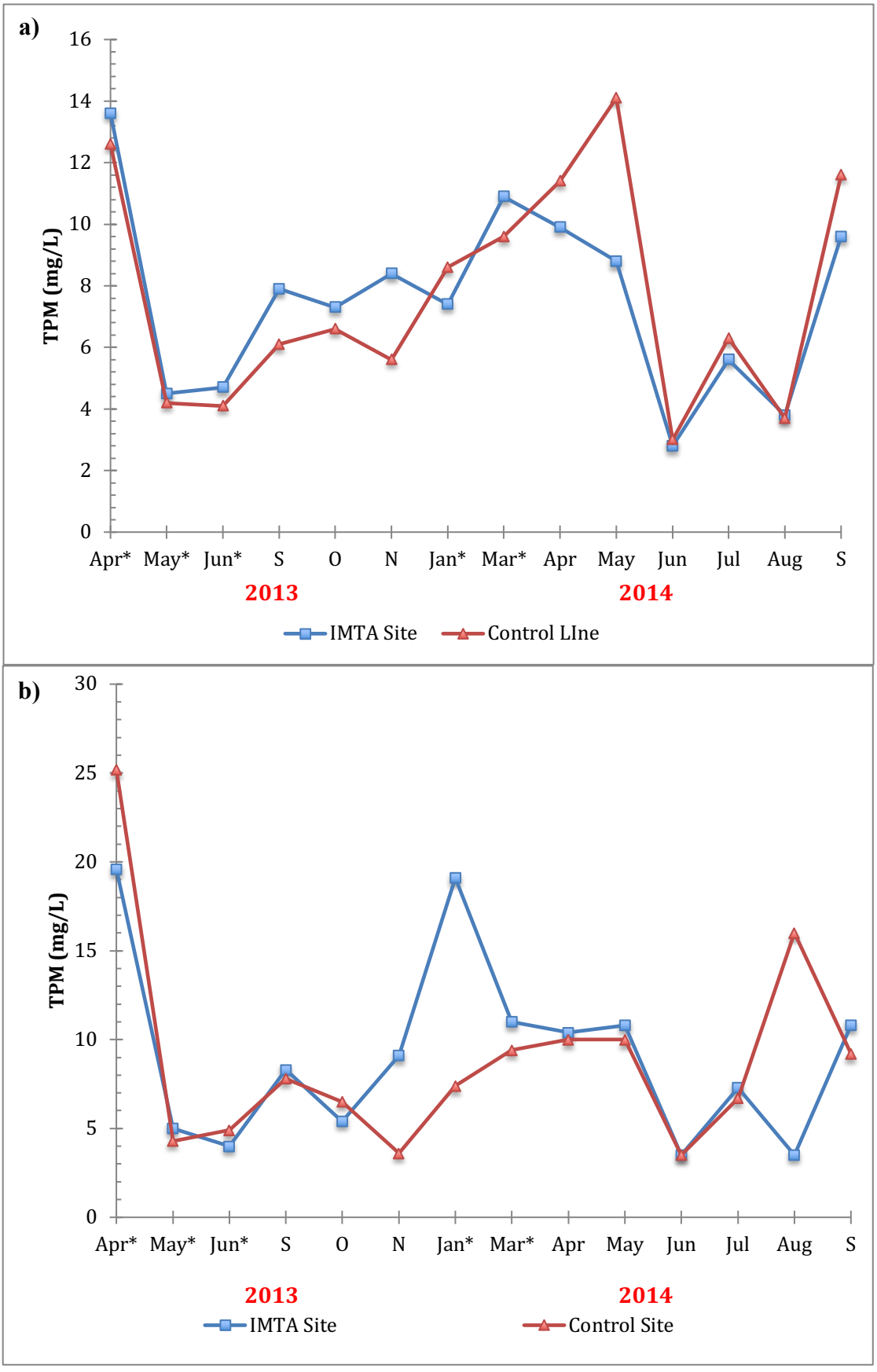


Figure 14: TPM levels at a) 0 m & b) 20 m depths at the IMTA and control site over the course of trial 1 & 2 (* = seaweed longlines present)

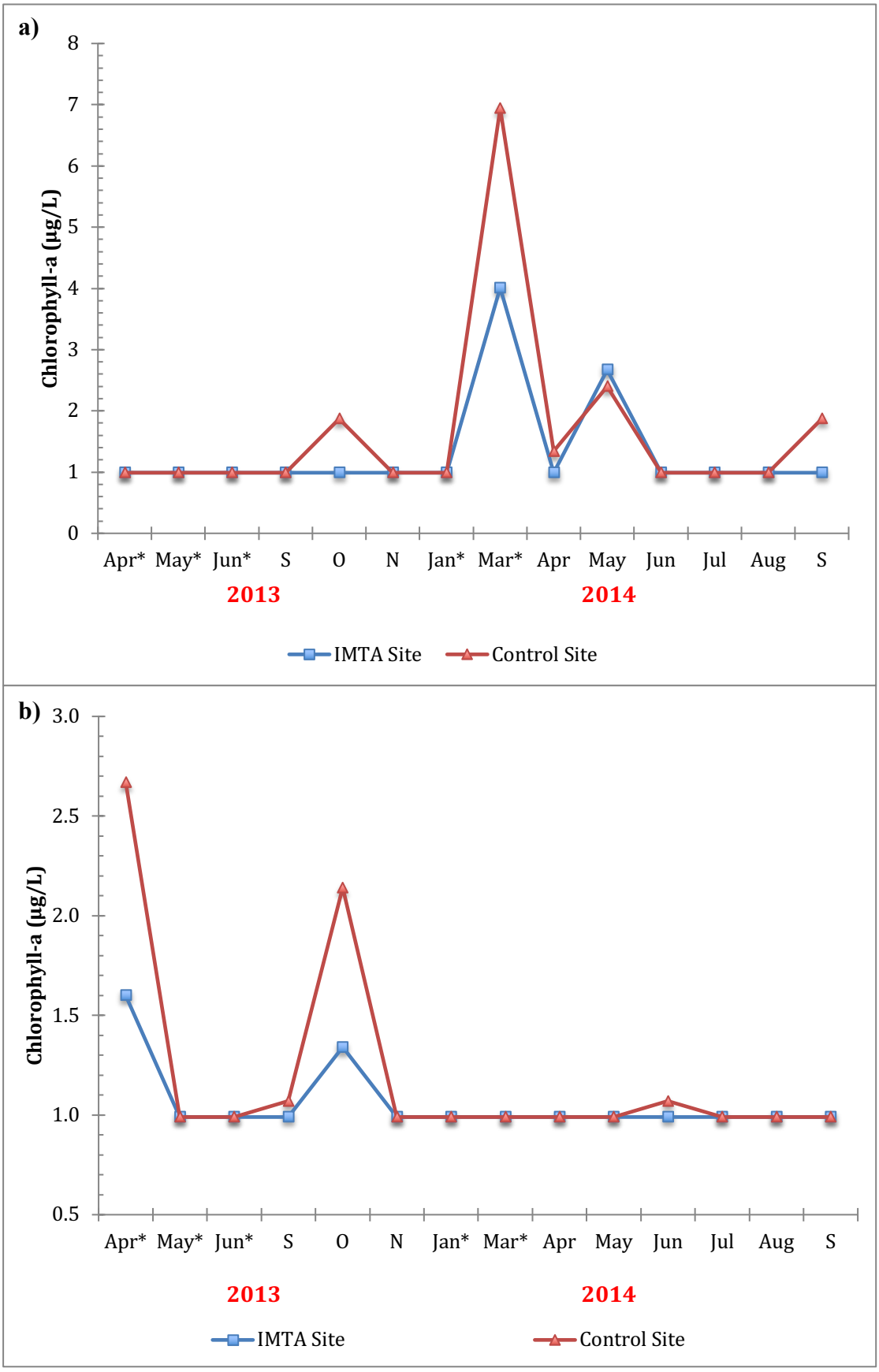
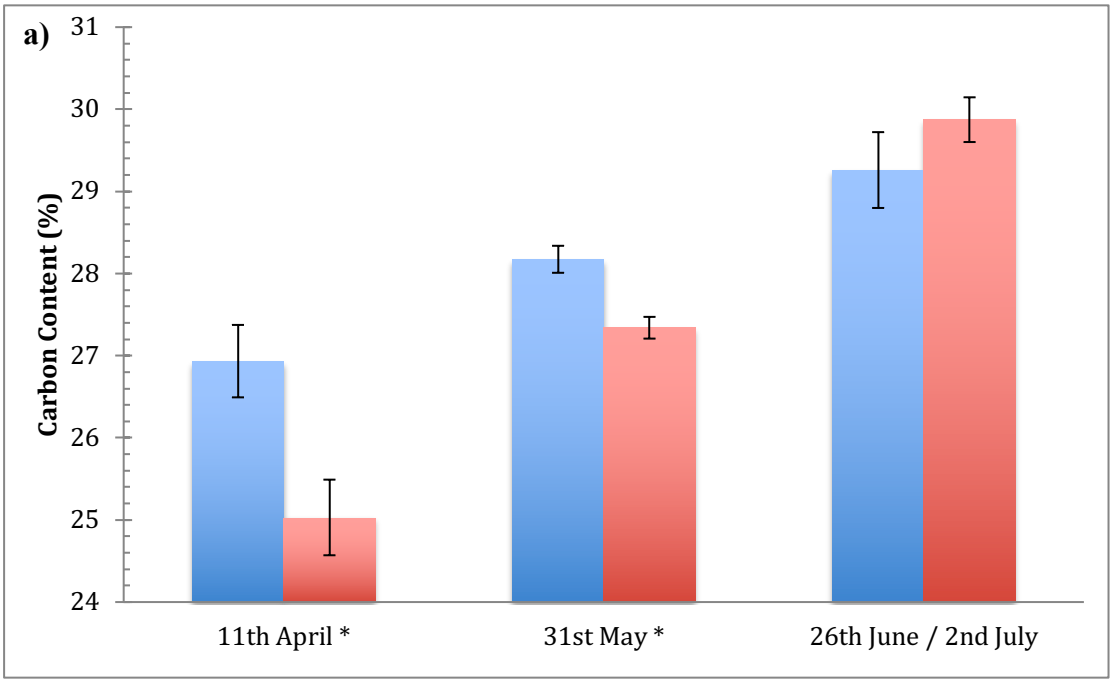


Figure 15: Chlorophyll-a levels at a) 0 m & b) 20 m depths at the IMTA and control site over the course of trial 1 & 2 (* = seaweed longlines present)

Carbon and Nitrogen content of A. esculenta (trial 1)

The carbon content of *A. esculenta* from the IMTA longline was higher than from the control line on the first two sampling dates ($p<0.01$), however, the differences were only minor. The largest difference was seen on the first sampling date (11/04/13), with *A. esculenta* from the IMTA longline having a carbon content 1.9% higher than the control longline. By the end of the trial, the carbon content of *A. esculenta* from the IMTA and control longlines was 29.26 ± 0.46% and 29.87 ± 0.27%, respectively ($p>0.05$) (Figure 16).

The nitrogen content of *A. esculenta* from the control longline was higher than from the IMTA line on all sampling dates, however, again the differences were only minor. The largest difference was seen on the second sampling date (31/05/13), with *A. esculenta* from the control longline having a nitrogen content 0.81% higher than the IMTA line ($p<0.01$). By the end of the trial, the nitrogen content of *A. esculenta* from the IMTA and control longlines was 2.22 ± 0.07% and 2.34 ± 0.43%, respectively ($p>0.05$) (Figure 16).



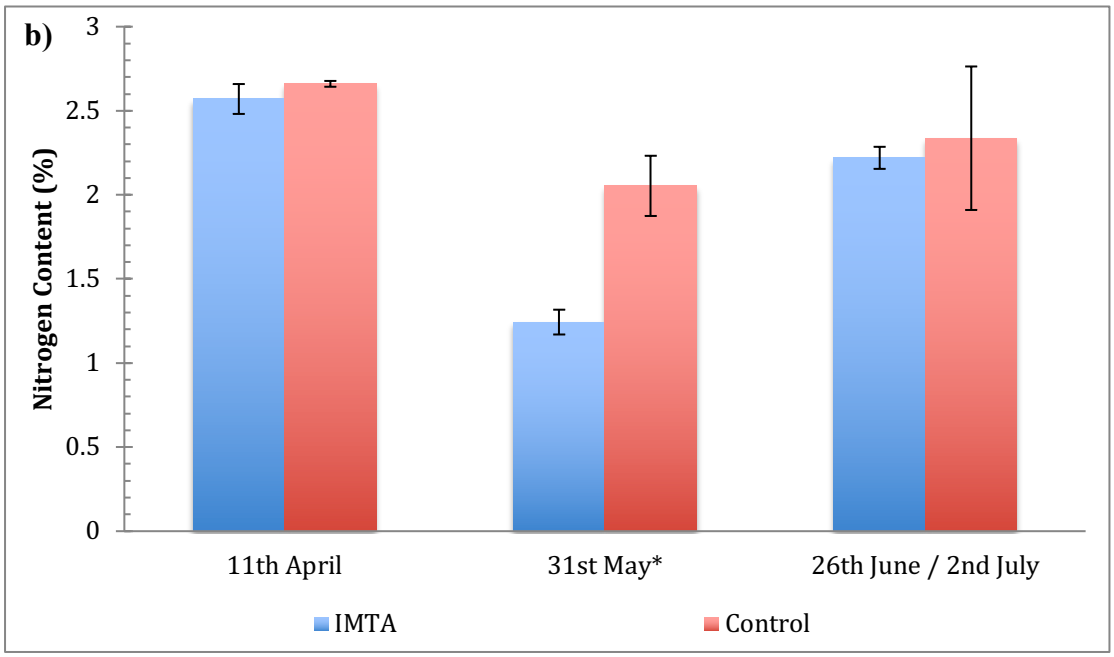


Figure 16: a) Carbon and b) Nitrogen content (%) of *A. esculenta* grown on the IMTA and control longlines over the course of trial 1 (mean ± SD; * = p<0.01)

Contaminant analysis (trial 3)

A. esculenta and *S. latissima* from the IMTA and control longlines did not exceed the EU threshold for arsenic, cadmium, mercury, and lead levels for human consumption or use as a feed ingredient (European Commission, 2006; European Commission, 2002). Although there is no EU regulation on the threshold levels of ICES-6 PCBs in seaweed, the levels found in both *A. esculenta* and *S. latissima* from the IMTA and control longlines did not exceed the threshold set for human consumption of fish and molluscs (European Commission, 2006) (Table 1). *E. coli* was not present on either seaweed species, irrespective of growth on the IMTA or control longlines.

Table 1: Contaminant levels in seaweeds from the IMTA and control longline (trial 3) (numbers in parentheses refer to content of heavy metal if seaweed was dried to a 12% moisture content – as per Directive 2002/32/EC threshold)

Analyte	*A.esculenta* – IMTA	*A.esculenta* – control	*S.latissima* - IMTA	*S.latissima* - control	EU threshold	
					Human Consumption[1]	Feed Ingredients[2] (Relative to 12% moisture content)
Heavy Metals (mg/kg WW)						
Arsenic	4.27 (25)	5.53 (32.37)	6.51 (38.07)	2.23 (13.07)	-	40
Cadmium	<0.1 (0.53)	0.15 (0.88)	<0.1 (0.26)	<0.1 (<0.1)	3	1
Mercury	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	0.1	0.1
Lead	<0.1 (<0.1)	<0.1 (<0.1)	0.11 (<0.1)	<0.1 (<0.1)	3	10
ICES-6 PCBs (ng/g WW)						
Sum of PCB 28, 52, 101, 138, 153, 180	1.8	1.9	0.6	0.2	75	-

[1]Directive 2006/1881/EC
[2]Directive 2002/32/EC

Harvesting

(a) Harvesting of co-cultured crop

The majority of *A. esculenta* and *S. latissima* harvested in trial 1 and/or 3 was either used on-site for feeding sea-urchins or discarded (Table 2). In trial 3, the control longline was left at sea to degrade naturally, due to a lack of demand. Nevertheless, a number of potential markets were identified over the course of the project and are detailed below.

Table 2: Quantity (kg), processing method, and utilisation of harvested seaweed (Trial 1 & 3)

Species	Quantity Harvested (kg WW)	Processing Method	Utilisation
Trial 1			
Alaria esculenta	IMTA line – c. 3440 Control line – c. 1810	(1) c. 300 kg (IMTA *A. esculenta*) sent to destination fresh and unprocessed (2) Remainder - unprocessed	(1) Horse feed additive – Irish horse feed company (2) Remaining biomass used on-site to feed sea-urchins or discarded
Trial 3			
A. esculenta	c. 952 (IMTA line)	Unprocessed	Used on-site to feed sea-urchins or discarded
Saccharina latissima	c. 1324.4 (IMTA line)	(1) c. 85 kg sent to destination fresh and unprocessed (2) Remainder - unprocessed	(1) Biogas research – Environmental Research Institute (UCC) (2) Remaining biomass used on-site to feed sea-urchins or discarded

(b) Difficulties encountered while harvesting

When the seaweed was ready to harvest the longline was quite heavy and a boat-operated crane was required to lift the line from the water. Murphy's Irish Seafood (MIS) had such a boat, however, it was regularly in use at the salmon farm and obtaining it to harvest the seaweed proved difficult. This issue was compounded by the fact that the weather patterns of Bantry Bay can be difficult to forecast and determining a suitable day for harvesting was tricky. This problem would be exacerbated if BMRS was to scale-up seaweed production and the purchase of a boat with a crane may be necessary in the future. Also, BMRS does not currently have any on-shore facilities for the processing of seaweed, therefore, harvested seaweed can only be sold fresh and unprocessed (discussed in more detail below).

(c) Steps required to achieved harvesting of co-cultured crops on a commercial scale

To achieve commercial scale harvesting of IMTA seaweed BMRS would need to deploy a lot more longlines. After a four year wait, BMRS received its seaweed licence in 2015, which allows for the deployment of seaweed longlines within a 6 hectare area. It is estimated that approximately 16 longlines of 200 m length could be positioned at a site of this size. The proposed area is located 200-300 m downstream of the organic salmon farm. BMRS would need to have a planning meeting with Cartron Point Shellfish Ltd to discuss the feasibility, requirements, and cost of producing seeded string at this scale. (Note: it was hoped that this seaweed licence would have been received before the IDREEM project began, and a larger IMTA infrastructure could have been implemented).

Currently BMRS does not have the required facilities on-site to process the harvested seaweed. The higher market price of selling seaweed in a processed form (e.g. milled, dried, etc.) would make commercial production of seaweed more financially viable for BMRS. If BMRS developed on-site seaweed processing facilities, there is the potential to target a greater variety of markets. For example, feed, cosmetic, and other companies may not have the facilities to process seaweed and therefore would not purchase the raw material. Also, as mentioned above, BMRS would need access to its own boat for the operation of a seaweed farm at such a scale to be feasible.

(d) Market & harvesting potential of chosen co-cultured crops

Over the course of the IDREEM project, BMRS has identified a number of potential markets for its IMTA crops (*A. esculenta* & *S. latissima*). These include:

- Health supplement in horse feed. In trial 1 BMRS supplied approximately 300 kg WW *A. esculenta* to an Irish horse-feed company.
- Cosmetics: The Irish branch of a famous high street cosmetic company expressed an interested in using BMRS' farmed seaweed crops in their beauty products.
- Supply of seaweed to research institutes: In trial 3 BMRS supplied approximately 83 kg WW *S. latissima* to the Environmental Research Institute (UCC) for biogas research.
- An additive to fish feed

- Health food market
- Bioplastics for use in, for example, eco-friendly packaging. BMRS were involved in the SEABIOPLAS project which researched the viability of using seaweeds as a source of bioplastics (http://cordis.europa.eu/project/rcn/110672_en.html).

The key issue with market potential is to aim towards a high-value market. Currently, far Eastern countries (e.g. China) can produce macro-algae very cheaply, therefore, to make this a potential line of business it is vital to go for either value-added products (e.g. derived ingredients) or quality-assured/certified products that command a higher market price (e.g. devise an IMTA-labelling system). With the new seaweed license allowing for approximately 16 longlines of 200 m each, a rough estimation of harvesting potential based on a conservative estimate of biomass (lower biomass of trial 3) can be made. With *A. esculenta* on the IMTA line reaching a final biomass of c. 12 kg/m in trial 3, with 16 longlines at 200 m each, BMRS could potentially achieve a total harvested biomass of 38,400 kg WW. As *S. latissima* has only been trialled for one year so far, further research is needed to realise its full harvesting potential. Unfortunately BMRS do not currently have the onshore facilities (e.g. drying or storage facilities) or staff requirements to achieve this harvesting potential.

7.4 Discussion

The large-scale growth of the European aquaculture industry has been constrained by a shortage of suitable sites, the ecological carrying capacity of existing sites, and consumers' increasing concerns about aquacultural products in relation to environmental impacts and food safety (Alexander *et al*. 2015; Simard *et al*. 2008; Kaiser and Stead, 2002). In 2012, the IDREEM (Increasing Industrial Resource Efficiency in European Mariculture) project was launched to investigate the feasibility of developing commercial-scale IMTA systems as a means to adopt a more environmentally friendly and economically efficient aquaculture industry for Europe.
Initially, Bantry Marine Research Station (BMRS) had planned to implement a large seaweed farm approximately 150-300 m downstream of the organic salmon farm; unfortunately, the required seaweed licence (applied for prior to the IDREEM project), was only approved after 4 years, which was towards the end of the IDREEM project. Applying for aquaculture licences in Ireland is a lengthy and complicated process (David O'Neill and John Murphy, Murphy's Irish Seafood, personal communication, 2014) and although Irish aquaculture policies pinpoint the need for strategic diversification of aquacultural activities and a need for innovative technology, there is no specific regulatory framework that deals with the implementation of IMTA aquaculture on a commercial scale. This lack of dedicated EU policy and legislation for IMTA could be a hindrance to its development in Europe. There is also a lack of public awareness of IMTA, and in BMRS' case, public consultations regarding the potential benefits of IMTA was believed to have helped in the granting of the seaweed licence (Alexander and Hughes, 2017; Alexander *et al*. 2016a; Hughes and Black, 2016; Alexander *et al*. 2015; Julie Maguire, personal communication, 2015). Following consultation with the Department of Agriculture, Food, and the Marine (DAFM), permission was granted for the deployment of two experimental-scale seaweed longlines without the need for a seaweed licence. This allowed for the deployment of one seaweed longline (maximum length of 300m) parallel to the salmon cages/coastline and one seaweed longline (maximum length of 100m) parallel to the coastline, 1 km east of the salmon farm. BMRS would have preferred to position the IMTA longline perpendicular to the most eastern salmon cage to maximise the flow of nutrients from the salmon cages based on the tidal flow patterns of Bantry Bay (Figure 1) (Maguire

and Burnell, 2001; Elliott *et al*. 1997; Edwards *et al*. 1996; Raines, 1996), however, DAFM would not allow this. Citing concerns about the high energy nature of Bantry Bay and the possibility of the longline detaching in the event of poor weather and impacting upon the existing salmon farm (Julie Maguire; David O'Neill; John Murphy, personal communication, 2015).

Over the course of this study, the results from both the IMTA and control site (i.e. seaweed biomass, carbon and nitrogen content of seaweeds, water parameters) were very similar. Both species of seaweed (*A. esculenta* and *S. latissima*) had a very similar rate of growth at both the IMTA and control site. In trial 1, the IMTA and control *A. esculenta* longlines produced a high amount of biomass, at 17.22 ± 1.69 kg/m WW and 18.13 ± 1.09 kg/m WW, respectively. For trial 3, the biomass of *A. esculenta* achieved at the IMTA and control longline were not as high as trial 1, at 11.90 ± 3.47 kg/m WW and 11.73 ± 0.66 kg/m WW, respectively. The biomass of *S. latissima* achieved at the IMTA and control longline in trial 3 was 6.02 ± 2.39 kg/m WW and 7.44 ± 0.84 kg/m WW, respectively. By comparison, other cultivation trials of *A. esculenta* and *S. latissima* conducted in Ireland (Strangford Lough, Ard Bay, Bantry Bay) have achieved mean biomasses of approximately 6-10 kg/m (Holdt and Edwards, 2014; Freddie O'Mahony, Cartron Point Shellfish Ltd, personal communication, 2013; Edwards and Watson, 2011; Arbona and Molla, 2006).

This similarity in biomass and carbon/nitrogen content may be due to the fact that the IMTA seaweed longline was not positioned in a location that would maximise the flow of nutrients from the salmon cages based on the tidal flow of Bantry Bay. However, due to the high flushing rate of Bantry Bay (approximately 70% of the water in the bay is exchanged over a 2-3 day period), even if the IMTA seaweed lines were positioned perpendicular to the salmon cages to maximise the flow of nutrients, these nutrients may not have been in the proximity of the seaweed longlines long enough to make a significant difference in growth to seaweed growing in a location of the bay not influenced by the salmon farm (AquaFact, 2012; Maguire and Burnell, 2001; Elliott *et al*. 1997; Edwards *et al.* 1996; Raines, 1996).

By the end of trial 1, the carbon and nitrogen content of *A. esculenta* was 29.26 ± 0.46% and 2.22 ± 0.07%, respectively, for the IMTA longline and 29.87 ± 0.27% and 2.34 ± 0.43%, respectively, for the control longline. The similarity of these results would suggest that positioning *A. esculenta* next to the salmon cages did not increase the rate in which it absorbed carbon and nitrogen. Unfortunately, as mentioned above, BMRS

was not able to position the IMTA longline in a location that would maximise the flow of excess nutrients from the cages. Nevertheless, even if the seaweed longlines were located in such a position, the high flushing rate of Bantry Bay (up to 70% of the water in the bay is exchanged over a 2 to 3 day period; Raines, 1996) may result in the nutrients from the farm being dissipated within a relatively short timeframe. This theory is supported by the water parameter data (i.e. TAN, nitrite, nitrate, phosphate, TPM, chlorophyll-a) that was collected over the duration of trial 1 and 2. Apart from a few exceptions, these water parameters were very similar at both the IMTA and control site. This included those months when the seaweed longlines were not present at either site, suggesting that the presence of the salmon cages does not have a detectable negative impact on the quality of the water in its immediate vicinity (approximately 50m north of the cages; i.e. the location that the water sampling took place).

Due to the small-scale nature of these trials (200-300 m IMTA longline and 100m control longline), and the positioning of the IMTA longlines, it was not possible to ascertain the extent to which the IMTA seaweed was uptaking excess nutrients from the salmon farm. However, it did show that both seaweeds grow well in Bantry Bay and that locating the seaweed longlines in close-proximity to the cages did not negatively impact upon growth. The level of contaminants (i.e. heavy metals and PCBs) did not differ greatly between the IMTA and control longlines either (trial 3), with *A. esculenta* and *S. latissima* from both sites falling below EU regulatory thresholds for human consumption and use in animal feeds (European Commission, 2006; European Commission, 2002). Only *S. latissima* from the IMTA line came close to the EU threshold for arsenic (40 mg/kg WW) for use as a feed ingredient (moisture content of 12%), with a level of 38.07 mg/kg WW (note: no EU regulatory threshold currently exists for arsenic levels in seaweed for human consumption) (European Parliament 2006). It is important to note, however, that we could also not be certain that this relatively high level of arsenic was as a result of cultivation in close proximity to the salmon cages, as these arsenic concentrations are consistent with those found in non-IMTA *S. latissima* (Raab *et al*. 2013; Llorente *et al*. 2011). Also, the concentration of cadmium in *A. esculenta* from the control line came close to the EU threshold (1 mg/kg WW) for use as a feed ingredient (moisture content of 12%), with a level of 0.88 mg/kg WW (European Parliament, 2006). This finding could be due to the high bioaccumulation behaviour of this genus. For example, recent studies of *A. esculenta* and the kelp, *Laminaria digitata*, showed that they had no active detoxification

mechanism for Cd (Reis *et al*. 2016; Ratcliff *et al*. 2015). Although there are no EU regulation for the presence of PCBs in seaweed, the levels of ICES-6 PCBs for all seaweeds at both the IMTA and control site fell well below the EU threshold for human consumption in fish and mollusc (European Parliament, 2002). *E. coli* was also not found on any seaweed sample.

From conducting these trials, BMRS identified five main steps that would need to be taken to produce seaweed as a co-cultured crop on a commercial scale: (1) positioning of the seaweed longlines next to the salmon cages in a location that receives the optimal flow of nutrients from the farm; (2) Acquisition of a boat for maintaining and harvesting the seaweed longlines; (3) Development of on-shore seaweed processing facilities (e.g. drying and blanching); (4) Identification of a market for the co-cultured seaweed - BMRS would most likely need to aim for high-value markets (e.g. cosmetic ingredients, processed seaweed for the health food market) as competing with Asian production will be difficult due to the difference in labour costs and volume of production (FAO, 2016; Granada *et al*. 2016; Neori *et al*. 2004; Lüning and Pang, 2003); and (5) Hiring of additional staff.

It must be noted, however, that this case study only examined the production of seaweed as a co-cultured crop in an offshore setting. Land-based IMTA offers a number of advantages over offshore IMTA, such as: 1) greater year-round control of the cultivation process from start to finish (e.g. temperature, nutrients; light); 2) more continuous and easier monitoring; 3) protection against adverse weather; 4) more effective control of disease and lice; 5) better control of waste production; and 6) avoidance of fish escapes (Badiola *et al*. 2012; Klinger and Naylor, 2012; Jeffery *et al*. 2010; Martins *et al*. 2010). A case study researching the viability of establishing land-based IMTA at the BMRS should be conducted in the future.

7.5 Conclusion

As IMTA incorporates ecologically based management practices, it has the potential to improve the social acceptability of aquaculture. There is a growing interest amongst consumers in sustainably produced seafood that they are willing to pay a premium for, particularly if the packaging contains eco-labels. Also, if IMTA operators were to

incorporate an eco-tourism venture into their farms, there is the opportunity to further the social acceptability of aquaculture, while also educating the community on food production techniques and ecological principles (Ma *et al*. 2013; Klinger and Naylor, 2012; Roheim *et al*. 2011; Culver and Castle, 2008).

7.6 Acknowledgements

Prof. Gavin Burnell provided feedback on this chapter. Freddie O' Mahony (Cartron Point Shellfish Ltd) provided the seeded seaweed string for the IMTA trials and also assisted in the monitoring of seaweed growth and the deployment and harvesting of seaweed. The trials for this study were designed by BMRS staff (Dr. Julie Maguire, Freddie O'Mahony, Marc Shorten, Mick Mackey, and Daryl Gunning), in conjunction with the owners of the salmon farm (Murphy's Irish Seafood, Ltd.) and the IDREEM consortium. A big thank you to Cat Smith, Fiona Moejes, Dee McElligot, Davide Monteggia, Thallis Ventura, and Dave Evans for their help in monitoring seaweed growth, deploying longlines, and harvesting. To Murphy's Irish Seafood staff, who operated the boats and assisted in the deployment and harvesting of seaweed. Finally, a thank you to the participants of the IDREEM project, who gave advice on these IMTA trials over the duration of the project.

7.7 References

Abreu, M.H., Varela, D.A., Henríquez, L., Villarroel, A., Yarish, C., Sousa-Pinto, I., and Buschmann, A.H. (2009). Traditional vs. integrated multi-trophic aquaculture of *Gracilaria chilensis* C.J. Bird, J. McLachlan & E.C. Oliveira: Productivity and physiological performance. *Aquaculture,* 293, pp. 211–220.

Alexander, K.A., and Hughes, A.D. (2017). A problem shared: technology transfer and development in European integrated multi-trophic aquaculture (IMTA). *Aquaculture*, 473, pp. 13-19.

Alexander, K.A., Angel, D., Freeman, S., Israel, D., Johansen, J., Kletou, D., Meland, M., Pecorino, D., Rebours, C., Rousou, M., Shorten, M., and Potts, T. (2016a). Improving sustainability of aquaculture in Europe: stakeholder dialogues on integrated multi-trophic aquaculture. *Environmental Science & Policy*, 55, pp. 96-106.

Alexander, K.A., Freeman, S., and Potts, T. (2016b). Navigating uncertain waters: European public perception of integrated multi trophic aquaculture (IMTA). *Environmental Science & Policy*, 61, pp. 230-237.

Alexander, K.A., Potts, T.P., Freeman, S., Israel, D., Johansen, J., Kletou, D., Meland, M., Pecorino, D., Rebours, C., Shorten, M., and Angel, D.L. (2015). The implications of aquaculture policy and regulation for the development of integrated multi-trophic aquaculture in Europe. *Aquaculture*, 443, pp. 16-23.

Aquafact. (2012). *Environmental survey at Gearhies aquaculture site, Bantry Bay, County Cork*. Aquafact International Services Ltd, 12 Kilkerrin Park, Tuam Road, Galway City, Galway, Ireland, pp. 1-64.

Arbona, J.F., and Molla, M. (2006). *Cultivation of brown seaweed* (*Alaria esculenta*). In: Watson, L. (eds.) *Aquaculture Explained No. 21*, Bord Iascaigh Mhara, Dublin, p. 50.

Badiola, M., Mendiola, D., and Bostock, J. (2012). Recirculating aquaculture systems (RAS) analysis: Main issues on management and future challenges. *Aquacultural Engineering,* 51, pp. 26–35.

Barsanti, L., and Gualtieri, P. (2014). *Algae: Anatomy, Biochemistry, and Biotechnology 2nd Edition*. CRC Press, Boca Raton, Florida, United States, p. 361.

Bridger, C.J., and Garber, A. (2002). Aquaculture escapement, implications, and mitigation: The salmonid case study. In *Ecological Aquaculture: The Evolution of the Blue Revolution*; Costa-Pierce, B.A., Ed.; Blackwell Science: Malden, MA, USA, pp. 77–102.

Brown, N. (2002). Flatfish farming systems in the Atlantic region. *Reviews in Fisheries Science,* 10, pp. 403–419.

Brown, J. J., Glenn, E. P., Fitzsimmons, K. M., and Smith, S. E. (1999). Halophytes for the treatment of saline aquaculture effluent. *Aquaculture*, 175, pp. 255-268.

Buhmann, A., and Papenbrock, J. (2013). Biofiltering of aquaculture effluents by halophytic plants: basic principles, current uses and future perspectives. *Environmental and Experimental Botany*, 92, pp. 122-133.

Bunker, F.Stp.D., Brodie, J.A., Maggs, C.A., and Bunker, A.R. (2010). *Seasearch Guide to Seaweeds of Britain and Ireland.* Marine Conservation Society, Ross-on-Wye, UK, p. 224.

Chopin, T., Cooper, J.A., Reid, G., Cross, S., and Moore, C. (2012). Open-water integrated multi-trophic aquaculture: environmental biomitigation and economic diversification of fed aquaculture by extractive aquaculture. *Reviews in Aquaculture*, 4, pp. 209-220.

Chopin, T. (2011). Progression of the integrated multi-trophic aquaculture (IMTA) concept and upscaling of IMTA systems towards commercialization. *Aquaculture Europe,* 36, pp. 5–12.

Chopin, T., Buschmann, A.H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G.P., Zertuche-González, J.A., Yarish, C., and Neefus, C. (2001). Integrating seaweeds into marine aquaculture systems: A key toward sustainability. *Journal of Phycology*, 37, pp. 975–986.

Culver, K., and Castle, D. (2008). *Aquaculture, Innovation, and Social Transformation*. Springer Science and Business Media: Berlin, Germany, 17, p. 344.

Edwards, M., and Watson, L. (2011). Aquaculture explained: cultivating *Laminaria digitata*. *Irish Sea Fisheries Board*, 26, p. 71. Available online: http://www.bim.ie/media/bim/content/publications/BIM%20Aquaculture%20Explained%20Issue%2026%20-%20Cultivating%20Laminaria%20digitata.pdf (Accessed on 1/03/17).

Edwards, A., Jones, K., Graham, J.M., Griffiths, C.R., MacDougall, N., Patching, J., Richard, J.M., and Raine, R. (1996). Transient coastal upwelling and water circulation in Bantry Bay, a ria on the south-west coast of Ireland. *Estuarine, Coastal, and Shelf Science*, 42(2), pp. 213–230.

Elliott, A.J., Barr, A.G., and Kennan, D. (1997). Diffusion in Irish coastal waters. *Estuarine, Coastal, and Shelf Science,* 44, pp. 15-23.

European Commission. (2006). Commission regulation (EC) No 1881/2006 of 19 December 2006 setting levels for certain contaminants in foodstuffs. Available online: http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32006R1881 (accessed on: 12/08/17). [Note: relevant amendments to this document can be found at: http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:02006R1881-20170728&qid=1504517810757&from=IT (accessed on: 12/08/17)].

European Commission. (2002). Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. Available online: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2002L0032:20061020:EN:PDF (accessed on 12/08/17).

Food and Agriculture Organization of the United Nations (2016). *The State of World Fisheries and Aquaculture 2016: Contributing to Food Security and Nutrition for All*; FAO: Rome, Italy, p. 200.

Granada, L., Sousa, N., Lopes, S., and Lemos, M.F.L. (2016). Is integrated multitrophic aquaculture the solution to the sectors' major challenges? - A review. *Reviews in Aquaculture*, 8, pp. 283–300.

Grigorakis, K., and Rigos, G. (2011). Aquaculture effects on the environmental and public welfare—The case of Mediterranean Mariculture. *Chemosphere*, 855, pp. 899–919.

Guiry, M.D. (2017). AlgaeBase – *Alaria esculenta* (Linnaeus) Greville, uses and compounds. Available online: http://www.algaebase.org/search/species/usage/?id=82 (accessed on: 08/08/17).

Hannah, L., Pearce, C.M., and Cross, S.F. (2013). Growth and survival of California sea cucumbers (*Parastichopus californicus*) cultivated with sablefish (*Anoplopoma fimbria*) at an integrated multi-trophic aquaculture site. *Aquaculture*, 406-407, pp. 34-42.

Holdt, S.L., and Edwards, M.D. (2014). Cost-effective IMTA: a comparison of the production efficiencies of mussels and seaweed. *Journal of Applied Phycology*, 26(2), pp. 933-945.

Hughes, A.D., and Black, K.D. (2016). Going beyond the search for solutions: understanding trade-offs in European integrated multi-trophic aquaculture development. *Aquaculture and Environment Interactions*, 8, pp. 191-199.

Huo, Y., Wu, H., and Chai, Z. (2012). Bioremediations efficiency of *Gracilaria verrucosa* for an integrated multi-trophic aquaculture system with *Pseudosciaena crocea* in Xiangshan Harbor, China. *Aquaculture*, 326–329, pp. 99–105.

Irish Seaweeds. (2016a). Irish Wakame (*Alaria esculenta*). Available online: http://www.irishseaweeds.com/irish-wakame-alaria-esculenta/ (accessed on: 12/08/17).

Irish Seaweeds. (2016b). Sweet Kelp (*Saccharina lattisima*). Available online: http://www.irishseaweeds.com/sweet-kelp-saccharina-lattisima-laminaria-saccharina/ (accessed on: 12/08/17).

Jeffery, K.R., Stone, D., Feist, S.W., and Verner-Jeffreys, D.W. (2010). An outbreak of disease caused by *Francisella* sp. in Nile tilapia *Oreochromis niloticus* at a recirculation fish farm in the UK. *Disease of Aquatic Organisms,* 91, pp. 161–165.

Kaiser, M., and Stead, S.M. (2002). Uncertainties and values in European Aquaculture: communication, management, and policy issues in times of "changing public perception". *Aquaculture International*, 10, pp. 469-490.

Klinger, D., and Naylor, R. (2012). Searching for solutions in aquaculture: Charting a sustainable course. *Annual Review of Environment and Resources,* 37, pp. 247–276.

Lander, T.R., Robinson, S.M.C., MacDonald, B.A., Martin, and J.D. (2013). Characterization of the suspended organic particles released from salmon farms and theirpotential as a food supply for the suspension feeder, Mytilus edulis inIntegrated Multi-trophic Aquaculture (IMTA) systems. *Aquaculture Reports*, 3, pp. 67-76.

Llorente-Mirandes, T., Ruiz-Chancho, M.J., Barbero, M., Rubio, R., and Lopez-Sanchez, J.F. (2011). Determination of water-soluble arsenic compounds in commercial edible seaweed by LC-ICPMS**.** *Journal of Agricultural and Food Chemistry*, 59(24), pp. 12963–12968.

Lüning, K., and Pang, S. (2003). Mass cultivation of seaweeds: current aspects and approaches. *Journal of Applied Phycology*, 15(2-3), pp. 115-119.

Ma, C., Zhang, X., Chen,W., Zhang, G., Duan, H., Ju, M., Li, H.. and Yang, Z. (2013). China's special marine protected area policy: Trade-off between economic development and marine conservation. *Ocean and Coastal Management*, 76, pp. 1–11.

Maguire, J.A., and Burnell, G.M. (2001). The effect of stocking density in suspended culture on growth and carbohydrate content of the adductor muscle in two population of the scallop (*Pecten maximus* L.) in Bantry Bay, Ireland. *Aquaculture*, 198, pp. 95-108.

Marinho-Soriano, E., Azevedo, C.A.A., Trigueiro, T.G., Pereira, D.C., Carneiro, M.A.A., and Camara, M.R. (2011). Bioremediation of aquaculture wastewater using macroalgae and Artemia (2011). *International Biodeterioration and Biodegradation,* 65, pp. 253–257.

Martins, C.I.M., Eding, E.H., Verdegem, M.C.J., Heinsbroek, L.T.N., Schneider, O., Blancheton, J.P., Roque d'Orbcastel, E., and Verreth, J.A.J. (2010). New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. *Aquacultural Engineering*, 43, pp. 83–93.

Naylor, R., and Burke, M. (2005). Aquaculture and ocean resources: Raising tigers of the sea. *Annual Review of Environment and Resources,* 30, pp. 185–218.

Naylor, R., Eagle, J., and Smith, W. (2003). Salmon aquaculture in the Pacific North-west: A global industry with local impacts. *Environment,* 45, pp. 18–39.

Neori, A., Chopin, T., and Troell, M. (2004). Integrated aquaculture: Rationale, evolution, and state of the art emphasizing seaweed biofiltration in modern Mariculture. *Aquaculture,* 231, pp. 361–391.

Primavera, J.H. (2006). Overcoming the impacts of aquaculture on the coastal zone. *Ocean and Coastal Management*, 49(9-10), pp. 531-545.

Raab, A., Newcombe, C., Pitton, D., Ebel, R., and Feldmann, J. (2013). Comprehensive Analysis of Lipophilic Arsenic Species in a Brown Alga (*Saccharina latissima*). *Analytical Chemistry*, 85(5), pp. 2817−2824.

Raines, R. (1996). Red tides and toxic phytoplankton in the southwest. *Aquaculture Ireland*, 10(11), pp. 26–27.

Ratcliff, J.J., Wan, A.H.L., Edwards, M.D., Soler-Vila, A., Johnson, M.P., Abreu M.H., and Morrison L. (2015). Metal content of kelp (*Laminaria digitata*) co-cultivated with Atlantic salmon in an Integrated Multi-Trophic Aquaculture system. *Aquaculture*, 450, pp. 234–243.

Reis, P.A., Gonçalves, J., Abreu, H., Pereira, R., Benoit, M., O'Mahony, F., Connellan, I., Maguire, J., and Ozório, R. (2016). Seaweed *Alaria esculenta* as a biomonitor species of metal contamination in Aughinish Bay (Ireland). *Ecological Indicators*, 69, pp. 19–25.

Roheim, C.A., Asche, F., and Santos, J.I. (2011). The elusive price premium for ecolabelled products: Evidence from seafood in the UK market. *Journal of Agricultural Eonomics*, 62, pp. 655–668.

Simard, F., Ojeda, J., and Haroun, R. (2008). The sustainable development of Mediterranean aquaculture: problems and perspectives. *Options Méditerranéennes*, 62, pp. 113-124.

Troell, M., Joyce, A., Chopin, T., Neori, A., Buschmann, A.H., and Fang, J.G. (2009). Ecological engineering in aquaculture—Potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. *Aquaculture*, 297, pp. 1–9.

Troell, M., Halling, C., Neori, A., Chopin, T., Buschmann, A.H., Kautsjy, N., and Yarish, C. (2003). Integrated mariculture: asking the right questions. *Aquaculture*, 226, pp. 69-90.

University of Coimbra. (2008a). Portuguese Seaweeds Website (MACOI) – *Alaria esculenta* (Linnaeus) Greville.
Available online: http://macoi.ci.uc.pt/spec_list_detail.php?spec_id=241 (accessed on: 1/09/17).

University of Coimbra. (2008b). Portuguese Seaweeds Website (MACOI) – *Saccharina latissima* (Linnaeus), C.E. Lane, C. Mayes, Druehl & G.W. Saunders. Available online: http://macoi.ci.uc.pt/spec_list_detail.php?spec_id=178 (accessed on 1/9/17).

Webb, J.M., Qunit., R., Papadimitriou, S., Norman, L., Rigby, M., Thomas, D.N., and Le Vay, L. (2012). Halophyte filter beds for treatment of saline wastewater from aquaculture. *Water Research*, 46, pp. 5102-5114.

Zhou, Y., Yang, H., Hu, H., Liu, Y., Mao, Y., and Zhou, H. (2006). Bioremediation potential of the macroalga *Gracilaria lemaneiformis* (Rhodophyta) integrated into fed fish culture in coastal waters of north China. *Aquaculture*, 252, pp. 264–276.

Concluding remarks

Our planet is currently experiencing a crisis of dwindling freshwater supplies and salinisation of soil and groundwater (Singh *et al*. 2014; Turcios and Papenbrock, 2014; Ventura & Sagi, 2013). Approximately one-third of the global terrestrial farmed area is affected by salinisation, and the freshwater shortage is expected to increase in the future due to a growing world population and rise in prosperity (US Census Bureau, 2017; De Vos *et al*. 2010; Ramani *et al*. 2006). Climate change is also influencing the ability of soils around the world to support crop production (Lal, 2004; Nearing *et al*. 2004). With this in mind, it is essential that novel food production methods are explored and developed, such as new aquaculture techniques, or that new crops are developed that have a greater salt resistance than conventional agricultural crops, especially those that can achieve high, economically lucrative yields and utilise crop production techniques that conserve water and energy consumption.

From the work detailed in this thesis, it has been found that *Salicornia europaea* has enormous potential as a biofilter of saline, aquaculture wastewater, removing up to 97.65%, 97.14%, 99.02%, and 83.34% of TAN, nitrite, nitrate, and orthophosphate, respectively, per week from the wastewater of an oyster hatchery. However, importantly, the level of harvestable biomass achieved was low (0.25 kg/m^2), which was due to the study limitations, i.e. the low nutrient levels of the wastewater from the oyster farm and/or the lack of a suitable infrastructure for the optimal development of *S. europaea* seedlings. Future work should further assess the biofiltering potential of *S. europaea* on a larger, commercial scale and with wastewater containing a higher level of nutrients.

A second important finding from this work is that seeds of *S. europaea* may require stratification (pre-germination treatment) to break dormancy and achieve successful germination. Future work should assess the impact of stratification on small and large *S. europaea* seeds, as it has been reported that larger seeds tend to have greater germination success (Philipupillai and Ungar, 1984; Ungar, 1979). Crop yield and resource use efficiency are dependent on successful plant establishment in the field, and the ability of seeds to germinate and establish seedlings rapidly is a critical objective of

the agricultural industry (Finch-Savage and Bassel, 2015). Increasing the germination success of *S. europaea*, or of any crop, has the potential for large economic savings and resource efficiency during crop production.

A third finding from this thesis is that a methodological problem exists in evaluation and monitoring of holothurian size. It is difficult to measure the length and weight of holothurians consistently (Watanabe, *et al*. 2012; Yamana *et al.* 2005; Battaglene *et al.* 1999; Sewell, 1990). Although it was found that the anaesthetic agent, 2% $MgCl_2$, significantly reduced the body weight variation of the holothurian, *Holothuria forskali*, by 84.45%, long periods of exposure to anaesthetics or handling of *H. forskali* lead to morphological and physiological stress. Nevertheless, the anaesthesia of marine species may have future potential in aquaculture in areas such as accurate size measurements and reducing stress during transport. It is recommended that the utilisation of anaesthesia in such areas is researched further.

This thesis has also found that Passive Integrated Transponder (PIT) tags are not an effective tagging method for *H. forskali*. Future studies should assess the efficacy of these tagging methods and other novel identification techniques (e.g. pattern recognition technology) (http://www.reijns.com/i3s/) on *H. forskali*. Photographic pattern recognition, in particular, has considerable potential for aquaculture research, as individual specimens can be tracked without the need for any stress-inducing physical contact.

Maraponic systems have potential as modelling systems for IMTA principles and as viable small-scale on-land saltwater food production systems. As these systems were prototypes, future studies are required to address a number of the limitations of this study. For example, modifications should be made to the design of the systems, particularly in relation to the housing of seaweeds. Further work is also required to identify a suitable ratio/biomass of extractive and inorganic for the efficient biofiltration of the water and the need for additional filtration and water exchange should also be assessed (this study was a zero-exchange system with no mechanical means of treating the water). In Ireland, the regulations surrounding experimentation with vertebrates are rigorous and the pressure to reduce the level of experimentation on animals is increasing globally. Therefore, the need to model the presence of vertebrate species in IMTA trials

(and any trial involving animals, particularly those that are vertebrate) may become increasingly necessary in the future.

This thesis has also examined the Fatty acid (FA) content of all species from trialled maraponic systems. There was strong evidence from this analysis that abalone were assimilating salmon waste, however, evidence for assimilation by mussels and sea cucumbers was less clear. The natural abundances of carbon and nitrogen stable isotopes (δ13C and δ15N) have been used to successfully identify sources of organic matter in aquatic food webs and to define the trophic positions of consumers (Post, 2002; Cabana and Rasmussen, 1996) and when used in conjunction with fatty acid analysis, may have the potential to provide a more reliable methodology for the research of trophic food webs and animal/plant nutrition (Ruess and Chamberlain, 2010; Alfaro *et al*. 2006; Hooker *et al*. 2001).

Finally, this thesis has examined pilot-scale IMTA systems (seaweed longlines containing *Alaria esculenta* and *Saccharina latissima*), which were trialled next to an operational organic salmon farm in Bantry Bay, Co. Cork, Ireland. This study demonstrated that both species of seaweed can achieve standard to high levels of biomass in Bantry Bay and that locating the seaweed longlines in close-proximity to the cages did not negatively impact upon growth or condition. From conducting these trials, five main practical steps have been identified that would need to be taken to produce seaweed as a co-cultured crop on a commercial scale: (1) positioning of the seaweed longlines next to the salmon cages in a location that receives the optimal flow of nutrients from the farm; (2) Acquisition of a boat for maintaining and harvesting the seaweed longlines; (3) Development of on-shore seaweed processing facilities (e.g. drying and blanching); (4) Identification of a market for the co-cultured seaweed - and (5) Hiring of additional staff. It must be noted, however, that this case study only examined the production of seaweed as a co-cultured crop in an offshore setting.

References

Alfaro, A.C., Thomas, F., Sergent, L., and Duxbury, M. (2006). Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. *Estuarine, Coastal, and Shelf Science,* 70(1-2), pp. 271-286.

Battaglene S. C., Seymour J. E., and Ramofa, C. (1999). Survival and growth of cultured juvenile sea cucumbers, *Holothuria scabra*. *Aquaculture,* 178, pp. 293-322.

Cabana, G., and Rasmussen, J.B. (1996). Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences.* 93, p. 10844.

De Vos, A. C., Broekman, R., Groot, M. P., and Rozema, J. (2010). Ecophysical response of *Crambe maritima* to airborne and soil-borne salinity. *Annals of Botany*, 105, pp. 925-937.

Finch-Savage, W.E., and Bassel, G.W. (2015). Seed vigour and crop establishment: extending performance beyond adaption. *Journal of Experimental Botany,* 67(3), pp. 567-591.

Hooker, S.K., Iverson, S.J., Ostrom, P., and Smith, S.C. (2001). Diet of northern bottlenose whales inferred from fatty-acid and stable isotope analyses of biopsy samples. *Canadian Journal of Zoology*, 79(8), pp. 1442-1454.

Lal, S. (2004). Soil carbon sequestration impacts on global climate change and food security. *Science*, 304, pp. 1623-1626.

Nearing, M.A., Pruski, F.F., and O'Neal, M.R. (2004). *Journal of Soil and Water Conservation*, 59(1), pp. 43-50.

Philipupillai, J., and Ungar, I. A. (1984). The effect of seed dimorphism on the germination and survival of *Salicornia europaea* L. populations. *American Journal of Botany*, 71(4), pp. 542-549.

Pleissner, D., Eriksen, N.T., Lundgreen, K., and Riisgård, H.U. (2012). Biomass compostion of blue mussels, *Mytilus edulis*, is affected by living site and species of ingest microalgae. *International Scholarly Research Notices – Zoology*, 2012, pp. 1-12.

Ramani, B., Reeck, T., Debez, A., Stelzer, R., Huchzermeyer, B., Schmidt, A., and Papenbrock, J. (2006). *Aster tripolium* L. and *Sesuvium portulacastrum* L.: two halophytes, two strategies to survive in saline habitats. *Plant Physiology and Biochemistry*, 44, pp. 395-408.

Ruess, L., and Chamberlain, P.M. (2010). The fat that matters: soil food web analysis using fatty acids and their carbon stable isotope signature. *Soil Biology and Biochemistry*, 42(11), pp. 1898-1910.

Singh, D., Buhmann, A. K., Flowers, T. J., Seal, C. E., and Papenbrock, J. (2014). *Salicornia* as a crop plant in temperate regions: selection of genetically characterised ecotypes and optimization of their cultivation conditions. *AoB PLANTS*, 6(plus071); doi:10.1093/aobpla/plu071.

Subasinghe, R., Soto, D., and Jia, J. (2009). Global aquaculture and its role in sustainable development. *Reviews in Aquaculture,* 1, pp. 2–9.

Turcios, A. E., and Papenbrock, J. (2014). Sustainable treatment of aquaculture effluents – what can we learn from the past for the future? *Sustainability*, 6, pp. 836-856.

Ungar, I.A. (1979). Seed dimorphism in *Salicornia europaea* L. *Botanical Gazette*, 140(1), pp. 102-108.

United States Census Bureau (2017). U.S. and World Population Clock. Available online: http://www.census.gov/popclock/ (accessed on 4th October 2017).

Ventura, Y., and Sagi, M. (2013). Halophyte crop cultivation: The case for *Salicornia* and *Sarcocornia*. *Environmental and experimental botany*, 92, pp. 144-153.

Watanabe, S., Zarate, J.M., Sumbing, J.G., Lebata-Ramos, M.J.H., and Nievales, M.F. (2012). Size measurement and nutritional condition evaluation methods in sandfish (Holothuria scabra Jaeger). *Aquaculture Research*, 43, pp. 940-948.

Yamana Y., Hamano T., and Yamamoto, K. (2005), Anesthetizer of the adult sea cucumber *Apostichopus japonicus. Nippon Suisan Gakkaishi,* 71, pp. 299- 306.

Appendix

Chapter 2:

Trial 1

Pre-trial *Salicornia europaea* germination attempts

The germination of *S. europaea* seeds was attempted on three occasions before successful germination was obtained and trial 1 could commence.

(a) Germination attempt 1

The first attempt at germinating *S. europaea* seeds took place indoors under natural light conditions (approximately 16 h days/8 nights; mean temperature: 18 ± 2.12 °C) at the Bantry Marine Research Station (BMRS) form 13th June to 11th July 2013.

S. europaea seeds (n=100) were sown onto the surface of individual plugs of 2 Garland® 84 plug-trays filled with Shamrock® multi-purpose compost and hand-watered (with a 2 L watering can) with freshwater every 2-3 days. Watering took place when the plugs appeared dry. The plugs were watered until they appeared damp. Care was taken to not overwater the compost to prevent the seeds from rotting. After 2 weeks, 14 seedlings germinated. At this stage, irrigation salinity was increased to 10 ± 0.34 ppt and included phostrogen plant feed (N:P:K 14:10:27 and trace elements; Bayer CropScience Ltd, Cambridge, UK). The seedlings were again irrigated every 2-3 days via the methods detailed above. After two weeks being irrigated at this salinity, 2 further seedlings emerged. Due to the low emergence of seedlings (16%), this trial was terminated at this stage.

(b) Germination attempt 2

The second attempt at germinating *S. europaea* seeds took place in a plant growth room at University College Cork (14 light:10 dark photoperiod; mean temperature: 18.51 ± 0.59 °C; mean humidity: 63.70 ± 7.32%) from 12th July to 19th August 2013. The light in the growth room was 5.3 ± 0.23 x10 μmol m^{-2} s^{-1} (measured with a Skye® PAR meter).

S. europaea seeds (n=350) were sown onto the surface of individual plugs of 5 Garland® 84 plug-trays filled with Shamrock® multi-purpose compost and transferred to the plant growth room. Half of the plugs (n=175; treatment 1) were given 9ml of freshwater per plug and the remaining plugs (n=175; treatment 2) were given 9ml of 10 ppt saltwater (30:70 seawater:freshwater) per plug. Then, for the following 2 weeks (until seedlings began to emerge), treatment 1 received 3ml of freshwater per plug every 2nd day and treatment 2 received 3ml of 10ppt water every 2nd day. After this 2 week period, treatment one and two received 3ml of 10 ± 0.65 ppt saltwater/phostrogen solution per plug every 2nd day for a further 23 days. At the end of this period, only 10.86% and 15.43% of seedlings emerged for treatment one and two, respectively. Due to this low emergence of seedlings, the trial was terminated at this stage.

(c) Germination attempt 3 and seedling development

The third attempt at germinating *S. europaea* seeds took place at UCC from 18th August to 5th November 2013.

Step 1: Pre germination stratification

S. europaea seeds (n=260) were distributed equally amongst 26 90 mm petri dishes (n=10 per dish). The petri dishes had two medium types: cotton wool (n=13; treatment 1) and 90 mm filter paper (n=13; treatment 2). A 0.5L mist-spray watering bottle was used to keep both mediums damp (approximately 0.5 ml of freshwater was required per petri dish). The lids of the petri dishes were taped shut and the petri dishes were then placed in a refrigerator at 5 ± 1.3 °C. The seeds were checked on a daily basis. If mould was present on the seeds, it was gently removed with a small paintbrush. If a large amount of mould had formed within the petri dish, the mould was removed and the respective medium replaced. Both mediums were kept damp throughout the stratification period and required dampening approximately every 3-4 days.

Step 2: Early germination

Following removal from the fridge, the petri dishes were kept indoors under natural light conditions (approximately 12 h days/12 h nights; mean temperature: 19 ± 1.9 °C) for 14 days. Salinity was introduced at this stage by dampening both medium types with approximately 0.5 ml of 10 ± 0.87 ppt saltwater (30;70 seawater:freshwater). The petri dishes were checked daily for mould formation and to see if their respective medium

required more saltwater solution. If mould formation was occurring it was removed with a small paintbrush. Each treatment's petri dishes were kept damp by adding 0.5 ml of the above solution when the respective medium appeared dry (required every 3-4 days for the duration of this stage). At the end of the early germination stage, germination on cotton wool and filter paper was 77.69 ± 13.01% and 65.39 ± 7.76%, respectively. It was also harder to remove seeds from the cotton wool medium.

Step 3: Seedling development

After the 14 day early germination stage, successfully seedlings and from both treatments were then transplanted into separate Garland® 84 plug trays containing a 50:50 mix of Shamrock® multi-purpose compost and Hortland® horticultural sand (2 trays per treatment), which were sitting on top of watering trays. They were maintained in a greenhouse at UCC under natural light conditions (approximately 10 h days/14 h nights; mean temperature of 19 °C ± 0.8) for a further 30 days. Every 3-4 days the watering tray was filled with a 10 ppt ± 0.21 saltwater (30:70 seawater: freshwater)/phostrogen solution. After 30 days, flowering of seedlings had begun and the growth of seedlings had ceased, therefore, this trial was terminated at this stage.

Chapter 3:

Trial 3

Table 1: Mean germination of seeds exposed to varying sterilisation times, based on agar type (n = 20 per agar percentage / sterilisation time) (mean ± SD)

Agar (%)	Sterilisation time (mins)				
	1	5	10	15	Control (0)
	% Germination				
0.4	80	75	75	85	70
0.5	70	80	60	60	75

Table 2: Mean root emergence of seeds exposed to varying sterilisation times, based on agar type (n = 20 per agar percentage / sterilisation time) (mean ± SD)

Agar (%)	Sterilisation time (mins)				
	1	5	10	15	Control (0)
	% Germination				
0.4	75	40	55	70	60
0.5	50	70	45	50	30

Note: there was no significant difference ($p>0.05$) in mean germination and root emergence between 0.4% and 0.5% agars

Chapter 4:

Trial 2

Table 3: Mean body weight (g) of sea cucumbers (n=4 per treatment) from 5 repeated measurements pre and post anaesthesia (mean ± SD)

2% $MgSO_4$	4% $MgSO_4$	1% $MgCl_2$	1.5% $MgCl_2$	2% $MgCl_2$
Pre				
177.51 ± 2.44	226.57 ± 5.73	225.65 ± 12.80	198.11 ± 7.75	206.27 ± 7.54
131.55 ± 3.28	136.77 ± 0.94	129.5 ± 17.71	108.81 ± 7.68	85.29 ± 3.88
157.73 ± 0.90	145.72 ± 2.29	178.4 ± 8.81	171.05 ± 9.51	133.32 ± 7.52
151.43 ± 3.56	142.95 ± 6.51	84.53 ± 9.88	76.44 ± 2.33	127.03 ± 4.28
Post				
206.68 ± 1.80	213.65 ± 0.31	254.13 ± 0.49	199.59 ± 0.90	186.18 ± 0.37
153.27 ± 1.71	139.42 ± 0.89	133.55 ± 2.41	102.83 ± 0.61	76.37 ± 0.47
187.21 ± 1.02	141.1 ± 0.77	222.88 ± 3.58	167.37 ± 1.07	131.99 ± 0.63
170.55 ± 0.65	141.16 ± 2.34	97.95 ± 0.90	74.99 ± 0.80	115.8 ± 0.29

Table 4: Mean body length (mm) of sea cucumbers (n=4 per treatment) from 5 repeated measurements pre and post anaesthesia (mean ± SD)

2% $MgSO_4$	4% $MgSO_4$	1% $MgCl_2$	1.5% $MgCl_2$	2% $MgCl_2$
Pre				
183.6 ± 19.62	217.2 ± 4.15	181 ± 9.57	199.6 ± 7.73	169.6 ± 17.36
164.6 ± 19.36	156.5 ± 7.3	119.8 ± 13.66	129 ± 5.34	111.2 ± 10.87
134.6 ± 10.78	149.8 ± 8.07	178.4 ± 11.3	186.8 ± 12.4	121.2 ± 10.87
164 ± 11.81	140.8 ± 15.51	102.6 ± 7.7	103.4 ± 4.04	115.6 ± 9.53
Post				
235 ± 13.21	238.2 ± 11.21	311.6 ± 13.97	215 ± 6.6	212 ± 12.17
177.8 ± 11.61	163.4 ± 18.08	201 ± 30.08	145 ± 13.17	116.6 ± 6.88
159.6 ± 20.67	187.6 ± 11.19	246 ± 32.29	173.8 ± 10.8	134.8 ± 7.05
173.2 ± 22.12	146.8 ± 13.81	150.8 ± 18.27	112.4 ± 6.35	139.4 ± 9.24

Trial 3

Table 5: Mean body weight (g) of sea cucumbers (n=10 per treatment) from 5 repeated measurements (mean ± SD) of control and anaesthesia treatments

Control	2% $MgSO_4$	4% $MgSO_4$	1% $MgCl_2$	1.5% $MgCl_2$	2% $MgCl_2$
73.33±2.29	69.24±4.69	150.09±0.72	73.73±0.43	113.06±0.75	68.48±0.13
87.76±1.64	51.27±0.89	99.90±0.38	121.01±1.14	51.92±0.28	64.78±0.92
97.56±0.44	156.87±0.7	100.27±0.48	101.39±0.13	85.48±0.13	87.54±0.38
153.6±1.26	62.8±0.55	128.5±1.65	130.53±1.47	85.32±2.21	93.36±0.15
76.47±2.12	128.11±0.2	93.5±0.64	150.65±0.42	107.08±0.23	100.78±0.54
93.67±0.59	107.81±0.61	79.58±0.36	62.84±0.34	127.4±0.87	68.42±0.23
90.58±3.52	43.14±0.26	103.9±10.88	87.87±1.19	69.16±0.11	93.04±0.29
92.48±2.72	67.13±0.67	120.09±2.59	89.2±0.55	116.22±0.4	66.04±0.25
104.85 ±5.28	77.58±1.64	47.76±1.12	104.4±0.16	75.96±0.18	109.96±0.21
118.38±8.01	100.02±0.99	176.83±0.68	112.75±1.7	66.46±0.17	83.92±0.37

Table 6: Mean body length (mm) of sea cucumbers (n=10 per treatment) from 5 repeated measurements (mean ± SD) of control and anaesthesia treatments

Control	2% $MgSO_4$	4% $MgSO_4$	1% $MgCl_2$	1.5% $MgCl_2$	2% $MgCl_2$
107.6±14.69	138±19.22	137.4±11.46	142±9.87	196.2±10.43	164.4±8.29
86.2±12.99	109.6±8.26	170±12.98	160.6±10.88	114.4±7.5	163.6±12.46
127.6±16.02	223.8±5.54	130.6±4.34	142.6±6.84	137±7.68	150.2±6.94
143.4±20.91	106±9.06	134.6±8.59	193.8±7.95	165±9.92	144.6±8.14
114±18.67	181±18.88	145±9.14	167.8±8.9	166.6±11.28	138.8±10.03
116±6.67	183.8±14.11	130.6±7.06	119.2±7.92	196.6±3.85	126±3.81
131.2±8.41	114.4±16.62	130.6±6.19	156.4±19.14	142.2±5.72	165.4±5.22
118.6±10.69	122.8±10.23	145.6±4.34	142±2.35	186.6±17.27	115±7.04
123.8±15.06	126±27.29	109±3.81	160.4±9.66	129.6±3.65	209.4±15.08
157.4±5.18	198±11.60	171.8±8.23	161±10.54	145±7.81	174.6±9.61

Chapter 6

The WinFish Model

AquaFish and WinFish are essentially the same modelling approach, the former being the underlying mathematical development of the model and the latter a console-based representation of the AquaFish model, that allows for simple additional calibration and validation through simulation.

Aquafish is the general process of determining how fish grow and applying state-of-the-art equations to describe, mathematically, the processes of feeding and feeding regulation, energy transfers (input, uptake and loss) through harvestable products, wastes and biological processes, oxygen consumption through anabolic and catabolic processes, and mass balance equations to account for energetic inputs and outputs to the growth of a single fish, in an aquaculture setting. Once internally calibrated, the model is coded as dynamic link libraries (DLL), directly usable in a variety of contexts. AquaFish was developed via application through visual software platforms including, for example, Powersim, a proprietary commercial simulation software platform; and InsightMaker, a free simulation software platform. Once the model is developed there needs to be a means with which to visualise the model outcomes in a succinct manner. Winfish, is one of the contexts in which DLLs can be used, and provides the visual representation platform through which this fish growth can be visualised, and outputs of the model presented using graphical, data, and summary formats. Run in Windows (32-bit or 64-bit), WinFish is coded using C++ to produce a console-based format / layout. These are used as workbenches to test the integrity of the models and provide simple ways of calibration and validation against measurements of individual growth, without the added complexity of a population- and environment-level modelling framework.

Having defined the growth model (AquaFish), simulations using WinFish can be adjusted for specific local circumstances by applying a limited number of additional parameters (such as changes in water temperature over the year, start weight and growth period). These additional parameters, representative of environmental and growth conditions and to some extent management decisions (e.g. on input date), can have a distinct effect on the potential for fish species to grow. Winfish can be used to simulate

variations in them, to see what effect this has on growth, and to determine the optimum period for aquaculture production.

Initial development

The model is developed through a series of modules that cover:

- How feed is used as it moves through stomach to gut, from gut to tissue growth, and out as waste
- Feed input, as affected by fish size, temperature and oxygen availability and demand
- Energy uptake in feed and use in maintenance, swimming, and digestion
- Mass balance of terms.

Feeding Module

The underlying feeding module encompasses all the processes involved in food intake, ingestion, and assimilation for fish growth or as waste to the environment. Feed input is driven through a calibrated Feed Conversion Ratio (FCR) table.

Understanding the biological processes involved is the first stage, allowing numerical equations and values to be added to control the processes. Table 7 provides an explanation of the processes involved in determining feeding and internal bio-processing of feed through the fish.

Table 7: AquaFish model feeding module parameter descriptors

Term	Description	Explanation
Feeding module determines the throughput of pelleted feed into the fish through the mouth and its transition through the stomach to the gut and out of the anus. It takes into account the assimilation of food through the gut into somatic tissue growth and release of faecal waste to the environment.		
Nominal Feed Conversion Ratio (FCR)	The amount of dry pelleted feed (in Kg) required to generate somatic growth of 1 kg in the fish.	FCR is one of the key means with which fish farmers evaluate the productivity of the culture system at individual sites.
Initial Fish weight (g)	The average weight at which fish are added to cages at sea.	The transfer takes place from freshwater cages or tanks, where salmon are grown for 6-9 months after leaving the hatchery. The size is variable depending on the time of year added to seacages, being less weight if added in spring, and heavier if left in freshwater facilities for a longer period and added to sea in the autumn.
Assimilation Efficiency	The percentage of food fed that gets assimilated in somatic growth, included in the model as a decimal.	Assimilation values represent the ability of the fish to take up the carbon (C), nitrogen (N), and phosphorus (P), which are present in differing quantities in feed pellets, depending on the proportions of protein, lipid, and carbohydrate present. Carbon makes up approximately 50% of feed and is assimilated at an efficiency of 0.8. Nitrogen assimilation is generally higher (0.85) but the amount present is significantly lower at 7.2%. P has the lowest assimilation efficiency as fish lack phytase in the gut to break it down efficiently, so that only half of the 1.6% of P added to feed is assimilated. The value used in the model represents a mean value assimilation of C, N, and P.
Elimination	An “IF” statement in the model which defines when elimination of waste feed, as faeces, is to take place and at what proportion of the original food intake.	If the gut content is greater than the value calculated to represent the gut being full, then the value for elimination is proportional to the gut content divided by the dump rate coefficient. Otherwise elimination is zero, and further input to the gut is created by the transfer of stomach contents to the gut. Elimination represents the mechanism by which feed intake is eliminated as waste faeces to the environment.

Table 7 continued

Elimination Rate Coefficient	Represents the speed with which feed in the gut is eliminated from the anus as waste.	
Elimination Rate	A function of time and on the assumption that faecal waste is produced all at once, once per day.	Timesteps in the model are measured in days, and the Dump Rate ensures all waste is rid before the start of the next feeding process.
Faeces wet weight (optional)	Wet weight of faecal material added to the environment.	Total faeces produced is processed in the model as dry weight and requires conversion to wet weight of material by the addition of percentage water content.
Faeces water content (optional)	Percentage of wet faecal matter that is eliminated through the anus, that is water, presented as a decimal.	Processing through the gut is managed as dry weight, so this converts dry matter to wet matter on a weight basis, based on a standardised faecal water content.

Allometric Control of Feeding

In any specific aquaculture application, feeding in fish is controlled tightly, not least because feed is the largest operating cost in fish cage culture. As fish aquaculture has developed and grown the control over feeding has been greatly improved and the quantity of feed added per kg increase in fish biomass (= Feed Conversion Ratio or FCR) has reduced significantly for a range of species, and more specifically for those species grown in Northern and Southern Europe. Thus, in the culture of salmon the fish are generally fed to satiation, the quantity of feed and the feeding rate varying with water temperature and water oxygen concentration, and most importantly with fish size. Control in feeding is an allometric function, varying with the size of the fish and its relative stomach size, which requires a progressive increase in food intake to achieve satiation. In the AquaFish model allometric control of feeding is a function of fish length or fish weight and variable temperatures, with the associated oxygen concentrations, throughout the growth cycle.

Table 8 provides an explanation of the process involved in controlling feeding rate for fish aquaculture within the model.

Table 8: AquaFish model feeding rate control parameter descriptors

Term	Description	Explanation
Defines the terms used to determine feeding and feeding rate, based on levels of dissolved oxygen in the water column and water temperature, both essential components in feeding, and controls in feeding as they relate to the size of the fish.		
Dissolved oxygen$_{critical}$ (mg l^{-1})	Critical value for dissolved oxygen concentration at which fish will no longer feed and growth will be nil or negative.	Fish have a critical value for oxygen concentration in the water column when functioning become difficult and no feeding takes place.
Dissolved oxygen$_{minimum}$ (mg l^{-1})	Absolute minimum dissolved oxygen concentration, the point at which fish will almost certainly die.	Below the critical oxygen concentration there is a minimum value affecting survival.
Dissolved oxygen (mg l^{-1})	Standard dissolved oxygen concentration of seawater at typical salinity and at the average temperature of seawater in Northern Europe.	Typical salinity of coastal seawaters in Europe is 30-35ppt.
DO Feeding Rate	The proportion / impact of feeding that is driven by the level of oxygen present in the water column	
Water temperature	Water temperature over time.	Predicted daily water temperature following a standardised sin-wave pattern, predicted based on the mean temperature and temperature amplitude and extrapolated over the production cycle.
Mean water temperature (°C)	Average seawater temperature in Northern Europe.	Values represent available information from Norway, Ireland and Scotland, the main salmon producing nations in Europe.
Water temperature amplitude (°C)	Variability in average seawater temperature around the mean.	In the model, the temperature of seawater in which the fish are growth is calculated, based on a standardised representation using a sin-curve. In order to develop the Sin-curve the mean and amplitude of the curve are needed, the amplitude represented by the difference between the minimum and maximum sea temperatures divided by two.

Table 8 continued

$Temperature_{maximum}$ (°C)	Maximum temperature at which the fish species concerned will survive.	
$Temperature_{minimum}$ (°C)	Minimum temperature at which the fish species concerned will survive.	
$Temperature_{optimum}$ (°C)	Optimal seawater temperature for growth and survival.	
Temperature related feeding rate	Is an exponential function of the optimum temperature minus the actual water temperature (as calculated from the sin curve outlined above) divided by the maximum temperature minus the minimum temperature raised to the power 4.	
Fmax	Maximum feeding rate.	Fmax is the maximum feeding rate in the fish species concerned. Is an allometric term and therefore calculated proportional to length and therefore to weight through the standard Length/Weight relationship.
Fish Length (cm)	Length of fish nose to tail.	The standardised relationship of fish length to fish weight is $L = aW^b$, where L = Length and W = weight, and a and b are constants.
A	Coefficient "a" to calculate standard fish LW relationship.	
B	Coefficient "b" to calculate standard fish LW relationship.	

Energy Balance for Growth

Through the AquaFish modelling simulation feed input and nutrient uptake (assimilation) are converted to energy units, before they are converted to fish biomass later in the simulation. In doing so the simulation allows loss terms to be generated; related to energy loss through:

1) Basal Metabolic Rate (BMR),
2) Specific Dynamic Action (SDA) of feeding, and
3) Swimming activity.

Each of these processes are affected by a number of factors that affect the energy utilised in maintaining their core internal processes, the energy used in processing food and in maintaining themselves in the water environment through swimming.

Fish are poikilotherms and as such are not able to control their body temperature, so internal temperatures reflect the water medium, which varies with location and varies seasonally as water temperatures change. Current speed is another controlling factor in which energy consumption through swimming increases with increased current speed through the cages, which will vary over time during the flood and ebb tides. Water at different temperatures has a specific density, the higher the density the more drag there is and the more energy is needed to swim through it, for example.

As top-level predatory fish in the wild, salmon are fed feed pellets, using their visual prowess to locate, gulp in and swallow these feed pellets, which are their only energy source. The extent to which the various components in the feed are assimilated and utilised for growth depends to a great extent on the feed composition and sources of those nutrients. Some elements in feed, such as phosphorus, needs to be added in excess because the assimilative capacity in fish is low, while other elements such as nitrogen components are more readily assimilated.

Table 9 provides an explanation of the energy loss terms and controlling factors within the Aquafish model.

Table 9: AquaFish model energy balance parameterisation descriptors

Term	Description	Explanation
Defines the energy inputs, in terms of fish and feed, and the costs in energy of maintenance, metabolism and swimming, assuming that all energy losses are described by these three terms or otherwise gets converted in to somatic growth.		
Calorie to Joule conversion	Base conversion of calories to energy units in Joules.	Mass balance of all terms is assessed in energy (Joules).
Energy of dry weight of feed	The energy present in feed pellets per kg.	Energy present in feed products is represented as Joules per kg feed added. The extent of energy in feeds is derived from minimum energy expectations in the fish and the associated energy present in the feed stuffs that make up the feed. This has varied in recent years as the quantity of fish meal and fish oil has been reduced by the feed manufacturers, replaced with meal and oil derived from plants.
Assimilation	In this context assimilation refers to a switch which ensures assimilation takes place within the model; if the fish is eating and food is being transferred to the stomach and through on into the gut.	
Initial Fish weight (g)	The average weight at which fish are added to cages at sea.	The transfer takes place from freshwater cages or tanks, where salmon are grown for 6-9 months after leaving the hatchery. The size is variable depending on the time of year added to sea cages, being less weight if added in spring, and heavier if left in freshwater facilities for a longer period and added to sea in the autumn.
Energy of fresh weight	The energy present in whole fish standardised to per gram (and therefore incorporating somatic tissues, skin, bones, internal organs etc).	Energy is partitioned between protein and lipids which themselves have different energy values. The proportions of protein and lipid present in salmon is multiplied by standard energy conversion for protein and lipid (4 and 9 kcal g^{-1}) to calculate the overall energy in one gram of fish.
Fish length	Fork length.	The standardised relationship of fish Length to Fish weight is $L = aW^b$, where L = Length and W = weight, and a and b are constants (see above).
Basal Metabolic Rate (BMR)	Minimum energy required that drive oxygen consumption and maintenance processes in the fish.	
Tmin	Minimum temperature at which the fish species concerned will survive.	
KT	Coefficient of catabolism.	A value calculated to assess the proportion of the energy intake through feeding is used up by BMR.
J	Temperature coefficient for processes related to basal metabolism.	

Table 9 continued

Kmin	basal metabolism at 0 ^{o}C	
Specific Dynamic Action (SDA)	Energy required to digest food (feeding catabolism).	Energy expended through SDA is variable throughout the day and peaks when food has been eaten and is being digested by the fish, before slowly reducing again as the food is consumed and assimilated. Overall energy use is dependent on the energy taken in as food and the SDA coefficient.
SDA coefficient	Coefficient describing the overall energy balance associated with digesting food.	Estimated to account for 9 - 25% of the overall energy balance in fishes. The model presumes a higher rate at specific points in the day when feed is present in the fish and is otherwise zero.
Swimming catabolism	Energy required for the fish to swim in the net-pen.	For modelling purposes swimming is not directly defined, instead is assumed to remain stationary, while water flows over the top of the fish at a rate equal to body lengths per second and is the energy required to swim against that water flow and to maintain a stationary position. Estimated from the drag coefficient generated by the fish, the frontal area of the fish over which the water flows based on the current speed and water density.
Drag coefficient (Cd)	The power output from fish swimming action. Dimensionless.	
Water density	Density of seawater.	Varies with sea water temperature, colder water being more viscous. Value used is based on average sea temperature and salinity taken from the literature for the local areas (being 9.4 ^{o}C and 30 ppt, respectively).
Water speed	current speed in ms^{-1}.	The combined speeds that occur over a production cycle, as it varies with the tidal regime present at any particular site. Assuming in shore waters the current speed is limited to a default of $0.1ms^{-1}$, unless measured data is available.
Frontal area	The frontal area of the fish.	The model presumes water flows over the fish head-on and that the fish has an elliptical frontal area. As this changes as fish grows, the model relies instead on the ratio of total length to height and height to width, plus the total length in meters to make the calculation.
Fish length in meters (optional)	Length of fish (always measured in cm in the model) converted to meters	
Ratio of total fish length to mean fish height	It is proposed that the H/L ratio in salmon is 0.2 (i.e. the fish is 5 times longer than it is in height).	

AquaFish Model Parameterisation: Atlantic salmon

Table 10 identifies the parameterisation of the model for Atlantic salmon (*Salmo salar*), which is grown extensively across Northern Europe (Norway, Scotland, and Ireland), along with sources of the information.

Table 10: Parameterisation in AquaFish model for Atlantic salmon (*salmo salar*) in maraponic units

Parameter	Coefficient Value	Source
Feeding Module		
Nominal Feed Conversion Ratio (FCR)	Output of model	Based on: Wang *et al*. 2012; Tacon and Metian, 2008; Asgard *et al*. 2007
Initial Fish Weight	100 g	Desired starting weight for trial
Assimilation Efficiency	0.8	Wang *et al*, 2012
Allometric Control of Feeding Rate		
Dissolved Oxygen (mg L^{-1})	9	Predicted DO of trial
Mean Temp (°C)	14	Predicted temperature of trial
Basal Metabolic Rate (BMR)		
Where Basal Metabolic Rate (BMR) = Coefficient of Catabolism (KT) / Fish Energy; and where the Coefficient of Catabolism (KT) = k_{min} * EXP (J*(Water temperature – $Temp_{min}$) (Yi, 1999)		
Energy in Fresh weight of whole salmon, calculated (cal g^{-1})	1491	Standard thermochemical conversions of protein, lipid and carbohydrate (Burr *et al*. 2013)
K_{min} (Basal Metabolism at °C)	0.0037	From and Ramussen, 1984
J (Temperature coefficient of processes related to metabolism)	0.0875	From and Ramussen, 1984
Water Temperature (°C)	14	Predicted temperature of the trial
$Temp_{min}$	12	Predicted $temp_{min}$ of the trial
Swimming Catabolism		
Swimming Catabolism = Drag Coefficient (Cd) * Frontal_area * Water_density * Current_speed^3/2/calorie_to_Joule * Seconds_to_days		
Table 10 continued		
Drag Coefficient (Cd)	0.012	Tang and Wardle, 1992

Frontal Area (an ellipse in m^2)	Calculated based on fish size and ratio of fish length (modelled) to fish height (= 5) and ratio of fish height to fish width (= 1.6).	Kirczuk and Domagala, 2011; Jones *et al.* 1999
Water Density (Kg m^3) at salinity = 30 ppt, temperature = 14 °C)	1235.19	Wang *et al.* 2012
Current Speed (ms^{-1})	0.1	
Calorie to Joule (a conversion)	4.184	
Feeding Catabolism		
Where Feeding Catabolism = Energy Input * Coefficient of Specific Dynamic Action (SDA)		
SDA (dimensionless)	0.3	Jobling, 1981
Energy Input (cal g^{-1})	4883	= Pellet energy (BMRS/salmon feed) / standard thermochemical conversions of energy

WinFish model runs and outputs

After coding in C++ the AquaFish model is integrated into a console-based application called WinFish. Within the application species can be selected for modelling (i.e. salmon), along with driver parameters in terms of temperature, salinity and current speed, dissolved oxygen concentration, and nutrient data that can be modified within the console windows.

Such data is typically applied as monthly data collected on a specific Julian day, which can also be identified. Model outputs show daily results. The model calculates parameters for a specific day through interpolation between the data points on the first Julian day used within the model (dependant on the start day) and the next data point available. As a minimum, however, the model can be run assuming one data point per driver (i.e. uniform temperature, salinity current speed etc. throughout the growth period).

WinFish operates on the basis of modelling growth and outputs from a single fish. Application for farm level populations is conducted through another application called the FARM model (Ferreira *et al*, 2012; Cubillo *et al*, 2016).

Critical inputs to run WinFish are the species, starting weight, and culture period. When the model is run the simulation uses the above determinant data to generate results in the form of a table of raw information that can be exported to Excel for further analysis. Critical outputs include:

1) Change in fish length and weight over the growth cycle,
2) A value for FCR covering the whole production period,
3) Specific Growth Rate (SGR) per day,
4) Feed supplied, consumed, and lost as direct waste (i.e. remained un-eaten),
5) Oxygen consumed,
6) Nutrients added to the environment (through waste faeces and urine).

To ensure that the outputs are reasonable and balanced the user is able to carry out a check via a summary mass balance output. Evaluation of this mass balance is critical to model validation, as errors here are magnified when population level is considered, for example.

References

Åsgård, T., Berge, G.M., Mørkøre, T., and Refstie, S. (2007). Flexibility in the use of feed ingredients turns the salmon industry sustainable. *USDA national Organic Program, Organic Aquaculture Symposium, Washington D C, USA*, 27 -28th November. Available online: http://www.ams.usda.gov/nop (accessed on 12/08/17).

Burr, G.S., Wolters, W.R., Barrows, F.T., and Donkin, A.W. (2013). Evaluation of a canola protein concentrate as a replacement for fishmeal and poultry by-product meal in a commercial production diet for Atlantic salmon (*Salmo salar*). *International Aquatic Research,* 5(5), pp. 1-8.

Cubillo, A.M., Ferreira, J.G., Robinson, S.M.C., Pearce, C.M., Corner, R.A., and Johansen. (2016). Role of deposit feeders in integrated mulit-trophic aquaculture – A model analysis. *Aquaculture*, 453, pp. 54-66.

From, J., and Rasmussen, G. (1984). A growth model, gastric evacuation, and body composition in rainbow trout, *Salmo gairdneri* Richardson, 1836. *Dana* 3, pp. 61-139.

Jobling, M. (1981). Mathematical models of gastric emptying and the estimation of daily rates of food consumption in fish. *Journal of Fish Biology*, 19, pp. 245 – 257.

Jones, R.E., Petrell, R.J. and Pauly, D. (1999). Using modified length-weight relationships to assess fish condition. *Aquacultural Engineering*, 20, pp. 261 – 276.

Kirczuk, L., and Domagala, J. (2011). Morphometric characterisation of reciprocal hybrids of Atlantic salmon *Salmo salar* L., and sea trout, *Salmo trutta* L., in the freshwater period of life. *Archives of Polish Fisheries*, 19, pp. 285 – 295.

Tacon, A.G.J. and Metian, M., (2008). Global overview on the use of fish meal and fish oil in indtrially compounded aquafeeds: Trends and future prospects. *Aquaculture*, 285, pp. 146 – 158.

Tang, J. and Wardle, C.S. (1992). Power output of two sizes of Atlantic salmon (*Salmo salar*) at their maximum sutained swimming speeds. *Journal of Experimental Biology*, 166, pp. 33 – 46.

Wang, X., Olsen, L.M., Reiten, K.I. and Olsen Y., (2012). Discharge if nutrient wastes from salmon farms: environmental effects, and potential for integrated multi-trophic aquaculture. *Aquaculture Environmental Interactions*, 2, pp. 267 – 283.

Yi, Y. (1999). Modelling growth of Nile tilapia (Ore*ochromis niloticus*) in a cage-cum-pond integrated culture system. *Aquacultural Engineering*, 21, pp. 113 - 133.

Water parameters of maraponic trial

Table 11: Water parameter averages from each maraponic system measured in the bottom tank, top tank, and seaweed buckets in the morning (09:00-11:59) and afternoon (12:00-18:00) (mean ± SD)

	Bottom Tank		**Top Tank**		**Seaweed Buckets**	
	Morning	*Afternoon*	*Morning*	*Afternoon*	*Morning*	*Afternoon*
System 1						
Salinity (ppt)	31.81±0.47	31.76±0.4	31.94±0.47	31.87±0.4	31.95±0.48	31.91±0.39
pH	7.96±0.06	8.03±0.1	7.99±0.07	8.06±0.10	7.99±0.06	8.05±0.08
Temp (°C)	14.22±1.74	14.87±1.4	14.20±1.74	14.92±1.37	14.14±1.71	14.85±1.38
DO (mg/L)	9.6±0.37	9.49±0.42	10.04±0.47	9.92±0.51	9.94±0.37	9.8±0.34
DO (% Sat)	97.36±2.38	98.12±2.96	101.31±4.39	101.95±4.78	99.27±1.56	99.68±1.88
TAN (mg/L)*	0.94±0.49*		-		-	
System 2						
Salinity (ppt)	31.73±0.47	31.67±0.38	31.73±0.47	31.7±0.36	31.76±0.48	31.7±0.35
pH	7.97±0.06	8.08±0.09	8.02±0.11	8.12±0.09	7.99±0.06	8.09±0.07
Temp (°C)	14.06±1.66	14.74±1.36	14.13±1.65	14.81±1.36	14.06±1.65	14.77±1.36
DO (mg/L)	9.74±0.39	9.80±0.37	10.17±0.50	10.10±0.41	10.05±0.41	9.91±0.34
DO (% Sat)	96.81±3.23	99.36±2.42	101.27±4.49	102.32±3.84	99.27±1.95	99.63±1.44
TAN (mg/L)*	0.74±0.47*		-		-	
System 3						
Salinity (ppt)	31.80±0.50	31.72±0.37	31.79±0.5	31.75±0.34	31.81±0.5	31.76±0.37
pH	7.98±0.06	8.11±0.09	8.01±0.07	8.13±0.09	8±0.06	8.1±0.08
Temp (°C)	13.74±1.71	14.38±1.39	13.83±1.74	14.49±1.37	13.77±1.73	14.39±1.39
DO (mg/L)	9.78±0.48	9.95±0.39	10.13±0.43	10.16±0.5	10.05±0.38	10±0.35
DO (% Sat)	96.38±3.71	99.69±2.45	99.69±3.71	101.68±4.21	98.62±1.63	99.76±1.74
TAN (mg/L)*	0.76±0.51*		-		-	

Fatty acid profiles

Table 12: Fatty acid composition (%) of mussels

%	Wild	Maraponics – top	Maraponics - bottom	P-value
Lipid	**2.43 ± 0.50**	**2.45 ± 0.61**	**2.14 ± 0.60**	**NS**
SFA				
14:0	2.20 ± 0.76[a]	1.17 ± 0.58[b]	1.39 ± 0.51[b]	<0.01
15:0	0.67 ± 0.11	0.65 ± 0.12	0.64 ± 0.14	NS
Iso 16:0	0.23 ± 0.03	0.28 ± 0.07	0.24 ± 0.05	NS
16:0	15.75 ± 1.17[a]	13.99 ± 2.20[a/b]	15.48 ± 1.14[b]	<0.05
1so 17:0	0.84 ± 0.18[a]	1.12 ± 0.31[b]	0.93 ± 0.12[a/b]	<0.05
Anti-Iso 17:0	0.84 ± 0.31[a]	1.37 ± 0.36[b]	1.24 ± 0.20[b]	<0.01
18:0	3.45 ± 1.00	4.07 ± 1.45	4.31 ± 1.17	NS
Total SFA	**23.98 ± 1.13[a/b]**	**22.67 ± 1.63[a]**	**24.23 ± 0.85[b]**	**NS**
MUFA				
16:1n-9	0	0.33 ± 0.65	0	n/a
16:1n-7	5.35 ± 1.75	4.20 ± 2.50	4.79 ± 2.26	NS
18:1n-9	2.15 ± 0.58	2.56 ± 0.48	2.89 ± 1.01	NS
18:1n-7	2.54 ± 0.36	2.69 ± 0.42	2.55 ± 0.55	NS
20:1n-11	1.36 ± 0.20[a]	1.85 ± 0.49[b]	1.68 ± 0.56[a/b]	NS
20:1n-9	2.69 ± 0.47[a]	3.61 ± 0.77[b]	3.63 ± 0.42[b]	<0.01
20:1n-7	0.95 ± 0.20	0.95 ± 0.15	0.97 ± 0.14	NS
22:1n-11	0.22 ± 0.45[a]	0.24 ± 0.18[a/b]	0.47 ± 0.22[b]	NS
22:1n-9	0.02 ± 0.04	0.17 ± 0.39	0.11 ± 0.24	NS
24:1n-9	0.01 ± 0.02[a]	0.10 ± 0.11[b]	0.05 ± 0.07[a/b]	NS
Total MUFA	**15.30 ± 1.62**	**16.70 ± 2.25**	**17.15 ± 3.94**	**NS**
n-6 PUFA				
18:2n-6	1.71 ± 0.29	1.39 ± 0.30	1.53 ± 0.28	NS
18:3n-6	0.08 ± 0.02	0.21 ± 0.43	0.07 ± 0.03	NS
20:2n-6	0.71 ± 0.12[a]	0.54 ± 0.11[b]	0.58 ± 0.11[a/b]	<0.05
20:3n-6	0.23 ± 0.06[a]	0.22 ± 0.10[a]	0.13 ± 0.06[b]	<0.01
20:4n-6 (ARA)	2.60 ± 0.73	3.34 ± 1.56	3.16 ± 0.66	NS
22:4n-6	0.29 ± 0.09	0.39 ± 0.16	0.33 ± 0.09	NS
22:5n-6	0.40 ± 0.06[a]	0.58 ± 0.18[b]	0.48 ± 0.05[a/b]	<0.01
Total n-6 PUFA	**6.03 ± 0.73**	**6.67 ± 1.52**	**6.28 ± 0.67**	**NS**
n-3 PUFA				
18:3n-3	1.42 ± 0.61	1.03 ± 0.28	1.09 ± 0.26	NS
18:4n-3	2.62 ± 0.95[a]	1.08 ± 0.60[b]	1.19 ± 0.45[b]	<0.001
20:3n-3	0.09 ± 0.05[a]	0.02 ± 0.03[b]	0.05 ± 0.04[b]	<0.01
20:4n-3	0.26 ± 0.09[a]	0.15 ± 0.07[b]	0.18 ± 0.07[b]	<0.05
20:5n-3 (EPA)	13.53 ± 1.16	11.93 ± 1.66	12.33 ± 2.25	NS
22:5n-3	1.19 ± 0.15[a]	1.48 ± 0.30[b]	1.38 ± 0.16[a/b]	<0.05
22:6n-3 (DHA)	19.18 ± 1.76	17.52 ± 2.17	18.14 ± 3.26	NS
Total n-3 PUFA	**38.29 ± 2.29[a]**	**33.20 ± 4.13[b]**	**34.35 ± 5.32[b]**	**<0.05**
Other PUFA				
16:2	0.33 ± .017[a]	0.13 ± 0.08[b]	0.16 ± 0.04[b]	<0.01
16:3	0.60 ± 0.38	0.92 ± 0.66	0.67 ± 0.30	NS
16:4	0.27 ± 0.17[a]	0.41 ± 0.05[b]	0.43 ± 0.10[b]	<0.05
Total other PUFA	**1.19 ± 0.40**	**1.47 ± 0.64**	**1.26 ± 0.30**	**NS**
Total PUFA	**45.51 ± 1.70[a]**	**41.34 ± 2.05[a/b]**	**41.89 ± 3.15[b]**	**<0.05**
DMA				
16:0 DMA	0.40 ± 0.14	0.48 ± 0.19	0.34 ± 0.05	NS
18:0 DMA	7.03 ± 1.70	8.86 ± 2.28	7.82 ± 1.56	NS
20:0 DMA	0.64 ± 0.31[a]	1.07 ± 0.57[b]	0.95 ± 0.21[a/b]	<0.05
Total DMA	**8.07 ± 1.84**	**10.41 ± 2.90**	**9.11 ± 1.72**	**NS**
NMID				
20:2 NMID	2.18 ± 0.47	2.78 ± 1.24	2.81 ± 0.83	NS
20:3NMID	0.47 ± 0.06	0.38 ± 022	0.43 ± 0.08	NS
22:2 NMID	3.07 ± 0.64[a]	4.23 ± 1.28[b]	3.17 ± 1[a]	<0.05
22:3NMID	1.42 ± 0.24	1.49 ± 0.29	1.21 ± 0.23	NS
Total NMID	**7.14 ± 1.32**	**8.88 ± 2.48**	**7.62 ± 1.98**	**NS**

Table 13: Fatty acid composition (%) of abalone

%	Farmed	Maraponics - top	Maraponics - bottom	P-value
Lipid	**1.14 ± 0.22[a]**	**1.12 ± 0.18[a]**	**1.47 ± 0.35[b]**	**<0.05**
SFA				
14:0	5.36 ± 0.23[a]	3.35 ± 0.24[b]	4.22 ± 0.41[c]	<0.001
15:0	0.67 ± 0.16	0.88 ± 0.08	0.83 ± 0.05	NS
16:0	12.16 ± 10.54	17.14 ± 0.91	17.88 ± 0.37	NS
18:0	5.09 ± 1.15[a]	5.85 ± 0.83[b]	4.37 ± 0.50[a]	<0.001
20:0	0.11 ± 0.04	0.10 ± 0.01	0.10 ± 0.01	NS
Total SFA	**23.39 ± 9.37**	**27.31 ± 1.53**	**27.41 ± 0.54**	**NS**
MFA				
16:1n-9	0.61 ± 0.15	0.93 ± 0.20	0.79 ± 0.10	NS
16:1n-7	3.62 ± 0.75[a]	2.09 ± 0.45[b]	3.36 ± 0.62[a]	<0.001
17:1n	0.56 ± 0.12	0.58 ± 0.09	0.46 ± 0.18	NS
18:1n-9	8.06 ± 0.70[a]	5.83 ± 0.72[b]	7.62 ± 0.78[a]	<0.001
18:1n-7	11.91 ± 0.63[a]	8.60 ± 0.62[b]	6.84 ± 0.80[c]	<0.001
20:1n-11	5.26 ± 0.20[a]	4.00 ± 0.57[b]	3.95 ± 0.27[b]	<0.05
20:1n-9	1.05 ± 0.91[a]	0.87 ± 0.12[a]	4.88 ± 1.26[b]	<0.001
20:1n-7	1.29 ± 0.50[a]	0.78 ± 0.11[b]	0.70 ± 0.05[b]	<0.001
22:1n-11	0.26 ± 0.11[a]	0.32 ± 0.10[a]	3.28 ± 0.94[b]	<0.001
24:1n-9	0	0.70 ± 2.03	0.39 ± 0.12	NS
Total MFA	**32.63 ± 1.34[a]**	**24.70 ± 2.17[b]**	**32.27 ± 2.54[a]**	**<0.001**
n-6 PUFA				
18:2n-6	2.48 ± 0.53[a/b]	1.64 ± 0.57[a]	3.11 ± 0.62[b]	<0.01
20:2n-6	0.54 ± 0.08	0.44 ± 0.40	0.47 ± 0.04	NS
20:3n-6	0.14 ± 0.05	0.16 ± 0.06	0.17 ± 0.02	NS
20:4n-6 (ARA)	5.01 ± 1.49[a]	5.94 ± 0.36[b]	3.35 ± 0.69[c]	<0.001
Total n-6 PUFA	**8.18 ± 2.10[a/b]**	**8.19 ± 0.60[a]**	**7.11 ± 0.15[b]**	**<0.01**
n-3 PUFA				
18:3n-3	2.11 ± 0.17	1.98 ± 0.45	1.86 ± 0.35	NS
18:4n-3	0.38 ± 0.08[a]	0.62 ± 0.25[a]	1.03 ± 0.29[b]	<0.01
20:3n-3	0.10 ± 0.03[a]	0.11 ± 0.02[a]	0.02 ± 0.04[b]	<0.01
20:4n-3	0.29 ± 0.03	0.28 ± 0.07	0.36 ± 0.08	NS
20:5n-3 (EPA)	10.09 ± 2.73[a]	11.18 ± 1.03[b]	9.54 ± 0.44[a]	<0.01
22:5n-3	6.78 ± 1.92[a]	7.85 ± 0.77[a]	4.83 ± 1.04[b]	<0.001
22:6n-3 (DHA)	0.09 ± 0.08[a]	0.88 ± 0.35[b]	4.08 ± 1.16[c]	<0.001
Total n-3 PUFA	**19.85 ± 4.96[a]**	**22.91 ± 1.65[b]**	**21.72 ± 0.52[b]**	**<0.001**
Total PUFA	**28.03 ± 7.91[a]**	**31.10 ± 1.37[b]**	**28.83 ± 0.91[a]**	**<0.001**
DMA				
16:0 DMA	0.72 ± 0.11	0.71 ± 0.36	0.55 ± 0.23	NS
18:0 DMA	6.42 ± 1.42[a]	7.52 ± 0.74[a]	4.52 ± 1.17[b]	<0.001
18:1 DMA	0.21 ± 0.04	0.26 ± 0.04	0.21 ± 0.06	NS
20:0 DMA	1.79 ± 0.56[a]	2.49 ± 0.27[b]	1.35 ± 0.39[a]	<0.001
Total DMA	**9.14 ± 2.12[a]**	**10.98 ± 0.91[b]**	**6.63 ± 1.61[a]**	**<0.001**
NMID				
20:2 NMID	0.56 ± 0.12[a]	0.28 ± 0.08[b]	0.18 ± 0.03[c]	<0.001
22:2 NMID	6.18 ± 0.78[a]	5.52 ± 0.58[a]	4.36 ± 0.83[b]	<0.01
22:3 NMID	0.08 ± 0.08[a]	0.10 ± 0.17[b]	0.32 ± 0.10[b]	<0.01
Total NMID	**6.81 ± 0.93[a]**	**5.90 ± 0.60[a/b]**	**4.87 ± 0.79[b]**	**<0.01**

Table 14: Fatty acid composition (%) of sea cucumber intestine

%	Wild	Maraponic systems	P-value
Lipid	**1.43 ± 0.45**	**1.86 ± 0.75**	**NS**
SFA			
14:0	1.59 ± 0.72	1.17 ± 0.94	NS
15:0	0.40 ± 0.18	0.32 ± 0.21	NS
Iso 15:0	0.15 ± 0.05	0.20 ± 0.24	NS
Anti-Iso 15:0	0.84 ± 0.71	0.88 ± 0.49	NS
Iso 16:0	0.54 ± 0.11	0.49 ± 0.24	NS
16:0	3.71 ± 1.17	3.58 ± 2.15	NS
1so 17:0	0.43 ± 0.01	0.34 ± 0.16	NS
Anti-Iso 17:0	0.78 ± 0.21	0.68 ± 0.30	NS
18:0	4.21 ± 0.90	4.82 ± 1.31	NS
19:0	1.45 ± 0.39	1.52 ± 0.20	NS
20:0	1.83 ± 0.14	2.09 ± 0.22	NS
21:0	1.58 ± 0.30	1.61 ± 0.40	NS
22:0	1.91 ± 0.38	2.04 ± 0.37	NS
23:0	0.26 ± 0.05	0.28 ± 0.08	NS
Total SFA	**19.67 ± 0.87**	**20.03 ± 4.28**	**NS**
MUFA			
16:1n-9	0.66 ± 0.48	0.44 ± 0.27	NS
16:1n-7	3.31 ± 0.59	3.20 ± 2.11	NS
18:1n-9	3.81 ± 2.25	3.40 ± 1.54	NS
18:1n-7	4.37 ± 0.35	4.31 ± 1.13	NS
19:1	0.53 ± 0.09	0.53 ± 0.15	NS
20:1n-11	4.49 ± 0.31	4.80 ± 1.09	NS
20:1n-9	1.89 ± 0.80	2.05 ± 1.13	NS
20:1n-7	0.87 ± 0.22	0.99 ± 0.31	NS
22:1n-11	0.91 ± 0.25	1.05 ± 0.36	NS
22:1n-9	1.21 ± 0.35	1.41 ± 0.34	NS
23:1n	8.07 ± 3.29	6.14 ± 1.83	NS
24:1n-9	1.59 ± 0.16	1.88 ± 0.37	NS
Total MUFA	**31.71 ± 6.85**	**30.19 ± 3.94**	**NS**
n-6 PUFA			
18:2n-6	0.34 ± 0.18	0.33 ± 0.08	NS
18:3n-6	0.19 ± 0.33	0	n/a
20:2n-6	1.55 ± 0.11	1.59 ± 0.21	NS
20:3n-6	0.20 ± 0.01	0.22 ± 0.07	NS
20:4n-6 (ARA)	14.10 ± 1.70	13.42 ± 3.96	NS
22:4n-6	0.46 ± 0.04	0.46 ± 0.09	NS
22:5n-6	1.27 ± 0.41	1.48 ± 0.27	NS
Total n-6 PUFA	**18.12 ± 2.29**	**17.50 ± 4.16**	**NS**
n-3 PUFA			
18:3n-3	0.72 ± 0.24	0.87 ± 0.53	NS
18:4n-3	1.50 ± 0.84	1.23 ± 0.85	NS
20:3n-3	0.38 ± 0.18	0.55 ± 0.17	NS
20:4n-3	0.42 ± 0.10	0.59 ± 0.27	NS

Table 14 continued

20:5n-3 (EPA)	16.37 ± 3.46	17.43 ± 4.94	NS
22:5n-3	0.61 ± 0.24	0.58 ± 0.35	NS
22:6n-3 (DHA)	2.30 ± 0.26	2.52 ± 0.71	NS
Total n-3 PUFA	**22.30 ± 4.40**	**23.78 ± 4.83**	**NS**
Other PUFA			
16:2	0.14 ± 0.06	0.14 ± 0.06	NS
16:3	0.29 ± 0.32	0.28 ± 0.18	NS
Total other PUFA	**0.43 ± 0.30**	**0.42 ± 0.15**	**NS**
Total PUFA	**40.84 ± 6.42**	**41.70 ± 6.37**	**NS**
DMA			
18:0 DMA	5.95 ± 0.99	6.26 ± 1.47	NS
19:0 DMA	1.83 ± 0.44	1.81 ± 0.70	NS
Total DMA	**7.78 ± 1.35**	**8.07 ± 1.96**	**NS**

Table 15: Fatty acid composition (%) of sea cucumber body wall

%	**Wild**	**Maraponic systems**	**P-value**
Lipid	**0.28 ± 0.12**	**0.32 ± 0.08**	**NS**
SFA			
14:0	0.59 ± 0.43	0.77 ± 0.41	NS
15:0	0.22 ± 0.10	0.27 ± 0.24	NS
Iso 15:0	0.08 ± 0.03	0.25 ± 0.40	NS
Anti-Iso 15:0	0.47 ± 0.26	0.85 ± 1.53	NS
Iso 16:0	0.27 ± 0.12	0.20 ± 0.07	NS
16:0	3.41 ± 1.35	4.52 ± 3.44	NS
1so 17:0	0.29 ± 0.06	0.37 ± 0.46	NS
Anti-Iso 17:0	0.41 ± 0.13	0.31 ± 0.16	NS
18:0	3.13 ± 0.42	3.54 ± 0.60	NS
19:0	1.45 ± 0.18	1.32 ± 0.13	NS
20:0	2.42 ± 0.07	2.36 ± 0.29	NS
21:0	2.07 ± 0.28	2.08 ± 0.41	NS
22:0	2.33 ± 0.07	2.55 ± 0.25	NS
23:0	0.32 ± 0.05	0.39 ± 0.18	NS
Total SFA	**17.46 ± 2.81**	**19.78 ± 8.11**	**NS**
MUFA			
16:1n-9	0.45 ± 0.14	0.57 ± 0.42	NS
16:1n-7	1.20 ± 0.77	1.15 ± 0.40	NS
18:1n-9	3.35 ± 0.53	3.96 ± 1.30	NS
18:1n-7	2.23 ± 0.38	2.05 ± 0.33	NS
19:1	0.43 ± 0.19	0.29 ± 0.11	NS
20:1n-11	8.21 ± 1.08	7.56 ± 1.59	NS
20:1n-9	0.55 ± 0.84	0.89 ± 0.52	NS
20:1n-7	0.49 ± 0.02	0.45 ± 0.10	NS
22:1n-11	0.74 ± 0.05	0.61 ± 0.09	NS
22:1n-9	1.95 ± 0.04	1.79 ± 0.25	NS
23:1n	9.45 ± 0.47	10.15 ± 0.69	NS
24:1n-9	2.89 ± 0.48	3.41 ± 0.82	NS
Total MUFA	**31.93 ± 1.27**	**32.87 ± 1.78**	**NS**

Table 15 continued

n-6 PUFA			
18:2n-6	0.23 ± 0.04	0.24 ± 0.16	NS
20:2n-6	1.57 ±0.16	1.34 ± 0.18	NS
20:3n-6	0.18 ± 0.02	0.15 ± 0.06	NS
20:4n-6 (ARA)	20.47 ± 2.50	17.98 ± 5.53	NS
22:4n-6	0.64 ± 0.08	0.50 ± 0.21	NS
22:5n-6	1.46 ± 0.04	1.41 ± 0.43	NS
Total n-6 PUFA	**24.54 ± 2.61**	**21.62 ± 6.28**	**NS**
n-3 PUFA			
18:3n-3	0.24 ± 0.17	0.36 ± 0.16	NS
18:4n-3	0.33 ± 0.08	0.47 ± 0.21	NS
20:3n-3	0.53 ± 0.10	0.49 ± 0.11	NS
20:4n-3	0.18 ± 0.02	0.24 ± 0.11	NS
20:5n-3 (EPA)	12.10 ± 1.26	11.46 ± 4.12	NS
22:5n-3	0.35 ± 0.03	0.20 ± 0.10	NS
22:6n-3 (DHA)	1.11 ± 0.27	0.92 ± 0.36	NS
Total n-3 PUFA	**14.85 ± 0.83**	**14.14 ± 4.81**	**NS**
Total PUFA	**40.08 ± 3.19**	**36.15 ± 9.96**	**NS**
Other PUFA			
16:2	0.12 ± 0.04	0.10 ± 0.05	NS
16:3	0.57 ± 0.11	0.29 ± 0.29	<NS
Total other PUFA	**0.69 ± 0.15**	**0.39 ± 0.33**	**<0.05**
DMA			
18:0 DMA	8.00 ± 0.75	8.73 ± 0.48	NS
19:0 DMA	2.53 ± 0.04	2.46 ± 0.39	NS
Total DMA	**10.52 ± 0.72**	**11.19 ± 0.83**	**NS**

Table 16: Fatty acid composition (%) of *A. nodosum*

%	**Wild**	**Maraponic Systems**	**P-value**
Lipid	**2.29 ± 0.16**	**1.75 ± 0.36**	**<0.05**
SFA			
14:0	7.95 ± 0.6	9.67 ± 1.44	NS
Anteiso 15:0	0.03 ± 0.05	0.14 ± 0.06	<0.05
15:0	0.20 ± 0	0.28 ± 0.08	<0.05
16:0	8.26 ± 0.38	10.16 ± 2.10	NS
18:0	0.59 ± 0.02	0.60 ± 0.10	NS
20:0	0.18 ± 0.01	0.16 ± 0.07	NS
22:0	0.12 ± 0.02	0.15 ± 0.06	NS
24:0	0.15 ± 0.01	0.19 ± 0.08	NS
Total SFA	**17.47 ± 1.03**	**21.35 ± 3.65**	**NS**
MUFA			
14:1	0.27 ± 0.01	0.17 ± 0.10	NS
16:1n-9	0.11 ± 0.01	0.07 ± 0.06	NS
16:1n-7	1.16 ± 0	1.13 ± 0.13	NS
17:1	0.29 ± 0.02	0.24 ± 0.10	NS
18:1n-9	42.08 ± 1.40	34.17 ± 7.43	<0.05
18:1n-7	0.10 ± 0.09	0.30 ± 0.09	NS

Table 16 continued

20:1n-11	0.23 ± 0.04	0.24 ± 0.07	NS
20:1n-9	0.14 ± 0.01	0.10 ± 0.08	NS
22:1n-11	0.22 ± 0.06	0.20 ± 0.16	NS
22:1n-9cis	0	0.11 ± 0.17	n/a
24:1	0.96 ± 0.06	1.58 ± 0.41	<0.01
Total MUFA	**45.54 ± 1.29**	**38.33 ± 7.11**	**<0.05**
n-6 PUFA			
18:2n-6	8.34 ± 0.36	8.60 ± 0.32	NS
18:3n-6	0.44 ± 0.01	0.53 ± 0.07	<0.05
20:2n-6	1.84 ± 0.18	1.77 ± 0.42	NS
20:3n-6	0.79 ± 0.02	0.71 ± 0.10	<0.05
20:4n-6 (ARA)	10.40 ± 0.33	12.05 ± 1.63	NS
22:4n-6	0.15 ± 0.04	0.10 ± 0.09	NS
Total n-6 PUFA	**21.97 ± 0.37**	**23.77 ± 1.47**	**NS**
n-3 PUFA			
18:3n-3	2.88 ± 0.27	3.09 ± 0.63	NS
18:4n-3	2.75 ± 0.48	2.87 ± 0.94	NS
20:3n-3	0.41 ± 0.02	0.30 ± 0.09	<0.01
20:4n-3	0.24 ± 0.01	0.20 ± 0.02	<0.01
20:5n-3 (EPA)	4.52 ± 0.11	4.96 ± 0.87	NS
Total n-3 PUFA	**10.80 ± 0.68**	**11.62 ± 2.70**	**NS**
Other PUFAs			
16:2	0.09 ± 0.01	0.04 ± 0.06	<0.05
20:3n-7	3.17 ± 0.48	2.82 ± 1.37	NS
Total other PUFAs	**3.26 ± 0.47**	**2.86 ± 1.36**	**NS**
Total PUFA	**36.02 ± 0.64**	**38.25 ± 3.13**	**NS**
Furan FAs	**0.97 ± 0.10**	**2.07 ± 0.74**	**<0.01**

Table 17: Fatty acid composition (%) of *L. digitata*

%	**Wild**	**Maraponic Systems**	**P-value**
Lipid	**1.21 ± 0.36**	**0.78 ± 0.22**	**<0.05**
SFA			
14:0	6.69 ± 0.44	6.25 ± 1.48	NS
Anteiso 15:0	0.40 ± 0.02	0.52 ± 0.21	NS
15:0	0.59 ± 0.02	0.53 ± 0.11	NS
16:0	19.88 ± 0.10	22.33 ± 3.32	NS
18:0	1.04 ± 0.03	1.15 ± 0.25	NS
20:0	0.94 ± 0.49	0.87 ± 0.36	NS
24:0	0	0.02 ± 0.07	n/a
Total SFA	**29.55 ± 1.03**	**31.67 ± 2.44**	**NS**
MUFA			
14:1	0	0.04 ± 0.11	n/a
16:1n-9	0.31 ± 0.01	0.11 ± 0.16	<0.01
16:1n-7	5.65 ± 0.17	5.74 ± 2.04	NS
17:1	0.43 ± 0.06	0.32 ± 0.18	NS
18:1n-9	17.40 ± 0.19	17.64 ± 1.47	NS

Table 17 continued

18:1n-7	0.54 ± 0.03	0.97 ± 0.34	<0.05
20:1n-11	0.40 ± 0.02	0.31 ± 0.26	NS
20:1n-9	0.30 ± 0	0.31 ± 0.32	NS
22:1n-11	0.35 ± 0.02	0.20 ± 0.37	<0.05
22:1n-9cis	0	0.08 ± 0.13	n/a
24:1	0.10 ± 0.17	0.68 ± 0.75	NS
Total MUFA	**25.48 ± 0.44**	**26.39 ± 3.10**	**NS**
n-6 PUFA			
18:2n-6	2.55 ± 0.21	4.15 ± 0.93	<0.01
18:3n-6	0.54 ± 0.01	0.49 ± 0.10	NS
20:2n-6	0.36 ± 0.02	0.25 ± 0.23	NS
20:3n-6	0.08 ± 0.14	0.30 ± 0.34	NS
20:4n-6 (ARA)	7.80 ± 0.10	9.84 ± 2.60	NS
Total n-6 PUFA	**11.33 ± 0.48**	**15.04 ± 3.49**	**<0.05**
n-3 PUFA			
18:3n-3	2.83 ± 0.07	3.34 ± 1.21	NS
18:4n-3	6.60 ± 0.53	4.75 ± 1	<0.05
20:3n-3	0.69 ± 0.18	0	n/a
20:4n-3	0.46 ± 0.01	0.38 ± 0.33	NS
20:5n-3 (EPA)	17.12 ± 0.75	9.57 ± 4.81	NS
22:5n-3	1.17 ± 0.20	0.93 ± 0.95	NS
22:6n-3 (DHA)	0.06 ± 0.11	0.04 ± 0.11	NS
Total n-3 PUFA	**28.93 ± 1.39**	**19.01 ± 6.14**	**<0.01**
Other PUFAs			
16:2	0.44 ± 0.30	0.18 ± 0.25	NS
20:3n-7	0	0.19 ± 0.19	n/a
Total other PUFAs	**0.44 ± 0.30**	**0.37 ± 0.24**	**NS**
Total PUFA	**40.70 ± 0.64**	**34.41 ± 7.62**	**<0.05**
Furan FAs	**4.29 ± 0.80**	**7.53 ± 5.32**	**NS**

Table 18: Fatty acid composition (%) of *F. vesiculosus*

%	**Wild**	**Maraponic Systems**	**P-value**
Lipid	**2.97 ± 1.14**	**1.36 ± 0.14**	**NS**
SFA			
14:0	10.76 ± 1.14	10.22 ± 0.33	NS
Anteiso 15:0	0.10 ± 0.09	0.24 ± 0.10	NS
15:0	0.33 ± 0.01	0.47 ± 0.04	<0.001
16:0	11.54 ± 1.56	16.84 ± 1.07	<0.001
18:0	0.90 ± 0.28	1.02 ± 0.60	NS
20:0	0.46 ± 0.02	0.18 ± 0.11	<0.05
22:0	0.16 ± 0.02	0.16 ± 0.10	NS
24:0	0.17 ± 0.06	0.29 ± 0.12	<0.05
Total SFA	**24.42 ± 2.80**	**29.42 ± 1.40**	**<0.01**
MUFA			
14:1	0.16 ± 0.01	0	n/a
16:1n-9	0.12 ± 0.02	0.04 ± 0.07	<0.05

Table 18 continued

16:1n-7	1.24 ± 0.25	1.26 ± 0.40	NS
17:1	0.28 ± 0.10	0.08 ± 0.10	<0.01
18:1n-9	35.94 ± 11.07	14.74 ± 2.63	NS
18:1n-7	0.24 ± 0.29	0.68 ± 0.35	<0.05
20:1n-11	0.42 ± 0.07	0.25 ± 0.04	<0.001
20:1n-9	0.20 ± 0.21	0.15 ± 0.24	NS
22:1n-11	0.65 ± 0.12	0.65 ± 0.54	NS
22:1n-9cis	0.04 ± 0.06	0.29 ± 0.46	NS
24:1	1.00 ± 0.18	2.25 ± 0.27	<0.001
Total MUFA	**40.29 ± 10.92**	**20.39 ± 3.48**	**<0.001**
n-6 PUFA			
18:2n-6	8.65 ± 1.19	6.55 ± 0.50	<0.01
18:3n-6	0.40 ± 0.08	0.47 ± 0.06	NS
20:2n-6	0.41 ± 0.04	0.59 ± 0.05	<0.001
20:3n-6	0.75 ± 0.14	0.53 ± 0.07	<0.01
20:4n-6 (ARA)	11.10 ± 1.38	14.12 ± 1.37	<0.01
Total n-6 PUFA	**21.31 ± 2.78**	**22.26 ± 1.79**	**NS**
n-3 PUFA			
18:3n-3	4.14 ± 1.59	6.68 ± 0.65	NS
18:4n-3	3.06 ± 1.99	6.49 ± 1.27	<0.01
20:3n-3	0.16 ± 0.01	0.13 ± 0.13	NS
20:4n-3	0.27 ± 0.10	0.44 ±0.07	<0.01
20:5n-3 (EPA)	4.58 ± 1.45	9.69 ± 1.30	<0.001
22:5n-3	0	0.02 ± 0.05	n/a
Total n-3 PUFA	**12.22 ± 5.13**	**23.44 ± 2.93**	**<0.01**
Other PUFAs			
16:2	0.10 ± 0.09	0	n/a
20:3n-7	0.28 ± 0.10	0.37 ± 0.24	NS
Total other PUFAs	**0.37 ± 0.17**	**0.37 ± 0.24**	**NS**
Total PUFA	**33.91 ± 7.71**	**46.07 ± 3.84**	**<0.01**
Furan FAs	**1.39 ± 0.86**	**4.12 ± 0.51**	**<0.001**

Table 19: Fatty acid composition (%) of *F. serratus*

%	**Wild**	**Maraponic Systems**	**P-value**
Lipid	**1.96 ± 0.12**	**1.44 ± 0.19**	**<0.01**
SFA			
14:0	8.56 ± 0.35	9.81 ± 0.98	NS
Anteiso 15:0	0.15 ± 0.01	0.24 ± 01.4	NS
15:0	0.31 ± 0.06	0.45 ± 0.08	<0.05
16:0	16.25 ± 1.26	17.80 ± 2.25	NS
ISO 18:0	0	0.02 ± 0.06	n/a
18:0	0.90 ± 0.42	0.86 ± 0.40	NS
20:0	0.36 ± 0.04	0.19 ± 0.13	<0.05
22:0	0.18 ± 0.01	0.16 ± 0.08	NS
24:0	0.13 ± 0.01	0.24 ± 0.05	<0.01
Total SFA	**26.86 ± 2.11**	**29.78 ± 1.88**	**NS**

Table 19 continued

MUFA			
14:1	0.08 ± 0.07	0.03 ± 0.07	NS
16:1n-9	0.14 ± 0.13	0	n/a
16:1n-7	1.82 ± 0.40	2.05 ± 0.46	NS
17:1	0.23 ± 0.02	0.27 ± 0.15	NS
18:1n-9	24.94 ± 0.94	19.84 ± 4.96	NS
18:1n-7	0.39 ± 0.35	0.90 ± 0.53	NS
20:1n-11	0.37 ± 0.04	0.12 ± 0.12	NS
20:1n-9	0.42 ± 0.26	0.09 ± 0.23	NS
22:1n-11	0.61 ± 0.17	0.44 ± 0.43	NS
22:1n-9cis	0	0.09 ± 0.16	n/a
24:1	1.50 ± 0.10	2.89 ± 0.69	<0.01
Total MUFA	**30.52 ± 1.58**	**26.71 ± 6.06**	**NS**
n-6 PUFA			
18:2n-6	8.80 ± 0.56	6.61 ± 0.89	<0.01
18:3n-6	0.53 ± 0.06	0.44 ± 0.06	NS
20:2n-6	0.31 ± 0.01	0.47 ± 0.05	<0.01
20:3n-6	1.06 ± 0.08	0.88 ± 0.16	NS
20:4n-6 (ARA)	12.47 ± 0.98	13.54 ± 2.60	NS
Total n-6 PUFA	**23.18 ± 1.68**	**21.94 ± 3.36**	**NS**
n-3 PUFA			
18:3n-3	4.70 ± 0.33	4.83 ± 0.97	NS
18:4n-3	4.42 ± 0.58	4.52 ± 1.38	NS
20:3n-3	0.39 ± 0.03	0	n/a
20:4n-3	0.51 ± 0.01	0.64 ± 0.22	NS
20:5n-3 (EPA)	7.47 ± 0.72	7.41 ± 1.91	NS
22:5n-3	0	0.01 ± 0.04	n/a
22:6n-3 (DHA)	0	0.23 ± 0.61	n/a
Total n-3 PUFA	**17.48 ± 1.65**	**17.66 ± 3.99**	**NS**
Other PUFAs			
16:2	0.14 ± 0.01	0.18 ± 0.33	NS
20:3n-7	0.15 ± 0.13	0.13 ± 0.13	NS
Total other PUFAs	**0.29 ± 0.14**	**0.31 ± 0.36**	**NS**
Total PUFA	**40.95 ± 3**	**39.91 ± 4.88**	**NS**
Furan FAs	**1.67 ± 0.23**	**3.61 ± 1.11**	**<0.05**

Table 20: Fatty acid composition (%) of *P. canaliculata*

%	**Wild**	**Maraponic Systems**	**P-value**
Lipid	**3 ± 0.16**	**1.53 ± 0.32**	**<0.001**
SFA			
14:0	7.95 ± 0.46	5.93 ± 2.27	NS
Anteiso 15:0	0.08 ± 0.07	0.49 ± 0.21	<0.01
15:0	0.31 ± 0.03	0.48 ± 0.18	<0.05
16:0	9.29 ± 0.71	13.40 ± 4.89	NS
ISO 18:0	0	0.88 ± 2.57	n/a
18:0	1.48 ± 0.16	2.08 ± 0.87	NS

Table 20 continued

20:0	0.35 ± 0.04	0.48 ± 0.21	NS
22:0	0.40 ± 0.04	0.68 ± 0.29	NS
24:0	0.32 ± 0.02	0.51 ± 0.22	NS
Total SFA	**20.18 ± 1.01**	**24.93 ± 5.50**	**NS**
MUFA			
14:1	0.09 ± 0.08	0.01 ± 0.04	<0.05
16:1n-9	0.09 ± 0.08	0.44 ± 0.38	NS
16:1n-7	1.19 ± 0.33	3.68 ± 1.63	<0.01
17:1	0.31 ± 0.02	0.38 ± 0.15	NS
18:1n-9	30.96 ± 1.13	33.46 ± 13.68	NS
18:1n-7	0.59 ± 0.50	4.53 ± 3.20	<0.05
20:1n-11	0.19 ± 0.02	0.14 ± 0.09	NS
20:1n-9	0.28 ± 0.22	0.13 ± 0.20	NS
22:1n-11	0.42 ± 0.21	0.28 ± 0.17	NS
22:1n-9cis	0	0.11 ± 0.19	n/a
24:1	1.01 ± 0.03	2.32 ± 0.92	<0.05
Total MUFA	**35.12 ± 0.85**	**45.49 ± 11.59**	**NS**
n-6 PUFA			
18:2n-6	8.06 ± 0.25	5.26 ± 1.01	<0.01
18:3n-6	1.33 ± 0.09	0.59 ± 0.18	<0.001
20:2n-6	0.73 ± 0.03	0.71 ± 0.20	NS
20:3n-6	1.58 ± 0.17	1.40 ± 0.64	NS
20:4n-6 (ARA)	17.13 ± 0.53	9.35 ± 3.70	<0.05
22:4n-6	0	0.05 ± 0.10	n/a
Total n-6 PUFA	**28.82 ± 1.02**	**17.36 ± 4.77**	**<0.001**
n-3 PUFA			
18:3n-3	4.60 ± 0.05	3.05 ± 3.86	NS
18:4n-3	3.42 ± 0.23	1.65 ± 2.61	NS
20:3n-3	0.20 ± 0.02	0.01 ± 0.04	<0.001
20:4n-3	0.31 ± 0.03	0.25 ± 0.21	NS
20:5n-3 (EPA)	4.86 ± 0.02	2.23 ± 0.66	<0.001
22:5n-3	0	0.27 ± 0.81	n/a
Total n-3 PUFA	**13.40 ± 0.23**	**7.46 ± 6.99**	**NS**
Other PUFAs			
14:2	0	0.07 ± 0.21	n/a
16:2	0.20 ± 0.03	0.51 ± 0.30	NS
16:3	0	0.49 ± 0.47	n/a
20:3n-7	0.47 ± 0.47	0.16 ± 0.17	<0.01
Total other PUFAs	**0.66 ± 0.06**	**1.23 ± 0.40**	**<0.01**
Total PUFA	**42.88 ± 1.05**	**26.05 ± 4.76**	**<0.001**
Furan FAs	**1.82 ± 0.18**	**3.54 ± 3.44**	**NS**

Table 21: Fatty acid composition (%) of *U. lactuca*

%	**Wild**	**Maraponic Systems**	**P-value**
Lipid	**1.35 ± 0.31**	**1.43 ± 0.13**	**NS**
SFA			

Table 21 continued

14:0	1.07 ± 0.34	0.97 ± 0.18	NS
Anteiso 15:0	0.61 ± 0.06	0.64 ± 0.05	NS
15:0	0.38 ± 0.08	0.27 ± 0.11	NS
16:0	35.79 ± 3.74	26.91 ± 3.33	<0.01
ISO 18:0	0	1.17 ± 2.33	n/a
18:0	0.89 ± 0.36	0.56 ± 0.20	NS
20:0	0.06 ± 0.10	0	n/a
22:0	1.66 ± 0.22	1.25 ± 0.23	<0.05
24:0	0.07 ± 0.12	0	n/a
Total SFA	**40.53 ± 4.48**	**31.77 ± 5.07**	**<0.05**
MUFA			
14:1	0.07 ± 0.11	0	n/a
16:1n-9	3.73 ± 5.48	0.23 ± 0.18	NS
16:1n-7	1.12 ± 0.16	1.60 ± 0.39	NS
17:1	0.57 ± 0.42	1.70 ± 0.55	P<0.01
18:1n-9	2.75 ± 19.1	2.16 ± 0.87	NS
18:1n-7	12.92 ± 1.41	13.07 ± 1.55	NS
20:1n-11	0	0.03 ± 0.08	n/a
20:1n-9	0.12 ± 0.21	0.02 ± 0.07	NS
22:1n-11	0.05 ± 0.09	0	n/a
22:1n-9cis	0.05 ± 0.09	0.18 ± 0.27	NS
24:1	0.17 ± 0.15	0.29 ± 0.47	NS
Total MUFA	**21.54 ± 6.26**	**19.29 ± 2.35**	**NS**
n-6 PUFA			
18:2n-6	3.51 ± 0.20	4.72 ± 1.42	NS
18:3n-6	0.27 ± 0.07	0.56 ± 0.25	NS
20:2n-6	0	0.03 ± 0.09	n/a
20:3n-6	0.07 ± 0.12	0.19 ± 0.12	NS
20:4n-6 (ARA)	0.30 ± 0.06	0.85 ± 0.34	<0.05
22:4n-6	0	0.21 ± 0.13	n/a
22:5n-6	0.36 ± 0.62	0	n/a
Total n-6 PUFA	**4.50 ± 0.91**	**6.56 ± 2.22**	**NS**
n-3 PUFA			
18:3n-3	10.68 ± 1.60	12.65 ± 1.96	NS
18:4n-3	6.11 ± 2.48	7.59 ± 1.12	NS
20:3n-3	0.19 ± 0.33	0	n/a
20:4n-3	0.48 ± 0.13	0.90 ± 0.24	<0.05
20:5n-3 (EPA)	0.49 ± 0.04	0.85 ± 0.16	<0.01
22:5n-3	1.44 ± 0.32	2.15 ± 0.58	NS
22:6n-3 (DHA)	0.05 ± 0.08	0	n/a
Total n-3 PUFA	**19.44 ± 4.32**	**24.13 ± 2.31**	**<0.05**
Other PUFAs			
16:2	0.48 ± 0.83	0.21 ± 0.40	NS
16:3	0.29 ± 0.50	0	n/a
16:4	2.01 ± 2.11	4.27 ± 3.88	NS
20:3n-7	0	0.27 ± 0.04	n/a
Total other PUFAs	**2.77 ± 1.27**	**4.75 ± 3.94**	**NS**
Total PUFA	**26.72 ± 5.28**	**35.44 ± 6.88**	**NS**
Furan FAs	**11.22 ± 6.91**	**13.50 ± 0.72**	**NS**

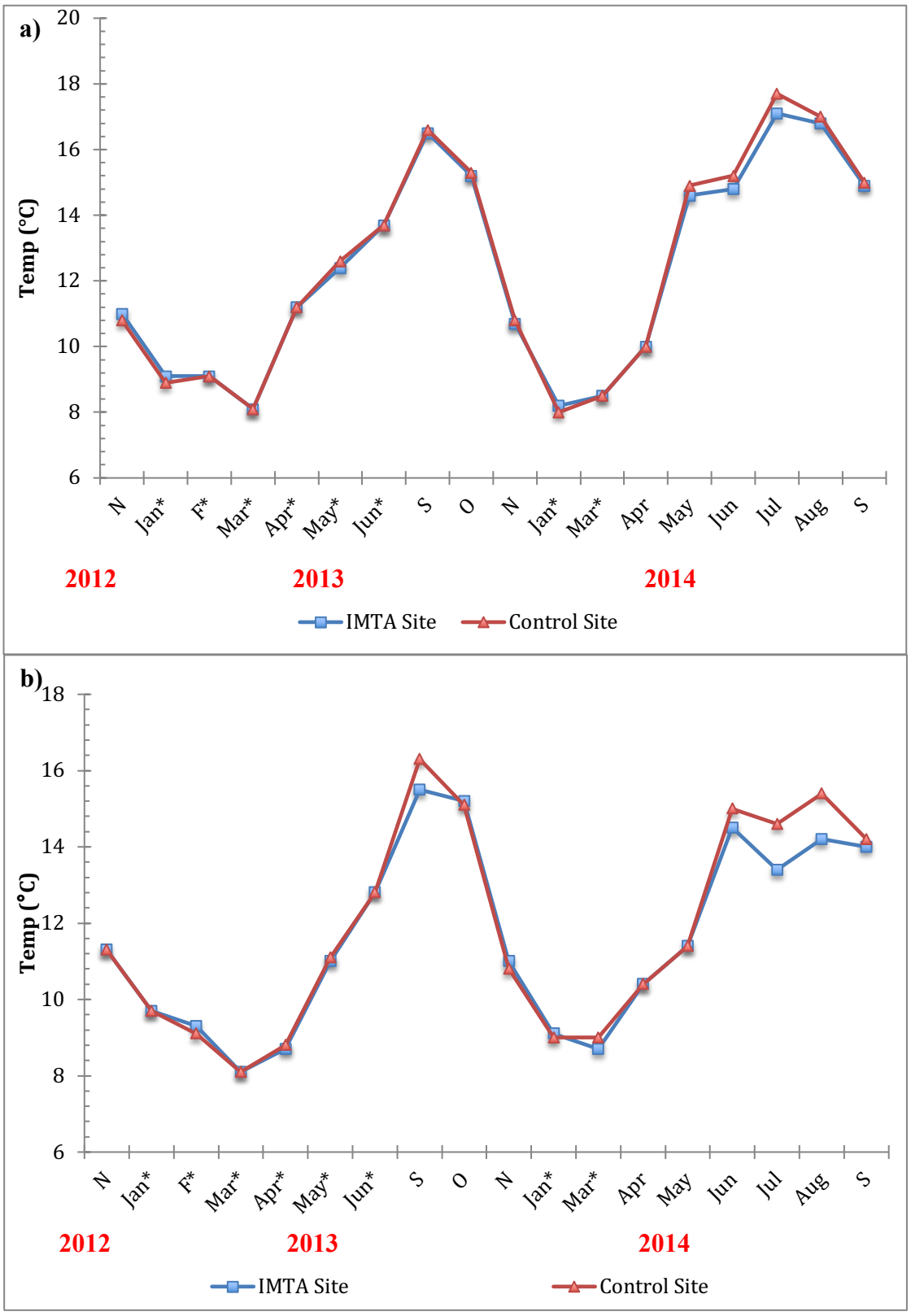


Figure 1: Temperature at a) 0 m & b) 20 m depths at the IMTA and control site over the course of trial 1 & 2 (* = seaweed longlines present)

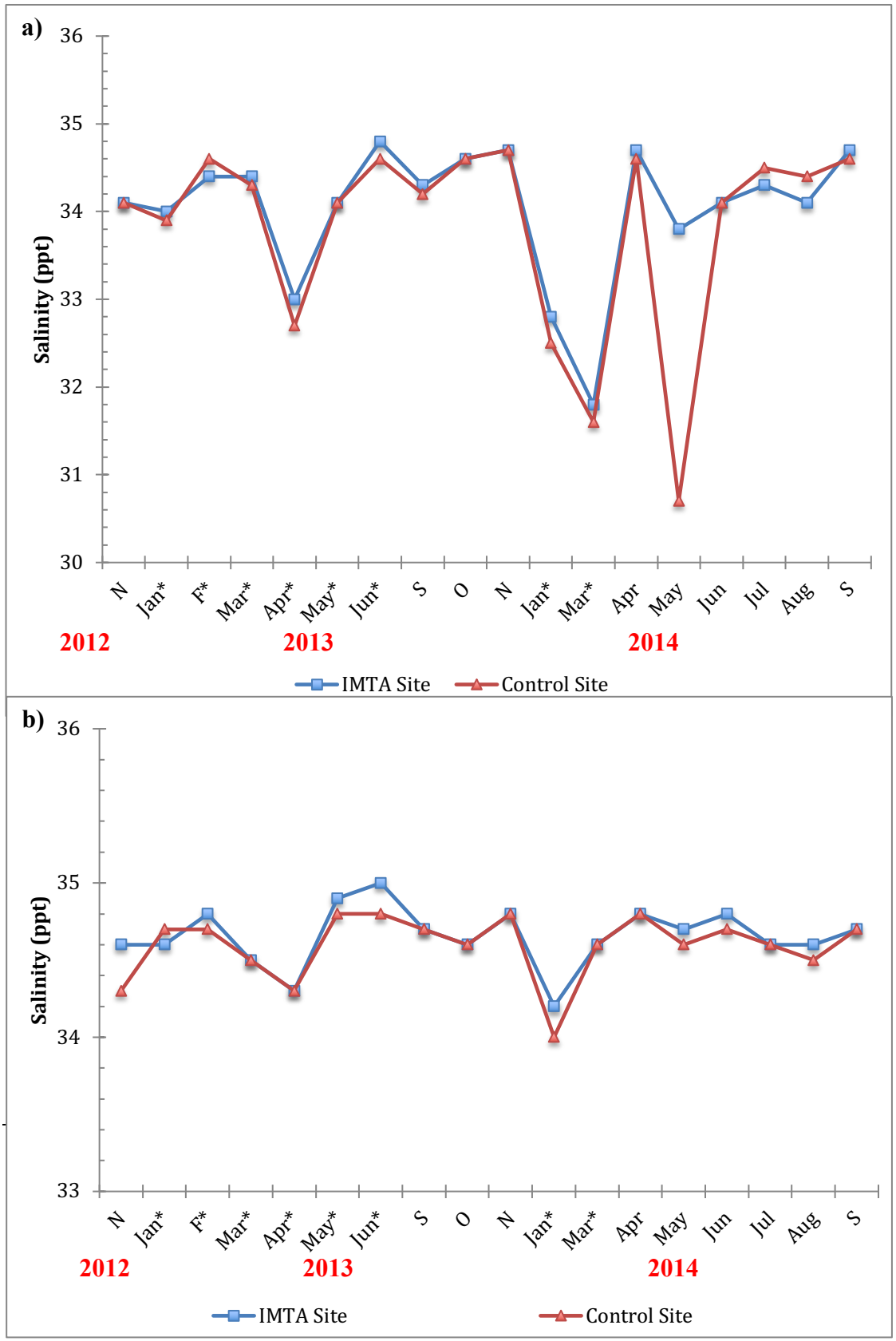


Figure 2: Salinity at a) 0 m & b) 20 m depths at IMTA and control site over the course of trial 1 & 2 (* = seaweed longlines present)

Acknowledgements

I would like to offer my most sincere gratitude to my supervisors Prof. Gavin Burnell (UCC). Without your continuous support this PhD thesis would not have been possible. A thank you to Dr. Timothy O'Sullivan, whom in the last few months of my PhD took the reigns as my main supervisor as a result of Gavin's retirement (good luck Gavin!).
To all the staff, past and present, at the Bantry Marine Research Station and Carton Point Shellfish Ltd (Freddie O'Mahony); a huge thank you for your continued support throughout my PhD. Your advice and emotional support was invaluable.
To Luke Harman and Mairead Kiely, your patience and hours of assistance involved in the development of many of my experiments that took place at UCC will not be forgotten.
A thank you to the staff of the Aquatic Services Unit, UCC (in particular Xie Quishi and Ger Morgan), Hensey Glan-Uisce Teo (Mary Hensey), and the micro-analytical laboratory, Chemistry Department, UCC (Barry O' Mahony).
I would also like to thank Ashlie Cipriano-Maack, Rory Nunan, Benjamin Horgan, Enya O' Reilly, Laura Barrett, Jessica Earl, Dean Murphy, Declan Sullivan, Colin Hannon, Cindy O' Brien, Richard Corner, Jao Ferreira, and Grace Flannery, whom in some way or other, assisted me at some point in my PhD. Also, a shout-out to all the participants of the IDREEM project. Ye were a great bunch of people and I hope our paths cross again in the future. Thank you for all your help and support.
To my parents, Breda and Derek Gunning, for supporting me (financially and emotionally) through 22 years of education. I love you both so much, you are amazing parents, and I promise I will never send you to TLC!
And finally, a huge thank you to my husband, Raymond Davidson Gunning. If it were not for your incredible cooking skills, patience, humour, love, and assistance with mathematic problems when my brain was on meltdown, I would not have made it this far. I love you so much.

This PhD project was funded by an Irish Research Council employment-based research grant (RS/2012/155)

Published Manuscripts

Gunning, D. (2016). Cultivating *Salicornia europaea* (Marsh Samphire). Bord Iascaigh Mhara – Irish Sea Fisheries Board, Dublin, Ireland, p. 95. Available online: http://bim.ie/our-publications/aquaculture/ (accessed on: 3/10/17).

Gunning, D., Maguire, J., and Burnell, G. (2016). The development of sustainable saltwater-based food production systems: a review of established and novel concepts. *Water*, 8(12), p. 598, DOI: 10.3390/w8120598.

Gunning, D., and Maguire, J. (2016). Developing IMTA across Europe. In: *Beyond fish monoculture – Developing Integrated Multi-Tropic Aquaculture in Europe*. M. Cochi, R. Corner and A. Hughes (Eds). ETA-Florence Renewable Energies, pp 12-19, ISBN: 9788889407400.

Jacob, A., Xia, A., Gunning, D., Burnell, G., and Murphy, J.D. (2016). Seaweed biofuel derived from integrated multi-trophic aquaculture. *International Journal of Environmental Science and Development*, 7(11), pp. 805-809.

Mariani, S., Griffiths, A.M., Velasco, A., Kappel, K., Jérôme, M., Perez-Martin, R.I., Schröder, U., Verrez-Bagnis, V., Silva, H., Vandamme, S.G., Boufana, B., Mendes, R., Shorten, M., Smith, C., Hankard, E., Hook, S.A., Weymer, A.S., Gunning, D., and Sotelo, C.G. (2015). Low mislabelling rates indicate marked improvements in European seafood market operations. *Frontiers in Ecology and the Environment*, 13(10), pp. 536-540.

Stanley, D.A., Gunning, D., and Stout, J.C. (2013). Pollinators and pollination of oilseed rape crops (*Brassica napus* L.) in Ireland: ecological and economic incentives for pollinator conservation. *Journal of Insect Conservation*, 17, pp. 1181-1189.