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Title	The effects of an invasive species on the structure of native ecosystems
Authors	Gallagher, Mary C.
Publication date	2017
Original Citation	Gallagher, M. C. 2017. The effects of an invasive species on the structure of native ecosystems. PhD Thesis, University College Cork.
Type of publication	Doctoral thesis
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Download date	2026-08-08 05:41:01
Item downloaded from	https://hdl.handle.net/10468/5345

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The effects of an invasive species on the structure of native ecosystems

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A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

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January 2017

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Appendix 1

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Modules and training courses undertaken during this research:

PG 6001 – STEPS: Scientific writing and presentation skills (5 credits)

ST6013 – Statistics for Postgraduate students (10 credits)

PG 6017- BEES Teaching and Learning (5 credits)

Statistics course: Data exploration, Regression, GLM and GAM with introduction to R. Highland Statistics, 4th -8th of May 2015, University of Palermo, Sicily.

Conference Presentations:

Gallagher MC, McAllen R, Culloty S and O'Riordan R (2016) "Does release from enemies facilitate the invasion success of a barnacle species?" *Environ 2016, University of Limerick, Limerick, 22nd – 24th of March 2016.*

Gallagher MC, McAllen R, Culloty S and O'Riordan R (2015) "Space, the final frontier! - Competitive colonisation between invasive and native species" *Aquatic Biodiversity and Ecosystems Conference, University of Liverpool, 30th of August – 4th of September 2015.*

Gallagher MC, McAllen R, Culloty S and O'Riordan R (2015) "Pushing the limits? An invasive species at its southern European range limit" *Environ 2015, Institute of Technology, Sligo, 8th – 10th of April 2015.*

Other presentations:

Invasive species – friend, enemy or frenemy? UCC Postgraduate Showcase 2016 (finalist in 3 minute thesis category)

Sleeping – Do not disturb! UCC doctoral showcase 2014 (second place in the Grand Plan category)

Non-Academic Publications:

Gallagher MC (2015) Alien versus native: The fight for free space. The Boolean: Snapshots of postgraduate research at University College Cork 2015.

I declare that the following work is my own and has not been submitted for another degree either at University College Cork or at another University.

Signed:

General Abstract

The Australasian barnacle species *Austrominius* (=*Elminius*) *modestus* (Darwin 1854) has been present in Europe for over seventy years. Despite the potential threats posed by non-native species to native biodiversity and native species, little is known about how *A. modestus* interacts with native species, or how its presence may alter ecosystem function. Recent recorded increases in the abundance of this species at some locations in Europe have been linked to increasing seawater temperatures. It has been suggested that *A. modestus* may be an "ecological sleeper", with the potential to undergo continued increases in abundance with predicted climate change. This study examined multiple factors that may play a role in determining the invasion success of *A. modestus.* Field sites were located in Ireland, representing the biogeographic boundary of northern and southern species and Scotland and Portugal, which represent the current northern and southern recorded limits of *A. modestus* in Europe. Long-term monitoring of the colonisation of space by *A. modestus* and native barnacle species in Ireland revealed dominance of the non-native species at the majority of study sites. However, *A. modestus* did not entirely displace native barnacle species at any site and a general pattern of coexistence was observed. A comparison of the parasite load of *A. modestus* and native barnacle species, showed that *A. modestus* experiences enemy release in comparison to native barnacle species, however this did not appear to be linked with increased reproductive success or abundance. Although *A. modestus* was recorded to be widespread at its northern and southern limits, it was outnumbered by native barnacle species at these locations. It appears that different factors may be controlling the abundance of *A. modestus* at its range limits, with competition playing an important role in the north and desiccation stress at the cypris stage controlling populations in the south. *A. modestus* was found to be the dominant barnacle species within Lough Hyne marine reserve and on artificial structures surveyed on the south-west coast of Ireland. Despite this dominance, native barnacle species continue to persist both in the marine reserve and on artificial structures of varying ages. The continuous production of broods is a key factor in the success of *A. modestus* as an invasive species. However, the continued availability of

free space on the shore, annual variation in recruitment and tolerance of wave-exposed conditions all contribute to the current persistence of native barnacle species in the presence of *A. modestus*. Future research should examine the total fecundity of this species throughout its invaded range, further investigate the factors limiting the recruitment of this species at its southern range limits and determine what impact the presence of *A. modestus* has on native ecosystem function.

Chapter 1

Introduction

1.1 Invasive species

Invasive species are recognised as one of the greatest threats facing ecosystems globally (Vitousek *et al.* 1997; Mack *et al.* 2000; Levin *et al.* 2002; Wright 2005; Seebens *et al.* 2013), resulting in reduced biodiversity, economic losses and potentially affecting human health (Bax *et al.* 2003). It has been noted that humans are responsible for altering the distribution of over one thousand marine species (Carlton 1989) and according to Sharma *et al.* (2005) the enhanced rate of invasion is one of the most significant impacts that humans have had on the Earth's ecosystem. The establishment of non-native species within a new range is not novel. However, the natural barriers that once restricted the movement of species are now commonly overcome via anthropogenic vectors (Ruiz *et al.* 1997; Lyons and Schwartz 2001; Levin *et al.* 2002; Witte *et al.* 2010). The globalised nature of our trade and transport systems has led to an increase in the rate at which species are being introduced to new areas (Seebens *et al.* 2013). It is often assumed that the niche occupied by a species in its native range represents the species' realised niche (Broennimann *et al.* 2007). However, this is not always the case, and it has been found that some species are present over wider climatic niches in their invaded range than in their native distributions. This can be due to local adaptation, phenotypic plasticity or barriers present in the native range that are absent in the invaded range (Beaumont *et al.* 2009).

While there are many different definitions for invasive species, the most basic criteria for a species to be termed invasive, alien, non-native or non-indigenous is that it is introduced to a new area outside of their historical biogeographic range (Ricciardi and Cohen 2007). While it has been proposed that the term invasive should be used to refer solely to the biogeographic status of a species without any connotation of impact (Richardson *et al.* 2000), some definitions specify that an invasive species must be an agent of change and pose a threat to native biodiversity (IUCN 1999), while others specify that invasive species must be capable of producing reproductive offspring that have the potential to spread over large areas (Colautti and MacIsaac 2004).

Not all non-native species become invasive. The rule of tens (Williamson and Fitter 1996), is well established in invasion ecology and states that approximately 10% of alien species will survive in a new range, 10% of these will become established and 10% of these will become invasive (Williamson and Fitter 1996; Levin *et al.* 2002; Bax *et al.* 2003). While some invasive species reach high abundances immediately, others exhibit a lag phase prior to undergoing exponential population growth (Mack *et al.* 2000; Bax *et al.* 2003; Lyons and Scheibling 2009; Witte *et al.* 2010). The ability to persist in a novel environment (Williamson and Fitter 1996), abiotic and biotic characteristics of the receiving environment (Schaffelke *et al.* 2006; Strong *et al.* 2009), propagule pressure (Williamson and Fitter 1996; Schaffelke *et al.* 2006; Witte *et al.* 2010), ecophysiological characteristics of the invasive species, interactions with other invaders (Witte *et al.* 2010) and disturbance in recipient ecosystems (Mack *et al.* 2000; Levin *et al.* 2002; Witte *et al.* 2010) are all cited as factors which can determine invasion success.

When an invasive species displaces a native species, this not only results in reduced biodiversity, but also changes in community structure and ecosystem processes such as productivity and nutrient cycling (Molnar *et al.* 2008). For example, the invasive ctenophore *Mnemiopsis leidyi* (A. Agassiz 1860) has been linked to the collapse of highly valuable coastal fisheries in the Black Sea, while the marine alga *Caulerpa taxifolia* (M. Vahl) C. Agardh 1817, has proliferated from the location of its establishment in Monaco, west to Toulon, France and east to Genes, Italy, to the extent that it now covers 97% of available surfaces (Bax *et al.* 2003). Invasive species can act as ecosystem engineers, altering resource availability through biotic and abiotic changes. Creating additional habitat is a common way in which invasive species can engineer habitats and this can promote the establishment of other non-native species, in a phenomenon known as “invasional meltdown” (Simberloff and Von Holle 1999), which can produce accelerated impacts in invaded ecosystems (Lang and Buschbaum 2010).

Elton (1958) proposed that more diverse communities would be less susceptible to invasion, due to efficient resource use, lack of available niches and competitive

ability. While there has been evidence for (Stachowicz *et al.* 1999) and against this theory (Levine and D'Antonio 1999), it is now generally accepted that a healthy, undisturbed native ecosystem has a high resilience to invasion (Occhipinti-Ambrogi and Savini 2003). More recently, invasibility has been linked to resource availability (Davis 2003; Romanuk and Kolasa 2005; Vaz-Pinto *et al.* 2013), where the ability of species to utilise resources maximally, with as little variability as possible, is more likely to produce invasion resistance than the number of species present (Romanuk and Kolasa 2005; Dunstan and Johnson 2007). Additionally, fluctuations of resource availability can alter competitive interactions with native species and thus increase or decrease invasion success (Davis 2003). Stress and disturbance can reduce the competitive ability of native species and are commonly associated with high levels of invasion. "Hot spots" for introductions arise in places with high levels of disturbance in conjunction with a high propagule pressure, such as ports and estuaries (Schaffelke *et al.* 2006; Schaffelke and Hewitt 2007). Invasive species often exhibit high tolerance to pollution, which also aids their establishment in such areas (Nyberg and Wallentinus 2005).

The process where ecosystems become dominated by opportunistic and generalist species, while native species decline, often due to establishment of invasive species, is termed biotic homogenisation (Olden *et al.* 2004; Schaffelke *et al.* 2006) and is generally accompanied by a decrease in taxonomic and functional diversity (Olden 2006). However, in some invaded communities, despite increases in the abundance of invasive species over time, the richness and diversity of native species have not undergone a significant decline (Heard and Sax 2013). Witte *et al.* (2010) noted, that independent of the effects on native species, a "homogenised" global ecosystem could be better adapted to endure future climate change than native ecosystems.

1.2 Barnacles

Over 1,200 known species of barnacles (cirripedes) exist globally. Adult barnacles are sessile suspension feeders, while the larval stages are planktonic (Tøttrup *et al*. 2010). Cirripedes are present in all oceans, from the upper intertidal zone to the abyssal plains, but are not found in freshwaters (Buckeridge 2012). Cirripede larvae

comprise a major seasonal component of zooplankton communities (Muxagata and Williams 2011), while adults are recognised as important members of the marine ecosystem and also as important fouling organisms, being able to colonise a wide variety of substrates, both living and inanimate (Buckeridge 2010). Intertidal barnacles make ideal study organisms, as they are exposed at low tide and occur in dense aggregations (Connell 1961). They can also be transplanted easily and are small enough that sufficient numbers can be attained for experimental and control treatments. Their sessile nature allows for recruitment and mortality to be measured and they can be photographed at different time intervals to compare growth and survival (Connell 1970).

Barnacles have been transported globally on the hulls of ships, giving them great potential to establish in new ranges, often with piers as opposed to shores being initially colonised (Barnes and Barnes 1961). Hosie and Ahyong (2008) reported only three successful invasions by barnacle species on a global scale, however, Carlton *et al.* (2011) reported that at least twelve species have been introduced to the Atlantic and Pacific coasts of North and South America alone. *Balanus glandula* (Darwin 1854) is one example of a very successful invasive barnacle species. Native to the Pacific coast of North America, this species was accidentally introduced to Argentina in the 1970s and has subsequently successfully invaded almost the entire Argentinian coast (Schwindt 2007), displacing native barnacle and mussel species in some locations (Vallarino and Elias 1997). *B. glandula* has also become established on parts of the Japanese (Kado 2003) and South African coastlines (Laird and Griffiths 2008; Simon-Blecher *et al.* 2008).

1.3 *Austrominius modestus*

Austrominius modestus (Darwin 1854), formerly *Elminius modestus*, is a species of barnacle native to New Zealand, Tasmania and the subtropical and temperate zones of Australia (Moore 1944; Buckeridge and Newman 2010). It is one of 279 barnacle species recorded in Australian waters, but one of only six that have an Australasian distribution (Jones 2012). *A. modestus* has been recorded as the most common fouling barnacle in New Zealand harbours (Foster 1982) and it has been suggested

that it was introduced to southern Australia from New Zealand via shipping (Foster and Anderson 1986). Bishop (1951) described this species as one of the "most geographically confined of all barnacles". Within its native range, this species is recognised as being able to penetrate deep into estuaries and harbours, as it can tolerate brackish and muddy waters. *A. modestus* is typically found in sheltered waters as opposed to wave-exposed coastlines (Moore 1944; Southward 1955; Crisp 1958) and can colonise a wide variety of surfaces including rock, wood, iron, algae and settled invertebrates, including other barnacles (Luckens 1975). This species is unusual in that, it not only colonises the top of rocks and other suitable surfaces but also has the ability to colonise the under side of suitable objects (Moore 1944). *A. modestus* is commonly found in the mid intertidal zone, where it can form dense populations.

The first record of *A. modestus* in Europe was from Chichester Harbour in 1945 (Bishop 1947). It is believed that this species was transported to Britain via ship or flying boat (Bishop 1947; Crisp 1958). Crisp and Chipperfield (1948) also recorded *A. modestus* from the south coast in the same year. The distance between the initial locations of colonisation, along with the subsequent extent of range expansion, led Stubbings (1950) to suggest that *A. modestus* was well established prior to 1945. According to Crisp (1958), the Second World War may have prevented the early stages of the introduction of this species to be recorded in British waters. Detailed examination of specimens from Portsmouth, pointed to 1943 as the time of establishment at this location (Stubbings 1950).

The spread of *A. modestus* along European coasts was facilitated by both remote and marginal dispersal (Crisp 1958). Barnes and Barnes (1963) noted that its establishment in Europe was one of the "best documented examples of a marine animal" to a new range and there have been many published records of its dispersal and abundance on European coasts, in particular from the time of its initial introduction to Europe until the late 1980s (e.g. Purchon 1947; Boschma 1948; Knight Jones 1948; den Hartog 1953; Bishop 1954; Beard 1957; Bishop and Crisp 1957; Crisp 1959; Moyse and Nelson-Smith 1963; Powell 1960; Davis 1967; Barnes

and Barnes 1968; Barnes and Barnes 1969; Barnes *et al.* 1972; Bennell 1981; Salman 1982; Hiscock 1985; Harms and Anger 1989). A single individual was recorded in South Africa in 1949, however the species did not become established in this area (Sandison 1950). Currently, the known European range of *A. modestus* extends from Scotland in the north (Nall *et al.* 2015) to southern Portugal in the south (O'Riordan and Ramsay 1999; O'Riordan and Ramsay 2013). Over seventy years since its establishment here, *A. modestus* is more widespread in Europe than its native range (Buckeridge and Newman 2010) and has been described as a "naturalised" member of European intertidal habitats (Tøttrup *et al.* 2010). Although it has not yet been reported from the coasts of America, the reason for which remains unclear, it is expected to become established, if it has not already done so, there in the future (Carlton *et al.* 2011).

The first Irish record of *A. modestus* was from near Lough Hyne in 1957 (Beard 1957), though it is believed that it was established in Cork Harbour prior to this (O'Riordan 2002), likely between 1954 and 1956 (Crisp and Southward 1959). As with the spread of this species in Britain, there are many detailed records regarding the spread of this species around the coast (Aitken and Hillis 1959; Crisp and Southward 1959; Boyd 1973; Crapp 1973; Myers *et al.* 1979; Myers *et al.* 1980; Seed and Harris 1980; Wilkinson *et al.* 1988; McGrath and King 1992; Sides *et al.* 1994; Minchin and Sheehan 1995; O'Riordan 1996; King *et al.* 1997; Allen *et al.* 2006).

The main factors which are contributing to the successful spread of *A. modestus* within European waters are euryhaline and eurythermal larval stages (Patel and Crisp 1960a; Patel and Crisp 1960b), high rates of cirral activity allowing for effective utilisation of food resources (Crisp 1964) and high levels of fecundity throughout the year (Harms 1999) coupled with a short generation time and prolonged settlement period (Crisp and Davies 1955). Additionally, the opportunistic nature of *A. modestus* facilitates its rapid colonisation of free space (Bracewell *et al.* 2012; Bracewell *et al.* 2013).

1.4 Competitive interactions between *A. modestus* and native species

One of the common negative implications of the presence of invasive species is often cited as the ability to outcompete native species. In reality there are very few recorded extinctions of native species due to competition from invasive species. However, competition may still play an important role in the overall extinction process (Davis 2003). While there have been reports of competitive interactions between *A. modestus* and native barnacle species (den Hartog 1953; Crisp 1960; Stubbings and Haughton 1964; Bennell 1981; Rainbow 1984) and commercially important oyster spat (Knight Jones 1948; Kochmann and Crowe 2014), many of these are anecdotal and the competitive interactions between *A. modestus* and native species have not been experimentally tested. It has also been reported that *A. modestus* has not outcompeted native barnacle species on the Belgian coast, despite being present in high abundances (Kerckhof and Cattrijsse 2001). It appears that the competitive effects of *A. modestus* on native species may be variable and possibly site specific. As highlighted by Torres *et al.* (2012), there is a need for studies to examine the impact of non-native barnacles on native species to gain an understanding of their population dynamics and annual successions. Additionally, it is important to examine resource conversion as well as resource exploitation of invasive species, as this can account for competitive superiority (Byers 2000).

1.5 Ecosystem structure and function

Invasive species are commonly reported to alter the structure and function of native ecosystems, however there have been few detailed analyses of such impacts. Baird *et al.* (2012) examined the effect of *A. modestus* and the non native oyster *Crassostrea gigas* on native ecosystem structure and function using Ecological Network Analysis (ENA) models of the Sylt-Rømø Bight in the North Sea at three different times, with differing densities of the invasive species. They found that the invasive species had impacts on lower trophic levels, mainly affecting phytoplankton abundance. While changes were detected in the ecosystem over their three time scales, they could not be solely linked to the presence of the invasive species. As shown by Schwartz *et al.* (2000) species richness is not necessarily an indicator of ecosystem function. Therefore, if the invasive species *A. modestus* reduces the

number of native barnacle species, this may not alter ecosystem function, provided that *A. modestus* plays the same functional role as those native species.

1.6 Research objectives

As noted by Ruiz *et al.* (1997), the effects of invasive species are generally poorly understood, and this is true in the case of *A. modestus.* While there is a wealth of information regarding the initial establishment and distribution of this species within its invaded range, there have been few such reports over the past three decades. In addition to this, while some authors have mentioned that *A. modestus* competes with native species, the extent and impacts of this competition have not yet been investigated. In areas where it has become abundant, little information is available regarding the implications of this on the native ecosystem. It is unknown whether *A. modestus* plays a different functional role to native species and if so, how its presence will affect ecosystem functioning. It has been suggested that recent increases in the abundance of *A. modestus* may be due to increasing seawater temperatures (Lawson *et al.* 2004; Witte *et al.* 2010) and that this species may be an "ecological sleeper", which persists in a location but only becomes abundant when environmental conditions are suitable. Given that predicted climate change will likely favour *A. modestus* it is particularly important to gain a better understanding of how this species interacts with native barnacle species and what factors control the abundance and distribution of *A. modestus.*

The objectives of my research were:

1) To experimentally examine the colonisation of space by *A. modestus* and native barnacle species to gain an insight into the role of space in the invasion of *A. modestus*;
2) To examine the recruitment of *A. modestus* and native barnacle species in Lough Hyne, which is Ireland's first and only marine nature reserve and a site where this species is known to be present at very high abundances;
3) To investigate the competitive impacts of *A. modestus* by comparing the growth and survival of native recruits in the presence and absence of the non-native species;

4) To compare the reproductive strategies of *A. modestus* and a native barnacle species from a single location over two distinct time periods, in order to determine whether or no differences in reproduction play a role in the invasion ecology of *A. modestus*;
5) To examine the factors controlling the northern and southern European limits of *A. modestus;*
6) To determine whether or not *A. modestus* experiences enemy release in comparison to native barnacle species and, if so, whether that gives it a competitive advantage;
7) To examine the long-term patterns of infection of *A. modestus* and a native barnacle species from two locations in Europe;
8) To compare the abundance of *A. modestus* and native barnacle species on artificial structures of differing ages;
9) To suggest potential future research directions for this species

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Chapter 2

Room for one more? Coexistence of native and non-indigenous barnacle species.

This chapter, in revised format, has been published in the journal *Biological Invasions*:

Gallagher MC, Culloty S, McAllen R and O'Riordan R (2016) Room for one more? Coexistence of native and non-indigenous barnacle species. Biological Invasions 18: 3033-3046. See Appendix 1.

2.1 Abstract

Space can be a limiting factor in intertidal communities and competition for this resource plays a role in determining community composition. Invasive species are known to be opportunistic, and are capable of rapidly colonising free space. Since its introduction to Europe over seventy years ago, the invasive barnacle species *Austrominius modestus* has become widespread on European coasts and is now the dominant barnacle species at numerous locations, in particular sheltered and estuarine locations. However, little is known about its competitive interactions with native barnacle species. During this study, 18 removal plots (25 cm^2) were created at each of 6 sites in south-west Ireland, encompassing a range of exposure levels and including both artificial structures and natural rocky shores. Monthly monitoring of these plots over 19 months revealed that, despite high recruitment of native species seasonally, *A. modestus* ultimately outnumbered natives in removal plots at four sites. With the provision of free space, the invasive species increased its relative abundance at three sites, but did not entirely displace natives at any site. Continued recruitment of the native species at high abundances on a seasonal basis in addition to the invasive species not entirely colonising all available space, ensure native species can persist and co-exist with *A. modestus* at the majority of sites at this location.

KEYWORDS: *Austrominius modestus*, invasive species, space, competition, barnacle, recruitment.

2.2 Introduction

In the intertidal zone, as in many habitats, space is limited, and competition for this resource plays an important role in determining overall community composition (Connell 1961; Dayton 1971; Dungan 1985; Sorte *et al.* 2010). Barnacles are sessile organisms, making space for attachment and for the facilitation of feeding and reproduction one of the most important requirements for these animals (Luckens 1975). Barnacles are well known for their fouling nature (Buckeridge 2010; Buckeridge 2012) and are capable of settling on a variety of substrates (Buckeridge 2010). In the marine environment, shipping has been identified as one of the main vectors for the movement of species to new ranges (Bax *et al.* 2003; Molnar *et al.* 2008; Seebens *et al.* 2013) and the rate of introductions has increased in line with globalisation and world trade (Seebens *et al.* 2013). Barnacles fouling the hulls of ships have been transported globally for centuries (Carlton *et al.* 2011), giving them great potential to establish in new ranges (Barnes and Barnes 1961).

Invasive species are described as a major threat facing ecosystems globally (Mack *et al.* 2000; Levin *et al.* 2002; Wright 2005; Seebens *et al.* 2013) and are commonly associated with reduced biodiversity (Bax *et al.* 2003; Molnar *et al.* 2008; McGeoch *et al.* 2010). The ability of an invasive species to compete with natives for resources may be a key determinant of their success (Byers 2000). Invasive species have been regarded as “opportunistic” (Occhipinti-Ambrogi and Savini 2003), in that they are capable of rapidly colonizing free space (Bracewell *et al.* 2012). For example, invasive barnacles have been recorded to initially colonise piers as opposed to shorelines (Barnes and Barnes 1961), probably due to the availability of space on novel structures.

Coastal areas have long been associated with large human populations and associated activities (Small and Nicholls 2003; Martínez *et al.* 2007) and populations in European coastal areas have been growing faster than those inland (EEA 2006). Additional pressure from tourism, and the need to protect these areas from environmental threats such as storm damage, have also contributed to the development of coastal areas, resulting in the construction of artificial structures

such as marinas, piers and breakwaters in the coastal environment (Bax *et al.* 2003). Not only do these artificial structures provide a novel habitat for fouling organisms to colonise, but they also contribute to habitat fragmentation (Bulleri and Chapman 2010). It has been found that invasive species are often more abundant on artificial structures than natural substrates (Tyrell and Byers 2007), and that artificial structures can differ significantly in community composition to that on surrounding natural substrates (Chapman and Underwood 2011). Where artificial structures are present in close proximity to each other, it is possible for them to act as stepping-stones, aiding the dispersal of invasive species (Bulleri and Airoldi 2005).

The Australasian barnacle species *Austrominius modestus* has been present in Europe since the 1940s (Bishop 1947; Crisp and Chipperfield 1948), and is now widespread on most European coasts (O'Riordan 1996; Allen *et al.* 2006; Arenas *et al.* 2006; Tøttrup *et al.* 2010). This species is commonly found in sheltered areas and estuaries as opposed to exposed coastlines (Moore 1944; Southward 1955; Crisp 1958). While *A. modestus* has become dominant at many locations within its invaded range (Franke and Gutow 2004; Lawson *et al.* 2004; Reichert and Buchholz 2006; Gomes-Filho *et al.* 2010; Witte *et al.* 2010, Bracewell *et al.* 2012), native species have been recorded to persist along with this species (Stubbings and Houghton 1964; Bennell 1981; Kerckhof and Cattrijsse 2001), and even remain the dominant species at some locations within this range (Southward 1991; Range and Paula 2001; Boaventura *et al.* 2002; Herbert *et al.* 2007, Herbert *et al.* 2009; Burrows *et al.* 2010; Gomes-Filho *et al.* 2010; Gallagher *et al.* 2015). Despite the long interval since its introduction to Europe, there have been no experimental studies investigating the competitive interactions between this alien and native barnacle species, though there have been anecdotal accounts of such competitive interactions (den Hartog 1953; Crisp 1958; Crisp 1960; Barnes and Barnes 1962; Bennell 1981).

Space has been highlighted by some authors as playing an important role in the invasion success of *A. modestus.* This invasive species breeds via a continuous succession of broods (Moore 1944; Moyse 1960; Patel and Crisp 1960; Moyse and Nelson-Smith 1963), potentially breeding throughout the entire year (Bennell 1981;

O'Riordan and Murphy 2000). This extended breeding period allows *A. modestus to* colonise any free space available during this time, thus limiting the space available for spat of the native arctic-boreal species, *Semibalanus balanoides* (Linnaeus 1767), to settle in the spring time (Rainbow 1984; Hui and Moyse 1987). However, provided there is space available at the time of *S. balanoides* spat fall, it is possible for the native species to utilise this prior to *A. modestus* settlement, allowing the native species to persist (Crisp and Davies 1955; Hui and Moyse 1987).

Whilst some recent studies have focused on the recruitment of *A. modestus* (Witte *et al.* 2010; Bracewell *et al.* 2012; Bracewell *et al.* 2013), there has been no research carried out on the recruitment and survival of this species in direct comparison to natives. This study examined the colonisation of free space by *A. modestus* and two native barnacle species *Semibalanus balanoides* and *Chthamalus montagui* Southward 1976, in south-west Ireland over a period of nineteen months. The south-west coast of Ireland is an ideal location for such a study, given its location at the western limit of the invaded range of *A. modestus* and at the biogeographic border of northern and southern species (Herbert *et al.* 2007). Additionally, *A. modestus* has been present at this location since the 1950s (Beard 1957), providing ample time for it to become well established. Examining the recruitment and survival of these barnacle species in removal plots and comparing it with recruitment occurring in free space available within the surrounding community, it was possible to determine how the native and invasive species interact in their utilisation of free space and the role of space in the invasion success of *A. modestus*.

2.3 Materials and methods

2.3.1 Study sites

Six field sites were located within two bays in south-west Ireland (Figure 1). Within each bay, one artificial structure such as a pier or slipway, and two natural shores, one sheltered and one semi exposed (Table 1) were selected as study sites (Figure 2). Criteria for site selection were: the presence of a vertical rock face, or concrete

structure, with a pre-existing barnacle cover, large enough for the establishment of numerous experimental plots and flat enough to ensure the surface could be totally cleared of all organisms.

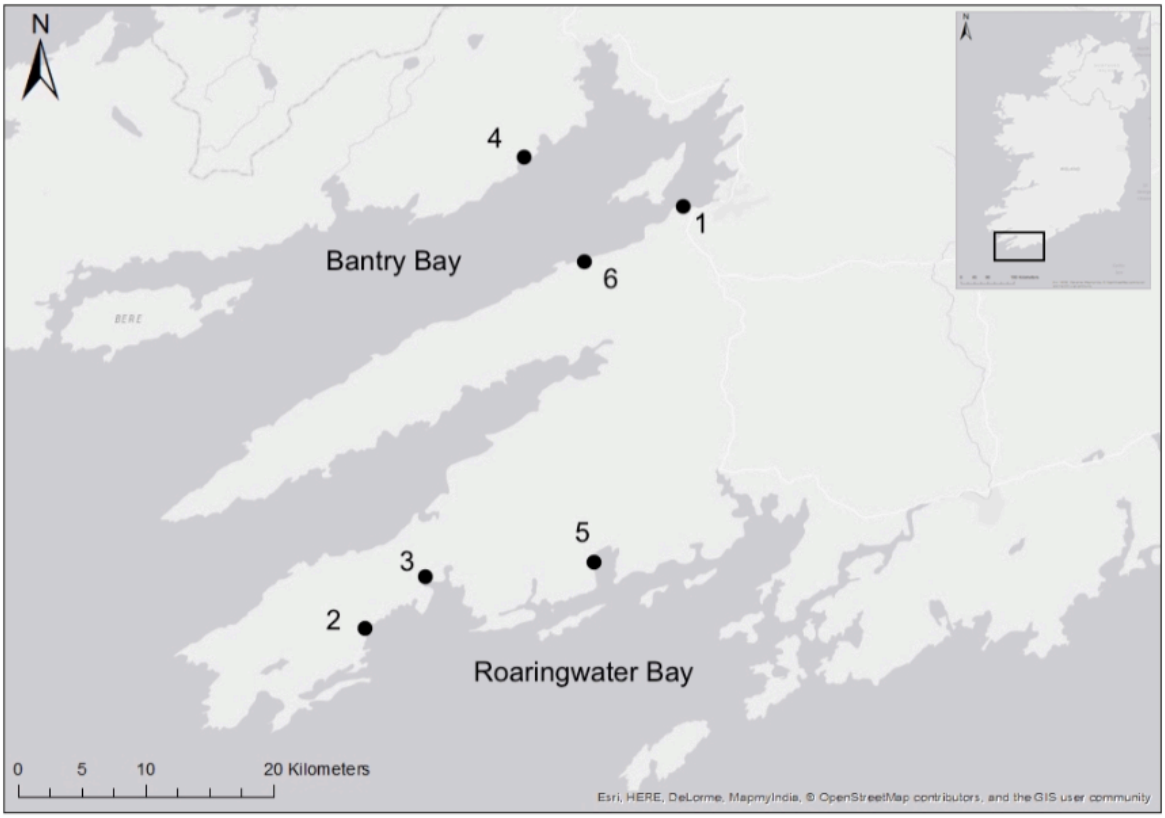

Figure 1. Location of six field sites in south-west Ireland. Inset map indicates enlarged area. See table 1 for site details. Map created using ArcGIS.

Table 1. Study site information (GPS coordinates, substrate type and exposure; determined using the Ballantine Scale (Ballantine, 1961)).

Number	Site name	GPS coordinates	Natural	Artificial	Ballantine Scale
1	Bantry Harbour	N 51°40.687’ W 009°28.396’		X	6
2	Goleen Pier	N 51°29.668’ W 008°42.402’		X	6
3	Ballyrisode	N 51°30.725’ W 009°39.724’	X		6
4	Zetland	N 51°41.644’ W 009°35.829’	X		5
5	Schull	N 51°31.331’ W 009°32.649’	X		4
6	Gearhies	N 51°33.811’ W 009°34.841’	X		2

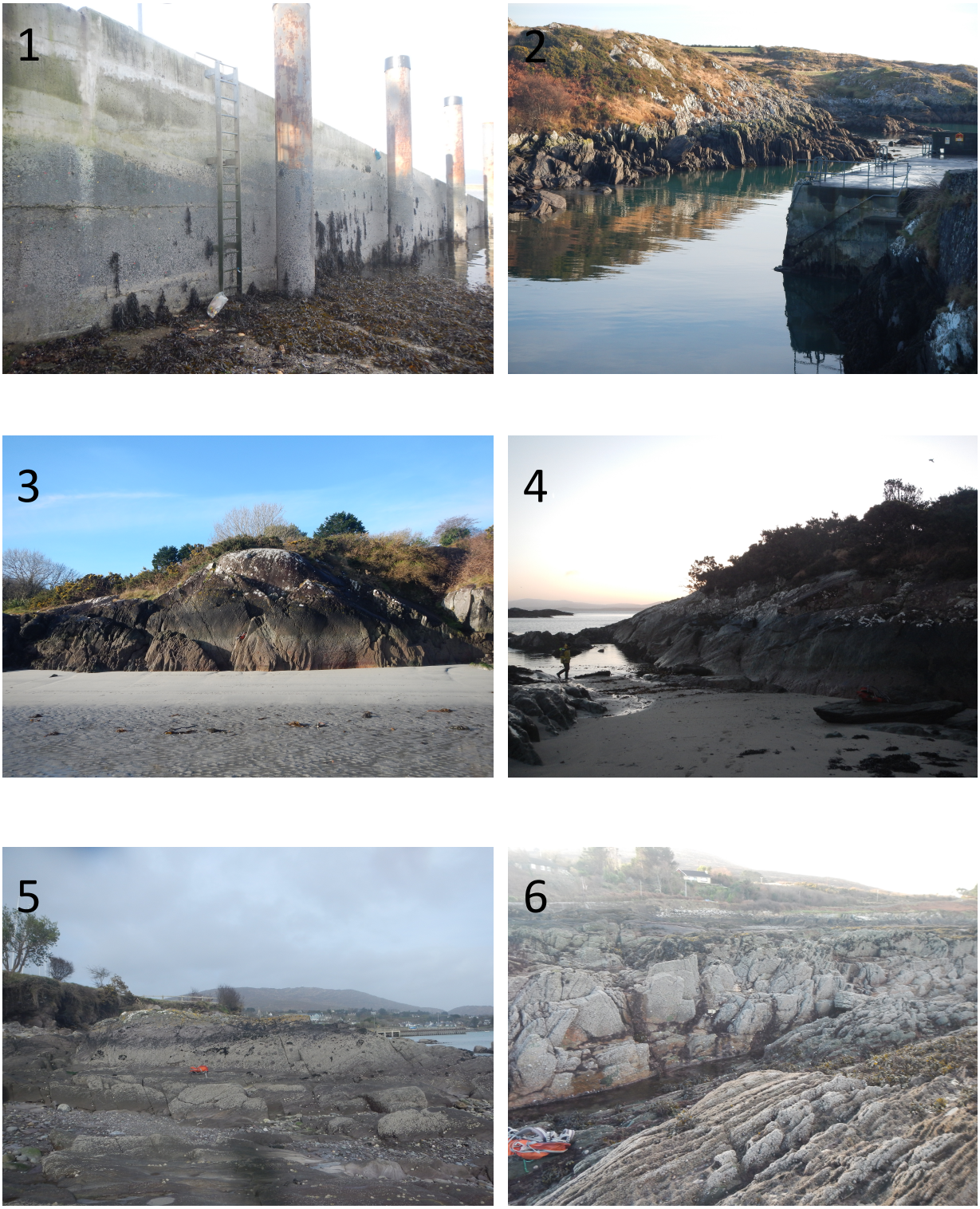

Figure 2. Photographs of field sites. 1 = Bantry Harbour, 2 = Goleen Pier, 3 = Ballyrisode, 4 = Zetland, 5 = Schull and 6 = Gearhies. For site locations see Figure 1 and for site details see Table 1. (Source: author's own).

2.3.2 Experimental plots

Experimental plots were established at three areas on the vertical rock face, approximate to the high, mid and low shore levels. High shore was taken as the area where barnacle cover began and low shore as the area where barnacle cover stopped, or where the rock itself stopped. Mid shore was determined as the area between these two zones. Six experimental plots and six control plots, each measuring 25cm^2 were established at each of these three areas at all sites (eighteen of each plot type per site). Plot location and subsequently plot type were randomly assigned using random numbers tables. Both plot types were initially photographed, using a 25cm^2 quadrat to frame the plot area, with a Nikon Coolpix AW110 digital camera. All images were taken using macro mode. These initial images were analysed in order to determine the community composition of the area prior to the establishment of experimental plots.

Areas designated as experimental or removal plots were cleared of all barnacles in the plot area, plus an edge of at least 1cm on each side of the plot, using a paint scraper. The cleared surface was then scoured with wire wool to ensure that all organisms had been removed. Removal plots were marked with a line of red enamel paint on the rock surface, along the top left hand corner of the quadrat, which provided a guide for replacement of the quadrat in the same position on each field visit. Half of the removal plots were randomly assigned as re-clearance plots, identified by a dot of paint next to the original line. These plots were re-cleared on a bi-monthly basis in order to examine colonisation of the area at distinct temporal scales, in comparison to long-term colonisation (19 months) in plots that were not re-cleared. Areas designated as control plots were not manipulated in any way, other than marking the location of the plot in the same way as that of the removal plots, using green enamel paint.

2.3.3 Monitoring and image analysis

Plots were established at sites between February and June 2014 and were monitored on a monthly basis until October 2015. On each field visit, all plots were photographed, using the paint mark as a guide for quadrat placement, which framed

the same area on each visit. Individuals that had settled and survived the period until observation were considered recruits (Keough and Downes 1982). The number of recruits within both removal and control plots were quantified and identified to species level from each image. As images were taken of the same plots for up to nineteen months, this provided data regarding the survival of recruits and the establishment of additional recruits to the plot area over time.

The amount of free space available for colonisation within each control plot was determined by estimating the percentage cover of bare rock within each individual control plot area. This was done using an acetate sheet with a grid of dots, at a density of four dots per cm^2. A density of 0.5 dots per $25cm^2$ has been shown to give consistent and reproducible estimates of percentage cover (Littler *et al.* 1986); however, given the small size of the organisms in this study a higher density was chosen. This acetate sheet was then placed over a laptop screen with the image of the plot area enlarged to "full screen". The number of dots that overlaid an area of bare rock was counted, and divided by the total number of dots covering the plot area, to calculate percentage cover. The number of recruits in each control plot could then be converted to represent number of recruits per $25cm^2$ of free space, to allow for direct comparison with removal plots.

2.3.4 Temperature Data

HOBO temperature loggers were placed at two of the field sites, Bantry Harbour and Ballyrisode. These sites were chosen due to the availability of suitable attachment points. Loggers were placed in the intertidal zone, as close to experimental plots as possible and were programmed to record the temperature (air or sea depending on the tidal state) at half hour intervals. The data were subsequently split into air and sea temperatures and mean temperatures for each month were calculated. It was not possible to retrieve data from loggers deployed during 2014, and so air temperature data from Met Éireann (daily values recorded at Sherkin Island weather station) have been utilised instead.

2.3.5 Statistical Analysis

Statistical analysis was carried out using R Studio software version 3.3.0 (R Core Team 2016). Due to a lack of independence and the unbalanced nature of the data, generalised linear mixed effects models (GLMMs) were constructed using the data with the package "lme4" (Bates *et al.* 2015). As the data were in the form of count data, and were found to be over dispersed, a negative binomial distribution was specified by running a negative binomial GLMM. Species, substrate and plot type were treated as fixed effects, while site and plot were treated as random effects. The effect of the fixed effects on abundance was determined overall and also at specific time intervals (0-120 days, 121-240 days, 241-360 days and 361-570 days), for all sites and also for each individual site, by creating subsets of the data. These time intervals correspond to the settlement period for *Semibalanus balanoides* (0-120), the main settlement period for *Austrominius modestus* (121-240), the settlement period for *Chthamalus montagui* (241-260) and the settlement period of both *S. balanoides* and *A. modestus* (361-570). Model validation was carried out using residual plots and QQ-plots.

2.4 Results

2.4.1 Initial Community composition

At the start of the study *A. modestus* was present at all six study sites. However it varied in abundance from 94.8 ind. $25cm^{-2}$ (± 3.79 ind. $25cm^{-2}$) (99% relative abundance) at Bantry Harbour to 0.5 ind. $25cm^{-2}$ (± 0.24 ind. $25cm^{-2}$) (< 1% relative abundance) at Gearhies (Table 2). Both native species, *S. balanoides* and *C. montagui*, were also present at all study sites, with the exception of Bantry Harbour, where *C. montagui* was absent from plots initially surveyed. There was also a large variation in the abundance of these two species between sites. *S. balanoides* was present in highest abundances at Zetland, with 16.9 ind. $25cm^{-2}$ (± 1.88 ind. $25cm^{-2}$) (49% relative abundance) and least abundant at Bantry Harbour (0.85 ind. $25cm^{-2}$ ± 0.22 ind. $25cm^{-2}$; 1% relative abundance) (Table 2). *C. montagui* had the highest abundance of any of the species across all sites at Gearhies (224.4 ind. $25cm^{-2}$ ± 32.32 ind. $25cm^{-2}$; 96% relative abundance), but was also recorded at very low

abundances (0.2 ind. 25cm^{-2} ± 0.08 ind. 25cm^{-2}; 1% relative abundance) at Goleen Pier (Table 1). *C. stellatus* was the least abundant of all species, and was only recorded at one site, Ballyrisode (0.9 ind. 25cm^{-2} ± 0.18 ind. 25cm^{-2}; 1% relative abundance) (Table 2).

2.4.2 Recruitment at distinct temporal scales

Recruits of three species, *A. modestus*, *S. balanoides* and *C. montagui* were recorded in both the removal and control plots during this study (Table 4; Figure 2). The three species differed in their timing and abundance during recruitment. Removal plots that were re-cleared, provide a more detailed insight into the timing of recruitment for each species. *A. modestus* was the only species recorded to recolonise removal plots following each bi-monthly re-clearance event, though this was only the case at two study sites, Ballyrisode and Goleen Pier (Table 3B). *A. modestus* recruits were present following the majority of re-clearance events at Bantry Harbour (90%), Zetland (80%) and Schull (66.66%), but only following 30% at Gearhies (Table 3B). The native species, *S. balanoides* and *C. montagui* displayed distinct periods of recruitment. *S. balanoides* recruits were recorded in re-cleared removal plots at all sites between April and July 2014 and April and June 2015 (Table 3A). *C. montagui* recruits were present in re-cleared removal plots at all sites from July to December 2014 and at three sites (Zetland, Schull and Gearhies) from July to October 2015 (Table 3C).

2.4.3 Cumulative recruitment and survival

Despite differences in the abundances of recruits, both between sites and between plot types, the same overall trends were displayed for each species at all locations over the duration of the study. *S. balanoides* recruits initially colonised free space in both plot types, reaching peak abundances in May 2014. Following this, the number of *S. balanoides* recruits declined quite rapidly until September 2014. Mean abundance of *S. balanoides* recruits continued to decline, but at a lower rate until April 2015, when an increase in recruits was again observed, peaking in June 2015 and subsequently declining again (Figure 3). Recruits of *A. modestus* were recorded from May 2014, and continued to increase in abundance until October 2014.

Following this, the number of recruits remained stable or underwent a slight decline, before increasing in abundance again from July 2015 until the end of the study (Figure 3). *C. montagui* recruits were recorded from August 2014, the number of recruits continued to increase, reaching peak abundances in November 2014. No further increase in the number of recruits was observed until September 2015; but little or no decline in recruit abundance was recorded during this time (Figure 3).

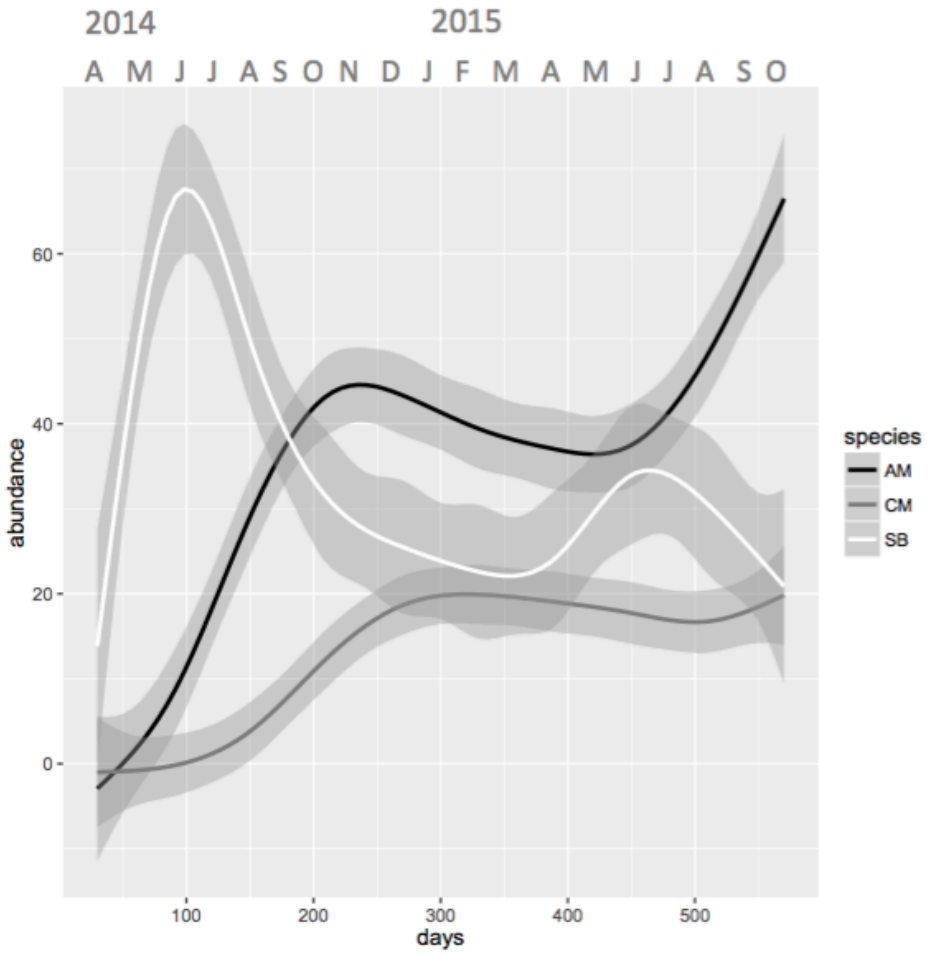

Figure 3. Smoothed mean abundance of recruits of three barnacle species *Austrominius modestus* (AM) (black), *Chthamalus montagui* (CM) (grey) and *Semibalanus balanoides* (SB) (white) from data recorded at six field sites in south-west Ireland from April 2014 to October 2015 (570 days). Shaded area surrounding each line represent the estimated standard error, smoothed abundances calculated using "loess" method (Cleveland 1979). Graph created using ggplot2 (Wickham 2009) in R Studio (R Core Team 2016).

Table 2. Mean abundance (ind. 25cm^{-2}) (± standard error) of four barnacle species, *Semibalanus balanoides*, *Austrominius modestus*, *Chthamalus montagui* and *C. stellatus* at six sites (n = 36 quadrats per site) in south-west Ireland between February and June 2014. Values in brackets represent the relative abundance of each species.

	Semibalanus balanoides	*Austrominius modestus*	*Chthamalus montagui*	*Chthamalus stellatus*
Bantry Harbour	0.9 ± 0.22 (1%)	94.8 ± 3.79 (99%)	0.0 ± 0.00 (0%)	0.0 ± 0.00 (0%)
Goleen Pier	14.5 ± 1.09 (52%)	12. 9 ± 1.02 (47%)	0.2 ± 0.08 (1%)	0.0 ± 0.00 (0%)
Ballyrisode	12.1 ± 1.71 (17%)	35.5 ± 2.43 (48%)	25.3 ± 3.53 (34%)	0.9 ± 0.18 (1%)
Zetland	16.9 ± 1.06 (49%)	1.9 ± 0.24 (6%)	15.3 ± 2.41 (45%)	0.0 ± 0.00 (0%)
Schull	4.4 ± 1.14 (3%)	65.6 ± 6.40 (54%)	52.4 ± 5.45 (43%)	0.0 ± 0.00 (0%)
Gearhies	7.6 ± 1.52 (3%)	0.5 ± 0.24 (<1%)	224.4 ± 32.32 (96%)	0.0 ± 0.00 (0%)

Table 3. Occurrence of *Semibalanus balanoides* (A), *Austrominius modestus* (B) and *Chthamalus montagui* (C) recruits in experimental plots at six sites in south-west Ireland at distinct spatial scales following re-clearance events from February 2014 to October 2015. Black boxes indicate the presence of recruits during the corresponding time period following clearance. Clear areas indicate that no recruits were recorded during the corresponding time intervals. N/A indicates that plots had not been established at this time.

A

Semibalanus balanoides	2014						2015			
	February-April	April-June	June-August	August-October	October-December	December-February	February-April	April-June	June-August	August-October
Bantry Harbour										
Goleen Pier	N/A	N/A								
Ballyrisode	N/A									
Zetland										
Schull	N/A									
Gearhies										

B

Austrominius modestus	2014						2015			
	February-April	April-June	June-August	August-October	October-December	December-February	February-April	April-June	June-August	August-October
Bantry Harbour										
Goleen Pier	N/A	N/A								
Ballyrisode	N/A									
Zetland										
Schull	N/A									
Gearhies										

C

Chthamalus montagui	2014						2015			
	February-April	April-June	June-August	August-October	October-December	December-February	February-April	April-June	June-August	August-October
Bantry Harbour										
Goleen Pier	N/A	N/A								
Ballyrisode	N/A									
Zetland										
Schull	N/A									
Gearhies										

A. modestus recruits were significantly more abundant than the two native species overall ($p < 0.001$; Table 4) and for three of the time intervals examined ($p < 0.001$; Tables 5-8). However, between day 0 and day 120, the native species, *S. balanoides* was significantly more abundant than *A. modestus* and *C. montagui* ($p < 0.001$; Table 5).

Abundances of recruits of all three species, were higher in control plots than removal plots overall ($p < 0.001$; Table 4) and at each of the four time intervals examined ($p < 0.05$; Tables 5-8). *S. balanoides* recruits displayed highest survival (40%) in control plots on natural substrate between May 2014 and April 2015. Survival of this species was 27% of maximum mean abundance in control plots on artificial substrate and 26% in removal plots on both substrate types. *C. montagui* recruits had very high survival levels, ranging from 97% in both plot types on natural substrate to 72% in removal plots and 76% in control plots on artificial substrate. Due to the continual breeding of *A. modestus* the survival of recruits was not quantified. At the end of the study, in October 2015, *A. modestus* was the most abundant species of recruit on both artificial (remove: 40.5 ind. per $25cm^{-2}$ ± 13.50 ind. per $25cm^{-2}$; Figure 4, control: 51.7 ind. per $25cm^{-2}$ ± 6.96 ind. per $25cm^{-2}$; Figure 5) and natural substrates (remove: 43.4 ind. per $25cm^{-2}$ ± 30.57 ind. per $25cm^{-2}$; Figure 6, control: 71.8 ind. per $25cm^{-2}$ ± 39.86 ind. per $25cm^{-2}$; Figure 7).

The type of substrate was found to have no significant effect on abundance overall, or at any of the time intervals examined. However, *C. montagui* was observed to be less abundant on the artificial substrate in comparison to the natural substrate. Maximum density of *C. montagui* recruits recorded on artificial substrate was 3.3 ind. per $25cm^{-2}$ ± 1.86 ind. per $25cm^{-2}$, in control plots in January 2015 (Figure 5), compared to a maximum of 40.3 ind. per $25cm^{-2}$ ± 33.99 ind. per $25cm^{-2}$ in removal plots on natural substrate in October 2015 (Figure 6).

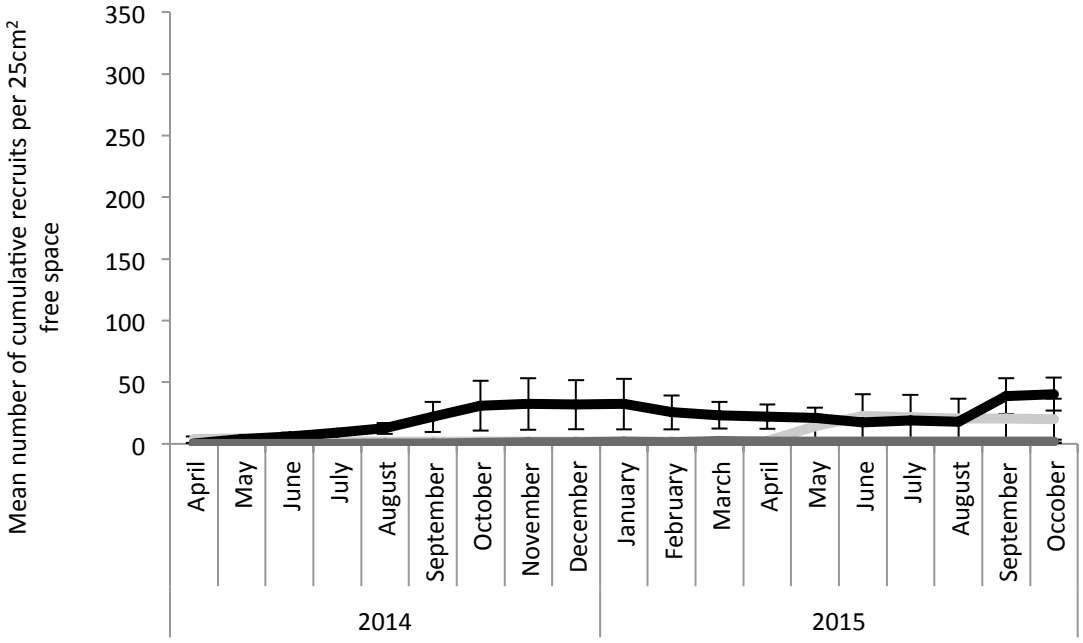

Figure 4. Mean number of cumulative recruits (± SE), per 25cm^2 free space, of three barnacle species, *Semibalanus balanoides* (light grey), *Austrominius modestus* (black) and *Chthamalus montagui* (dark grey), in removal plots on artificial structures at two study sites from April 2014 to October 2015.

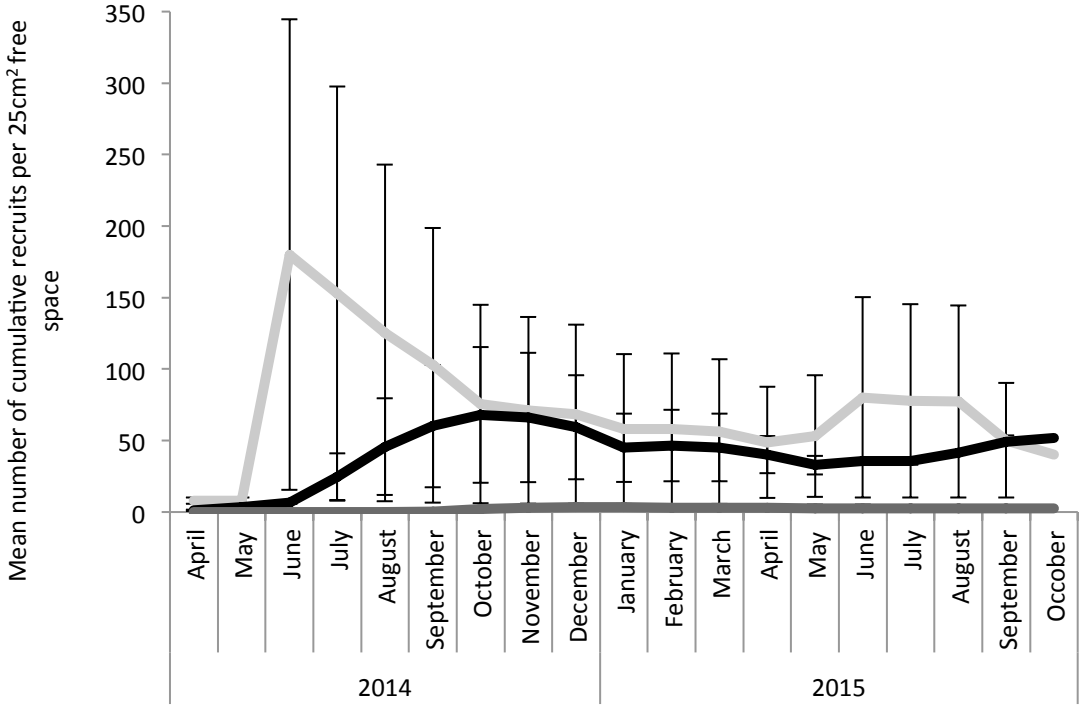

Figure 5. Mean number of cumulative recruits (± SE), per 25cm^2 free space, of three barnacle species, *Semibalanus balanoides* (light grey), *Austrominius modestus* (black) and *Chthamalus montagui* (dark grey), in control plots on artificial structures at two study sites from April 2014 to October 2015.

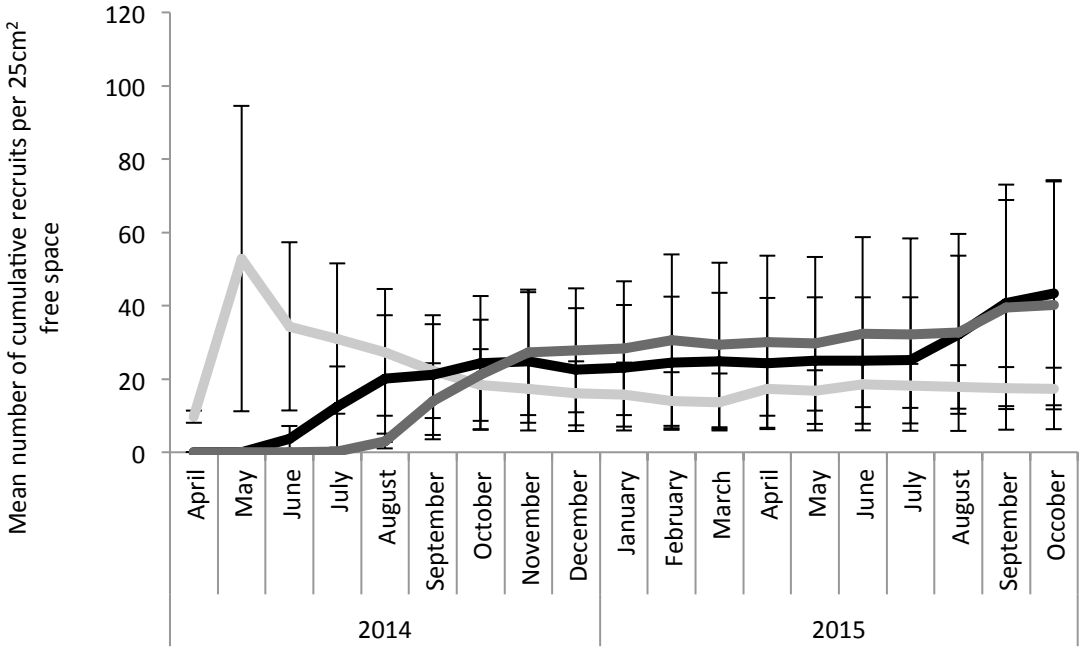

Figure 6. Mean number of cumulative recruits (± SE), per 25cm^2 free space, of three barnacle species, *Semibalanus balanoides* (light grey), *Austrominius modestus* (black) and *Chthamalus montagui* (dark grey), in removal plots on natural structures at four study sites from April 2014 to October 2015.

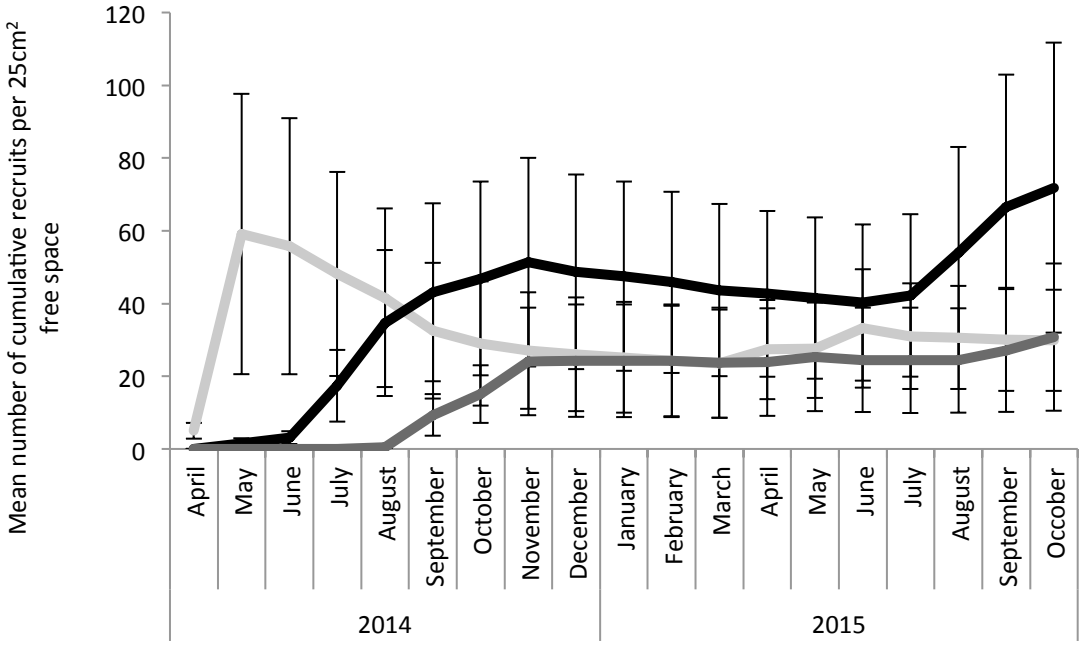

Figure 7. Mean number of cumulative recruits (± SE), per 25cm^2 free space, of three barnacle species, *Semibalanus balanoides* (light grey), *Austrominius modestus* (black) and *Chthamalus montagui* (dark grey), in control plots on natural structures at four study sites from April 2014 to October 2015.

Table 4. Generalised Linear Mixed Model estimates for recruit data collected at six field sites in south-west Ireland from April 2014 to October 2015. Variables in bold italics represent the value for comparison. Estimate values are log values and should be compared to the variable in bold italics. For example, recruits of *Chthamalus montagui* (CM) are estimated to be log(-1.11) ± log(0.08) less abundant than those of *Austrominius modestus* and this difference is significant ($p < 0.001$). Significant p values ($p < 0.05$) are highlighted in bold.

	Estimate	Standard error	z value	p
AM				
CM	-1.11	0.08	-13.55	**< 0.001**
SB	-0.33	0.06	-5.34	**< 0.001**
AM remove				
CM remove	-0.70	0.12	-5.62	**< 0.001**
SB remove	-0.53	0.09	-5.48	**< 0.001**
AM control				
CM control	-1.32	0.1	-12.87	**< 0.001**
SB control	-0.31	0.07	-4.04	**< 0.001**
AM artificial				
CM artificial	-2.34	0.08	-26.95	**< 0.001**
SB artificial	-0.12	0.08	-1.42	0.15
AM natural				
CM natural	-0.18	0.11	-1.53	0.12
SB natural	-0.21	0.08	-2.58	**< 0.01**

Table 5. Generalised Linear Mixed Model estimates for recruit data collected at six field sites in south-west Ireland from April 2014 – August 2014. Variables in bold italics represent the intercept value for comparison. Estimate values are log values. Significant p values ($p < 0.05$) are highlighted in bold.

	Estimate	Standard error	z value	p
AM				
CM	-5.70	0.37	-15.27	**< 0.001**
SB	2.17	0.1	14.72	**< 0.001**
AM remove				
CM remove	-3.95	0.42	-9.26	**< 0.001**
SB remove	1.98	0.24	8.14	**< 0.001**
AM control				
CM control	-42.76	1	-3406	**< 0.001**
SB control	2.28	0.01	182	**< 0.001**
AM artificial				
CM artificial	-29.01	0.01	-1459.10	**< 0.001**
SB artificial	1.00	0.01	50.60	**< 0.001**
AM natural				
CM natural	-4.90	0.38	-12.63	**< 0.001**
SB natural	2.72	0.18	14.69	**< 0.001**

Table 6. Generalised Linear Mixed Model estimates for recruit data collected at six field sites in south-west Ireland from August 2014 – December 2014. Variables in bold italics represent the intercept value for comparison. Estimate values are log values. Significant p values ($p < 0.05$) are highlighted in bold.

	Estimate	Standard error	z value	p
AM				
CM	-1.36	0.17	-7.79	**< 0.001**
SB	-0.33	0.12	-2.69	**< 0.001**
AM remove				
CM remove	-1.05	0.28	-3.75	**< 0.001**
SB remove	-0.84	0.22	-3.70	**< 0.001**
AM control				
CM control	-1.61	0.21	-7.63	**< 0.001**
SB control	-0.31	0.15	-2.06	**< 0.05**
AM artificial				
CM artificial	-2.85	0.19	-14.56	**< 0.001**
SB artificial	-0.1	0.17	-0.58	0.56
AM natural				
CM natural	-0.32	0.16	-1.31	0.18
SB natural	-0.24	0.24	-1.44	0.14

Table 7. Generalised Linear Mixed Model estimates for recruit data collected at six field sites in south-west Ireland from December 2014 – April 2015. Variables in bold italics represent the intercept value for comparison. Estimate values are log values. Significant p values ($p < 0.05$) are highlighted in bold.

	Estimate	Standard error	z value	p
AM				
CM	-0.80	0.14	-5.39	**< 0.001**
SB	-0.77	0.11	-6.74	**< 0.001**
AM remove				
CM remove	-0.5	0.23	-2.16	**< 0.05**
SB remove	-1.25	0.19	-6.33	**< 0.001**
AM control				
CM control	-1.02	0.18	-5.42	**< 0.001**
SB control	-0.74	0.14	-5.15	**< 0.001**
AM artificial				
CM artificial	-2.00	0.17	-11.51	**< 0.001**
SB artificial	-0.57	0.16	-3.45	**< 0.001**
AM natural				
CM natural	0.01	0.2	0.023	0.98
SB natural	-0.72	0.14	-4.84	**< 0.001**

Table 8. Generalised Linear Mixed Model estimates for recruit data collected at six field sites in south-west Ireland from April 2015 – October 2015. Variables in bold italics represent the intercept value for comparison. Estimate values are log values. Significant p values ($p < 0.05$) are highlighted in bold.

	Estimate	Standard error	z value	p
AM				
CM	-1.38	0.11	-11.78	**< 0.001**
SB	-0.73	0.09	-7.95	**< 0.001**
AM remove				
CM remove	-0.96	0.17	-5.355	**< 0.001**
SB remove	-0.72	0.13	-5.24	**< 0.001**
AM control				
CM control	-1.54	0.14	-10.54	**< 0.001**
SB control	-0.73	0.11	-6.19	**< 0.001**
AM artificial				
CM artificial	-2.28	0.11	-20.35	**< 0.001**
SB artificial	-0.29	0.11	-2.48	**< 0.05**
AM natural				
CM natural	-0.68	0.17	-3.93	**< 0.001**
SB natural	-0.78	0.12	-6.36	**< 0.001**

2.4.4. Recruitment patterns at individual sites

There were large differences in the abundances of recruits of the three species among sites, however patterns observed in both removal and control plots at each site were very similar. Over the entire study, *A. modestus* was the most abundant barnacle recruit in removal plots at four of the six study sites (Bantry Harbour, $p < 0.001$; Goleen, $p < 0.001$; Ballyrisode, $p < 0.01$ and Schull, $p < 0.001$) (Figures 8, 10, 12 and 16; Tables 9, 10, 11 and 13). In removal plots at the remaining two sites, Zetland and Gearhies, recruits of both native species were significantly more abundant than *A. modestus* ($p < 0.001$) (Tables 12 and 14). By the end of the study, both native species *S. balanoides* and *C. montagui* were present in similar abundances (SB = 17.6 ind. per $25cm^{-2}$ ± 5.10 ind. per $25cm^{-2}$, CM = 14.2 ind. per $25cm^{-2}$ ± 5.95 ind. per $25cm^{-2}$) at Zetland (Figure 14; Table 4), while at Gearhies, *C. montagui* was recorded at much higher densities (141.9 ind. per $25cm^{-2}$ ± 36.18 ind. per $25cm^{-2}$) than *S. balanoides* (9.6 ind. per $25cm^{-2}$ ± 4.62 ind. per $25cm^{-2}$) (Figure 18; Table 4). Very low densities of *A. modestus* recruits were recorded in removal plots at both sites, with just 6.0 (± 3.16) ind. per $25cm^{-2}$ recorded at Zetland (Figure 14; Table 4) and 1.9 (± 0.84) ind. per $25cm^{-2}$ recorded at Gearhies (Figure 18; Table 4) by October 2015.

In control plots, *A. modestus* recruits were significantly more abundant than *C. montagui* at Goleen ($p < 0.001$; Table 10), Bantry ($p < 0.001$; Table 9), Ballyrisode ($p < 0.001$; Table 11) and Schull ($p < 0.001$; Table 13) over the entire study (Figures 9, 11, 13 and 17). However, *S. balanoides* recruits were significantly more abundant than *A. modestus* in control plots at Goleen ($p < 0.001$; Table 10), Gearhies ($p < 0.001$; Table 14) and Zetland ($p < 0.001$; Table 12). *S. balanoides* recruits were also more abundant than *A. modestus* in control plots at Ballyrisode throughout the study, though this was not found to be significant ($p = 0.83$; Table 11). By the end of the study, lowest abundances of *A. modestus* recruits were recorded at Zetland (6.6 ind. per $25cm^{-2}$ ± 0.83 ind. per $25cm^{-2}$) (Figure 15; Table 4) and Gearhies (0.8 ind. per $25cm^{-2}$ ± 0.38 ind. per $25cm^{-2}$) (Figure 19; Table 4), following the same trend as seen in removal plots. Recruits of the native species also showed a similar trend with control plots at Gearhies dominated by *C. montagui* recruits (89.2 ind. per $25cm^{-2}$ ±

30.56 ind. per 25cm^{-2}) (Figure 19; Table 4) and recruits of both *S. balanoides* (26.1 ind. per 25cm^{-2} ± 4.84 ind. per 25cm^{-2}) and *C. montagui* (27.1 ind. per 25cm^{-2} ± 12.33 ind. per 25cm^{-2}) recorded at similar abundances in control plots at Zetland (Figure 15; Table 4).

Even at sites where *A. modestus* was the dominant recruit either overall, or by the end of the study, recruits of the native species were abundant seasonally (Figures 8 – 19). At all six study sites, *S. balanoides* recruits were significantly more abundant ($p < 0.001$; Tables 9-14) than those of *A. modestus* from the start of the study until day 120 (July 2014) (Figures 8-19), while *C. montagui* recruits were significantly more abundant than *A. modestus* at Zetland (Figures 15 and 16) and Gearhies (Figures 18 and 19) from day 241 (November 2014) until the end of the study (Tables 13 and 14).

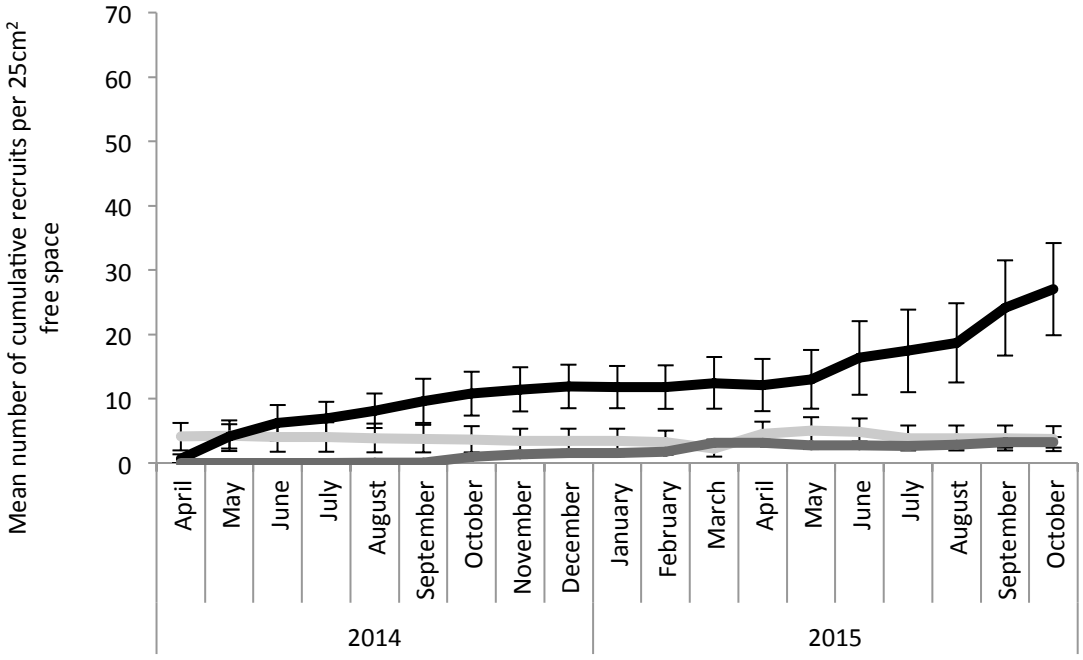

Figure 8. Mean number of cumulative recruits (± SE), per 25cm^2 free space, of three barnacle species, *Semibalanus balanoides* (light grey), *Austrominius modestus* (black) and *Chthamalus montagui* (dark grey), in removal plots on an artificial substrate in Bantry Harbour, Bantry Bay from April 2014 to October 2015.

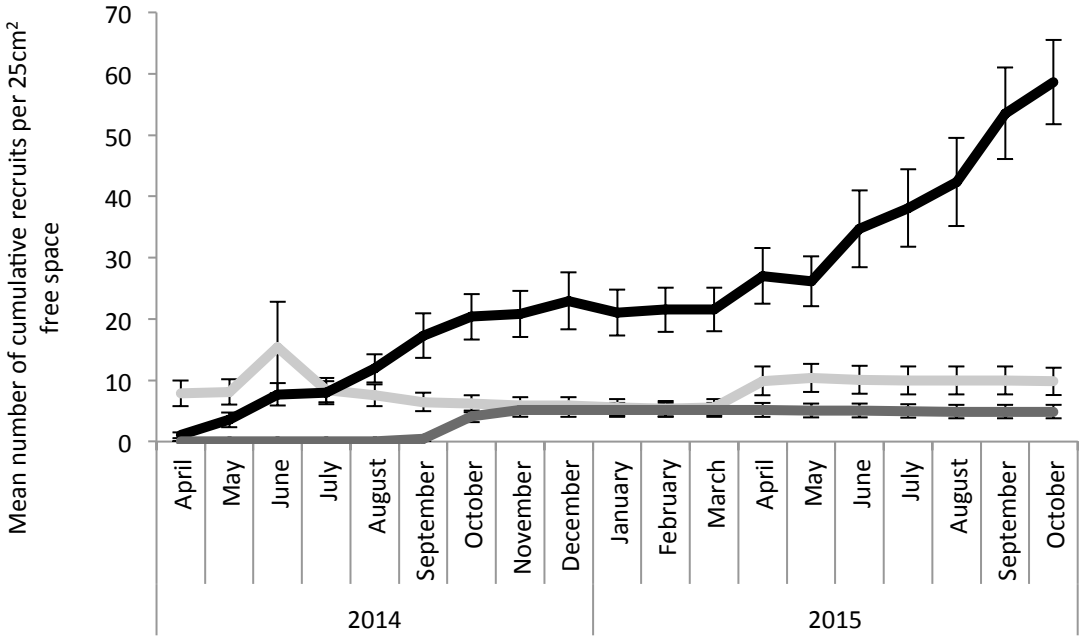

Figure 9. Mean number of cumulative recruits (± SE), per 25cm^2 free space, of three barnacle species, *Semibalanus balanoides* (light grey), *Austrominius modestus* (black) and *Chthamalus montagui* (dark grey), in control plots on an artificial substrate in Bantry Harbour, Bantry Bay from April 2014 to October 2015.

Table 9. Generalised Linear Mixed Model estimates for recruit data collected at Bantry Harbour, Bantry Bay from April 2014 – October 2015. Variables in bold italics represent the intercept value for comparison. Estimate values are log values. Significant p values ($p < 0.05$) are highlighted in bold.

	Estimate	Standard error	z value	p
AM				
CM	-1.96	0.08	-22.56	**< 0.001**
SB	-1.11	0.08	-13.02	**< 0.001**
AM remove				
CM remove	-1.99	0.15	-12.55	**< 0.001**
SB remove	-1.31	0.16	-8.21	**< 0.001**
AM control				
CM control	-1.93	0.09	-19.41	**< 0.001**
SB control	-1.07	0.09	-11.03	**< 0.001**
days 0-120				
AM				
CM	-20.10	104.51	-0.19	0.84
SB	0.48	0.23	2.08	**< 0.05**
days 121-240				
AM				
CM	-2.12	0.19	-11.06	**< 0.001**
SB	-0.99	0.18	-5.44	**< 0.001**
days 241-360				
AM				
CM	-1.48	0.14	-10.03	**< 0.001**
SB	-1.37	0.14	-9.29	**< 0.001**
days 361-570				
AM				
CM	-2.03	0.10	-19.08	**< 0.001**
SB	-1.42	0.10	-13.47	**< 0.001**

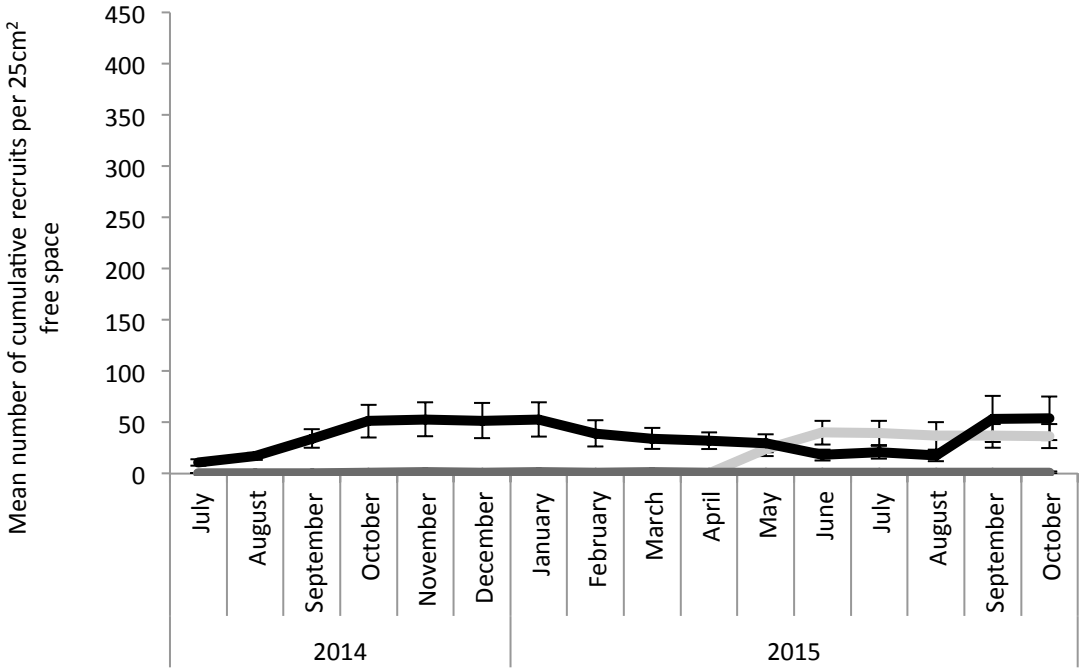

Figure 10. Mean number of cumulative recruits (± SE), per 25cm^2 free space, of three barnacle species, *Semibalanus balanoides* (light grey), *Austrominius modestus* (black) and *Chthamalus montagui* (dark grey), in removal plots on an artificial substrate at Goleen, Roaringwater Bay from July 2014 to October 2015.

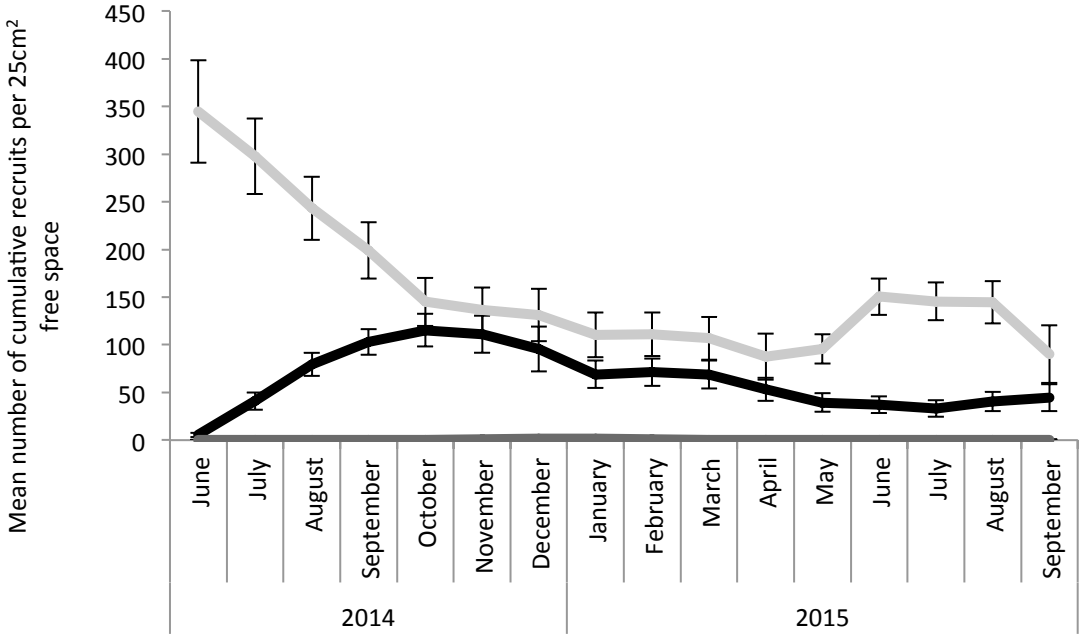

Figure 11. Mean number of cumulative recruits (± SE), per 25cm^2 free space, of three barnacle species, *Semibalanus balanoides* (light grey), *Austrominius modestus* (black) and *Chthamalus montagui* (dark grey), in control plots on an artificial substrate at Goleen, Roaringwater Bay from June 2014 to October 2015.

Table 10. Generalised Linear Mixed Model estimates for recruit data collected at Goleen, Roaringwater Bay from June 2014 – October 2015. Variables in bold italics represent the intercept value for comparison. Estimate values are log values. Significant p values ($p < 0.05$) are highlighted in bold.

	Estimate	Standard error	z value	p
AM				
CM	-4.37	0.15	-29.07	**< 0.001**
SB	0.69	0.12	5.47	**< 0.001**
AM remove				
CM remove	-3.71	0.29	-12.70	**< 0.001**
SB remove	-1.22	0.24	-4.94	**< 0.001**
AM control				
CM control	-4.95	0.15	-31.65	**< 0.001**
SB control	1.01	0.10	9.97	**< 0.001**
days 0-120				
AM				
CM	-21.72	0.00	-3915	**< 0.001**
SB	2.49	0.00	454	**< 0.001**
days 121-240				
AM				
CM	-5.12	0.30	-16.84	**< 0.001**
SB	0.35	0.24	1.47	0.14
days 241-360				
AM				
CM	-3.89	0.33	-11.58	**< 0.001**
SB	-0.05	0.28	-0.17	0.86
days 361-570				
AM				
CM	-4.09	0.21	-19.23	**< 0.001**
SB	0.82	0.16	5.09	**< 0.001**

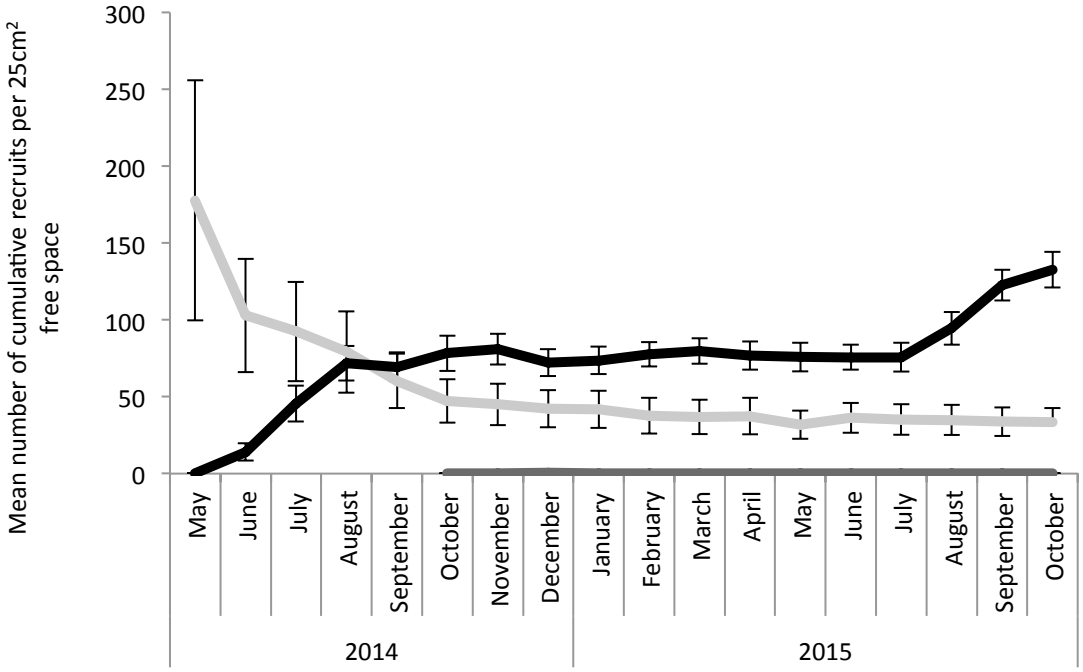

Figure 12. Mean number of cumulative recruits (± SE), per 25cm^2 free space, of three barnacle species, *Semibalanus balanoides* (light grey), *Austrominius modestus* (black) and *Chthamalus montagui* (dark grey), in removal plots on a natural substrate at Ballyrisode, Roaringwater Bay from May 2014 to October 2015.

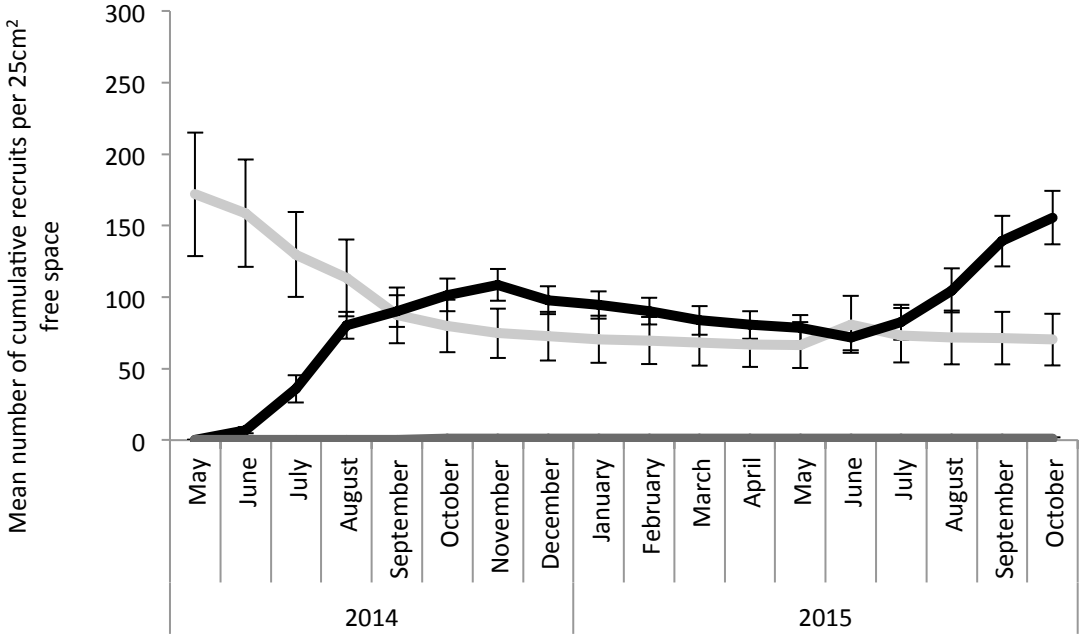

Figure 13. Mean number of cumulative recruits (± SE), per 25cm^2 free space, of three barnacle species, *Semibalanus balanoides* (light grey), *Austrominius modestus* (black) and *Chthamalus montagui* (dark grey), in control plots on a natural substrate at Ballyrisode, Roaringwater Bay from May 2014 to October 2015.

Table 11. Generalised Linear Mixed Model estimates for recruit data collected at Ballyrisode, Roaringwater Bay from May 2014 – October 2015. Variables in bold italics represent the intercept value for comparison. Estimate values are log values. Significant p values ($p < 0.05$) are highlighted in bold.

	Estimate	Standard error	z value	p
AM				
CM	-5.04	0.11	-44.19	**< 0.001**
SB	-0.01	0.08	-0.21	0.83
AM remove				
CM remove	-6.54	0.26	-24.71	**< 0.001**
SB remove	-0.26	0.10	-2.50	**< 0.05**
AM control				
CM control	-4.93	0.15	-31.60	**< 0.001**
SB control	0.06	0.12	0.519	0.60
days 0-120				
AM				
CM	-3.22E+01	2.81E+05	0	1
SB	2.18E+00	2.86E-01	7.62	**< 0.001**
days 121-240				
AM				
CM	-5.27	0.20	-25.48	**< 0.001**
SB	-0.11	0.15	-0.8	0.42
days 241-360				
AM				
CM	-4.63	0.19	-23.55	**< 0.001**
SB	-0.35	0.15	-2.27	**< 0.05**
days 361-570				
AM				
CM	-4.97	0.16	-29.61	**< 0.001**
SB	-0.49	0.13	-3.75	**< 0.001**

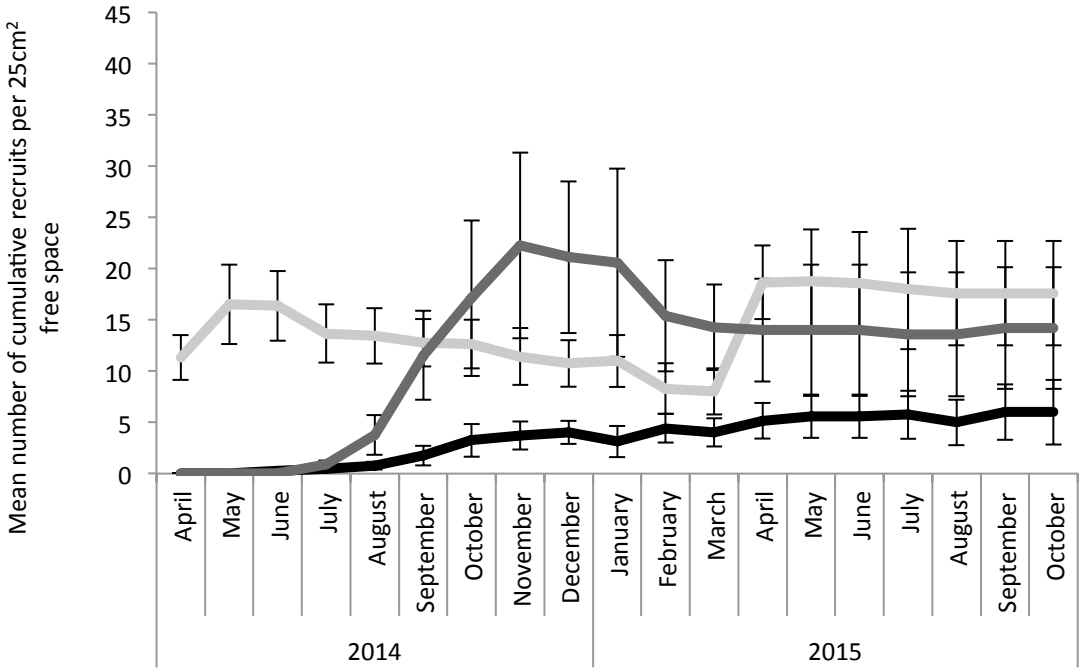

Figure 14. Mean number of cumulative recruits (± SE), per 25cm^2 free space, of three barnacle species, *Semibalanus balanoides* (light grey), *Austrominius modestus* (black) and *Chthamalus montagui* (dark grey), in removal plots on a natural substrate at Zetland, Bantry Bay from April 2014 to October 2015.

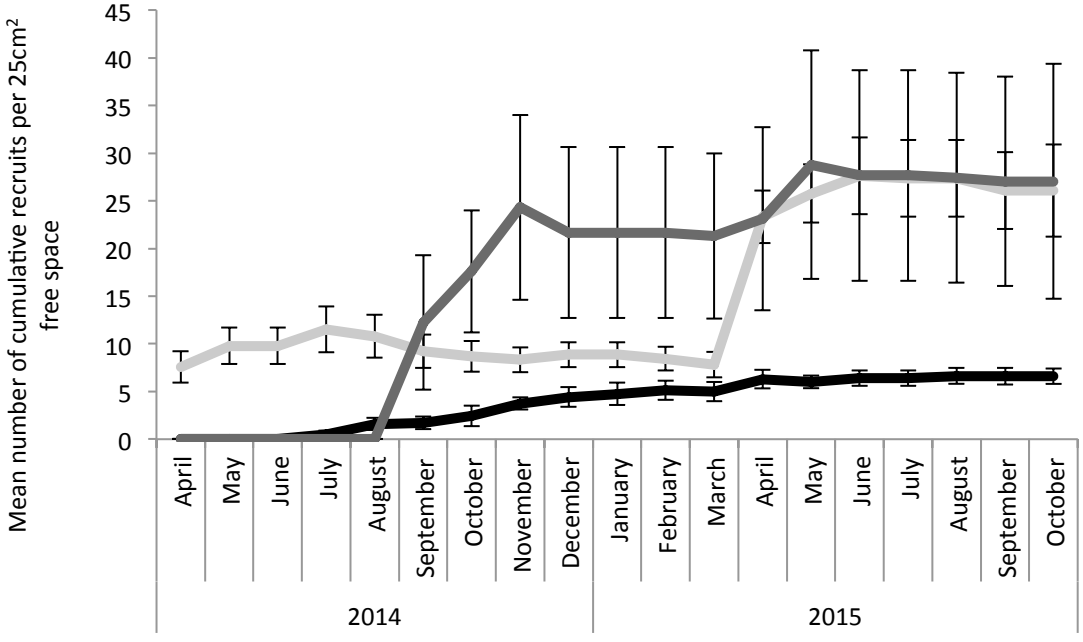

Figure 15. Mean number of cumulative recruits (± SE), per 25cm^2 free space, of three barnacle species, *Semibalanus balanoides* (light grey), *Austrominius modestus* (black) and *Chthamalus montagui* (dark grey), in control plots on a natural substrate at Zetland, Bantry Bay from April 2014 to October 2015.

Table 12. Generalised Linear Mixed Model estimates for recruit data collected at Zetland, Bantry Bay from April 2014 – October 2015. Variables in bold italics represent the intercept value for comparison. Estimate values are log values. Significant p values ($p < 0.05$) are highlighted in bold.

	Estimate	Standard error	z value	p
AM				
CM	1.36	0.11	12.37	**< 0.001**
SB	1.50	0.11	13.64	**< 0.001**
AM remove				
CM remove	1.23	0.13	9.41	**< 0.001**
SB remove	1.62	0.13	12.4	**< 0.001**
AM control				
CM control	1.33	0.14	9.08	**< 0.001**
SB control	1.41	0.14	9.52	**< 0.001**
days 0-120				
AM				
CM	-0.21	0.48	-0.45	0.65
SB	4.55	0.33	13.67	**< 0.001**
days 121-240				
AM				
CM	1.70	0.20	8.30	**< 0.001**
SB	1.63	0.20	7.82	**< 0.001**
days 241-360				
AM				
CM	1.45	0.15	9.51	**< 0.001**
SB	0.64	0.15	4.13	**< 0.001**
days 361-570				
AM				
CM	1.21	0.12	9.48	**< 0.001**
SB	1.31	0.12	10.35	**< 0.001**

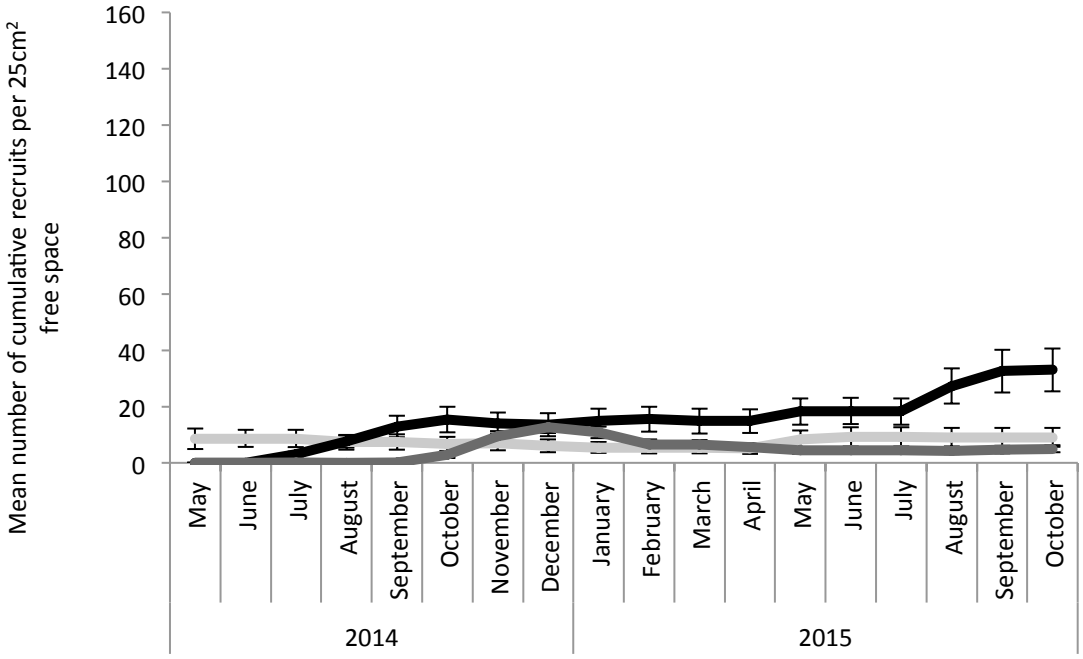

Figure 16. Mean number of cumulative recruits (± SE), per 25cm^2 free space, of three barnacle species, *Semibalanus balanoides* (light grey), *Austrominius modestus* (black) and *Chthamalus montagui* (dark grey), in removal plots on a natural substrate at Schull, Roaringwater Bay from May 2014 to October 2015.

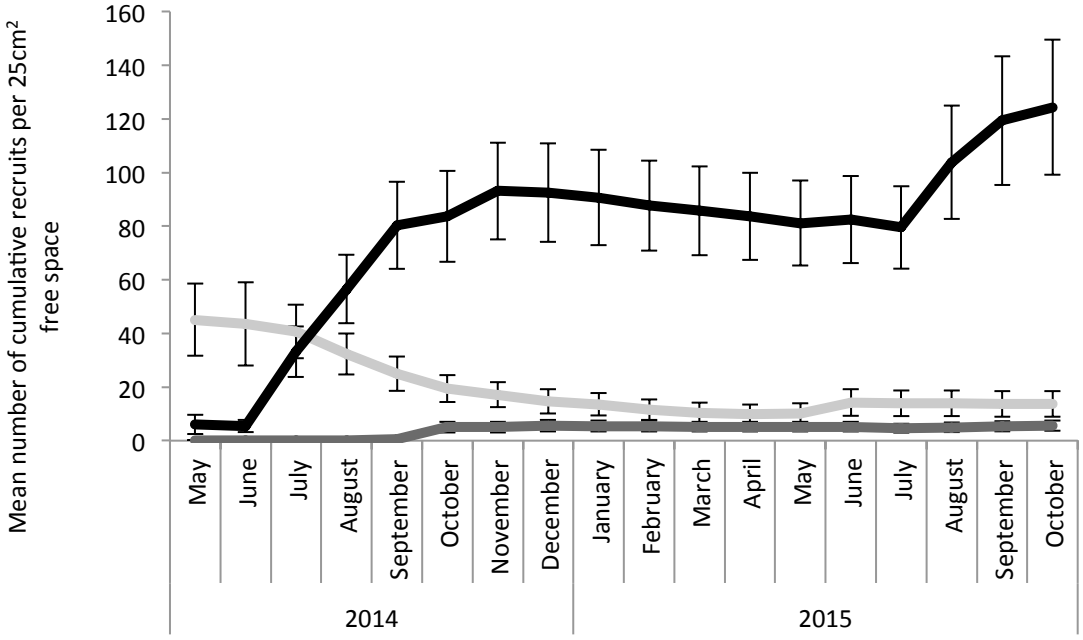

Figure 17. Mean number of cumulative recruits (± SE), per 25cm^2 free space, of three barnacle species, *Semibalanus balanoides* (light grey), *Austrominius modestus* (black) and *Chthamalus montagui* (dark grey), in control plots on a natural substrate at Schull, Roaringwater Bay from May 2014 to October 2015.

Table 13. Generalised Linear Mixed Model estimates for recruit data collected at Schull, Roaringwater Bay from April 2014 – October 2015. Variables in bold italics represent the intercept value for comparison. Estimate values are log values. Significant p values ($p < 0.05$) are highlighted in bold.

	Estimate	Standard error	z value	p
AM				
CM	-2.56	0.12	-19.78	**< 0.001**
SB	-1.27	0.13	-9.75	**< 0.001**
AM remove				
CM remove	-1.05	0.15	-6.62	**< 0.001**
SB remove	-0.81	0.16	-5.00	**< 0.001**
AM control				
CM control	-3.00	0.17	-17.03	**< 0.001**
SB control	-1.36	0.17	-7.70	**< 0.001**
days 0-120				
AM				
CM	-6.72	1.07	-6.23	**< 0.001**
SB	1.11	0.40	2.78	**< 0.01**
days 121-240				
AM				
CM	-2.9	0.26	-10.77	**< 0.001**
SB	-1.12	0.26	-4.24	**< 0.001**
days 241-360				
AM				
CM	-2.15	0.22	-9.45	**< 0.001**
SB	-1.81	0.22	-7.97	**< 0.001**
days 361-570				
AM				
CM	-2.64	0.18	-14.51	**< 0.001**
SB	-1.86	0.18	-9.84	**< 0.001**

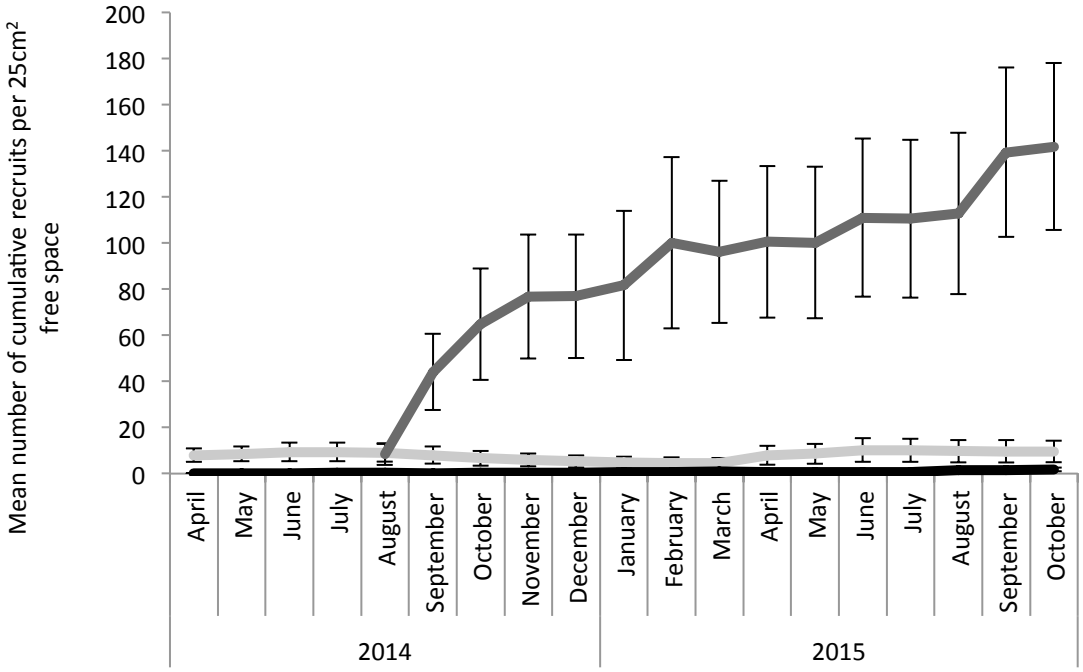

Figure 18. Mean number of cumulative recruits (± SE), per 25cm^2 free space, of three barnacle species, *Semibalanus balanoides* (light grey), *Austrominius modestus* (black) and *Chthamalus montagui* (dark grey), in removal plots on a natural substrate at Gearhies, Bantry Bay from April 2014 to October 2015.

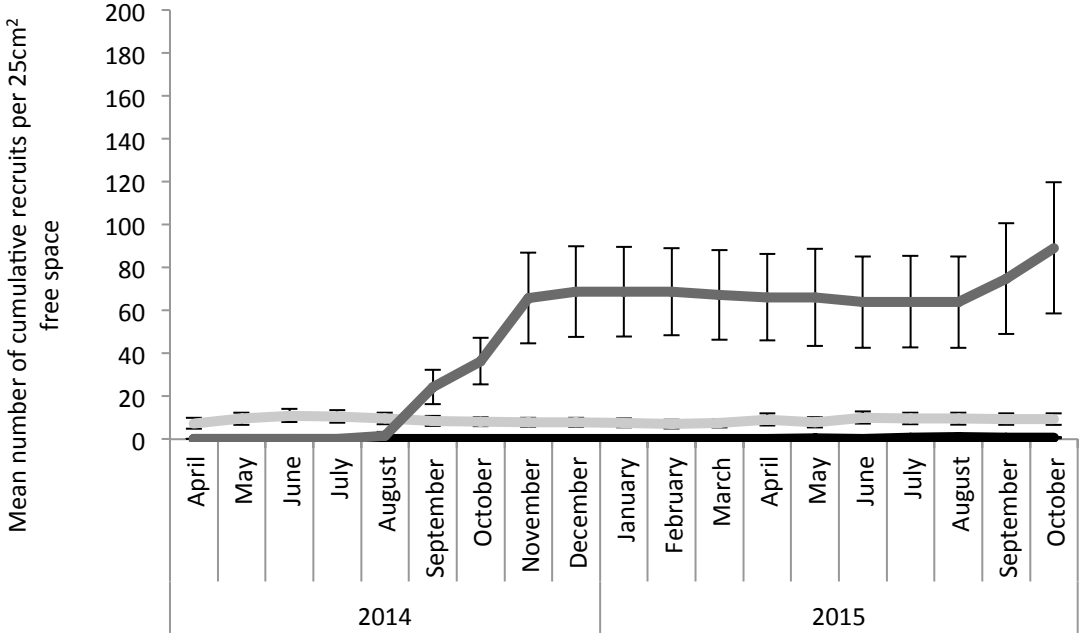

Figure 19. Mean number of cumulative recruits (± SE), per 25cm^2 free space, of three barnacle species, *Semibalanus balanoides* (light grey), *Austrominius modestus* (black) and *Chthamalus montagui* (dark grey), in control plots on a natural substrate at Gearhies, Bantry Bay from April 2014 to October 2015.

Table 14. Generalised Linear Mixed Model estimates for recruit data collected at Gearhies, Bantry Bay from April 2014 – October 2015. Variables in bold italics represent the intercept value for comparison. Estimate values are log values. Significant p values ($p < 0.05$) are highlighted in bold.

	Estimate	Standard error	z value	p
AM				
CM	4.96	0.15	32.49	**< 0.001**
SB	3.2	0.15	20.86	**< 0.001**
AM remove				
CM remove	4.84	0.26	18.61	**< 0.001**
SB remove	2.74	0.25	10.62	**< 0.001**
AM control				
CM control	5.15	0.19	27.03	**< 0.001**
SB control	3.60	0.19	18.64	**< 0.001**
days 0-120				
AM				
CM	-33.80	0.03	-1031.60	**< 0.001**
SB	4.51	0.03	139.00	**< 0.001**
days 121-240				
AM				
CM	5.28	0.34	15.14	**< 0.001**
SB	3.72	0.35	10.62	**< 0.001**
days 241-360				
AM				
CM	5.27	0.26	19.58	**< 0.001**
SB	2.80	0.27	10.26	**< 0.001**
days 361-570				
AM				
CM	4.86	0.21	23.05	**< 0.001**
SB	2.78	0.21	13.06	**< 0.001**

2.4.5 Temperature

Lowest temperatures recorded during this study were in January and February 2015, when air temperatures were just 6.6^{o}C (± 0.48^{o}C) and 6.1^{o}C (± 0.18^{o}C), respectively. Highest temperatures were recorded in July 2015 (17.2^{o}C ± 0.13^{o}C) (Figure 20). Recorded air temperatures were higher than seawater temperatures from April to August 2015. The largest difference between air and seawater temperatures was recorded for February 2015, when seawater temperatures were 2.3^{o}C higher than air

temperatures (Figure 20). Overall air temperatures for 2015, seemed to be slightly higher than those for 2014 (Figure 20), however this could be attributed to the different recording location.

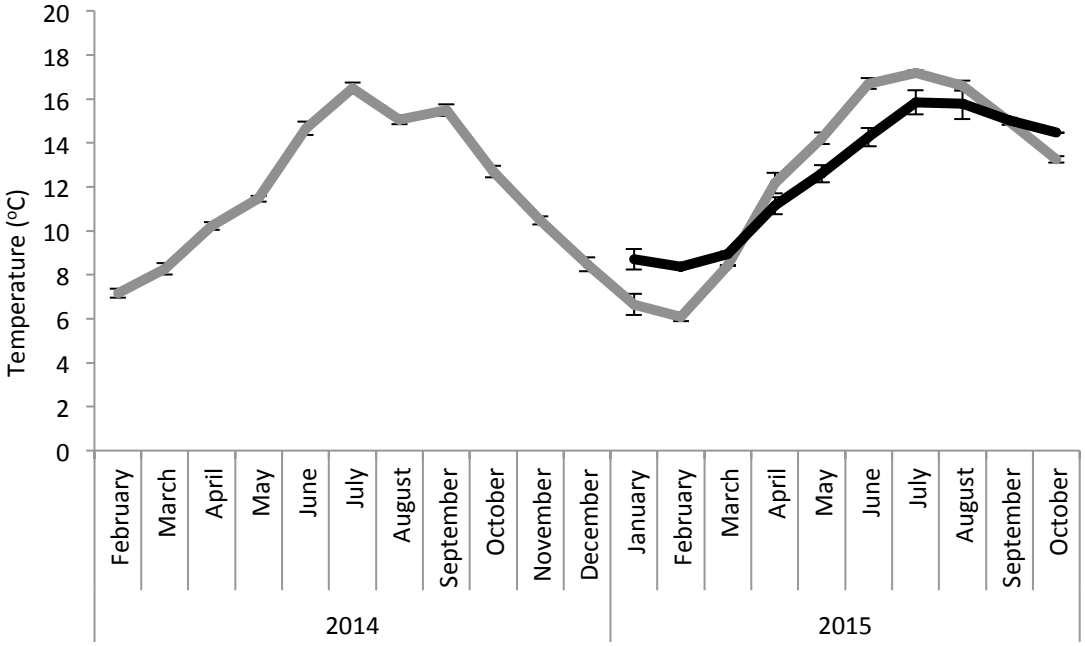

Figure 20. Mean monthly air (grey) and sea (black) temperatures (°C) collected at two study sites (Bantry harbour and Ballyrisode) in 2015. Air temperature data from 2014, was collected at Sherkin Island weather station and was obtained from Met Éireann. Error bars represent standard error.

2.4.6 Changes in relative abundance

Comparison of initial community composition (Table 2) to the community which became established in removal plots and control plots by October 2015 (Table 15), showed that *A. modestus* had increased in relative abundance in removal plots at four of the six study sites (Goleen, Ballyrisode, Zetland and Schull) and in control plots at three sites (Ballyrisode, Zetland and Schull). At Bantry Harbour, the site that had the highest actual and relative abundance of *A. modestus* in the starting community, *A. modestus* decreased from 99% relative abundance in the initial community (Table 2) to 79% in removal plots and 80% in control plots (Table 15), while *S. balanoides* and *C. montagui* increased in relative abundance by 7% - 10% in removal and control plots, in comparison to the initial community (Tables 2 and 15).

Table 15. Mean abundance (converted to ind. 25cm^{-2}) (± standard error) of three barnacle species, *Semibalanus balanoides*, *Austrominius modestus*, *Chthamalus montagui* in removal (n = 18 per site) and control plots (n = 18 per site), established between February and June 2014, at six sites in south-west Ireland in October 2015. Values in brackets are the relative abundance of each species.

	Removal Plots			Control Plots		
	SB	**AM**	**CM**	**SB**	**AM**	**CM**
Bantry Harbour	3.8 ± 1.96 (11%)	27.0 ± 7.15 (79%)	3.2 ± 0.83 (10%)	9.9 ± 2.25 (13%)	58.6 ± 6.86 (80%)	4.9 ± 1.09 (7%)
Goleen Pier	36.7 ± 11.70 (40%)	54.0 ± 21.29 (59%)	1.2 ± 1.10 (1%)	90.2 ± 30.20 (67%)	44.7 ± 14.30 (33%)	0.4 ± 0.42 (<1%)
Ballyrisode	33.3 ± 9.26 (20%)	132.8 ± 11.66 (80%)	0.0 ± 0.00 (0%)	70.4 ± 18.11 (31%)	152.5 ± 18.62 (68%)	1.2 ± 0.83 (1%)
Zetland	17.6 ± 5.10 (46%)	6 .0 ± 3.16 (16%)	14.2 ± 5.95 (38%)	26.1 ± 4.84 (44%)	6.6 ± 0.83 (11%)	27.1 ± 12.33 (45%)
Schull	9.0 ± 3.30 (19%)	33.0 ± 7.60 (70%)	5.0 ± 1.28 (11%)	13.8 ± 4.81 (10%)	124.4 ± 25.17 (86%)	5.6 ± 1.89 (4%)
Gearhies	9.6 ± 4.62 (6%)	1.9 ± 0.84 (1%)	141.9 ± 36.18 (93%)	9.3 ± 2.64 (9%)	0.8 ± 0.38 (1%)	89.2 ± 30.56 (90%)

C. montagui recruits were either absent or present at very low densities in removal and control plots at Ballyrisode and Schull, despite being quite abundant in the initial community (Ballyrisode = 34% relative abundance, Schull = 43% relative abundance; Table 2). At these two sites, both the native *S. balanoides* and the alien *A. modestus* increased in relative abundance, in the absence of high densities of *C. montagui* (Tables 2 and 15). *C. montagui* only increased in relative abundance at one site, Bantry Harbour (absent from initial community survey; Table 2, 10% relative abundance in removal plots and 7% relative abundance in control plots; Table 15). *S. balanoides* was recorded to increase in its relative abundance in removal plots at four study sites (Bantry Harbour, Ballyrisode, Schull and Gearhies) and in control plots at five sites (Bantry Harbour, Goleen, Ballyrisode, Schull and Gearhies) (Tables 2 and 15). The largest increases in relative abundance for this species were in control plots at Ballyrisode (14% increase compared to initial community; Tables 2 and 15) and removal plots in Schull (16% increase compared to initial community; Tables 2 and 15), both sites also experienced very low levels of *C. montagui* recruitment.

2.5 Discussion

While recruits of the invasive *Austrominius modestus* became the dominant barnacle species in removal plots at four of the six study sites, it did not entirely displace native barnacle species at any location. Native barnacle species also colonised experimental plots and even increased in relative abundance in these plots at five of the study sites, including Bantry Harbour, an artificial structure dominated by *A. modestus*. This, in conjunction with the fact that *A. modestus* has been present in Ireland since the 1950s (Beard 1957; Crisp and Southward 1959; O'Riordan 2002), indicates that this species is more likely to coexist with native barnacle species than to displace or outcompete them, at this location. While invasive species have the potential to outcompete native species, there have been many reports of native and invasive species coexisting (Buschbaum *et al.* 2006; Briggs 2010; Davies *et al.* 2011), even over long temporal scales (Heard and Sax 2013).

Hui and Moyse (1987) noted that the native barnacle *Semibalanus balanoides* would continue to persist in the presence of *A. modestus*, provided that the invasive

species had not colonised all available space before *S. balanoides* spatfall. In reality, it is rare for all space to be utilised, in particular at exposed coastal locations (Pannacciulli 1995). In this study the amount of free space available in control plots at the start of the study ranged from < 1cm^2 to 20cm^2 (4% to 80% of plot area). The successful recruitment of *S. balanoides* in removal and control plots during this study, in spring 2014 and 2015, indicates that *A. modestus* is not colonising all available space and it is still possible for the native species to recruit, even at locations where *A. modestus* is abundant. Density dependent mortality (Barnes and Powell 1950; Menge 2000; Hills and Thomason 2003; Kent *et al.* 2003; Jenkins *et al.* 2008), in addition to predation by dog whelks (Connell 1961) and limpet bulldozing (Dayton 1971; Hawkins 1983) all contribute to the maintenance of free space at sites.

Although the invasive species was initially dominant on one of the two artificial structures in this study (Bantry Harbour), recruits of the native species *S. balanoides* and *Chthamalus montagui*, were recorded in both removal and control plots at this site. It is worth noting that removal plots were not created on the second artificial structure, Goleen Pier, until June 2014, when *S. balanoides* was no longer settling. *A. modestus* was recorded to dominate the removal plots at this site until May 2015. At this point *S. balanoides* successfully recruited to removal plots, in relatively high numbers, becoming the most abundant barnacle species in removal plots at this site until September 2015, when *A. modestus* recruits became more abundant again. This illustrates, that despite the ability of the invasive *A. modestus* to utilise free space through the year, when natives are unable to do so, it is still possible for native species to recruit at high densities seasonally allowing them to persist. It is likely that artificial structures will be initially dominated by the invasive species (Bracewell *et al.* 2012; Bracewell *et al.* 2013), given the short window of opportunity for native species to colonise the new structure, and the extended breeding period of the invasive species (Crisp and Davies 1955; O'Riordan and Murphy 2000). However, it is still possible for the native species to become established on such structures.

There have been numerous reports of abundances of *S. balanoides* decreasing in the presence of *A. modestus* at various locations (den Hartog 1953; Stubbings and Houghton 1964; Southward 1967; Bennell 1981), possibly indicating competitive interactions between the two species. In this study, following initial recruitment, abundances of recruits of the native *S. balanoides* underwent a decline at most sites. The same was not true, however, for the invasive *A. modestus*. While it is possible that this decline in *S. balanoides* is due to competitive pressure from recruits of *A. modestus*, it may also be attributed to post settlement mortality, which is known to occur in barnacle species (Menge 2000; Hills and Thomason 2003). Intraspecific competition is one factor that can result in post settlement mortality, in particular affecting areas with high densities of recruits (Hills and Thomason 2003). In this study, Ballyrisode, the site with highest densities of *S. balanoides* recruits, also showed the largest decrease in recruit density for this species. Hills and Thomason (2003), identified mid-June to mid-August as a period during which high density dependent mortality was observed for *S. balanoides* recruits in Millport, Scotland and Southward (1991) described heavy density independent mortality of *S. balanoides* juveniles during the summer due to temperature at Cellar Beach, England.

In the present study, decreases in the abundance of *S. balanoides* recruits were observed during the same time period. This coincides with the timing of the establishment of *A. modestus* recruits in experimental plots. While it is possible that some of the mortality of *S. balanoides* recruits observed was due to competition from *A. modestus* spat, the fact that mortality of the native species during the same time period has previously been observed in the absence of *A. modestus* (Hills and Thomason 2003), and at sites with low abundances of *A. modestus* in this study, indicates that this mortality is not entirely due to competitive pressure from *A. modestus*. It is likely that *A. modestus* recruits are also experiencing some post settlement mortality, however the continued production of larvae allows any recruits which failed to survive to be in effect “replaced” by new recruits, and overall numbers of recruits may even increase. The second native species *C. montagui* displays lower levels of post settlement mortality than *S. balanoides*, and also

experiences some limited replacement of recruits due to the production of multiple broods (Burrows *et al.* 1992).

Reduced biodiversity and extinction of native species due to competitive superiority over natives have been cited as the effects of invasive species (Bax *et al.* 2003; Molnar *et al.* 2008). However, the extinction of native species due to the presence of invaders is rare (Davies *et al.* 2011), especially in the marine environment (Briggs 2010). The current situation observed at the majority of sites during this study was one of coexistence between the native barnacle species and *A. modestus*. Generally when two species are competing for the same resources, which are limited, competitive exclusion of one species will occur if there is no niche differentiation between the native and invasive species. If these species display niche partitioning, or if the native species can adapt to fulfill an alternative niche, it is possible for both native and invasive species to coexist (Bøhn *et al.* 2008). The fact that *A. modestus* is coexisting with native species at the majority of sites in this study, indicates that there is some form of niche partitioning between the invasive and native species. This could be facilitated in various ways, for example, the three barnacle species display distinct patterns of zonation and generally occupy different parts of the shore. In this study, at sites where *C. montagui* was present, this species dominated plots at the high shore level, while *A. modestus* and *S. balanoides* were more abundant at the mid and low shore. On both artificial structures where *C. montagui* was absent, *A. modestus* was abundant at the high and mid shore, while *S. balanoides* was more common at the low shore and overlapped in distribution with *A. modestus* at the mid shore level (Gallagher pers. obs.). Colonisation of different parts of the shore reduces direct competition between the species allowing them to coexist. Differences in the timing and extent of the reproductive periods of the native and invasive species, is also likely a contributing factor in the coexistence of these species, with *A. modestus* and native barnacle species essentially utilising different temporal niches.

However, it has previously been noted by Olenin *et al.* (2007) that *A. modestus* carries out the same functional role as the native barnacle species. While it has not

been investigated in detail, there is some evidence that the invasive species may differ from the native species in its utilisation of food resources (Southward 1955; Moyse 1963; Stone 1989; Harms 1999), which could facilitate niche partitioning. Shinen and Navarrete (2014) noted that for coexistence to truly occur, each species must be capable of recovering from periods of rarity. The increase in relative abundance of native species at Bantry Harbour, during the present study, may be an indication for the ability of native species to recover from periods of low abundances in the presence of *A. modestus*.

A. modestus is known to favour sheltered locations (Moore 1944; Southward 1955; Crisp 1958; Rainbow 1984, Gomes-Filho *et al.* 2010), with lower abundances of this species recorded at more exposed coastal locations (Southward 1991; Gomes-Filho *et al.* 2010). The two most exposed shores in this study, Zetland and Gearhies, had the lowest initial abundance of *A. modestus* and lowest levels of *A. modestus* recruits were recorded here. Additionally, at Gearhies, which is the most exposed site, there was no change in the relative abundance of this species with the provision of free space. *A. modestus* recruits only successfully recolonised free space after approximately one third of re-clearance events at this location, much lower than the other field sites. This illustrates the importance of environmental conditions such as exposure, in determining the abundance of *A. modestus*, despite the availability of space for recruitment. There are many areas of exposed shoreline along the south-west coast of Ireland, and it seems likely that natives will continue to remain dominant at these locations with *A. modestus* being absent or present at very low densities at such locations.

The presence of *A. modestus* at many sites, despite the continued persistence of native species, is still a cause for concern, as changing environmental conditions may increase the threat posed by the invasive species (Hellmann *et al.* 2008; Witte *et al.* 2010). It is likely that future climate change will favour the invasive *A. modestus* in addition to the warm water adapted native *C. montagui*, which both reproduce and recruit during the warmest parts of the year, while *S. balanoides* will decline (Simkanin *et al.* 2005; Svensson *et al.* 2005; Svensson *et al.* 2006; Hawkins *et al.*

2008; Poloczanska *et al.* 2008; Mieszkowska *et al.* 2014). The ability of *C. montagui* to recruit at very high densities and to survive at such densities, will probably allow the two species to coexist at some locations, however at sites where *C. montagui* is absent or scarce, it is likely that *A. modestus* will be the dominant barnacle species. Of particular concern are artificial substrates. In this study, *C. montagui* was present in very low abundances on artificial as opposed to natural substrates. In the absence of *S. balanoides*, it is possible that artificial substrates could become dominated by the non-native, *A. modestus*. Additionally, although both *C. montagui* and *A. modestus* are warm water adapted, *A. modestus* may be better able to cope with increasing sea water temperatures (Buckeridge 2012), giving it an advantage over the native.

Although *A. modestus* became the most abundant barnacle species in removal plots at four sites by October 2015, compared to three sites for control plots, native barnacle species increased in relative abundance in both plot types at more sites than the alien species. This indicates that the provision of free space does not necessarily facilitate the establishment of *A. modestus.* The non-native species did not dominate free space to the exclusion of native species at any site, despite its ability to reproduce throughout the entire year, which gives it a competitive advantage over natives. Barnacle recruitment is known to be variable, both between years and locations (Connell 1961; Hawkins and Hartnoll 1982; Jenkins *et al.* 2000; Burrows *et al.* 2010) often due to differences in timing of larval release and the phytoplankton bloom (Connell 1961; Hawkins and Hartnoll 1982) or other water column processes, such as currents (Kendall *et al.* 1985) and winds (Hawkins and Hartnoll 1982). The relatively short time scale of the present study does not provide information on the long-term variability of recruitment of the three species in this study. These fluctuations, which will include some years with successful recruitment of the native species, in addition to the invasive species not entirely colonising all available space, are contributing to native species currently co-existing with *A. modestus* at the majority of sites. Additionally, the presence of many areas of coastline which are very exposed where native barnacle species are abundant, but

are unfavourable to *A. modestus*, currently limit the extent to which *A. modestus* can dominate in the intertidal zone at locations in south-west Ireland.

2.6 References

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Chapter 3

Recruitment of *Austrominius modestus* and native barnacle species in Lough Hyne Marine Nature Reserve

This chapter, in revised format with additional data has been submitted to the Journal *Coastal, Estuarine and Shelf Science*

3.1 Abstract

The invasive barnacle species *Austrominius modestus* was first recorded in Ireland, close to Lough Hyne marine nature reserve in 1957. This species was not recorded inside the Lough until 1980, but by 2001, was the dominant intertidal barnacle within the reserve. Although *A. modestus* was found to experience higher levels of mortality than native barnacle species during the cold winters of 2009/2010 and 2010/2011, this species remains dominant within the Lough. Native species continue to persist, but generally at low abundances, with the exception of the Rapids, where turbulent water conditions are not suitable for *A. modestus*. Monitoring the recruitment of intertidal barnacles within Lough Hyne during 2014-2015 revealed that *A. modestus* was the dominant recruit at all study sites, in both removal plots and in space within the pre-existing community. Despite recruits of the native *Semibalanus balanoides* being initially dominant at Barloge Creek, the one study site not within the Lough itself, *A. modestus* recruits ultimately outnumbered natives here too. Recruits of native barnacle species were recorded at all sites, but at low abundances. While the impact of continued *A. modestus* dominance within Lough Hyne is unknown, it is possible that it may be altering trophic interactions and energy flow within the reserve.

KEYWORDS: barnacle, recruitment, *Austrominius modestus*, invasive, marine reserve

3.2 Introduction

Austrominius modestus was first recorded in Ireland, close to Lough Hyne, in 1957 (Beard 1957). However, it is believed to have been established in Cork Harbour prior to this (O'Riordan 2002), likely between 1954 and 1956 (Crisp and Southward 1959). Lough Hyne was the first designated statutory marine reserve in Europe and remains Ireland's only marine reserve (Anon 1981a; Anon 1981b). The fauna and flora of Lough Hyne have been extensively studied for almost a century (Kitching 1987) and long-term datasets exist for many species within the Lough, including barnacles. Survey work within the Lough during 1955 and 1958, did not record *A. modestus*, but found that *Chthamalus stellatus* (not yet divided into *Chthamalus montagui* Southward and *Chthamalus stellatus* (Poli)) and, to a lesser extent, *Semibalanus balanoides* were widespread within the Lough (Ebling *et al.* 1960). Increased abundances of *Chthamalus stellatus* were recorded at other locations in Ireland and the UK during the 1950s, coinciding with increased temperatures (Southward and Crisp 1954).

It was not until 1980 that *A. modestus* was recorded inside Lough Hyne (Holmes 1980), prior to its designation as a marine reserve. In the early 1990s, Little *et al.* (1992) reported that over-all barnacle cover had increased in the Lough since surveys carried out by Ebling *et al.* (1960), due to increases in the abundance of *S. balanoides*, which coincided with a cooling period from the 1960s to the 1980s (Hiscock *et al.* 2004), and the introduction of *A. modestus.* By 2001, *A. modestus* was recorded as the dominant barnacle within the Lough, and the sole species of intertidal barnacle found at some locations there (Lawson *et al.* 2004). This coincided with reports of increasing abundance of this warm water species at other locations in Europe (Franke and Gutow 2004; Simkanin *et al.* 2005; Reichert and Buchholz 2006; O'Riordan *et al.* 2009; Gomes-Filho *et al.* 2010; Witte *et al.* 2010), possibly linked to increasing sea surface temperatures (Lawson *et al.* 2004; Witte *et al.* 2010). Witte *et al.* (2010) suggested that *A. modestus* could be an "ecological sleeper", a species that survives in an area, but does not become abundant there until environmental conditions - increasing seawater temperatures in the case of *A. modestus* - become favourable.

Like many invasive species in Europe, *A. modestus* is warm water adapted (Witte *et al.* 2010), with a physiological optimum above 20°C (Harms 1999) and low levels of recruitment and survival have been recorded for *A. modestus* following cold winters (den Hartog 1953; Crisp 1964; Harms and Anger 1989; Witte *et al.* 2010). In Lough Hyne, this species was found to experience higher levels of mortality in comparison to native barnacle species following the extremely cold winters of 2009/2010 and 2010/2011 (O'Riordan *et al.* unpublished data). However, surveys carried out in the summer of 2011 found that *A. modestus* remained the dominant species within the Lough, despite this cold mortality (O'Riordan *et al.* unpublished data). The present study examined the current status of *A. modestus* within Lough Hyne by monitoring recruitment and survival of *A. modestus* and native barnacle species at four sites within the reserve from April 2014 – April 2015 in removal plots and in space within the preexisting community.

3.3 Materials and Methods

3.3.1 Study site

Lough Hyne Marine Nature Reserve (51°30'N, 9°18'W) is a marine lough located in County Cork, on the south-west coast of Ireland. It measures 0.8 km x 0.6 km and is connected by a narrow channel called the Rapids to Barloge Creek, which opens into the Atlantic (Kitching 1987) (Figure 1). The rapids are approximately 150m long, 12m wide and less than 1m deep at low water, resulting in an asymmetrical tidal pattern (4 hour flood and 8.5 hour ebb). The tidal range within the Lough is less than 1.5m, compared with one of >4m on the adjacent Atlantic coastline (Bassindale *et al.* 1948) and the reduced intertidal zone within the Lough is sheltered (De Grave and Holmes 1998). Castle Island partially divides the Lough into northern and southern basins. The Western Trough is the deepest part of the Lough, reaching depths of almost 50m. The Lough has an overall flushing time of twenty-two tidal cycles (Johnson *et al*. 1995) and salinity within Lough Hyne is comparable to the adjacent coastal Atlantic (Watson *et al.* 2005).

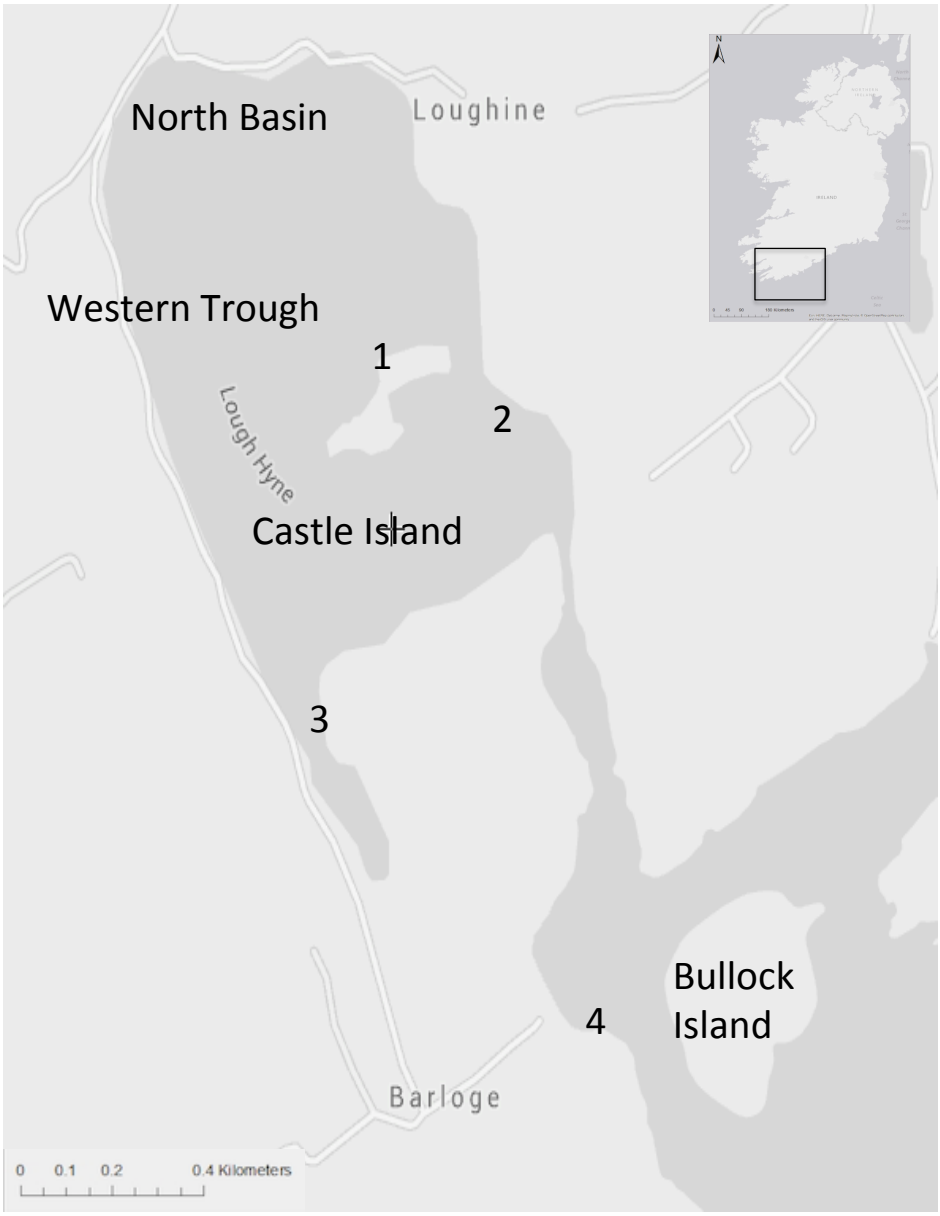

Figure 1.The location of study sites within Lough Hyne Marine Nature Reserve. For site details see Table 1. Inset map shows area enlarged.

Table 1. Name and GPS coordinates of survey sites within Lough Hyne Marine Nature Reserve. For site locations see Figure 1.

Name	Site	GPS
1	Northwest Castle	N 51°30.081’ W 009°18.030’
2	Whirlpool Cliff	N 51°30.041’ W 009°17.443’
3	Goleen Cliff	N 51°29.880’ W 009°18.145’
4	Barloge Creek	N 51°29.508’ W 009°17.455’

3.3.2 Recruitment monitoring

Monitoring of barnacle recruitment in space within the pre-existing community and in cleared removal plots was carried out between April 2014 and April 2015 in the Marine Reserve. Four sites (Whirlpool Cliff, Goleen Cliff, Northwest Castle and Barloge Creek) were selected for monitoring due to the presence of a vertical rock face suitable for the establishment of monitoring plots and a pre-existing barnacle community consisting of both *A. modestus* and native barnacle species. In February 2014, three removal and three control plots were established at areas approximate to the high, mid and low shore at each site (n = 9 of each plot type per site). Removal plots measured 25cm^2 and were created by clearing the plot area of all organisms, plus an additional edge of 1cm on each side of the plot, using a paint scraper. To ensure that all organisms had been removed, the rock surface was then scoured using wire wool. To allow for continued monitoring of the re-colonisation of these plots over time, the location of the plot was marked on the rock surface using a red enamel paint mark. On each monthly field visit, the top left hand corner of a 25cm^2 quadrat was aligned with the paint mark, and a photograph was taken of the plot area using a Nikon Coolpix AW10 digital camera in macro mode. From these images the recruitment and survival of different barnacle species was determined. Control plots also measured 25cm^2 but were not manipulated in any way. Control plot location was using green enamel paint.

The amount of free space present within each control plot was calculated by estimating the percentage cover of bare rock within the plot and converting this to area (cm^2) as described in Chapter 2 (page 28). The number of recruits in each control plot could then be converted to represent number of recruits 25cm^{-2} of free space, to allow for comparison with removal plots.

3.3.3 Statistical Analysis

Statistical analysis was carried out using R Studio software 3.3.0 (R Core Team 2016). As barnacle recruitment was in the form of count data, with a high amount of zeros, a zero inflated model with a negative binomial distribution was constructed using the package “pscl” (Zeileis *et al.* 2008). A negative binomial distribution was chosen

as it was found to have significantly better fit for the data than a Poisson distribution (Vuong's test, $p < 0.01$). Model validation was carried out by plotting the model residuals against the fitted values and QQ – plots.

3.4 Results

Recruits of *A. modestus* were recorded at all four study sites, while those of *S. balanoides* were absent from Northwest Castle and *C. montagui* recruits were not recorded at Barloge Creek (Figures 2 and 3). No recruits of *C. stellatus* were recorded in experimental plots during this study. Overall, recruits of *A. modestus* were significantly more abundant than those of *S. balanoides* ($p < 0.001$) or *C. montagui* ($p < 0.001$) (Table 2).

A very similar trend in recruit abundance was observed in both removal and control plots at all four study sites, although recruits of all three species were more abundant in control plots (Figure 3) than removal plots ($p < 0.001$) (Figure 2). Recruits of *A. modestus* were significantly more abundant than both *S. balanoides* and *C. montagui* in both plot types ($p < 0.001$) (Figures 2 and 3; Table 2). *C. montagui* was the least abundant recruit in both plot types ($p < 0.001$), with a maximum density of 2.08 (± 0.94) ind. 25 cm^{-2} recorded from control plots (Figure 3) and 0.33 (± 0.13) ind. 25 cm^{-2} in removal plots (Figure 2). Maximum density of *S. balanoides* recruits recorded was 5.81 (± 3.43) ind. 25 cm^{-2} in control plots (Figure 3) and 1.99 (± 1.44) ind. 25 cm^{-2} in removal plots (Figure 2). *A. modestus* recruits were recorded at a maximum density of 32.61 (± 14.59) ind. 25 cm^{-2} in control plots (Figure 3) and 9.96 (± 3.88) ind. 25 cm^{-2} in removal plots (Figure 2).

Significantly lower abundances of recruits were recorded at Barloge Creek in comparison to the three sites within the Lough itself ($p < 0.001$) (Figures 2 and 3; Table 2). *S. balanoides* recruits were initially dominant in both plot types at this site, but ultimately *A. modestus* recruits outnumbered native species in both plots types at this site (Figures 2 and 3). *A. modestus* recruits peaked in abundance in June 2014 and following this maintained a stable level of abundance until the end of the study (Figure 2 and 3). A slight peak in the abundance of *S. balanoides* recruits was

observed between May 2014 and July 2014 at Barloge Creek (both plot types) (Figures 2 and 3) and Whirlpool Cliff (control plots) (Figure 3).

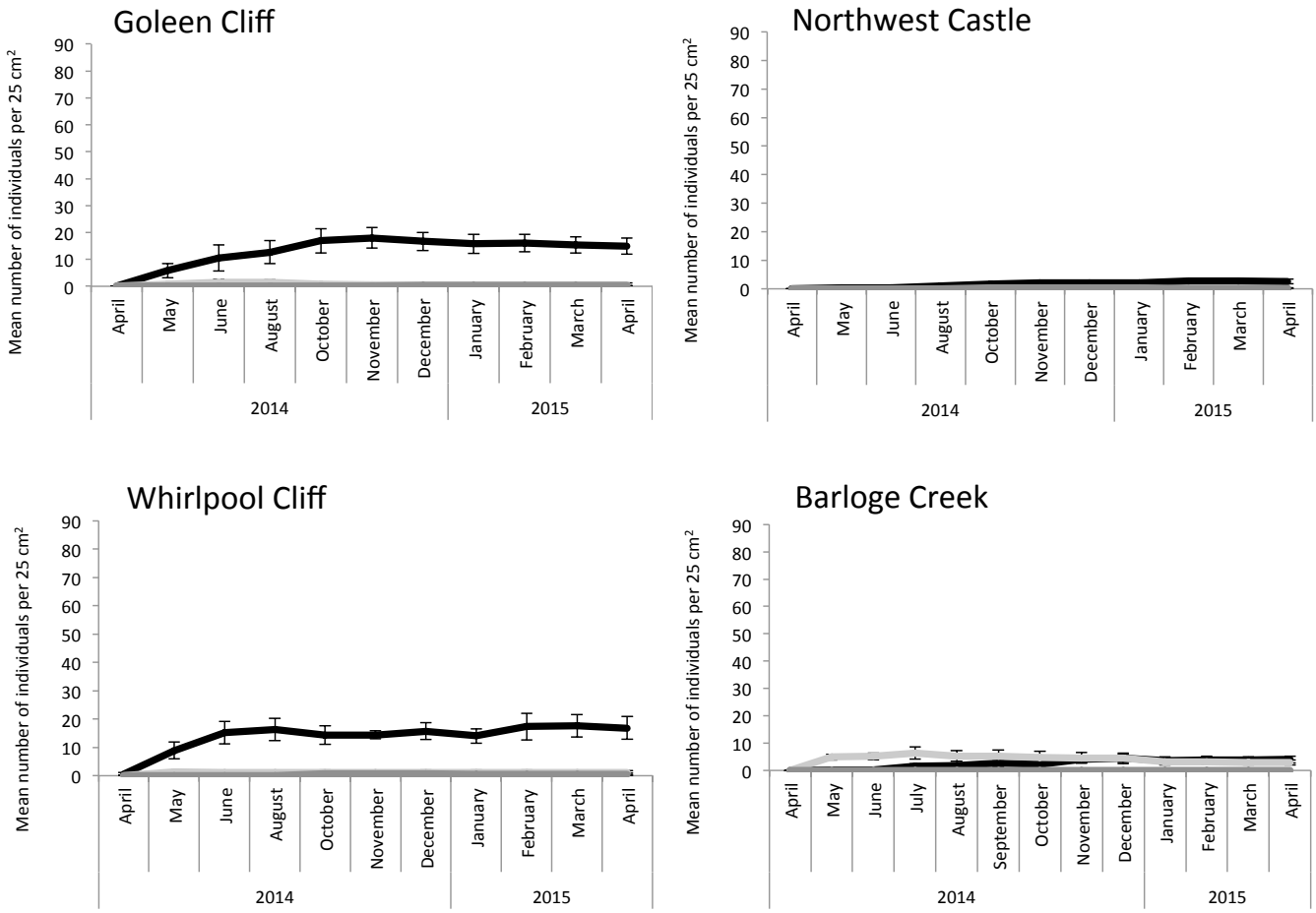

Figure 2. Mean number of recruits (± SE), per 25cm^2 free space, of three barnacle species, *Austrominius modestus* (black), *Semibalanus balanoides* (light grey) and *Chthamalus montagui* (dark grey), in removal plots at four sites, Barloge Creek, Whirlpool Cliff, Goleen and Castle Island, within Lough Hyne Marine Nature Reserve from April 2014 to April 2015.

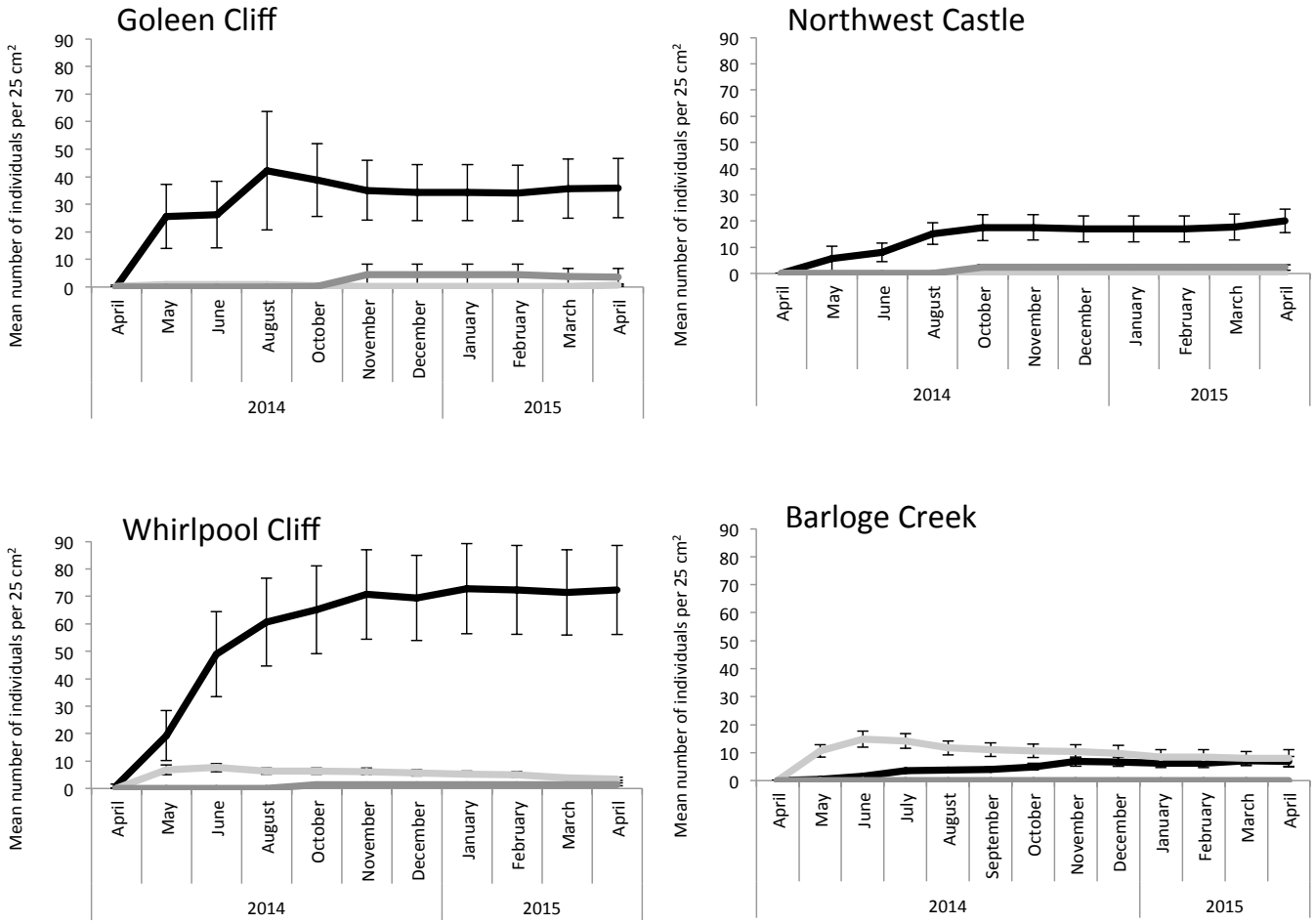

Figure 3. Mean number of recruits (± SE), per 25cm^2 free space, of three barnacle species, *Austrominius modestus* (black), *Semibalanus balanoides* (light grey) and *Chthamalus montagui* (dark grey), in control plots at four sites, Barloge Creek, Whirlpool Cliff, Goleen and Castle Island, within Lough Hyne Marine Nature Reserve from April 2014 to April 2015.

Table 2. Zero inflated negative binomial model estimates for recruit data collected at Lough Hyne Marine nature reserve on a monthly basis from April 2014 – April 2015. Variables in bold italics are represent the intercept value for comparison. Estimate values are log values. Significant p values ($p < 0.05$) are highlighted in bold.

	Estimate	Standard error	z value	p
AM				
CM	-1.97	0.14	-13.81	**< 0.001**
SB	-1.50	0.09	-15.64	**< 0.001**
Barloge Creek				
Whirlpool Cliff	0.99	0.11	8.97	**< 0.001**
Goleen	1.30	0.13	9.78	**< 0.001**
Castle Island	0.76	0.15	4.43	**< 0.001**
AM control				
Remove	-1.20	0.11	-10.81	**< 0.001**
CM control	-2.77	0.12	-21.7	**< 0.001**
SB control	-0.64	0.14	-4.59	**< 0.001**
AM remove				
CM remove	-3.50	0.23	-14.82	**< 0.001**
SB remove	-0.38	0.14	-2.60	**< 0.001**

3.5 Discussion

Recruits of *Austrominius modestus* were dominant at the three survey sites within the Lough in 2014-2015. Ultimately, this species also became dominant at Barloge Creek, located outside the Lough, despite native *Semibalanus balanoides* recruits being more abundant here initially. The pattern of recruitment observed in removal plots and in the surrounding community was very similar and the creation of free space did not promote the abundance of *A. modestus*. The ability of *A. modestus* to reproduce throughout the entire year (O'Riordan and Murphy 2000) via continuous broods (Moore 1944; Crisp and Davies 1955; Moyse 1960; Patel and Crisp 1960; Moyse 1963), in addition to the low flushing time in the Lough promote high abundances of *A. modestus* (Jessopp and McAllen 2008) and contribute to a storage effect within the Lough, which contributes to continued successful recruitment over time (Warner and Chesson 1982). Larvae of this species have been found to be more abundant inside Lough Hyne than waters outside the reserve (Jessopp and McAllen 2007) and *A. modestus* has been noted to be more successful inside the marine

reserve than in non-protected areas (Burfeind *et al.* 2013). It is possible that juvenile stages of *A. modestus* may be constantly present within the waters of the Marine Reserve, which could act as a reservoir for this non-native species. The long retention time within the Lough and the presence of the Rapids (Jessopp 2007) limit connectivity to the adjacent coastline (Jessopp and McAllen 2007) and no export of *A. modestus* larvae from the reserve has been recorded (Jessopp and McAllen 2007). This is important given the potential for marine reserves to act as exporters of larvae to surrounding waters (Jessopp and McAllen 2007; Hoskin *et al.* 2010), potentially playing a role in the spread of invasive species.

Although *A. modestus* was the dominant recruit at study sites, recruits of at least one native barnacle species were also recorded in both removal and control plots at all sites. It seems likely that *C. montagui* and *C. stellatus*, will continue to persist in the marine reserve, in particular at locations in the Rapids, where the turbulent conditions are not suitable for *A. modestus* (Lawson *et al.* 2004). However, the survival of *S. balanoides* is not certain at this location, despite species generally having a lower risk of extinction in marine reserves (Lubchenco *et al.* 2003). It has been found that the contraction of the southern range limit of *S. balanoides* in Europe is linked to warm winter temperatures that inhibit reproduction (Wethey and Woodin 2008) and *S. balanoides* population declines have previously been observed during warm periods (Southward and Crisp 1954; Southward 1967; Southward 1991; Hawkins *et al.* 2008; Hawkins *et al.* 2009). While the occurrence of cold winter events should favour *S. balanoides*, the low abundance of adults within the Lough could preclude recruitment at high densities here. Import of larvae from waters outside the Lough may be required for significant population growth, though the low flushing time within the Lough, in particular in the North Basin (Bassindale *et al.* 1957), make this unlikely.

Since *S. balanoides,* just like *A. modestus* is a cross-fertilising hermaphrodite (Barnes and Crisp 1956; Rainbow 1984) individuals need to be in close proximity to allow them to breed, to ensure further increases in abundance. Additionally, conditions within Lough Hyne during the summer months may not be optimal for *S. balanoides.*

It has been found that there is a distinct plankton community within the Lough, in comparison to adjacent coastal waters due to restricted tidal flushing. In particular high abundances of dinoflagellates and barnacle nauplii have been recorded here during the summer months (Johnson and Costello 2002). *A. modestus, C. montagui* and *C. stellatus* all feed on flagellates which are common in warmer waters. However, the northern, cold water adapted species *S. balanoides* feeds on larger diatoms found in northern waters (Moyse 1963; Stone 1989) and so may not be feeding optimally here during the summer months, which could possibly impact growth, survival and reproduction.

A. modestus remains the dominant barnacle within Lough Hyne, although native barnacles continue to persist. Natives are generally present at low densities, but are dominant at some locations within the Lough that are not suitable for *A. modestus*. Despite its abundance, Watson *et al.* (2005) stated that this invasive species had "little discernible effect" on native species at this location, while Lawson *et al.* (2004) noted that while the impact of *A. modestus* in Lough Hyne remains unclear, it is likely to cause ecological harm. Non-native species have the potential to compromise marine protected areas as they alter the ecosystem or reduce biodiversity (Bax *et al.* 2003). As a suspension feeder, barnacles are responsible for the transfer of energy between overlying waters and the intertidal community (Baird *et al.* 2012), if *A. modestus* differs in diet to the native species, it is possible that high abundances of this species would alter trophic interactions within the enclosed ecosystem of Lough Hyne. Ecological Network Analysis carried out by Baird *et al.* (2012) the Sylt-Rømø Bight in the North Sea examined the effect of *A. modestus* and the non-native oyster *Crassostrea gigas* on native ecosystem structure and function and found that the presence of the invasive species had impacts on lower trophic levels, mainly effecting phytoplankton abundance and a similar effect is possible in Lough Hyne. The potential presence of *A. modestus* larvae within the plankton throughout the entire year could also alter trophic dynamics within the system, having knock-on effects at multiple trophic levels. Climatic warming, despite being punctuated by extreme cold events, is likely to produce an intertidal community composed of *A. modestus*, *C. montagui* and *C. stellatus*. The impact that this may have on energy

flow and trophic interactions in the Lough warrants future investigation, especially given its status as a marine reserve.

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Chapter 4

Preliminary investigations into the competitive interactions between *Austrominius modestus* and native barnacle species

4.1 Abstract

Competition for resources can play an important role in determining the distribution of species on the shore. In particular, in the case of invasive species, the way in which they compete with natives for resources can determine invasion success. References have been made to competitive interactions between the non-native *Austrominius modestus* and native barnacle species, but have not been tested experimentally. A comparison of the growth of recruits of *A. modestus* and native barnacle species in removal plots across six sites found that *A. modestus* ultimately attained a larger opercular diameter than natives, despite the native *Semibalanus balanoides* exhibiting very fast growth rates following settlement. *Chthamalus montagui* grew much more slowly than either *A. modestus* or *S. balanoides*, which is not surprising given its smaller size. A comparison of the growth and survival of *S. balanoides* in the presence and absence of *A. modestus* found that the presence of *A. modestus* did not have a negative impact on the growth or survival of the native species. Fast growth following settlement gives *S. balanoides* an advantage, possibly facilitating its survival even in areas where it is outnumbered by *A. modestus.*

KEYWORDS: competition, survival, growth, invasive species

4.2 Introduction

Competition for space and other resources can shape the distribution of species on the shore and, in the case of invasive species, its ability to compete with native species for resources, may be paramount in determining their success (Byers 2000). While references have been made to competitive interactions between the non-native *Austrominius modestus* and native species (Knight Jones 1948; Crisp 1960; Barnes and Barnes 1962; Bennell 1981; Kochmann and Crowe 2014), these have been anecdotal as opposed to experimental and it remains unknown how these species compete with each other. Competitive interactions control the distribution of barnacle species on the shore (Connell 1961; Dungan 1985) and so it is important to understand how the presence of *A. modestus* is shaping the intertidal barnacle community within its invaded range.

Field manipulation experiments can be a useful method in determining competitive interactions between organisms (Gurevitch *et al.* 1992) and the intertidal zone has been the site of much ecological work over the years, due to its accessibility and the potential to observe sessile species (Paine 1974). Connell's classic removal experiments showed that competition was the factor controlling the zonation of *Chthamalus* spp. (called *C. stellatus* at that time, but most likely *C. montagui*) and *Semibalanus balanoides* on the shore, with *Chthamalus montagui* dominant at the high shore and *S. balanoides* dominant at lower levels of the shore. Connell revealed that in the absence of *S. balanoides*, *Chthamalus* spp. were capable of surviving at lower shore levels, thus proving that competitive interactions between *S. balanoides* and *Chthamalus* spp. could account for the distribution displayed by these two species (Connell 1961).

Where *A. modestus* occurs along with native barnacle species in the intertidal zone, the native *Chthamalus montagui* generally remains dominant at the high shore level. Both *A. modestus* and the native *S. balanoides* are found at the mid and low shore and so the non-native species can be seen as posing a more direct threat to *S. balanoides* as it competes with it directly for space. In order to demonstrate the competitive interactions between two species, it is necessary to show that one

species is more successful in the absence of the other (Luckens 1975), usually in terms of survival or growth. Individuals with higher growth rates are generally recognised as being competitively superior, as they will occupy space at the expense of slower growing individuals, often causing them to be displaced (Crisp 1964). However, Crisp (1964) stated that for sessile animals, with a limited amount of available space, success is also dependent on total biomass and the reproductive output as opposed to the size of the animal.

This study aimed to examine the competitive interactions between recruits of the native barnacle species *Semibalanus balanoides* and the non-native *A. modestus* by examining the abundance and growth of each species in isolation and when present together. Recruits as opposed to adults were examined as monitoring of adult populations revealed very slow growth of both species (Gallagher pers. obs.). It seems that the settlement and recruitment stage is of greater importance in terms of monitoring competitive interactions, as recruits are fast growing and have the potential to outcompete each other for space at this stage.

4.3 Materials and Methods

4.3.1 Growth of recruits in removal plots April 2014 – April 2015

To examine the competitive interactions between *Austrominius modestus* and native barnacle species the growth of recruits in clearance plots at six field sites in south west Ireland was monitored from April 2014 to April 2015. For details of field sites and plot clearance please see the materials and methods section of Chapter 2 (page 24). Measurements of the opercular diameter of a maximum of ten individuals of each species present in each plot photograph were obtained using the measure tool in ImageJ image analysis software. This measurement was then converted to millimeters using the scale bar present on the side of the quadrat for conversion. Individuals of each species were randomly selected from each image each month, however the same plots were photographed each month.

4.3.2 Growth and survival in competition plots at Ballyrisode

To examine the competitive interactions between *A. modestus* and *S. balanoides* in more detail, in March 2015 forty-five removal plots (25cm^2) were created at one of the six field sites, Ballyrisode. This site was chosen as it is a sheltered shore (for site details see page 25) and has rocky outcrops with a vertical aspect, which are ideal for the creation of removal plots. As *A. modestus* is known to preferentially settle in sheltered locations, it may pose more of a threat to native species at such locations and so it is of particular importance to understand the interactions between *A. modestus* and native species at these locations. This site was also found to have high levels of settlement of *A. modestus* and the native species *S. balanoides* with both species present at high abundances.

Fifteen plots were created on three areas of the rock face with a pre-existing barnacle cover (> 80%), approximate to the high, mid and low shore levels. High shore was taken to be the area where barnacle cover started, low shore where barnacle cover ended or where the rock itself met the sand and mid shore was taken to be the area in between. All organisms within the plot area plus an additional edge of 1cm on each side were removed using a paint scraper and the rock surface was then scoured with wire wool to ensure all organisms had been removed. The location of each plot was marked on the rock face using a paint mark to indicate where the top left hand corner of the quadrat should be place on each field visit.

Plots were randomly designated to be “mixed” plots (*A. modestus* and *S. balanoides* recruits), “SB only” plots (*S. balanoides* recruits only, any *A. modestus* individuals which established in the plots were removed on each monthly field visit) and “AM only” plots (*A. modestus* recruits only, any *S. balanoides* individuals which were present in the plots were removed until the end of the *S. balanoides* settlement season. The number of recruits of each species present in each plot was recorded from images taken of each plot on monthly field visits. This allowed for a calculation of the survival of recruits of the two species in the different plot types. A maximum of ten individuals of each species were randomly selected from each image to determine the growth rate of *A. modestus* and *S. balanoides* in the presence and

absence of each other. Using the measure tool in ImageJ image analysis software the opercular diameter of recruits obtained. This measurement was converted to millimeters using the scale bar present in each image. Plots were monitored until October 2015.

4.3.2 Statistical Analysis

Statistical analysis was carried out using R Studio Software 3.3.0 (R Core Team 2016). Due to a lack of independence and the unbalanced nature of the measurement data collected from April 2014 – April 2015, linear mixed effects models (LMMs) were constructed using the package "nlme" (Pinheiro *et al.* 2016). Species (three levels, unbalanced), time (fourteen levels, unbalanced) and substrate (two levels, unbalanced) were treated as fixed effects. Site (six levels), shore height (three levels) and plot (six levels) were treated as random effects. T-tests were also used to compare the opercular diameter of species in different plot types in October 2015.

4.4 Results

4.4.1 Growth of recruits in removal plots April 2014 – April 2015

Recruits of *Semibalanus balanoides* were first recorded in removal plots in April 2014, while *Austrominius modestus* recruits did not become established until May 2014. Despite the native *S. balanoides* having an additional month of growth, by the end of the study, 13 months later, *Austrominius modestus* had attained a significantly ($p < 0.001$) larger mean opercular diameter than the native species. *A. modestus* had become larger than *S. balanoides* by October 2014, and it maintained this larger size until the end of the study (Figure 1). *A. modestus* also had a larger initial mean opercular diameter (0.56mm ± 0.13mm) compared to *S. balanoides* (0.47mm ± 0.05mm) (Figure 1). While *S. balanoides* exhibited large increases in mean opercular diameter between April and July (0.26mm, 0.21mm and 0.14mm), the largest increase in opercular diameter was recorded for *A. modestus* between July and August (0.37mm) (Table 1). Subsequently *A. modestus* displayed a larger increase in opercular diameter than *S. balanoides* at each interval until the end of the study (Table 1). *Chthamalus montagui* was significantly smaller than both *A. modestus* ($p < 0.001$) and *S. balanoides* ($p < 0.001$). This species had both the

smallest initial mean opercular diameter (0.21mm ± 0.02mm) (Figure 1) and lowest growth rate, not exceeding an increase of 0.12mm during any interval throughout the study (Table 1).

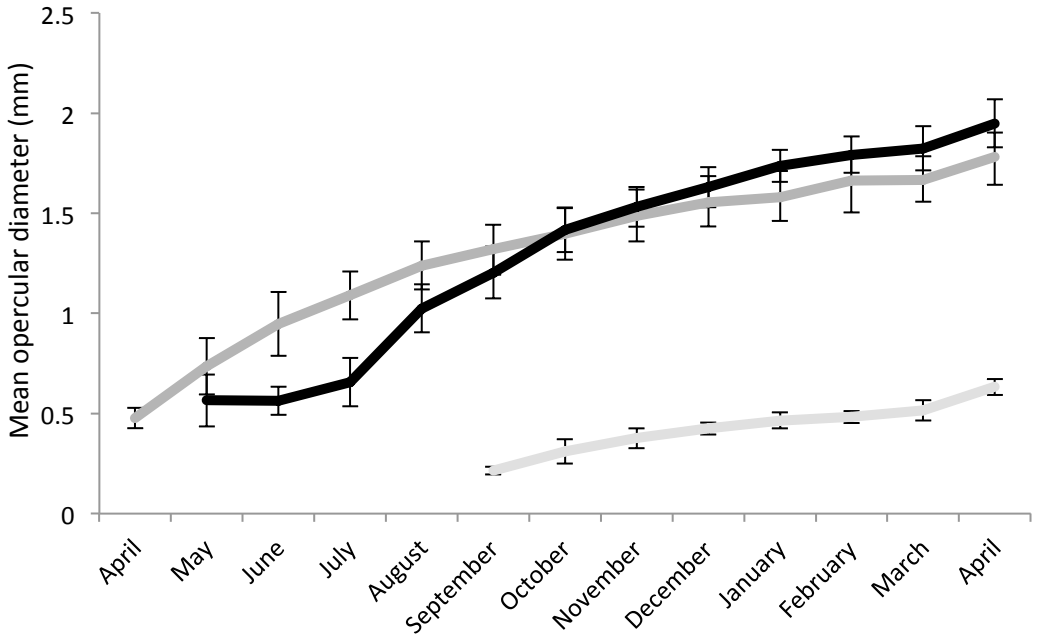

Figure 1. Mean (± SE) opercular diameter (mm) of *Semibalanus balanoides* (dark grey), *Austrominius modestus* (black) and *Chthamalus montagui* (light grey) at monthly interval from April 2014 to April 2015. Mean values were calculated from recruits in removal plots across six sites (n=108 plots) in south-west Ireland.

Table 1. Increases in mean opercular diameter (mm) of *Semibalanus balanoides*, *Austrominius modestus* and *Chthamalus montagui* at monthly intervals, from April 2014 to April 2015. The value under a given month represents the increase in mean opercular diameter (mm) from the previous month.

	2014									2015			
	April	May	June	July	August	September	October	November	December	January	February	March	April
Semibalanus balanoides		0.26	0.21	0.14	0.15	0.08	0.08	0.09	0.07	0.03	0.08	0.00	0.12
Austrominius modestus			0.00	0.09	0.37	0.18	0.21	0.12	0.10	0.11	0.06	0.03	0.12
Chthamalus montagui							0.10	0.07	0.05	0.04	0.02	0.03	0.12

4.4.2 Competition plots at Ballyrisode

Abundance and Survival

Maximum mean (± SE) abundance of *S. balanoides* recruits in "SB only" plots was recorded in July 2015 with a density of 12 (± 5.18) ind. $25cm^{-2}$ (Figure 2). Recruits of *S. balanoides* were more abundant in "mixed" plots, with a density of 27.7 (± 5.82) ind. $25cm^{-2}$ recorded in June 2015 (Figure 4). Mean number of *S. balanoides* recruits decreased from August 2015 until October 2015 in both plot types (Figure 2 and 4). Recruits of *A. modestus* were more abundant than those of *S. balanoides* in both plot types and increased throughout the study, though there was a slight decline in mean recruit abundance between September and October 2015 (Figures 3 and 4). Highest densities were recorded in September 2015 with 65.0 (± 25.8) ind. $25cm^{-2}$ recorded in "AM only" plots (Figure 3) and 129.3 (± 28.25) ind. $25cm^{-2}$ in "mixed" plots (Figure 4).

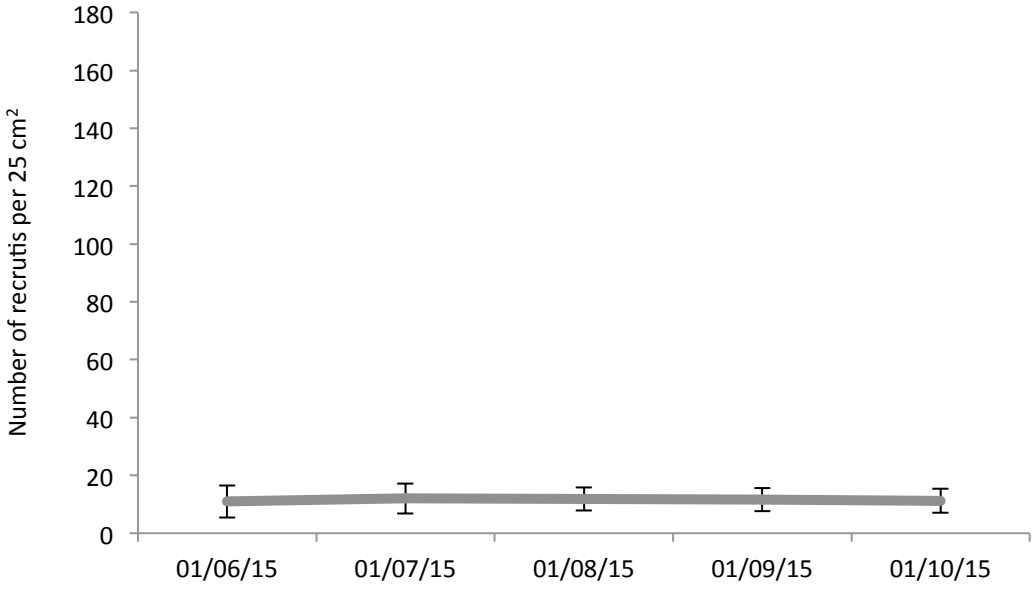

Figure 2. Mean number of *Semibalanus balanoides* recruits per 25cm^2 on five sampling dates between June and October 2015 in "SB only" plots from which *Austrominius modestus* recruits were removed. Error bars represent standard error.

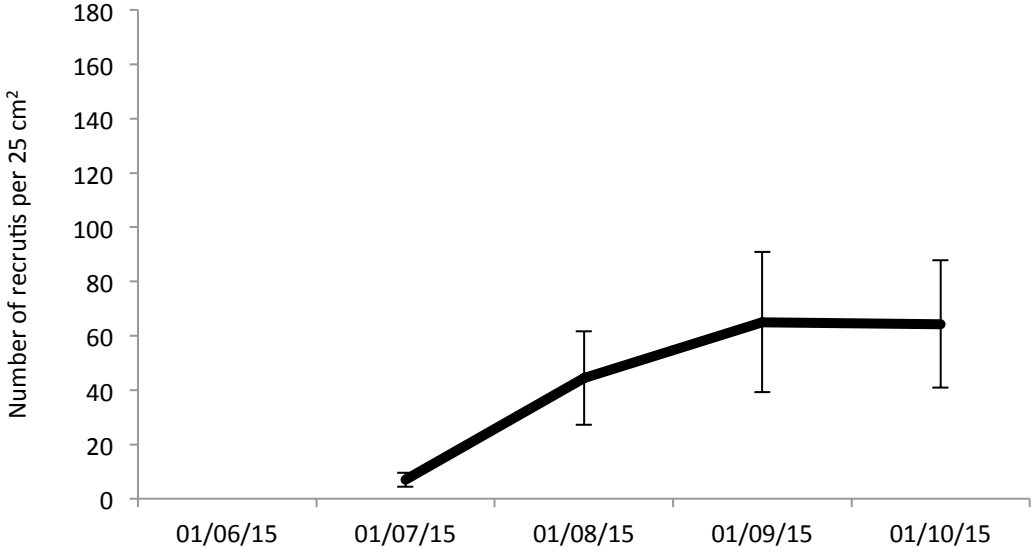

Figure 3. Mean number of *Austrominius modestus* recruits per 25cm^2 on five sampling dates between June and October 2015 in "AM only" plots from which *Semibalanus balanoides* recruits were removed. Error bars represent standard error.

Comparing mean abundances in July 2015 to those at the end of the study in October 2015, *S. balanoides* recruits had a mean survival rate of 86 (± 4.5)% in mixed plots and 83 (±11.7)% in plots where *A. modestus* was removed. *A. modestus* displayed a slightly lower percentage increase in mean recruit abundance in "mixed"

plots (173 ± 23.4%) compared to plots where *S. balanoides* had been removed (182 ± 29.7%).

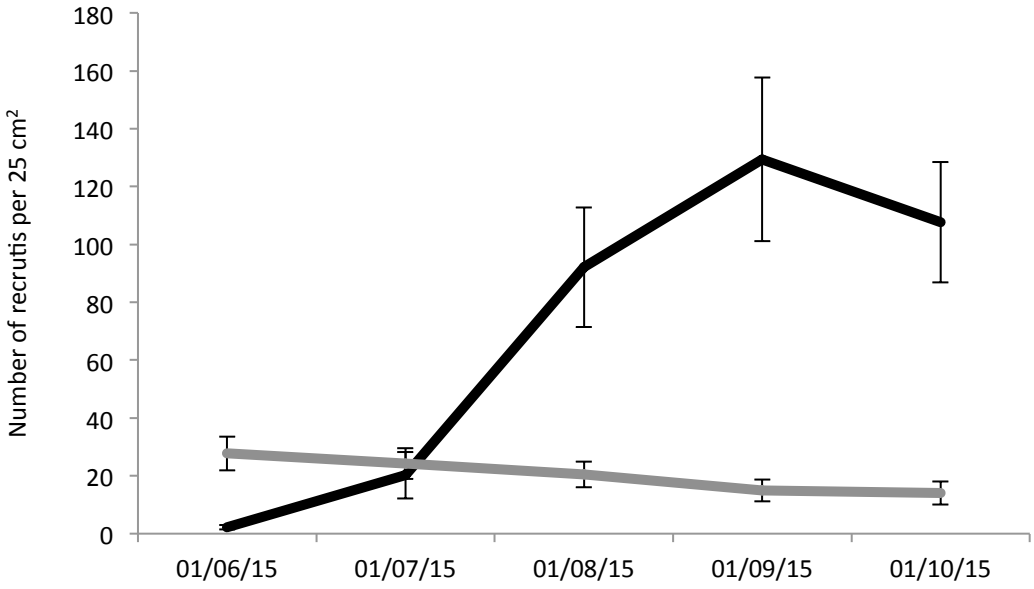

Figure 4. Mean number of *Austrominius modestus* (black) and *Semibalanus balanoides* (dark grey) recruits per 25cm^2 at four sampling dates between June and September 2015 in "mixed" plots where both species were present. Error bars represent standard error.

Growth

S. balanoides had a larger mean opercular diameter than *A. modestus* on all sampling dates in both plot types (Figures 5 and 6). Although *S. balanoides* had a larger mean opercular diameter (3.1 mm ± 0.08 mm) than *A. modestus* (2.7 mm ± 0.07 mm) in mixed plots (Figure 6) by October 2015, this difference was not found to be statistically significant (t = -0.43, 188 df, p = 0.66). In "AM only" plots and "SB only" plots, *S. balanoides* again had a larger mean opercular diameter (2.62 mm ± 0.08 mm) in comparison to *A. modestus* (2.4 mm ± 0.08 mm) in October 2015 (Figure 5), however this difference was not statistically significant (t = 0.48, 148 df, p = 0.62). Throughout the study both species had a larger mean opercular diameter in "mixed" plots. By October 2015 *A. modestus* recruits had a mean opercular diameter of 2.7 (± 0.07) mm in "mixed" plots (Figure 6) in comparison to 2.4 mm (± 0.08) mm in "AM only" plots (Figure 5). Similarly, *S. balanoides* recruits had a mean opercular diameter of 3.1 (± 0.08) mm in "mixed" plots (Figure 6) compared to 2.6 (± 0.08) mm

in “SB only” plots (Figure 5). This difference was only found to be significant in the case of *S. balanoides* (t = -2.00, 168 df, p = 0.04).

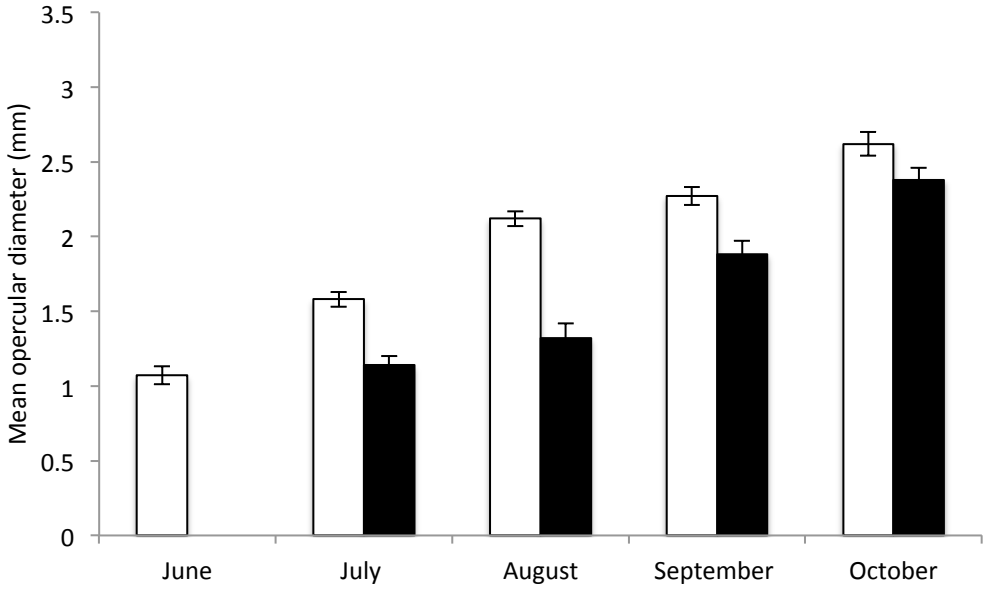

Figure 5. Mean opercular diameter (mm) of *Semibalanus balanoides* (white bars) and *Austrominius modestus* (black bars) recruits in “SB only” and “AM only” plots at Ballyrisode from June to October 2015.

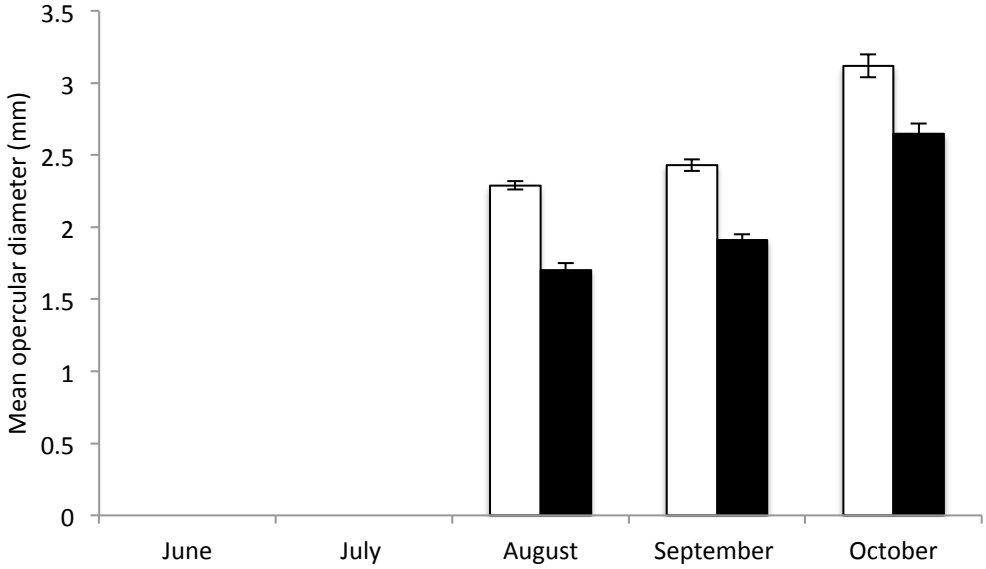

Figure 6. Mean opercular diameter (mm) of *Semibalanus balanoides* (white bars) and *Austrominius modestus* (black bars) recruits in “mixed” plots at Ballyrisode from August to October 2015.

4.5 Discussion

Long term monitoring of recruit growth across six sites from April 2014 – April 2015 revealed that although recruits of the native species *Semibalanus balanoides* displayed a fast initial growth rate at a time when *Austrominius modestus* was not present at high abundances, the non-native species had attained a larger mean opercular diameter by the end of the monitoring period. Fast growth rates of *S. balanoides* recruits was also found during monitoring of recruit growth from June 2015 to October 2015. However at this location, *S. balanoides* recruits were found to have a larger mean opercular diameter than those of *A. modestus*. However, it is worth noting that during the long-term monitoring of recruit growth from April 2014 – April 2015, *A. modestus* did not attain a larger mean opercular diameter until October 2014, and so continued monitoring of recruit growth at Ballyrisode may have found that over the following months *A. modestus* grew to a larger size than *S. balanoides.*

A higher number of both *S. balanoides* and *A. modestus* recruits were recorded in "mixed" plots at Ballyrisode in comparison to "AM only" plots. This indicates that the presence of the native species did not preclude settlement and recruitment of the invasive species. Survival rates of both species was slightly lower in "mixed" plots when compared to "SB only" and "AM only" plots, however these differences were very small and not enough to signify that the presence of one species is having an effect on the survival of the other. In terms of growth, recruits of both species had a larger mean opercular diameter in mixed plots as opposed to "SB only" and "AM only" plots, indicating that both species grow larger when in competition with each other.

The fast growth of *S. balanoides* observed during both parts of the study illustrates how this species is utilising a temporal niche. By growing quickly at a time when *A. modestus* recruits are not abundant, it is able to colonise space on the rock while attaining a large size, making it is less susceptible to being undercut (where an individual is removed from the rock surface by another barnacle growing underneath

it) or smothered by recruits of the invasive species and limiting the space available for this species to settle.

Figure 7. Photograph of a "mixed" plot at the low shore level at Ballyrisode in October 2015 illustrating recruits of *Austrominius modestus* (grey coloured individuals) that have settled between and on top of recruits of *Semibalanus balanoides* (cream coloured individuals).

Previous work by Crisp (1960) reported that *S. balanoides* spat have a faster growth rate than the invasive species, resulting in displacement of recruits of the invasive species that settle amongst *S. balanoides*. In experimental plots at Ballyrisode, *A. modestus* recruits were observed to settle on or adjacent to *S. balanoides* recruits in "mixed" plots (Figure 7), however this did not result in the displacement of invasive recruits at this location. Neither did it appear to have a negative effect on the growth or survival of native recruits, however continued monitoring would be required to ensure that this was indeed the case.

Overall it would appear that despite being more abundant than the native *S. balanoides* and potentially attaining a larger opercular diameter than the native species over time, there is little evidence to suggest that *A. modestus* is outcompeting *S. balanoides* at the study location. This may in part be sue to the fact that all space was never entirely saturated in the experimental plots during this study, and strong competitive interactions may only be observed when the resource in question, space, is truly limiting. Despite being outnumbered by *A. modestus* recruits in plots at Ballyrisode, *S. balanoides* recruits displayed relatively high survival rates, comparable to those seen in the absence of the non-native species. Additionally, neither species was observed to displace or undercut the other, even though the non-native species settled on top of and adjacent to *S. balanoides.* The fast initial growth rate of *S. balanoides* gives this species a competitive advantage that may facilitate its survival, despite becoming outnumbered by *A. modestus* as multiple broods allow recruits to continue to settle throughout the year.

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Chapter 5

Can differences in reproductive strategy facilitate the persistence of a non-native species alongside natives?

This chapter in revised format is being prepared for submission as a short note to the Journal *Marine Biology*

5.1 Abstract

The non-native barnacle species *Austrominius modestus* can breed throughout the entire year, reproducing via continuous broods. In contrast, the native barnacle species *Semibalanus balanoides* only produces a single brood per year. Previous studies have examined the reproductive cycle of *A. modestus,* however, this is the first study to provide information on the stage of embryo development of both *A. modestus* and a native barnacle species from the same location over two extended time periods. Samples of *A. modestus* and *S. balanoides* were collected on a monthly basis from June 2006 – November 2007 and again from June 2014 – November 2015 at Bullens Bay, Co. Cork, Ireland. A proportion of *A. modestus* individuals were found to have embryos that were ready to hatch in seventeen of eighteen months sampled in 2006-2007 and fifteen of eighteen months in 2014-2015. This is in contrast to *S. balanoides*, which only had embryos at this stage during a distinct five-month period in 2006-2007 and in just two months during 2014-2015. Both species had a significantly lower proportion of individuals with embryos that were ready to hatch during 2014-2015 in comparison to 2006-2007. In such years where *S. balanoides* has low recruitment, the continual addition of *A. modestus* recruits to the population will be of particular advantage to the non-native species.

KEYWORDS: *Austrominius modestus*, *Semibalanus balanoides*, barnacle, reproduction, recruitment, invasive

5.2 Introduction

The Australasian barnacle species *Austrominius modestus* has become well established on most Atlantic European coasts (Harms 1999; Tøttrup *et al.* 2010) since it was first recorded in Europe in the early 1940s (Bishop 1947; Crisp and Chipperfield 1948; Stubbings 1950). The successful persistence of *A. modestus* in Europe and dominance over natives at some locations in its invaded range (Franke and Gutow 2004; Lawson *et al.* 2004; Reichert and Buchholz 2006; Gomes-Filho *et al.* 2010; Witte *et al.* 2010, Bracewell *et al.* 2012), has been attributed to multiple factors, including differences in reproductive traits of this species in comparison to native barnacle species (Harms 1999).

Austrominius modestus is an obligatory cross-fertilising hermaphrodite (Southward 1955; Barnes and Crisp 1956), which reproduces via continuous succession of broods throughout the entire year (Moore 1944; Crisp and Davies 1955; Moyse 1960; Patel and Crisp 1960; Moyse 1963). This is in contrast to the native arctic-boreal species *Semibalanus balanoides* which produces a single brood per year (Anderson 1994) and the native warm water adapted *Chthamalus* spp. which produces multiple broods (Burrows *et al.* 1992), mainly during the summer months. In addition to being able to reproduce throughout the entire year, *A. modestus* is capable of becoming mature within 8-10 weeks of settling (Crisp and Davies 1955; Barnes 1956), while native species take longer to become mature. For example, individuals of *S. balanoides* do not become mature until they are at least one year old (Barnes and Barnes 1954) and *Chthamalus* spp. do not begin to breed for nine to ten months after settlement (Southward and Crisp 1954; O'Riordan 1992). With a generation time of only 14 weeks, it has been estimated that an individual *A. modestus* is capable of producing up to 20,000 young over its lifetime (Crisp and Davies 1955). Having a short time to maturity and small time intervals between the production of large broods has previously been reported to increase the invasiveness of plant species by ensuring rapid population growth (Rejmánek and Richardson 1996).

Although the broods of *A. modestus* are non-seasonal, there is a difference in the rate of breeding with season (Crisp & Davies 1955; O'Riordan and Murphy 2000). *A.*

modestus can breed at temperatures ranging from 6 °C to 20 °C and has eurythermal embryos capable of tolerating temperatures ranging from 3°C to 30°C (Patel and Crisp 1960). However, during the colder parts of the year the brood period and rate of embryonic development are extended in comparison to the warmer summer months (Crisp & Davies 1955). As with most sessile barnacles, fertilised egg masses are brooded within the mantle cavity until hatching (Hill *et al.* 1988). Following liberation, the planktonic nauplii go through a series of developmental stages, before metamorphosing into a cypris stage, which will settle on a substrate (Knight Jones and Waugh 1949; Pineda *et al*. 2002; Ross *et al.* 2003). Survival during these developmental stages will influence the rate of spat-fall and thus population size. Temperature, salinity and food availability are known to influence the development of nauplii (Harms 1999), while water column processes, such as wind driven dispersion, play an important role in the provision of larvae to suitable habitats for settlement (Kendall *et al.* 1985; Alexander and Roughgarden 1996). In particular, the survival of barnacle nauplii is dependent on the timing of hatching coinciding with a period of food availability, which can lead to a delay between the embryos becoming fully developed and the time of naupliar release (Patel and Crisp 1960). *S. balanoides* is known to liberate its single annual brood around the same time as the spring phytoplankton bloom, ensuring a plentiful food supply for the nauplii and enhancing their chances of survival (Moyse and Knight Jones 1967; Crisp 1974).

Despite the long time interval since the introduction of *A. modestus* to Europe, there is little published information regarding its reproductive cycle within its invaded range, in particular in direct comparison to that of a native barnacle species at the same location. This study compares the proportions of individuals of *A. modestus* and *S. balanoides* brooding embryos at various stages of development, over two eighteen month periods in 2006-2007 and 2014-2015 at Bullens Bay, Ireland. Data collected over two distinct time periods provides an insight into differences in the breeding cycle of these species between years and potential implications for the abundance of the two species. Such information is important in order to better understand the invasion ecology of *A. modestus*, in particular in comparison to native species.

5.3 Materials and Methods

5.3.1 Sample collection and screening

Sampling was carried out on a monthly basis at Bullens Bay, Co. Cork, Ireland (N51°38.578' W008°33.092'), a relatively sheltered shore (5 on the Ballantine Scale (1961)), from June 2006 – November 2007 inclusive and again from June 2014 – November 2015 inclusive. Small rocks and stones with barnacles growing on them were collected and transported to the laboratory and kept in tanks with aerated seawater until they were examined (never more than 48 hours). Sixty individuals of *Austrominius modestus* and of the native barnacle species *Semibalanus balanoides* were screened each month (n=120 each month).

Individual barnacles were removed from the substrate using a scalpel and the body mass of the barnacle was then removed from the shell using a forceps and placed in the well of a cavity slide with 70% ethanol. The specimen was then examined under the microscope (Nikon SM 2645; X 5 magnification). The presence of embryos was recorded and staged following O'Riordan and Murphy (2000) for *A. modestus* and Barnes and Barnes (1974) for *S. balanoides*. Hourly seawater temperature data recorded by buoys M3 and M5 off the south coast of Ireland were obtained from Met Éireann (The Irish Meteorological Service) for the duration of the sampling periods and mean (± SE) monthly values were calculated.

5.3.4 Statistical Analysis

The proportion of individuals of *S. balanoides* and *A. modestus* recorded with embryos at all stages of development and with embryos at stages 4-4h from each month in 2006-2007 was compared to the corresponding month during 2014-2015. Analysis was carried out separately for each species, given their different reproductive cycles, using the "prop.test" function in R Studio version 3.3.0 (R Core Team 2016). Sequential Holm-Bonferroni corrections were applied to the p values in order to reduce the probability of Type 1 error (Holm 1979; Getano 2013) using a Holm-Bonferroni sequential correction EXCEL calculator (Gaetano 2013).

5.4 Results

Individuals of *Austrominius modestus* were found to possess embryos on each sampling occasion in 2006-2007 and 2014-2015, with the exception of December 2006 and November 2014 (Figures 1a and 1b). Highest proportions of individuals bearing embryos were recorded in the summer and early autumn (Figures 1a and 1b), when seawater temperatures were highest (Figure 2). In June, July and August 2006, 54%, 44% and 47% of individuals were found with embryos respectively. Similarly 58% of individuals were recorded with embryos in July 2007 and 44% in August 2007 (Figure 1a). 65% of individuals were recorded with embryos in June 2014 and 42% in July 2014. A significantly higher proportion of individuals were recorded with embryos in November and December 2014 and June 2015May, June, July comparison to the same months in 2006-2007 (Table 1) (Figure 1b).

In every month where *A. modestus* was recorded with embryos during 2006-2007, a proportion of individuals (12% - 48%) were brooding embryos at developmental stages 4-4h, which is ready to hatch (Figure 1a). This was also the case for the majority of months in 2014-2015 (2% - 45%), with the exception of November 2014, February 2015 and June 2015 (Figure 1b). The proportion of individuals with embryos at stages 4-4h was significantly higher in 2006-2007 in comparison to 2014-2015 for eight of the eighteen sampling months (Table 1).

During 2006-2007, there were never less than 12% of individuals recorded with embryos at stages 4-4h (Figure 1a), however during 2014-2015, more than 10% of *A. modestus* individuals screened were found to have embryos at stages 4-4h in only three months, June 2014 (45%), July 2014 (37%) and September 2015 (12%) (Figure 1b). A proportion of individuals were recorded to have embryos at the remaining developmental stages (1-3), throughout the majority of all sampling months in 2006-2007 (Figure 1a) and 2014-2015 (Figure 1b), indicating the continual production of broods throughout the year.

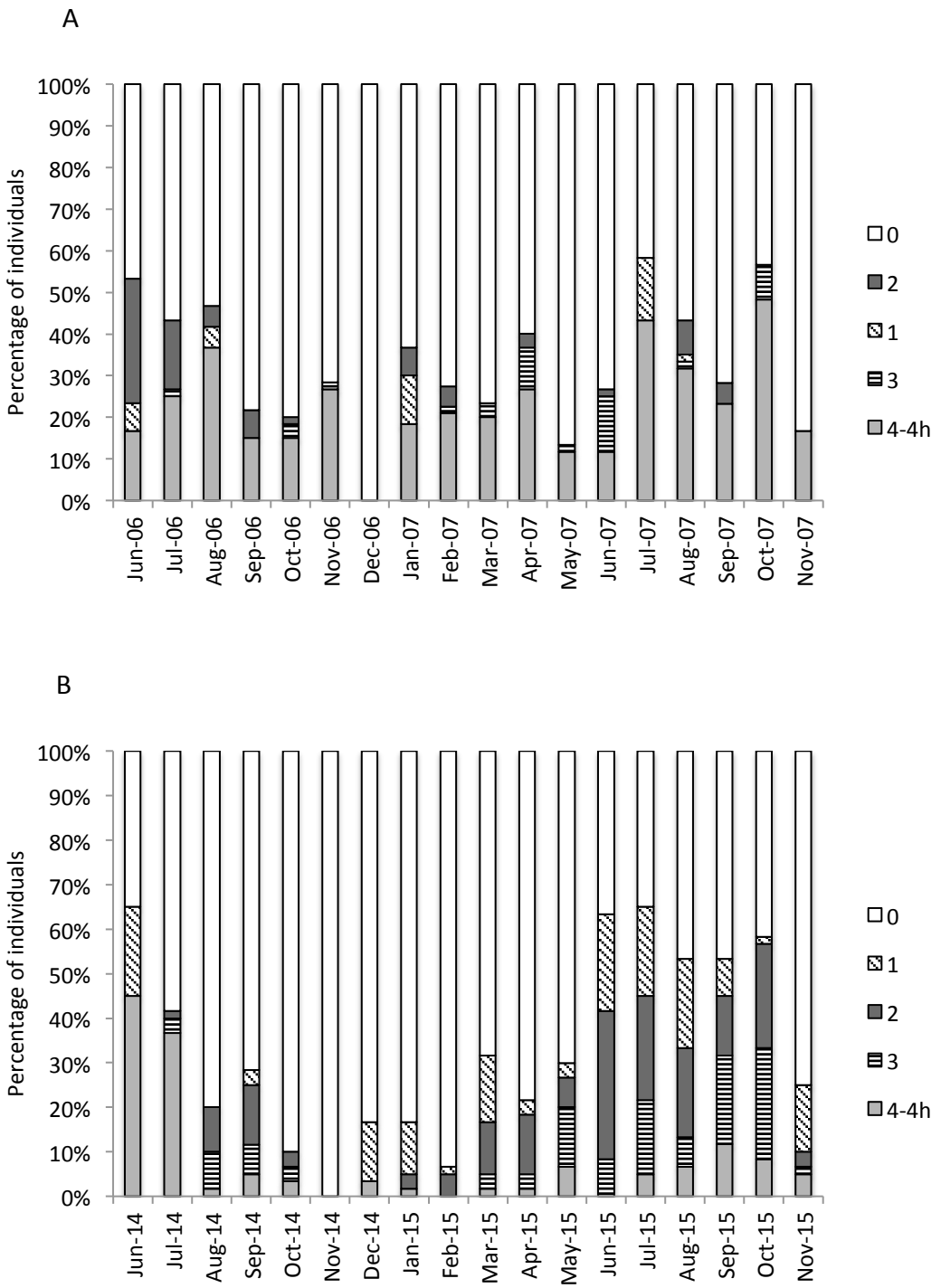

Figure 1. Percentage of individuals of *Austrominius modestus* from Bullens Bay, Co. Cork, Ireland, without embryos (0) or with embryos at developmental stages 1-4h (following O'Riordan and Murphy 2000) at monthly sampling intervals from (A) June 2006 – November 2007 inclusive and (B) June 2014 – November 2015 inclusive (n=60 per month).

Table 1. "Prop.test" results with Holm-Bonferroni sequential correction (Holm 1979; Gaetano 2013), comparing the proportion of individuals of *Austrominius modestus* with embryos during 2006-2007 to the corresponding months during the 2014-2015 sampling period and the proportion of individuals with embryos at stages 4/4h during 2006-2007 in comparison to 2014-2015. Analysis was carried out using R Studio version 3.3.0 (R Core Team 2016) and Holm-Bonferroni sequential correction EXCEL calculator 1.2 (Getano 2013). Significant p values (< 0.05) are highlighted in bold.

	Austrominius modestus					
	All embryos			**Stage 4-4h**		
	χ^2	df	p	χ^2	df	p
June	1.24	1	1.00	10.00	1	**0.01**
July	<0.01	1	1.00	1.41	1	0.60
August	8.43	1	0.05	21.51	1	**0.01**
September	0.40	1	1.00	2.31	1	0.60
October	1.63	1	1.00	3.60	1	0.34
November	17.54	1	**0.01**	16.22	1	**0.01**
December	8.83	1	**0.03**	0.50	1	0.70
January	5.15	1	0.25	8.62	1	**0.03**
February	8.31	1	0.05	12.42	1	**0.01**
March	0.66	1	1.00	8.62	1	**0.03**
April	3.90	1	0.46	13.42	1	**0.01**
May	3.97	1	0.46	0.40	1	0.95
June	13.51	1	**0.01**	5.46	1	0.15
July	0.31	1	1.00	22.00	1	0.05
August	0.83	1	1.00	10.54	1	**0.01**
September	6.76	1	0.10	2.07	1	0.60
October	< 0.01	1	1.00	21.71	1	**0.01**
November	0.80	1	1.00	3.10	1	0.46

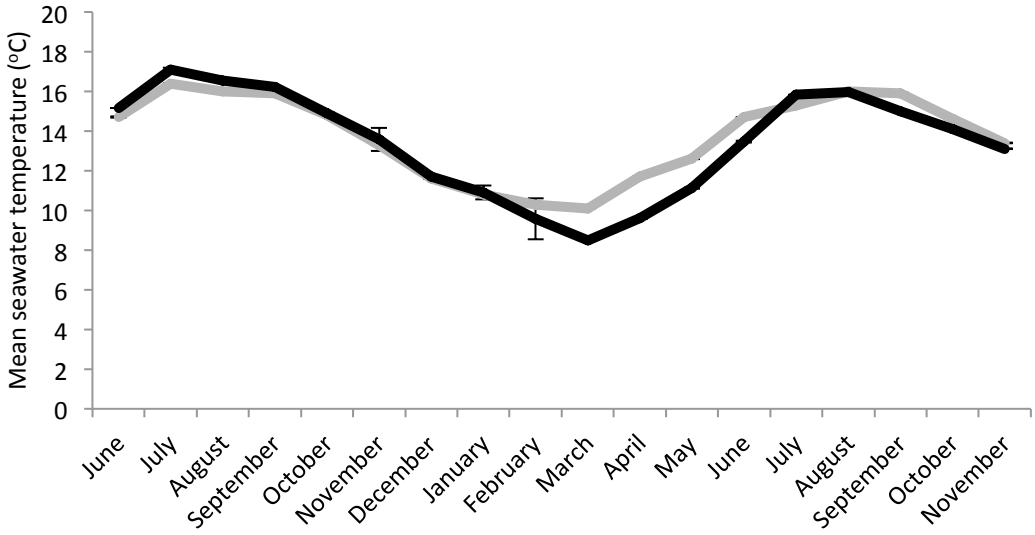

Figure 2. Mean seawater temperature (°C) recorded by buoys M3 and M5 located off the south coast of Ireland during June 2006-November 2007 (grey line) and June 2014-November 2015 (black line). Error bars represent standard error from the mean.

During 2006-2007, individuals of *Semibalanus balanoides* were found to possess embryos from December 2006 to July 2007. A significantly higher proportion of individuals were recorded with embryos in 5 months during 2006-2007 compared with the corresponding months during 2014-2015 (Table 2). The development of a single brood can be seen, with the majority of embryos recorded at developmental stage 1 in December 2006 (63%) (Figure 3). 12% of individuals had embryos at stage 2 in the following month and subsequently the majority of embryos recorded were at stages 4-4h (2% - 54%). A significantly higher proportion of individuals were found with embryos at stages 4-4h during two of the six months where embryos at this stage were recorded during 2006-2007 in comparison to 2014-2015 (Table 2). The main breeding period for this species seemed to have ended by May 2007, with only 2% of individuals recorded with embryos until July 2007. The onset of the breeding period for this species was again observed in October and November 2007, with 8% and 3% of individuals recorded with embryos in these months (Figure 3a).

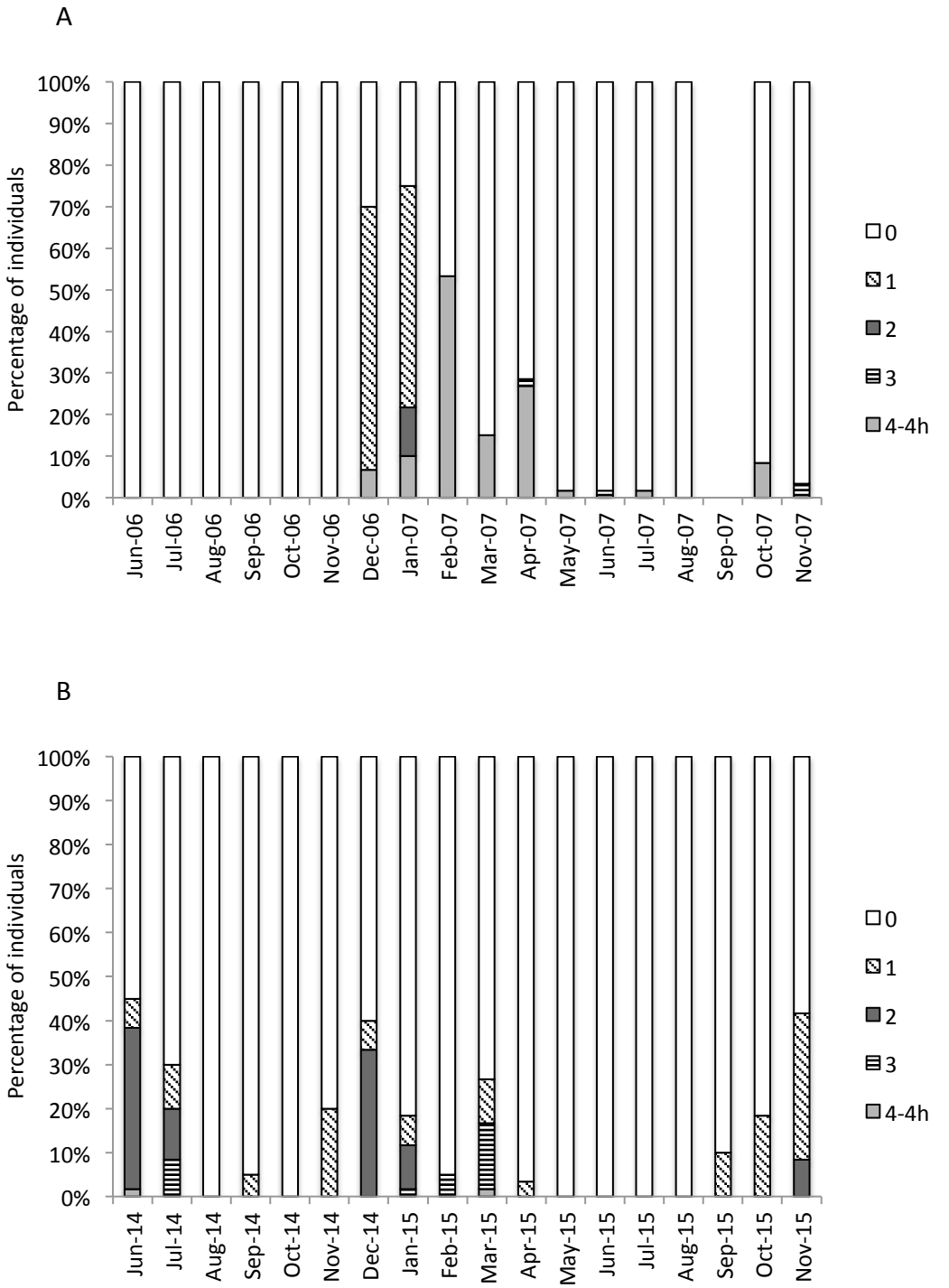

Figure 3. Percentage of individuals of *Semibalanus balanoides* from Bullens Bay, Co. Cork, Ireland, without embryos (0) or with embryos at developmental stages 1-4h (following O'Riordan and Murphy 2000) at monthly sampling intervals from (A) June 2006 – November 2007 inclusive and (B) June 2014 – November 2015 inclusive (n=60 per month).

Table 2."Prop.test" results with Holm-Bonferroni sequential correction (Holm 1979; Gaetano 2013), comparing proportion of individuals of *Semibalanus balanoides* with embryos during 2006-2007 to corresponding months during the 2014-2015 sampling period and the proportion of individuals with embryos at stages 4/4h during 2006-2007 in comparison to 2014-2015. Analysis was carried out using R Studio version 3.3.0 (R Core Team 2016) and Holm-Bonferroni sequential correction EXCEL calculator 1.2 (Getano 2013). Significant p values (< 0.05) are highlighted in bold.

	Semibalanus balanoides					
	All embryos			**Stage 4-4h**		
	χ^2	df	p	χ^2	df	p
June						
July						
August						
September						
October						
November						
December	9.73	1	**0.03**	2.32	1	0.20
January	36.46	1	**0.01**	4.38	1	0.20
February	31.62	1	**0.01**	40.92	1	**0.01**
March	1.81	1	0.34	5.34	1	0.20
April	12.256	1	**0.01**	16.22	1	**0.01**
May						
June						
July						
August						
September						
October	1.8	1	0.34	3.33	1	0.20
November	23.13	1	**0.01**			

During 2014-2015, 45% of *S. balanoides* screened were recorded with embryos in June 2014, 30% in July 2014 and 5% in September 2014, representing the end of the 2014 breeding period for this species. However, embryos at stages 4-4h were only recorded in June 2014 (2%). The start of the next breeding period was in November 2014, with 20% of individuals with embryos at stage one. Over the following months a decreasing proportion of individuals were recorded with embryos at stage one, with an increasing proportion recorded at stages 2 and 3. By March 2015, embryos at stages 4-4h were recorded (Figure 3b), although only 2% of individuals were recorded brooding embryos at this developmental stage (Figure 3b), which was lower than March 2007 (Figure 3a). Individuals of *S. balanoides* were again found to be brooding embryos in September 2015 (10% at stage 1), earlier than 2006, 2007 (Figure 3a) and 2014 (Figure 3b). A significantly higher proportion of *S. balanoides* individuals were recorded with embryos in November 2015 in comparison to November 2007 ($\chi^2 = 23.13$, 1 df, $p = 0.01$).

Seawater temperatures in in 2006-2007 ranged from 10.0 ^{o}C (± 0.02 ^{o}C) in April 2006 to 16.4 ^{o}C (± 0.03 ^{o}C) in July 2006 and showed a similar trend in 2014-2015, ranging from 8.5 ^{o}C (± 0.01^{o}C) in March 2015 to 17.1 ^{o}C (± 0.08 ^{o}C) in July 2014 (Figure 2). Highest proportions of *A. modestus* recorded with embryos coincided with times of highest seawater temperatures during both sampling periods (Figures 1a and 1b), while the onset of brooding in *S. balanoides* coincided with declining seawater temperatures (Figures 3a and 3b).

5.5 Discussion

A proportion of individuals of *Austrominius modestus* were recorded to possess embryos that were ready to hatch (stages 4-4h) throughout the majority of this study. In contrast, the native barnacle species *Semibalanus balanoides* was recorded with embryos at stages 4-4h from December 2006 to May 2007 and in only two months during the 2014-2015 sampling period. As would be expected, highest proportions of the warm water adapted *A. modestus* brooding embryos were recorded during times of warmest seawater temperature, while highest proportions of the arctic-boreal species *S. balanoides* brooding embryos were recorded during

colder parts of the year. This peak in the proportion of *A. modestus* brooding embryos occurred at the same time as previously recorded by O'Riordan and Murphy (2000) in Ireland.

A similar trend in the proportions of *A. modestus* brooding embryos was observed in 2006-2007 and 2014-2015, although the proportion of the sampled population with embryos at stages 4-4h was consistently higher in 2006-2007 than 2014-2015. This may be linked with higher seawater temperatures from January to June 2007, in comparison with the same period in 2015. Proportions of *S. balanoides* brooding embryos and in particular with embryos at developmental stages 4-4h were also lower during 2014-2015 than 2006-2007. It is possible that this is due to sampling bias or that embryos had been released between field visits. However, it seems more likely that 2014-2015 was an unsuccessful year for reproduction of *S. balanoides* at this site. The high proportion of individuals recorded with developing embryos at the end of the study, in October and November 2015 indicate that a higher proportion will have embryos at stages 4-4h by spring 2016 than spring 2015. This may be due to the fact that seawater temperatures during summer 2014 were higher than those of summer 2006 and 2015 and may have been unfavourable to the cold water adapted species at the start of its reproductive cycle, which requires temperatures below 10^{o}C for successful reproduction (Crisp and Clegg 1960; Barnes 1963).

The reproductive strategy of *A. modestus* gives it an advantage over the native *S. balanoides*, as it is not reliant on the success of just one brood for the population to persist and can potentially colonise free space throughout the entire year (Rainbow 1984; Hui and Moyse 1987). This is of particular importance in years where *S. balanoides* does not recruit at high densities. As illustrated in the present study, both *S. balanoides* and *A. modestus* had lower proportions of individuals with embryos at stages 4-4h in during 2014-2015 in comparison to 2006-2007. For *S. balanoides* this translates into very low levels of recruitment and although recruitment must also have been relatively low for *A. modestus* it had multiple opportunities to recruit at low densities throughout the year, limiting the space available for *S. balanoides* recruits to colonise the following spring. However, years where *S. balanoides* has

successful settlement and recruitment, resulting in a high density of fast growing recruits facilitates the persistence of this species in addition to *A. modestus.* Despite the differences in reproductive strategy of the two species, unless *S. balanoides* fails to recruit in multiple consecutive years, it seems more likely that the two species will continue to persist for some time.

Barnacle larvae are a common component of the zooplankton, and are one of the dominant groups in this community seasonally (Pineda *et al.* 2002; Muxagata and Williams 2011). The single annual brood produced by *S. balanoides* represents a significant pulse in the zooplankton community, with up to 5,000 eggs produced per adult per brood (Barnes and Barnes 1968). However, the presence of *A. modestus* larvae throughout the year, while at a lower abundance (Crisp and Davies 1955), may represent an additional energy source, which would have been unavailable prior to the introduction of this species. It is possible that this may have an impact on trophic dynamics within the ecosystem and could warrant future study. The availability of food for developing barnacle nauplii is important for their survival (Moyse and Knight Jones 1967; Crisp 1974). The diet of nauplii has been shown to be closely linked to their geographical distribution. The nauplii of the northern, cold-water species *S. balanoides* feed on large diatoms common in northern waters using widely spaced setules, while nauplii of the southern species *C. montagui* and *C. stellatus* have closely spaced setules suitable for feeding on small flagellates which are common in warmer waters (Moyse 1963; Stone 1989). The nauplii of *A. modestus* are capable of feeding on both large diatoms and small flagellates, facilitating their survival at a range of temperatures (Stone 1989) and locations. This would also suggest that regardless of the time of year that nauplii are released into the plankton, they should be capable of feeding on the food that is available.

It was previously found that despite producing multiple broods throughout the summer in Scotland, the warm water adapted *Chthamalus* spp. only had successful spatfall from broods produced in the late summer and early autumn (Barnes 1956). Similarly, O'Riordan (1992) recorded that *Chthamalus* settlers recorded in Ireland in August did not survive, while settlers recorded from October onwards did. This could

also be the case for *A. modestus* in Ireland, even though broods are being produced throughout the entire year, only those produced at specific times, most likely the late summer and early autumn, may result in a successful spatfall. Additionally, even if multiple broods do result in successful spatfall, it is possible that environmental conditions at that time of year may not be favourable for the young *A. modestus*. For example, low levels of recruitment and high levels of post-settlement mortality have been recorded for this species following cold winters (den Hartog 1953; Crisp 1964; Harms and Anger 1989; Witte *et al.* 2010). Settlement of this species has been recorded throughout the majority of the year at field sites in south-west Ireland, however at very low levels from November to March (Gallagher, unpublished data).

Like many invasive species, *A. modestus* is warm water adapted (Witte *et al.* 2010), with a physiological optimum above 20^{o}C (Harms 1999). Nauplii of both *A. modestus* and *S. balanoides* have been found to be capable of surviving temperatures up to 35°C and 36°C respectively. However, *A. modestus* was much more tolerant of cyclic fluctuations in temperature and had a greater survival rate at high temperatures (Cawthorne 1980), possibly giving it an advantage with predicted climate change. *A. modestus* will be better able to acclimatize to future environmental conditions than native barnacle species (Buckeridge 2012) and increases in seawater temperature (IPCC 2014) may even further promote the year-round reproduction of this species, with optimal conditions for embryo development present for longer parts of the year. However, it is worth noting that O'Riordan and Murphy (2000) found significantly lower proportions of *A. modestus* individuals brooding embryos at a warm water site, in comparison to sites subject to normal seawater temperatures. Rising temperatures are likely to negatively impact the reproductive success of *S. balanoides*, which may even fail to reproduce at some locations in some years. However, the occurrence of occasional cold winters (Wang *et al.* 2010) could continue to facilitate the persistence of *S. balanoides* (Wethey *et al.* 2011). Ultimately, the production of continuous broods by *A. modestus* is likely to be one of the main factors contributing to its invasion success. Multiple broods act as a buffer for post settlement mortality allowing numbers to remain stable or even increase. Even if some broods are not successful, continuous production will likely ensure that

there will always be some success. The ability to have some level of successful recruitment every year is critical in allowing *A. modestus* to persist within its invaded range.

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Chapter 6

Blurred Lines: The non-native species *Austrominius modestus* at its northern and southern European range limits - current status and factors limiting its abundance

Part of this chapter in revised format has been published in the journal *Estuarine, Coastal and Shelf Science*:

Gallagher MC, Davenport J, Gregory S, McAllen R and O'Riordan R (2015) The invasive barnacle species, *Austrominius modestus*: Its status and competition with indigenous barnacle species on the Isle of Cumbrae, Scotland. Estuarine, Coastal and Shelf Science 152: 134-141. See Appendix 2

A second part of this chapter in revised format (as a short note) is under review at the journal *Marine Biodiversity*:

Gallagher MC, Borges TC, Culloty SC, McAllen R ,and O'Riordan R (2016) Pushing the limits? A non-native species at its southern invaded limit

6.1 Abstract

Despite the threats posed by non-native species, information regarding their distribution, abundance and impacts are often lacking. This type of information is of particular importance at the range limits of non-native species, in order to predict future changes in their abundance and distribution. The current recorded invaded range of the Australasian barnacle *Austrominius modestus* in Europe extends from the Shetland Islands in the North to the Algarve in southern Portugal. Surveys carried out on the Isle of Cumbrae, Scotland and in the Algarve, Portugal, revealed that *A. modestus* is widespread at both locations, but remains less abundant than native barnacle species. Despite a high proportion of *A. modestus* individuals producing embryos, at both locations at temperatures ranging from 7^{o}C - 22^{o}C, native barnacle species were found to dominate removal plots at both locations. However, low levels of recruits of the native species *Semibalanus balanoides* on the Isle of Cumbrae during 2015-2016 did coincide with increased abundances of *A. modestus* recruits in comparison to the previous year. It seems likely that different factors are controlling the abundance of *A. modestus* at its northern and southern limits, with competition playing an important role in the north and desiccation stress potentially limiting abundances in the south. Predicted climate change should favour *A. modestus* and the warm water adapted native species *Chthamalus montagui*. It remains to be seen whether intertidal ecosystems which are dominated by these species will differ in function from those where cold water adapted barnacle species are also present.

KEY WORDS: *Austrominius modestus, Semibalanus balanoides, Chthamalus montagui,* range limits, climate change, temperature, competition, environmental tolerance

6.2 Introduction

Despite the potential threats that invasive species pose to native biodiversity and ecosystems (Vitousek *et al.* 1997; Mack *et al.* 2000; Levin *et al.* 2002; Bax *et al.* 2003; Wright 2005), these species often remain unrecorded for years following their introduction to a new area (Delaney *et al*. 2008) and there is often a general lack of information regarding their distribution, in particular in the marine environment (Ruiz *et al.* 1997). The impacts of invasive species often remain unknown, and when such information is available, it is often restricted spatially (Kochmann *et al.* 2013).

Following the initial introduction of *Austrominius modestus* to Britain, over seventy years ago (Bishop 1947; Crisp and Chipperfield 1948; Stubbings 1950), this species spread rapidly along European coasts (Harms and Anger 1989), facilitated by both remote and marginal dispersal (Crisp 1958). There are many published records of the establishment and distribution of *A. modestus* on European coasts and it has been noted as one of the "best documented examples" of a marine animal to a new range (Barnes and Barnes 1963). This species is now more widespread in Europe than in its native Australasian range (Buckeridge and Newman 2010) of New Zealand, Tasmania and the subtropical and temperate zones of Australia (Moore 1944; Buckeridge and Newman 2010).

Since its introduction, the extent of the invaded limit of *A. modestus* in Europe has fluctuated, as has its abundance at these range limits. Such fluctuations are common at the geographical range limits of a species and are often linked to environmental changes (Southward and Crisp 1954; Southward and Crisp 1956; Brown *et al.* 1996). Temperature, or the tolerance of a species to hot or cold conditions, has traditionally been cited as the main factor controlling the geographic range of a species (Hutchins 1947). However, while temperature can play an important role in determining the northern and southern limits of a species, it may not be the sole determining factor (Davies *et al.* 1998; Helmuth *et al.* 2006), with competition and interspecific interactions also playing an important role (Wethey 2002). In 1958, the range limits of *A. modestus* in Europe were recorded as south-west Scotland in the north and France in the south (Crisp and Southward 1958). By 1963, the southern limit had

been extended to São Martinho de Porto, Portugal (Barnes and Barnes 1963). The current recorded southern limit of this species in Europe is the south coast of the Algarve (O'Riordan and Ramsay 1999; O'Riordan and Ramsay 2013) where it co-occurs in the intertidal zone with the warm water adapted *Chthamalus montagui, C. stellatus* and *Amphibalanus amphitrite* (Southward 2008). The recorded northern limit of *A. modestus* in Europe is the Shetland Islands, where it was first reported in 1978 (Hiscock *et al.* 1978). Although *A. modestus* has persisted on Shetland (Nall *et al.* 2015), it has not become common there (Moore *et al.*, 1995; Moore and Howson 2014). At the northern limits of *A. modestus* the native species *C. montagui* and the cold water adapted *Semibalanus balanoides* are commonly found (Southward 2008). A single individual was recorded in South Africa in 1949, however, the species did not become established in this area (Sandison 1950).

Climate change is predicted to favour non-native species (Zerebecki and Sorte 2011; Buckeridge 2012), which are often able to cope with a wider range of temperatures than native species and have a greater capacity to deal with thermal stress (Zerebecki and Sorte 2011). Despite the importance of temperature tolerances, Ibáñez *et al.* (2006) noted the importance of including differences in reproductive potential, dispersal, changes in biotic interactions and future biodiversity sources when predicting species distribution in a changing climate.

Data regarding species at their invaded range limits are of particular importance, in terms of understanding the factors that determine these limits and predicting how they may change in the future (Herbert *et al.* 2009). The objectives of this study were 1) to assess the abundance and distribution of *A. modestus* close to its northern and southern European limits, in comparison to native barnacle species and 2) to compare the reproduction and recruitment of *A. modestus* with native barnacle species at these locations. Thus, trying to elucidate some factors which may play a role in controlling the invaded range limits of this species and how these limits may change under future climate warming.

6.3 Materials and Methods

6.3.1 Study locations

The Isle of Cumbrae, Scotland and the Algarve, Portugal (Figure 1) were chosen as study sites given their proximity to the northern and southern limits (respectively) of *Austrominius modestus* in Europe and the presence of some pre-existing data on *A. modestus* and native barnacle species at these locations (O'Riordan and Ramsay 1999; O'Riordan *et al.* 2009; O'Riordan and Ramsay 2013).

Figure 1. The location of study sites on the Isle of Cumbrae, Firth of Clyde, Scotland and study sites in the Algarve, Portugal. Map created using ArcGIS.

The Isle of Cumbrae lies in the Firth of Clyde, on the west coast of Scotland. The Clyde Sea is a large fjordic sea lough system (Thomason *et al.* 1997), comprising a

deep, partially enclosed basin, which only experiences limited exchange with adjacent shelf seas over a shallow sill (approx. 45m) (Simpson and Rippeth 1993) and receives a considerable freshwater input (60-700 ms^{-1}) from the River Clyde and other rivers (Poodle 1986). The Isle of Cumbrae is known to experience very high levels of recruitment, in particular of the cold water adapted *Semibalanus balanoides* (Connell 1961; Hills and Thomason 2003). *A. modestus* was discovered on the Isle of Cumbrae in 1955 (Connell 1955) and by 2009, was recorded to be widespread around the island (O'Riordan *et al.* 2009), in contrast to the Shetland Islands where *A. modestus* has persisted (Nall *et al.* 2015) since first being recorded in 1978 (Hiscock *et al.* 1978), but has not become common (Moore *et al.* 1995; Moore and Howson 2014).

The Ria Formosa natural park, which is an area of international ecological importance is located on the coast of the Algarve. It is known as a nursery ground for commercially important fish species and is an important location for aquaculture and tourism (Newton and Mudge 2003; Gamito and Erzini 2005). The Ria Formosa has been described as a coastal lagoon, with two sand spits and a chain of islands separating the lagoon from the sea (Asmus *et al.* 2000). Despite its Atlantic coastal location, the Ria Formosa experiences a Mediterranean climate, with hot dry summers and warm wet winters. Annual seawater temperatures range from 12^{o}C to 24^{o}C (Falcao and Vale 1990). The majority (50% - 75%) of water within the Ria is exchanged daily (Newton and Mudge 2003), producing a low retention time (Asmus *et al.* 2000). Within the Ria Formosa, there is a lack of natural rocky substrates, with artificial structures such as pier pilings and pontoons and small rocks comprising the majority of the area available for sessile invertebrates to colonise. The most southerly published record of *A. modestus* in Europe is Praia de Faro, in the Ria Formosa (O'Riordan and Ramsay 2009; O'Riordan and Ramsay 2013).

6.3.2 Current status of Austrominius modestus *at its European range limits*

Southern limit

Prior to study site selection, sixteen locations along the coast of the Algarve were surveyed for the presence of *Austrominius modestus* in July 2014 (Table 1; Figure 2).

Rapid assessment surveys (RAS) of artificial structures have previously been used to assess the distribution of invasive species in the south of England (Arenas *et al.* 2006) and a modified RAS was carried out at these sites. This modified version focused solely on recording the presence or absence of *A. modestus* at the survey sites. Once *A. modestus* had been encountered at a site it was recorded as present and if *A. modestus* was not found during a thirty - minute search, it was recorded as absent from the site. This allowed for many sites to be surveyed over a short time period.

In addition to this, at seven of the survey sites, twenty quadrats (25 cm^2) were randomly surveyed to produce an estimate of the barnacle cover and species composition at the sites, by counting the abundance of each barnacle species present in each quadrat. Locations where barnacles were only present on small stones or railings, or areas which were not easily accessed, were considered unsuitable for an abundance survey. O'Riordan and Ramsay (2013) had previously carried out surveys at Alvor and Albuferia and their data regarding the presence of *A. modestus* have been utilised here (Table 1).

Table 1. Name, GPS location, occurrence or non-occurrence of abundance estimate for *Austrominius modestus* and source of data for eighteen sites along the coast of the Algarve, Portugal.

Site number	Site name	Latitude, Longitude (GPS)	Abundance estimate	Source
1	Fàbrica	N 37°09.20' W 7°33.24'	X	2014 survey
2	Cabanas	N 37°08.03' W 7°36.11'	X	2014 survey
3	Quatro Aguas	N 37°06.96' W 7°37.80'	✓	2014 survey
4	Ihla de Tavira	N 37°06.99' W 7°37.82'	X	2014 survey
5	Santa Luzia	N 37°06. 99' W 7°37. 82'	X	2014 survey
6	Moimho de Maré	N 37°01. 84' W 7°48. 89'	X	2014 survey
7	Ihla de Armona	N 37°01.40' W 7°48.37'	✓	2014 survey
8	Olhão	N 37° 01.36' W 7°50.20'	X	2014 survey
9	Farol (Ihla de Culatra)	N 36°58.66' W 7°51.96'	✓	2014 survey
10	Faro	N 37°00' 57' W 7°56'17'	X	2014 survey
11	Praia de Faro	N 37°05.17' W 7°59.663'	✓	2014 survey
12	Quarteira	N 37°04.07' W 8°06.50'	✓	2014 survey
13	Praia de Oura	N 37°05'4.8'' W 8°13'0.9''	X	O'Riordan and Ramsay (2013)
14	Alvor Harbour	N 37°07'40.6'' W 8°35'45''	X	O'Riordan and Ramsay (2013)
15	Meia Praia	N 37°07.23' W 8°37.21'	✓	2014 survey
16	Meia Praia (Lagos end)	N 37°06.02' W 8°40.06'	✓	2014 survey
17	Lagos	N 37°06' 18' W 8°40' 21'	X	2014 survey
18	Praia da Dona Ana	N 37°05.83' W 8°40.09'	X	2014 survey

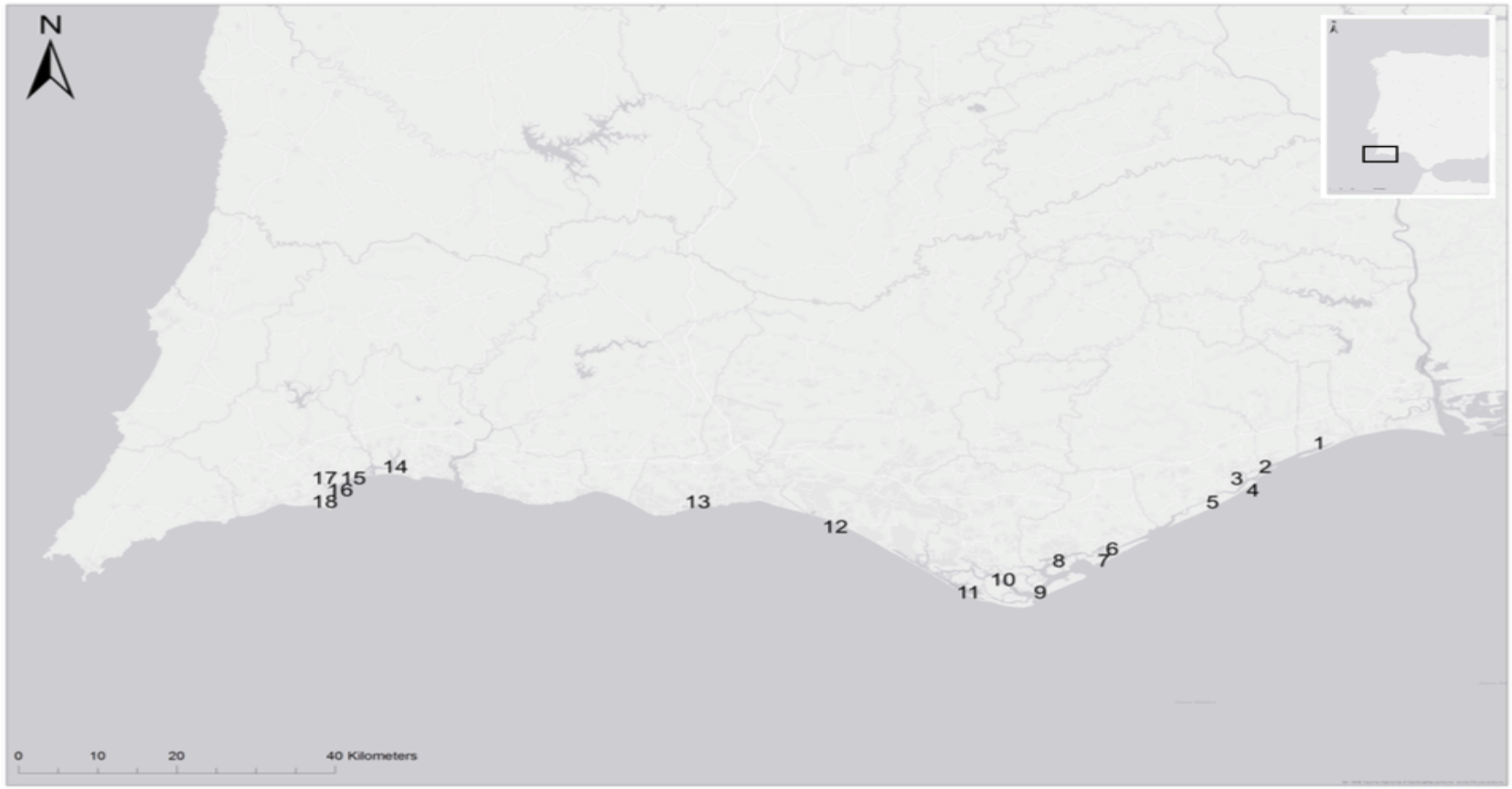

Figure 2. Location of eighteen survey sites on the coast of the Algarve, Portugal. Inset map shows location of enlarged area. For site names and details see Table 1. Map created using ArcGIS.

Northern limit

On the Isle of Cumbrae, Scotland, thirteen sites had previously been surveyed in March 2013 (Table 2; Figure 3). Three line transects were carried out at each site, starting where barnacles first appeared on the shore and ending at the low tide mark. In the case of piers and slipways, transects were conducted along the vertical side of the structure. Stations were located along the transect at 40cm drops in vertical height which were measured using the 'two pole method', where one person stood at the upper shore of the transect line holding a 0.6 m pole in a vertical position, while a second person moved down the transect line with a 1 m pole. At the point where the tops of the two poles were in line with each other, and with the horizon, a drop in shore height of 40 cm had been reached and the next station could be established. At each station ten 10cm x 10cm quadrats were sampled haphazardly for each of the three barnacle species separately (i.e. 30 quadrats per station). Dead barnacles or spat were not recorded.

Table 2. Name and GPS location of thirteen sites surveyed on the Isle of Cumbrae, Scotland in March 2013.

Site number	Site name	Latitude, Longitude (GPS)
1	White Bay	N55°47.50' W4°54.88'
2	Ferry Slipway	N55°47.91' W4°53.90'
3	Watersports slip (North)	N55°46.58' W4°53.55'
4	Watersports slip (South)	N55°46.58' W4°53.55'
5	Butter Lump	N55°45.55' W4°56.42'
6	Farland Bight	N55°44.44' W4°54.41'
7	Farland Point	N55°47.05' W4°54.84'
8	Kames Bay	N55°42.01' W4°55.00'
9	Millport Harbour Wall	N55°45.08' W4°55.95'
10	West Bay	N55°44.95' W4°56.10'
11	Sherriff's Point	N55°45.16' W4°57.02'
12	Fintry Bay	N55°45.60' W4°56.42'
13	Skate Point	N55°47.05' W4°55.39'

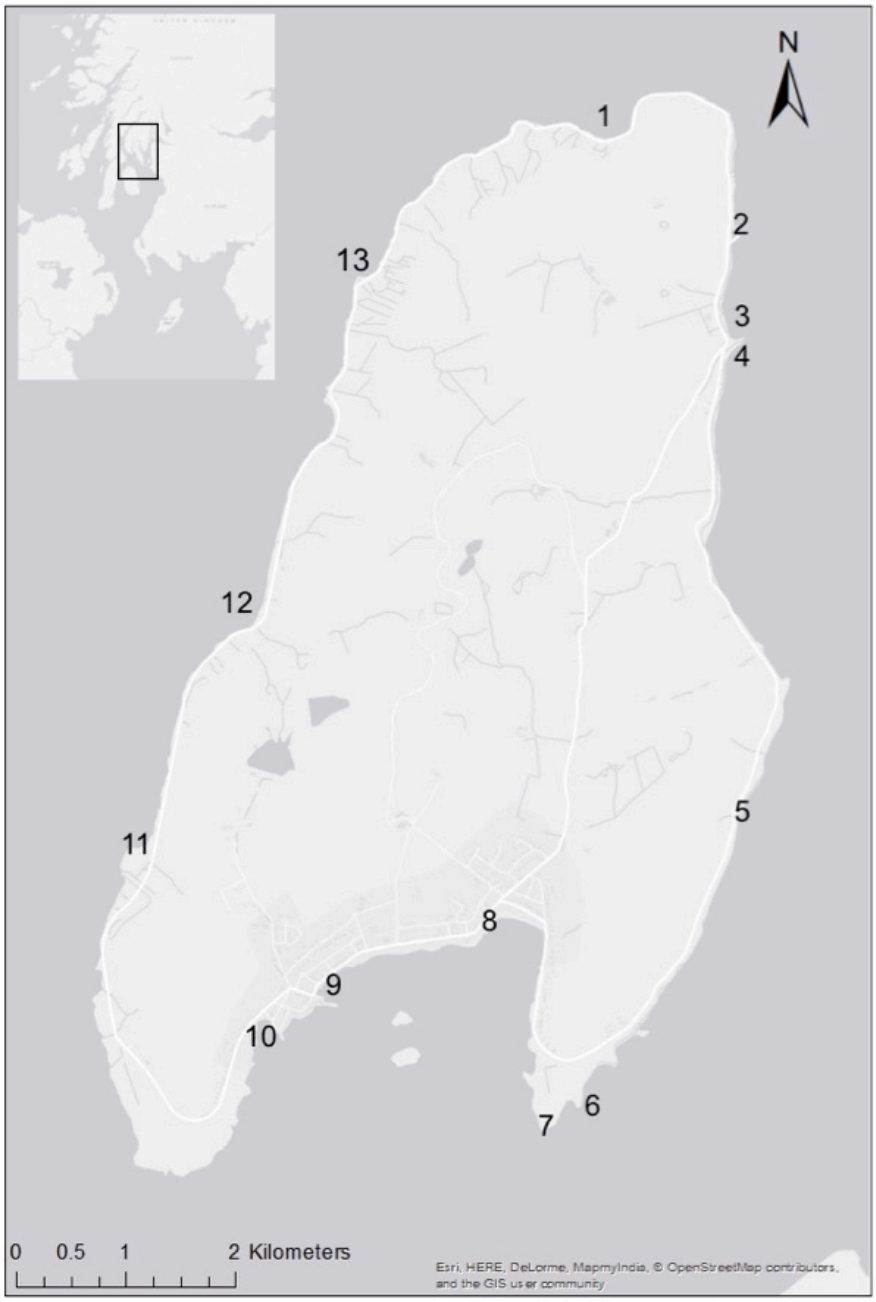

Figure 3. Location of thirteen survey sites on the Isle of Cumbrae, Scotland. Inset map shows location of enlarged area. For site names and details see Table 2. Map created using ArcGIS.

6.3.3 Recruitment of Austrominius modestus *at its northern and southern limits*

Study sites

To examine the recruitment of *A. modestus* in comparison to native barnacle species, four field sites were each established in the Algarve and on the Isle of

Cumbrae. In the Algarve, study sites were located within the Ria Formosa natural park, given its ecological importance and the potential effects of *A. modestus* on native species. Ihla da Armona, Quatro Águas, Praia de Faro and Farol (Figure 4), were selected due to the relative abundance of *A. modestus* in comparison to native species and their suitability for the establishment of experimental plots.

Figure 4. Study sites in the Algarve, Portugal: Ihla da Armona (1), Quatro Águas (2), Praia de Faro (3) and Farol (4). Source: author's own.

On the Isle of Cumbrae, Millport Harbour Wall, Watersports Slipway, Butterlump and Kames Bay (Figure 5) were selected based on the abundance of *A. modestus* recorded during the 2013 survey, their suitability for the establishment of experimental plots and the fact they comprised both sheltered and semi-exposed shores.

Figure 5. Study sites on the Isle of Cumbrae, Scotland: Millport Harbour Wall (1), Watersports Slipway (2), Butterlump (3) and Kames Bay (4). Source: author's own.

Fieldwork was carried out at each location (Portugal and Scotland) on a seasonal, biannual basis, with the exception of July – August 2014. During this eight week period fieldwork was carried out on a fortnightly basis in the Algarve, to collect detailed data regarding *A. modestus* at its southern range limits, during the period of warmest seawater temperatures. Subsequent visits to southern field sites were carried out in February and August 2015 and February 2016. At the northern sites, fieldwork was carried out in March and September 2014, March and September 2015 and March 2016.

Removal plots

In order to examine the recruitment of both *A. modestus* and native barnacle species, removal plots were established at all of the study sites. Removal plots measured 25 cm^2 (5cm x 5cm). Six removal plots were located at areas approximate to the high, mid and low shore levels at each site (i.e. eighteen plots per site). High

shore was taken to be the area where barnacle cover began, low shore where barnacle cover ended and mid shore as the area in between. A photograph recording the area prior to clearance was taken. The plot area, plus an edge of at least 1cm on each side of the plot, was cleared using a paint scraper. The cleared surface was then scoured with wire wool to ensure that all organisms had been removed. A red paint mark was placed on the rock surface indicating the location of the top left hand corner of the quadrat and was used to relocate removal plots. These plots were resurveyed on each field visit and were photographed on each occasion, using the paint mark to ensure the same area was photographed on each visit. The number of recruits of each species present in each plot was subsequently counted from these images. To examine recruitment at distinct spatial scales in addition to long-term recruitment, half of the removal plots were re-cleared in autumn 2014 (Scotland) and spring and autumn 2015 (Scotland and Portugal). In Scotland in March 2015, all removal plots were re-cleared as original removal plots from March 2014 were becoming difficult to distinguish from the surrounding community and paint marks identifying the location of the plot area were not surviving the six-month interval between field visits.

6.3.4 Reproductive state

Samples of *A. modestus* and a native barnacle species (*Chthamalus montagui* in Portugal and *Semibalanus balanoides* in Scotland) were collected on each field visit, in order to record whether or not they were brooding embryos and if embryos were present, at what stage of development. In the Algarve, Farol was selected for sample collection as it had the highest abundance of *A. modestus* of all sites surveyed and both *A. modestus* and *C. montagui* were abundant on small rocks which could be collected and brought to the laboratory. Although *C. montagui* co-occurs intertidally with *A. modestus* throughout its entire invaded range, it was not chosen for screening in Scotland. This was because *C. montagui* was rarely found growing on small rocks, or any suitable substrate, which could be brought to the laboratory, so *S. balanoides* was selected instead. However this species is not present as far south as Portugal, and so could not be examined at the southern field sites. On the Isle of Cumbrae, samples were collected from two sites, Fintry Bay and Millport Harbour.

Screening of specimens from both of these sites had previously been carried out, so data collected during the present study could be used for comparison.

On each field visit, a selection of small rocks and limpet shells with both barnacle species growing on them were collected from the mid level of the shore and brought to the laboratory. Samples were stored in tanks with aerated seawater and were always examined within 72 hours of collection. Sixty individuals of each species from each field site were examined under the microscope (Portugal: Zeiss Stemi 2000 – C x 5 magnification; Scotland: Olympus BH-2 x 5 magnification). Each individual was removed from the rock using a scalpel and placed on the edge of a well side. The rostro-carinal diameter and opercular diameter were measured using digital calipers (Silver Line Tools, digital vernier calipers). The body mass of the individual was removed with fine forceps and placed in the well of the slide with some ethanol. The inside of the shell was scraped out using fine forceps and dipped into the ethanol to ensure the entire specimen was removed. Using a pointer, the body mass was manipulated to allow for thorough examination under the microscope. If embryos were present their developmental state was scored using the scale provided in O'Riordan and Murphy (2000) for *A. modestus*, O'Riordan *et al.* (1995) for *C. montagui* and Barnes and Barnes (1974) for *S. balanoides*.

6.3.5 Seawater Temperature

Seawater temperature data recorded for the Isle of Cumbrae during the study period was obtained from Marine Scotland. Mean (± SE) monthly values were then calculated. Mean monthly seawater temperatures for the Algarve were obtained from the global seawater temperature website (www.1).

6.3.6 Statistical Analysis

Data analysis was carried out using RStudio Software 3.3.0 (R Core Team 2016). Differences in the proportion of individuals with embryos at all developmental stages and at stages 4-4h were compared using the "prop.test" function in RStudio. Sequential Holm-Bonferroni corrections were applied to the p values in order to reduce the probability of Type 1 error (Holm 1979; Getano 2013) using a Holm-

Bonferroni sequential correction EXCEL calculator (Gaetano 2013). Abundance of recruits was analysed using negative binomial regression, using site and shore height as predictors. This test was used as number of recruits is count data and there was an excess of zeros in the data. Negative binomial distribution was used as opposed to a Poisson distribution as it had a better fit for the data (lower AIC and better residual plots). A negative binomial distribution also accounts for over dispersion. Model validation was carried out by plotting the residuals against the fitted values and creating histograms and QQ-plots of the residuals.

6.4 Results

6.4.1 Status of Austrominius modestus *at its range limits*

Southern limit

Austrominius modestus was found to be widespread along the coast of the Algarve in July 2014, being recorded at fifteen out of eighteen sites surveyed (Figure 6). The native *C. montagui* was the dominant barnacle species at all sites surveyed, ranging from 92% to 100% relative abundance (Figure 6). The lowest mean abundance of this species was 660.4 (± 42.56) ind. 100 cm^{-2} at Quatro Águas (Table 3). Despite being widespread, *A. modestus* was present at much lower abundances than *C. montagui*, ranging from a mean of 0.8 (± 0.80) ind. 100 cm^{-2} to 79.2 (± 12.68) ind. 100 cm^{-2} at sites where it was present (Table 3), with a maximum relative abundance of 8% at Farol (Figure 6). *C. stellatus* and *Ampibalanus amphitrite* were also recorded at a number of survey sites, but at relatively low abundances (Table 3).

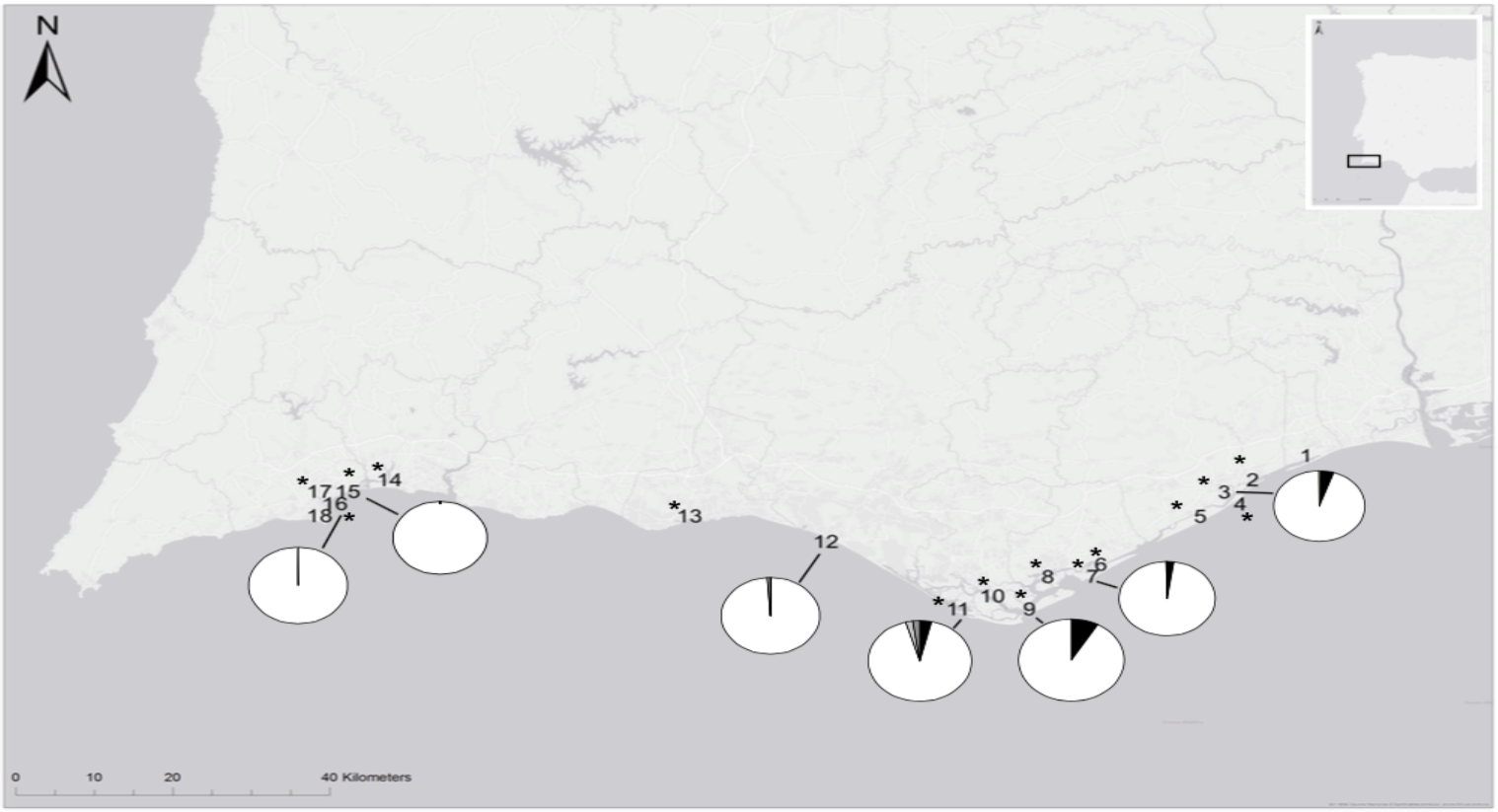

Figure 6. Distribution of *Austrominius modestus* on the southern coast of the Algarve in July 2014. An asterix denotes the presence of *A. modestus* at a survey site. Mean relative abundance of *Austrominius modestus* (black), *Chthamalus montagui* (white), *C. stellatus* (light grey) and *Amphibalanus amphitrite* (dark grey) is shown for seven sites where abundance estimates were carried out. For site details see Table 1.

Table 3. Mean abundance (± standard error) per 100cm^2 of four barnacle species (*Austrominius modestus, Chthamalus montagui*, *C. stellatus* and *Amphibalanus amphitrite*) at seven sites surveyed in the Algarve Portugal in July 2014.

	Austrominius modestus	*Chthamalus montagui*	*Chthamalus stellatus*	*Amphibalanus amphitrite*
Meia Praia (Lagos)	0.8 ± 0.80	782.0 ± 86.88	2.0 ± 0.96	0.0 ± 0.00
Meia Praia	0.0 ± 0.00	795.2 ± 73.12	0.0 ± 0.00	0.0 ± 0.00
Quarteira	0.0 ± 0.00	791.6 ± 65.00	8.8 ± 3.36	0.0 ± 0.00
Praia de Faro	40.4 ± 6.04	1004.8 ± 60.36	24.4 ± 0.12	24.4 ± 6.80
Farol (Ihla de Culatra)	79.2 ± 12.68	868.4 ± 57.16	0.0 ± 0.00	0.1 ± 0.12
Ihla da Armona	17.6 ± 3.48	752.0 ± 116.36	0.0 ± 0.00	2.4 ± 1.96
Quatro Águas	35.6 ± 5.84	660.4 ± 42.56	1.2 ± 0.40	0.0 ± 0.00

Northern limit

A. modestus was also found to be widespread on the Isle of Cumbrae and was recorded at all thirteen survey sites on the island in 2013. The native *S. balanoides* was the most abundant barnacle species at twelve of the thirteen survey sites, with a maximum abundance of 132.3 (± 9.32) ind. 100 cm^{-2} at Watersports Slip (South) (Table 4; Figure 7). At the remaining survey site, Farland Point, *C. montagui* was the most abundant barnacle species 57.4 (± 8.16) ind. 100 cm^{-2} (Table 4; Figure 8). *A. modestus* was not the dominant barnacle species at any of the survey sites (Figure 7), but was present at relatively high abundances at sites in the north east of the island, with a maximum of 45.5 (± 6.73) ind. 100 cm^{-2} at Watersports Slip (south) (Table 4; Figure 7).

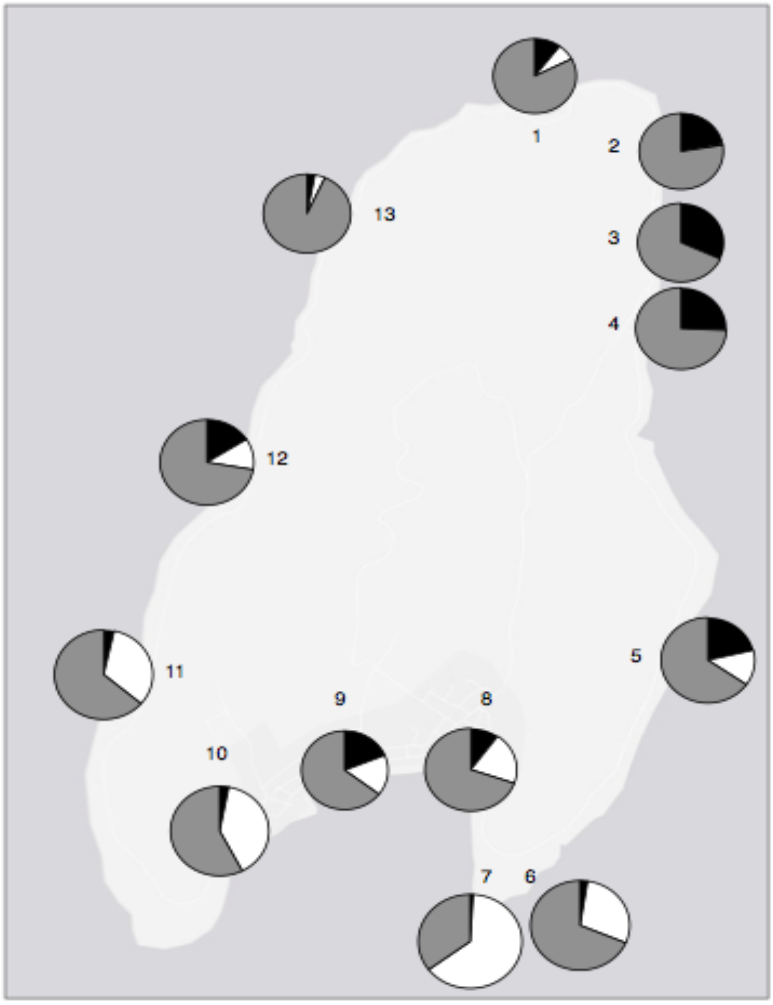

Figure 7. Relative abundance of *Austrominius modestus* (black), *Chthamalus montagui* (white) and *Semibalanus balanoides* (grey) at thirteen sites on the Isle of Cumbrae, Scotland, surveyed from the 3rd – 6th of March 2013.

Table 4. Mean abundance (± standard error) per 100cm^2 of three barnacle species (*Austrominius modestus, Chthamalus montagui* and *Semibalanus balanoides*) at thirteen sites surveyed on the Isle of Cumbrae in March 2013.

	Austrominius modestus	*Chthamalus montagui*	*Semibalanus balanoides*
White Bay	11.8 ± 1.95	8.2 ± 1.72	94.6 ± 5.66
Ferry Slipway	15.1 ± 1.46	0.4 ± 0.14	53.9 ± 3.17
Watersports Slip (North)	38.0 ± 6.38	0.0 ± 0.00	81.1 ± 5.56
Watersports Slip (South)	45.5 ± 6.73	0.3 ± 0.23	132.3 ± 9.32
Butterlump	6.6 ± 0.86	4.2 ± 1.08	20.2 ± 2.35
Farland Bight	2.4 ± 0.44	28.3 ± 4.98	66.9 ± 8.28
Farland Point	0.9 ± 0.19	57.4 ± 8.16	31.7 ± 3.22
Kames Bay	9.2 ± 2.11	18.8 ± 3.65	64.3 ± 4.66
Millport Harbour Wall	14.1 ± 1.58	12.5 ± 2.21	48.2 ± 3.54
West Bay	3.3 ± 0.83	42.9 ± 5.57	62.7 ± 4.20
Sheriff's Point	6.0 ± 0.87	57.1 ± 7.55	111.0 ± 6.40
Fintry Bay	13.6 ± 2.02	10.1 ± 1.41	61.7 ± 4.34
Skate Point	2.2 ± 0.48	3.0 ± 0.71	72.1 ± 6.00

6.4.2 Reproductive state

Southern limit

On each sampling occasion during July and August 2014 both *A. modestus* and *C. montagui* were found to have a proportion of individuals with embryos at developmental stages 2-4h. A higher proportion of *A. modestus* individuals were recorded with embryos on each date (85%, 45%, 50% and 66.66% respectively) in comparison to *C. montagui* (53%, 42%, 43% and 42% respectively) (Figure 8). However, these differences were only found to be significant on the first sampling date (p = 0.01; Table 5). A higher proportion of *A. modestus* individuals were also recorded to have embryos at stages 4-4h on each sampling date (58%, 10%, 18% and 28% respectively) in comparison to *C. montagui* (20%, 17%, 5% and 7% respectively) (Figure 8). These differences were statistically significant on three of the sampling occasions (Table 5).

Table 5. ."Prop.test" results with Holm-Bonferroni sequential correction (Holm 1979; Gaetano 2013), comparing the proportion of individuals of two barnacle species *Austrominius modestus* and *Chthamalus montagui* collected from Farol, Ihla de Culatra, Algarve Portugal with embryos at all developmental stages and embryos at stages 4-4h (ready to hatch) at different time intervals between July 2014 and February 2016 and for each species in August 2014 and 2015 and February 2015 and 2016. Significant p values ($p < 0.05$) are highlighted in bold.

	All embryo stages			Stages 4-4h		
	χ2	df	p	χ2	df	p
CM/AM						
21/07/14	12.66	1	**0.01**	16.928	1	**0.01**
01/08/14	0.033	1	1.00	0.109	1	1.00
18/08/14	0.301	1	1.00	3.962	1	0.32
28/08/14	6.579	1	0.10	8.311	1	**0.02**
22/02/15	2.727	1	0.63	0.606	1	1.00
17/08/15	4.071	1	0.36	0.000	1	1.00
10/02/16	3.497	1	0.48	7.687	1	**0.04**
2014/2015/2016						
AM Aug	1.259	1	1.00	10.14	1	**0.01**
CM Aug	0.317	1	1.00	0.175	1	1.00
AM Feb	0.133	1	1.00	0.727	1	1.00
CM Feb	0.337	1	1.00	0.508	1	1.00

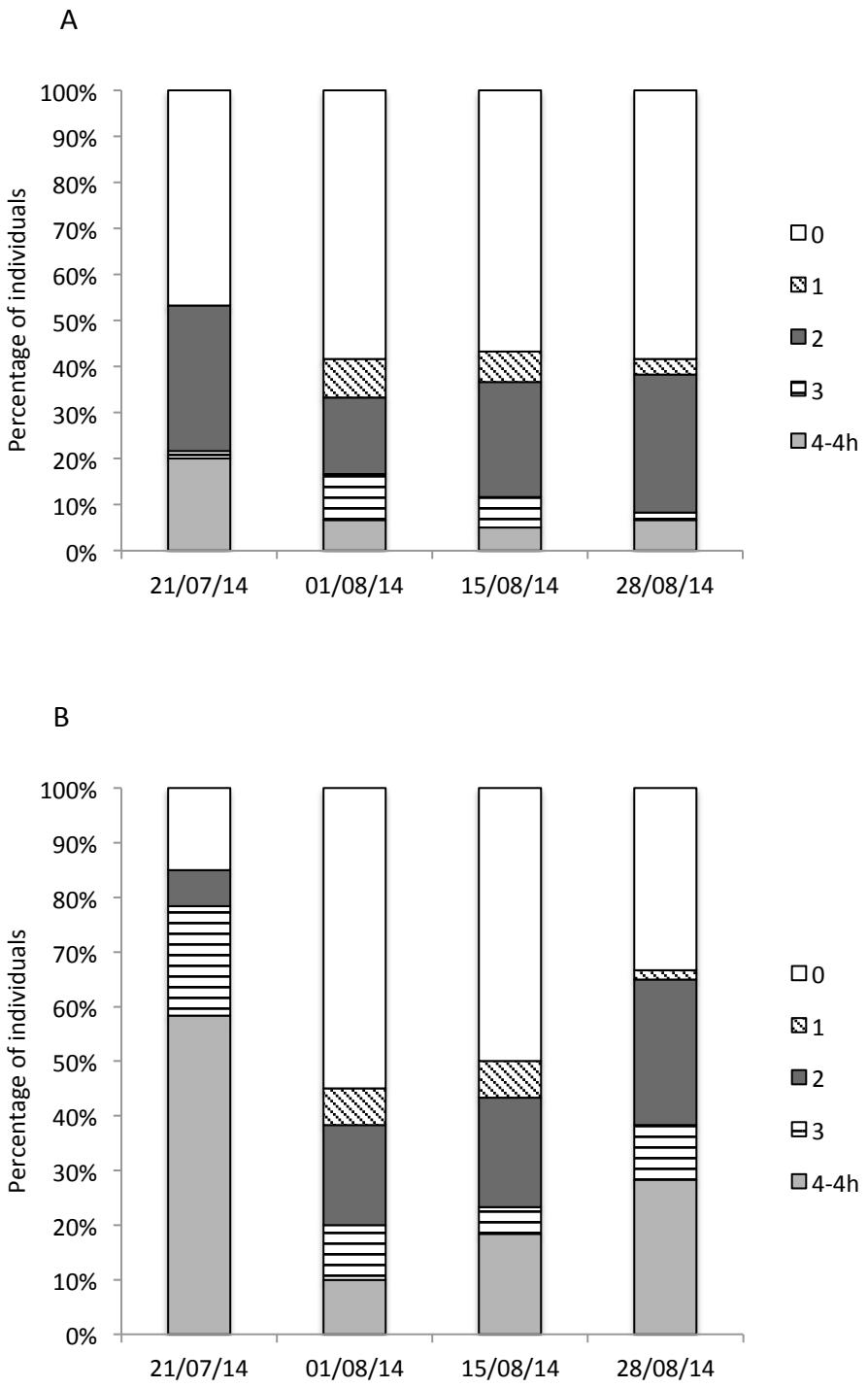

Figure 8. Percentage of individuals of *Chthamalus montagui* (A) and *Austrominius modestus* (B) without embryos (0) or with embryos at developmental stages 1-4h (following O'Riordan and Murphy 2000) from Farol, Ihla de Culatra, Portugal in July and August 2014, (n = 60 individuals of each species on each sampling occasion).

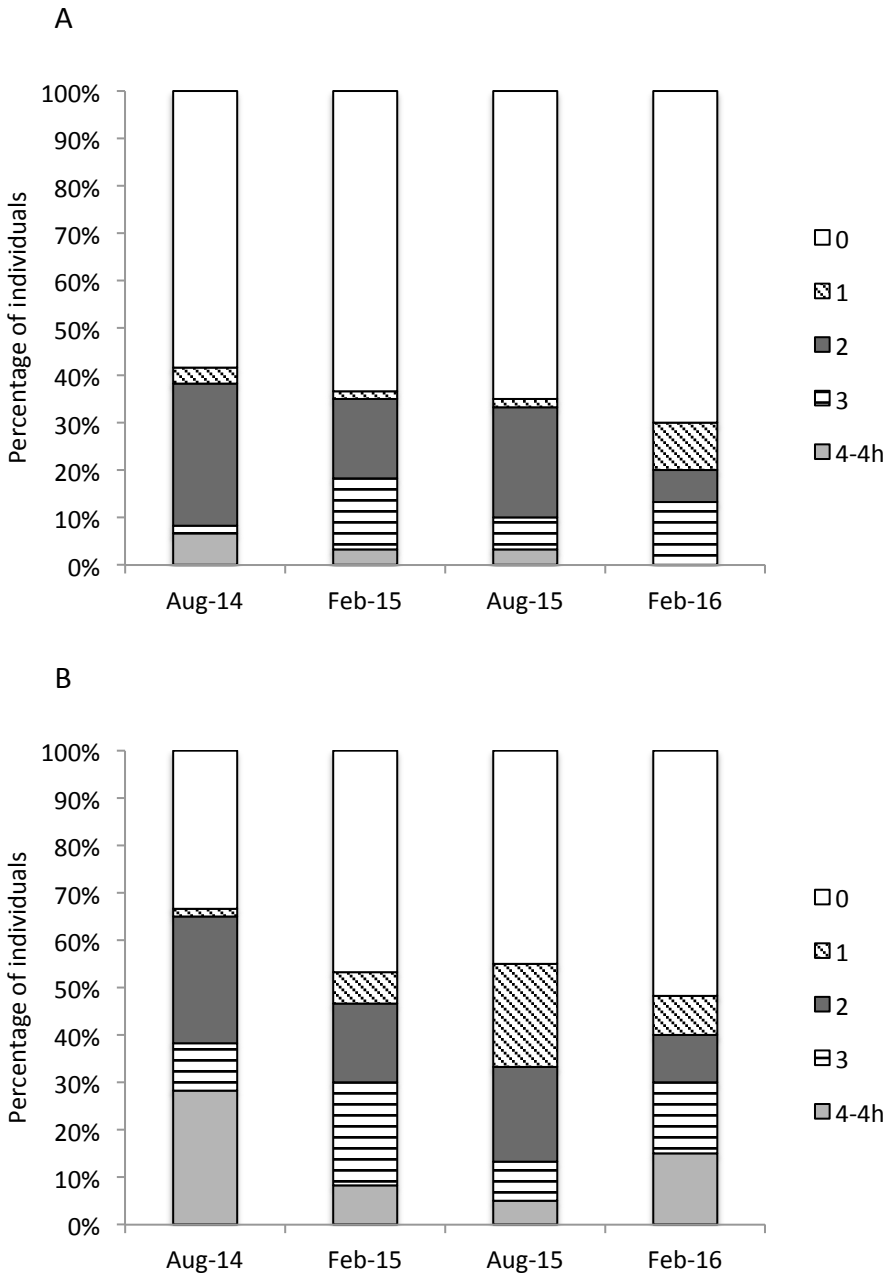

Figure 9. Percentage of individuals of *Chthamalus montagui* (A) and *Austrominius modestus* (B) without embryos (0) or with embryos at developmental stages 1-4h (following O'Riordan and Murphy 2000) from Farol, Ihla de Culatra, Portugal in August 2014, February and August 2015 and February 2016 (n = 60 individuals of each species on each sampling occasion). Note that 28/08/2014 (Figure 9) was used to represent August 2014 in this Figure.

A proportion of individuals of both species were also recorded to possess embryos at all developmental stages in February and August 2015 and February 2016, with the exception of *C. montagui* which had no individuals with embryos at stages 4-4h in February 2016 (Figure 9). On each sampling occasion a higher proportion of *A.*

modestus individuals were recorded with embryos (67%, 53%, 55% and 48% respectively) in comparison to 42%, 37%, 35% and 30% respectively for *C. montagui* (Figure 9). A higher proportion of *A. modestus* individuals were also recorded with embryos at stages 4-4h (28%, 8%, 5% and 15%) in comparison to individuals of *C. montagui* (7%, 3%, 3% and 0% respectively). This difference was found to be significant in August 2014 and February 2016 (Table 5). There were no significant differences in the proportion of individuals of either *A. modestus* or *C. montagui* recorded with embryos in August 2014 in comparison to August 2015 or February 2015 in comparison to February 2016 (Table 5). A significantly ($p < 0.01$) lower proportion of *A. modestus* individuals were recorded with embryos at stages 4-4h in August 2015 (5%) in comparison to August 2014 (28%). There was no significant difference in the proportion of individuals of *C. montagui* recorded with embryos at stages 4-4h between these two time periods, or for either species between February 2015 and February 2016 (Table 5).

Northern limit

The majority (97% - 100%) of *S. balanoides* individuals screened from both Millport Harbour and Fintry Bay were found to be without embryos in September 2014 and September 2015 (Figure 10), however a proportion of individuals of this species were recorded brooding embryos at a range of developmental stages including 4-4h in March 2014, 2015 and 2016 from both sampling sites (Figure 10). There were differences in the proportion of *S. balanoides* individuals brooding embryos at all developmental stages and embryos at stages 4-4h both between years and between sampling location (Table 6). In March 2014, a significantly higher proportion of *S. balanoides* individuals were recorded to be brooding embryos (all stages) and embryos at stages 4-4h from Fintry Bay in comparison to Millport Harbour ($p = 0.01$; Table 6). There was no significant difference found between locations in March 2015, however, at Fintry Bay significantly fewer individuals were recorded with embryos (all stages and at stages 4-4h) in comparison to March 2014 ($p = 0.01$; Table 6). There were also fewer individuals recorded with embryos at Millport Harbour in March 2015 (37%) in comparison to March 2014 (50%) (Figure 10), but this difference was not found to be significant (Table 6). Only 7% of individuals were

recorded with embryos at stages 4-4h from Millport Harbour in March 2015, significantly lower than March 2014 (50% of individuals) ($p = 0.01$; Table 6) (Figure 11). In March 2016, 100% of *S. balanoides* individuals from Fintry Bay were recorded with embryos (all stages), significantly higher than Millport Harbour where 85% of individuals were recorded with embryos ($p = 0.04$; Table 6). In contrast, a higher proportion of individuals were recorded with embryos at stages 4-4h from Millport Harbour (40%) in comparison with Fintry Bay (22%) at this time.

A proportion (3% - 28%) of *A. modestus* individuals were recorded to have embryos at stages 4-4h from both sampling locations at all time periods, with the exception of Fintry Bay in March 2015 (Figure 11). There were no significant differences in the proportion of *A. modestus* individuals recorded brooding embryos (all stages) or embryos at stages 4-4h at any time period at Millport Harbour or Fintry Bay (Table 7). In March 2014, all *A. modestus* individuals recorded with embryos at Fintry Bay were at stages 4-4h (28%). This was significantly higher than March 2015, when no individuals were found with embryos at this developmental stage at this location ($p = 0.01$; Table 7) and only 5% of individuals were recorded with embryos at any developmental stage, compared to 28% in March 2014 ($p = 0.01$; Table 7) (Figure 11). Over the study period, *S. balanoides* had a higher proportion of individuals with embryos in March as opposed to September (Figure 12), while a higher proportion of *A. modestus* individuals were recorded with embryos in September compared to March (Figure 11).

Table 6. "Prop. test" results with Holm-Bonferroni sequential correction (Holm 1979; Gaetano 2013), comparing the proportion of individuals of *Semibalanus balanoides* from two sites, Millport Harbour and Fintry Bay on the Isle of Cumbrae with embryos at all developmental stages and embryos at stages 4-4h (ready to hatch) at different time intervals between March 2014 and March 2016. Significant p values (< 0.05) are highlighted in bold.

	All embryo stages			Stages 4-4h		
	χ2	df	p	χ2	df	p
Harbour/Fintry						
Mar-14	28.25	1	**0.01**	28.25	1	**0.01**
Sep-14	0.50	1	1.00		NA	
Mar-15	0.85	1	1.00	2.32	1	0.50
Sep-15	0.00	1	1.00		NA	
Mar-16	7.68	1	**0.04**	3.90	1	0.24
Sept 14/Sept 15						
Harbour	0.00	1	1.00	0.00	1	1.00
Fintry	0.00	1	1.00	0.00	1	1.00
March 14/March 15						
Harbour	1.66	1	1.00	25.65	1	**0.01**
Fintry	31.62	1	**0.01**	104.8	1	**0.01**
March 15/March 16						
Harbour	27.42	1	**0.01**	16.81	1	**0.01**
Fintry	40.95	1	**0.01**	12.42	1	**0.01**
March 14/March 16						
Harbour	15.19	1	**0.01**	0.84	1	1.00
Fintry	1.36	1	1.00	63.39	1	**0.01**

Table 7. "Prop. test" results with Holm-Bonferroni sequential correction (Holm 1979; Gaetano 2013), comparing the proportion of individuals of *Austrominius modestus* from two sites, Millport Harbour and Fintry Bay on the Isle of Cumbrae with embryos at all developmental stages and embryos at stages 4-4h (ready to hatch) at different time intervals between March 2014 and March 2016. Significant p values (< 0.05) are highlighted in bold.

	All embryo stages			Stages 4-4h		
	χ2	df	p	χ2	df	p
Harbour/Fintry						
Mar-14	3.23	1	0.79	3.23	1	0.64
Sep-14	2.85	1	0.81	28.25	1	1.00
Mar-15	0.48	1	1.00	0.00	1	1.00
Sep-15	0.03	1	1.00	1.92	1	1.00
Mar-16	0.00	1	1.00	0.00	1	1.00
Sept 14/Sept 15						
Harbour	0.85	1	1.00	3.60	1	0.57
Fintry	0.91	1	1.00	0.40	1	1.00
March 14/March 15						
Harbour	0.08	1	1.00	4.32	1	0.40
Fintry	10.14	1	**0.01**	17.54	1	**0.01**
March 15/March 16						
Harbour	2.94	1	0.80	0.25	1	1.00
Fintry	6.85	1	0.09	0.50	1	1.00
March 14/March 16						
Harbour	1.39	1	1.00	1.60	1	1.00
Fintry	0.17	1	1.00	12.25	1	**0.01**

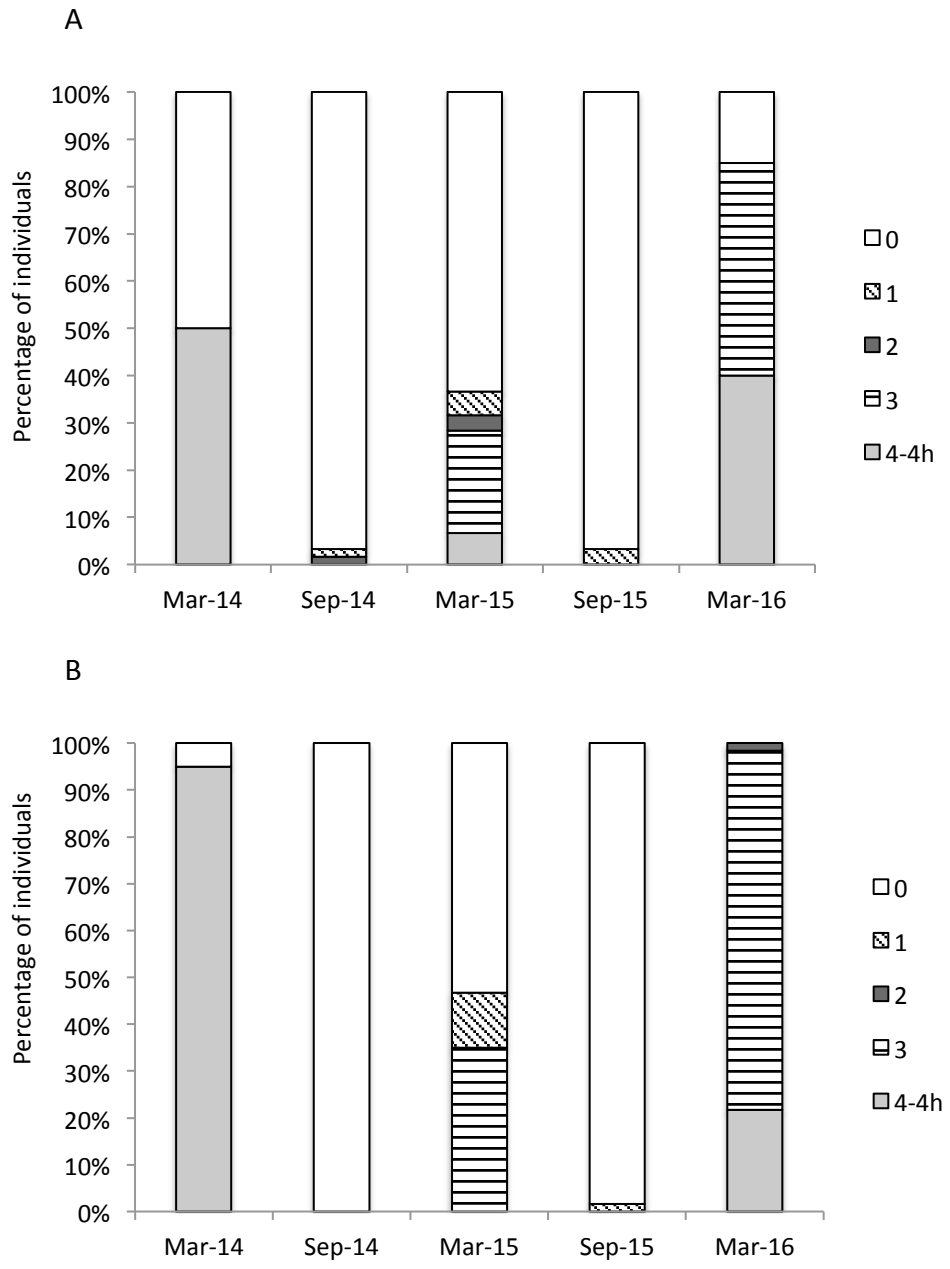

Figure 10. Percentage of individuals of *Semibalanus balanoides* at Millport Harbour (A) and Fintry Bay (B), Isle of Cumbrae, Scotland, without embryos (0) or with embryos at developmental stages 1-4h (following O'Riordan and Murphy 2000) in March and September 2014, March and September 2015 and March 2016 (n = 60 individuals of each species on each sampling occasion).

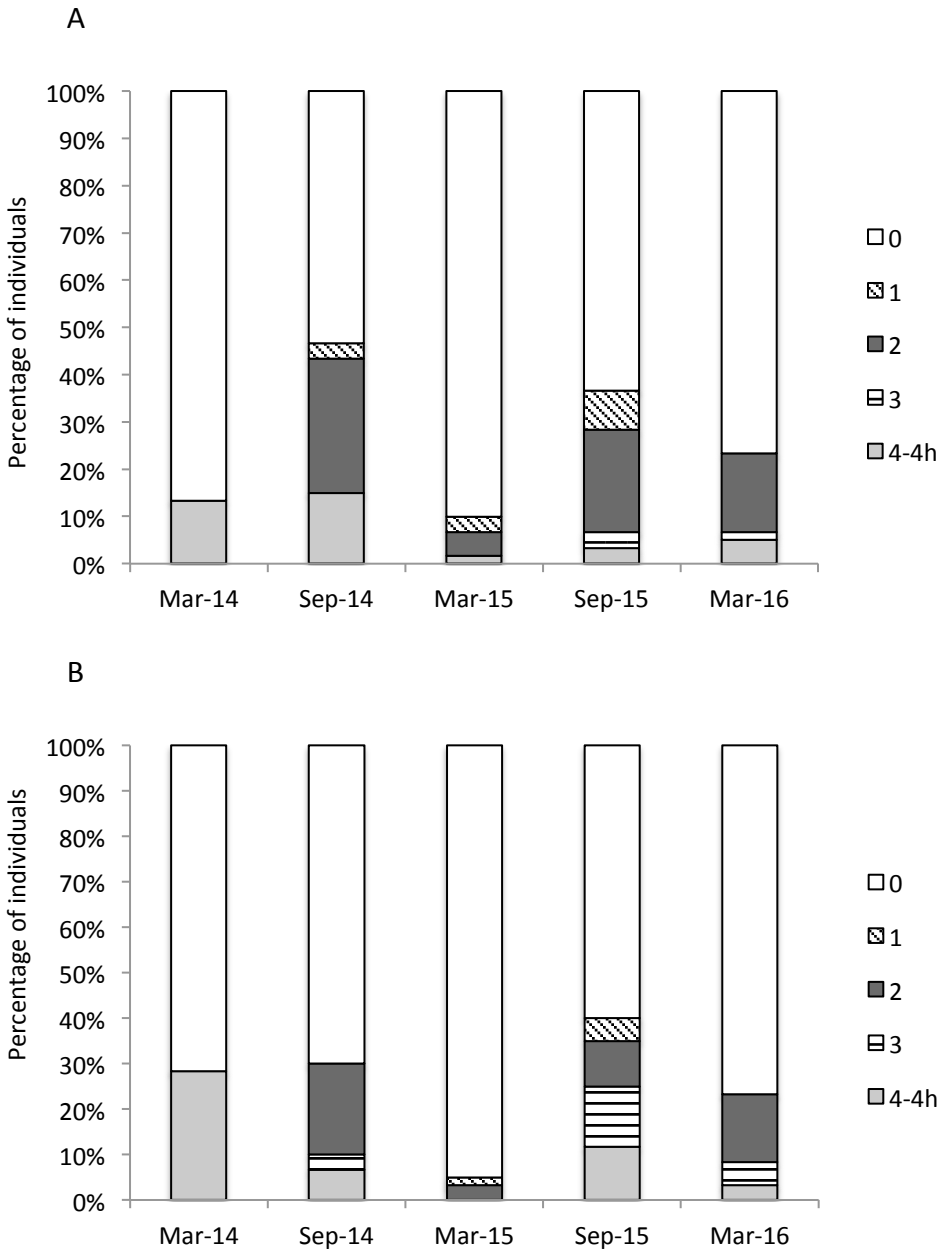

Figure 11. Percentage of individuals of *Austrominius modestus* at Millport Harbour (A) and Fintry Bay (B) Isle of Cumbrae, Scotland, without embryos (0) or with embryos at developmental stages 1-4h (following O'Riordan and Murphy 2000) in March and September 2014, March and September 2015 and March 2016 (n = 60 individuals of each species on each sampling occasion).

6.4.3 Recruitment

Southern limit

The native *C. montagui* was the dominant recruit in removal plots at all sites throughout the entire study (Figures 12-19). A maximum density of 510.3 (± 115.61)

individuals per 25cm^2 was recorded at Farol, in August 2014 (Figure 15). During July and August 2014, *C. montagui* recruits were most abundant at Farol and Ihla da Armona (Figures 12 and 15), while lowest densities were recorded at Praia da Faro (0.2 ± 0.12 individuals per 25cm^2 – 4.5 ± 1.46 individuals per 25cm^2) (Figure 14). Recruits of *A. modestus* were only recorded at Farol (0.2 ± 0.15 individuals per 25cm^2) on a single sampling date during this period (Figure 15), and were significantly less abundant than those of the native *C. montagui* during this time ($p < 0.001$; Table 8). Recruits were most abundant at the low shore level and were significantly less abundant at the mid shore and high shore level ($p < 0.01$; Table 8).

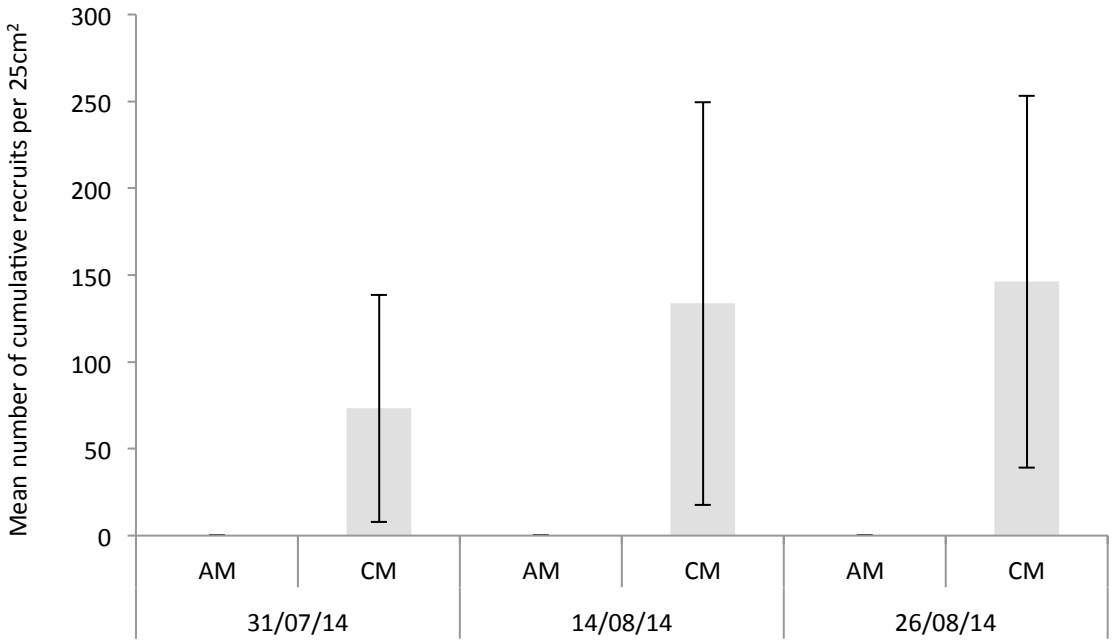

Figure 12. Mean (± SE) abundance of recruits of three barnacle species *Chthamalus montagui* (light grey), *Austrominius modestus* (black) and *Amphibalanus amphitrite* (dark grey) per 25cm^2 free space in removal plots (n=18 per site) at Ihla da Armona, Algarve, Portugal on three sampling dates in July and August 2014.

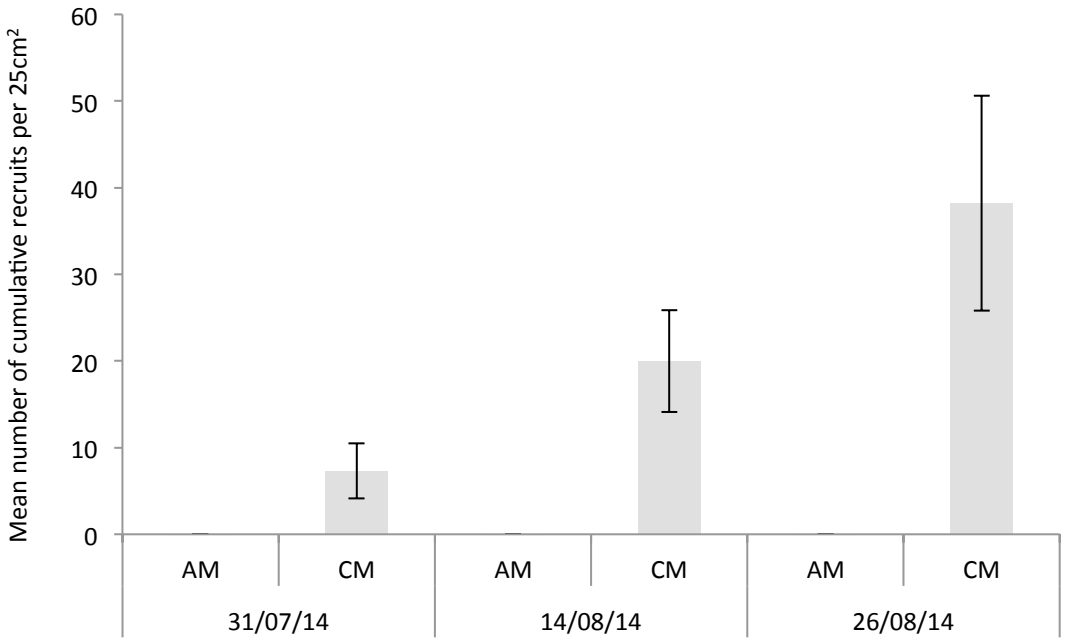

Figure 13. Mean (± SE) abundance of recruits of three barnacle species *Chthamalus montagui* (light grey), *Austrominius modestus* (black) and *Amphibalanus amphitrite* (dark grey) per 25cm^2 free space in removal plots (n=18 per site) at Quatro Águas, Algarve, Portugal on three sampling dates in July and August 2014.

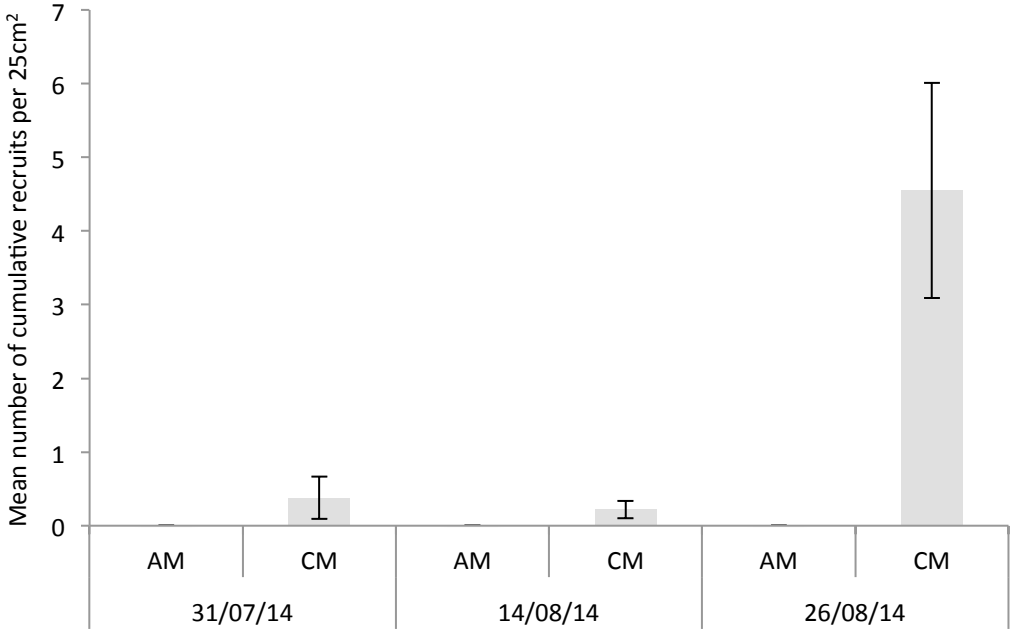

Figure 14. Mean (± SE) abundance of recruits of three barnacle species *Chthamalus montagui* (light grey), *Austrominius modestus* (black) and *Amphibalanus amphitrite* (dark grey) per 25cm^2 free space in removal plots (n=18 per site) at Praia de Faro, Algarve, Portugal on three sampling dates in July and August 2014.

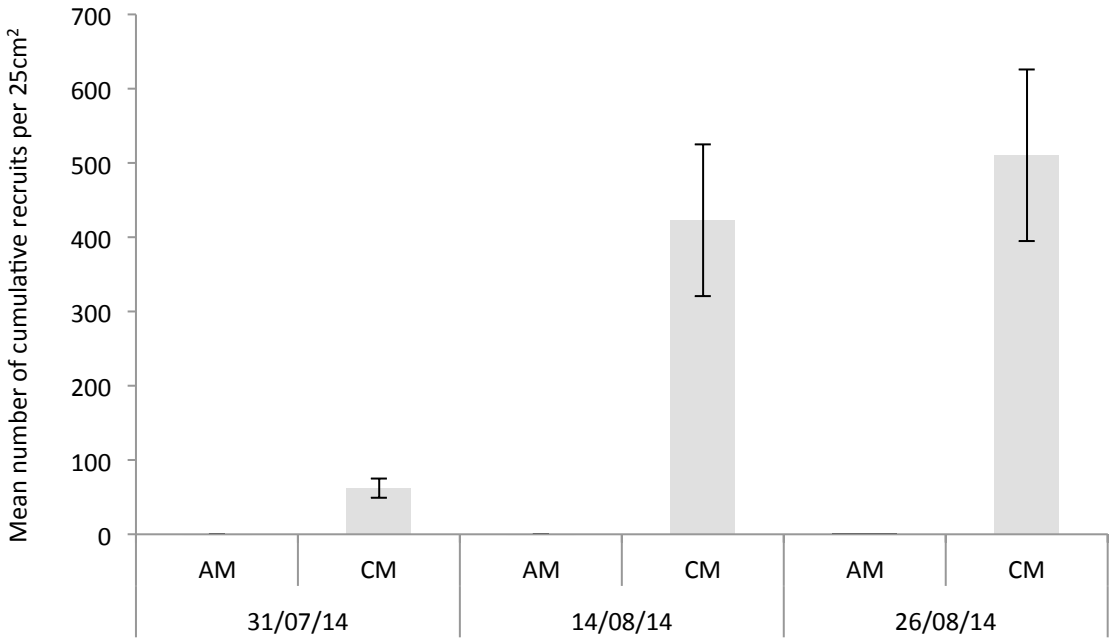

Figure 15. Mean (± SE) abundance of recruits of three barnacle species *Chthamalus montagui* (light grey), *Austrominius modestus* (black) and *Amphibalanus amphitrite* (dark grey) per 25cm^2 free space in removal plots (n=18 per site) at Farol, Algarve, Portugal on three sampling dates in July and August 2014.

Table 8. Negative binomial regression statistics for abundance data of recruits of two barnacle species, *Chthamalus montagui* (intercept) and *Austrominius modestus* (AM) from removal plots at high (1/intercept), mid (2) and low (3) shore four field sites in the Algarve in July and August 2014. Species, site and shore height were used as predictors for abundance of recruits. Significant p values (< 0.05) are highlighted in bold.

	estimate	**± SE**	**z value**	**p**
Intercept	5.74	0.383	14.9	**< 0.001**
Species AM	-7.08	0.379	-18.6	**< 0.001**
Quatro Águas	-3.9	0.446	-8.7	**< 0.001**
Praia da Faro	-6.48	0.474	-13.6	**< 0.001**
Farol	-1.53	0.407	-3.7	**< 0.001**
Height 2	1.12	0.368	3.0	**< 0.010**
Height 3	1.71	0.326	5.2	**< 0.001**

Between August 2014 and February 2015 *A. modestus* became established at all four sites, but remained significantly less abundant than *C. montagui* ($p < 0.001$; Table 9). By the end of the study, Farol was the site with highest densities of both *A. modestus* (6.1 ± 1.87 individuals per 25cm^2) and *C. montagui* (400.2 ± 71.45 individuals per 25cm^2) recruits (Figure 19). Lowest abundances of *A. modestus* were recorded at Ihla

da Armona (0.5 ± 0.37 individuals per 25cm^2) (Figure 16) while Praia da Faro was the site with lowest abundances of *C. montagui* (15.1 ± 3.27 individuals per 25cm^2) (Figure 18). Recruits of a third species, *Amphibalanus amphitrite* were also recorded at Quatro Águas (0.1 ± 0.05 individuals per 25cm^2) (Figure 17) and Praia da Faro (0.6 ± 0.33 individuals per 25cm^2) (Figure 18) at this time.

Highest densities of both species were also recorded at Farol in August 2015 and February 2016 (Figure 19) while lowest abundances of *A. modestus* continued to be recorded at Ihla da Armona (Figure 16) and at Praia da Faro (Figure 18) for *C. montagui*. *A. modestus* increased in abundance at two sites, Praia de Faro (Figure 18) and Farol (Figure 19) between February 2015 and August 2015, however by February 2016 abundances recorded at these locations had decreased to levels recorded prior to February 2015. A similar trend was observed for *C. montagui*, with a decrease in the number of recruits recorded at the majority of sites between August 2015 and February 2016, however densities were still much higher than those of either *A. modestus* or *A. amphitrite* which also decreased in abundance during this time period, and by February 2016 was only recorded at one site, Praia da Faro (0.1 ± 0.11 individuals per 25cm^2) (Figure 18). Total abundance of recruits became more similar between sites as the study progressed, with only Praia de Faro showing a significant difference ($p < 0.001$), with less recruits than the other three sites (Figure 18; Table 9). Lowest abundances of recruits were recorded at the high shore level, with significantly higher abundances found at the mid ($p < 0.001$) and low shore ($p < 0.001$; Table 9).

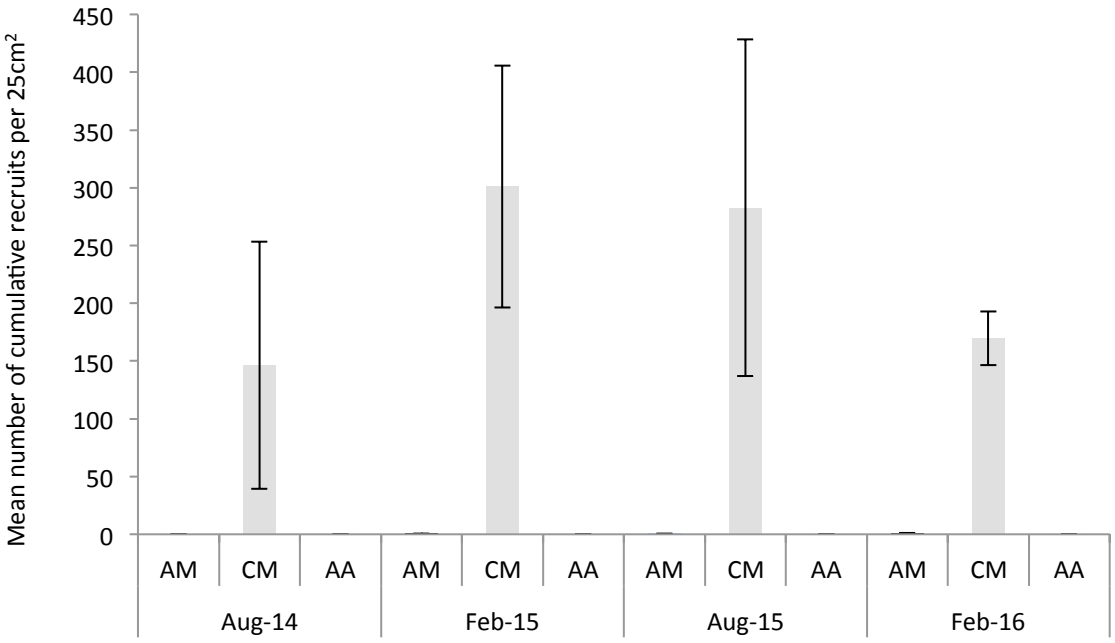

Figure 16. Mean (± SE) number of cumulative recruits of three barnacle species *Chthamalus montagui* (CM; light grey), *Austrominius modestus* (AM; black) and *Amphibalanus amphitrite* (AA; dark grey) per 25cm^2 free space in removal plots (n=9 per site) at Ihla da Armona, Algarve, Portugal in August 2014, February and August 2015 and February 2016.

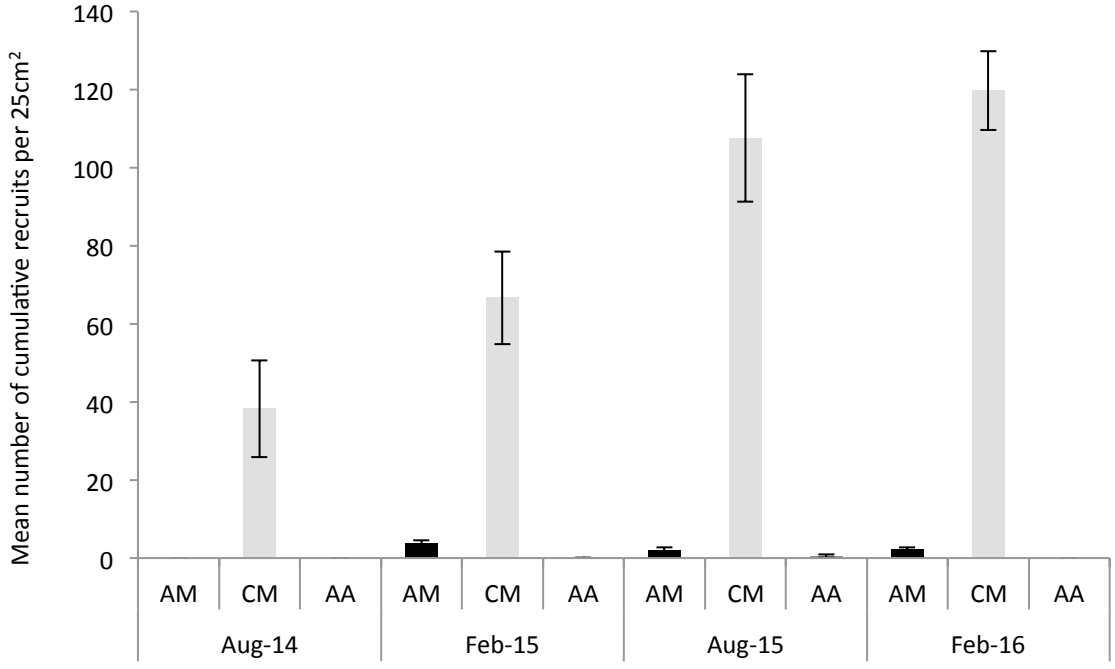

Figure 17. Mean (± SE) number of cumulative recruits of three barnacle species *Chthamalus montagui* (CM; light grey), *Austrominius modestus* (AM; black) and *Amphibalanus amphitrite* (AA; dark grey) per 25cm^2 free space in removal plots (n=9 per site) at Quatro Águas, Algarve, Portugal in August 2014, February and August 2015 and February 2016.

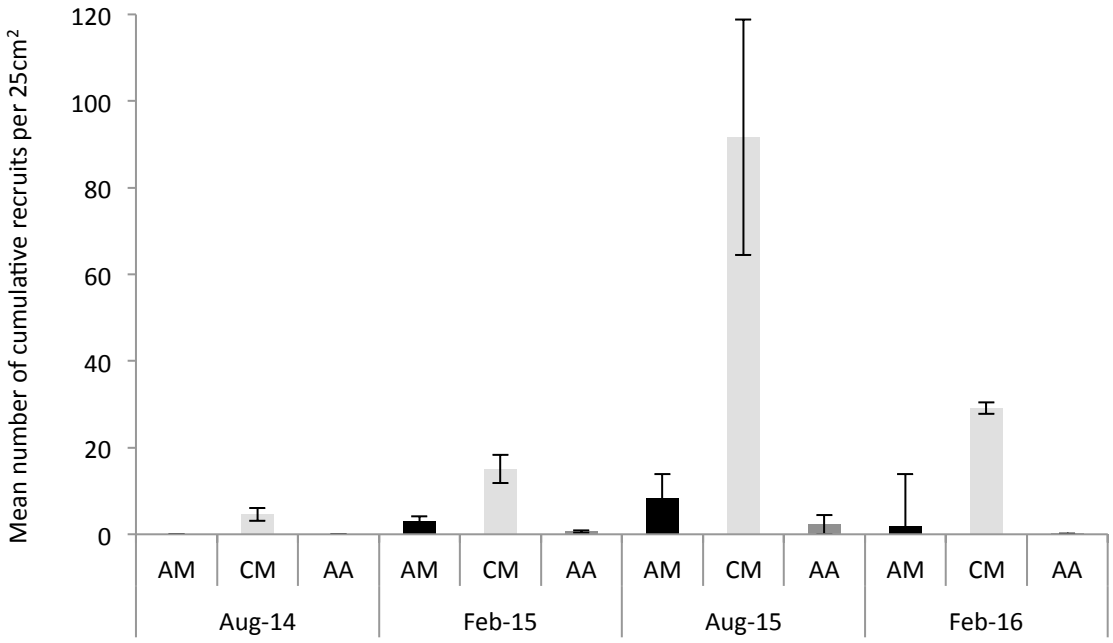

Figure 18. Mean (± SE) number of cumulative recruits of three barnacle species *Chthamalus montagui* (CM; light grey), *Austrominius modestus* (AM; black) and *Amphibalanus amphitrite* (AA; dark grey) per 25cm^2 free space in removal plots (n=9 per site) at Praia de Faro, Algarve, Portugal in August 2014, February and August 2015 and February 2016.

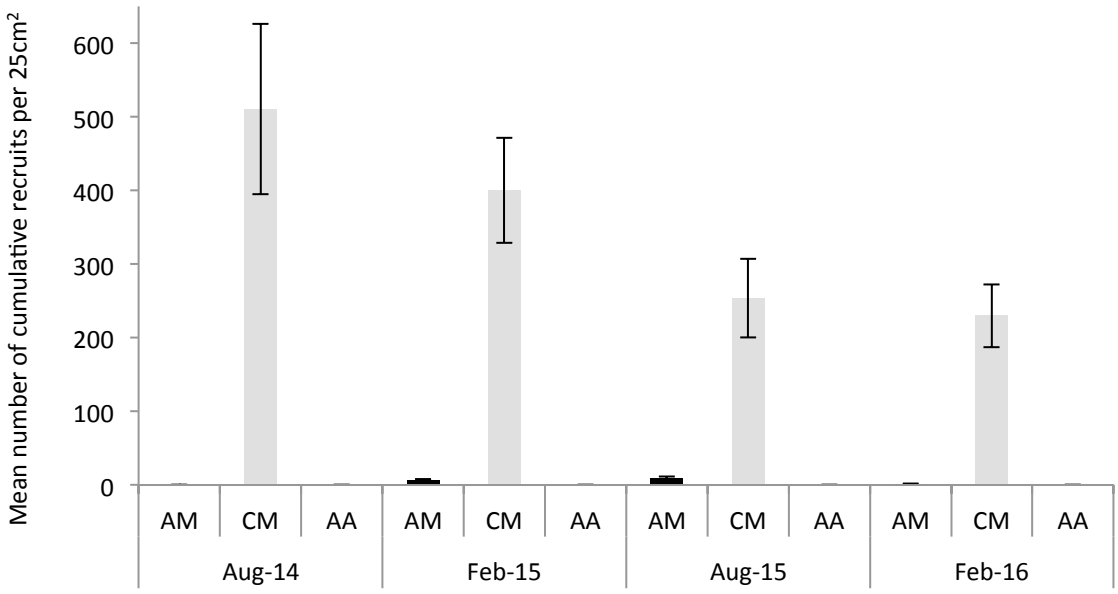

Figure 19. Mean (± SE) number of cumulative recruits of three barnacle species *Chthamalus montagui* (CM; light grey), *Austrominius modestus* (AM; black) and *Amphibalanus amphitrite* (AA; dark grey) per 25cm^2 free space in removal plots (n=9 per site) at Farol, Algarve, Portugal in August 2014, February and August 2015 and February 2016.

Table 9. Negative binomial regression statistics for abundance data of recruits of two barnacle species, *Chthamalus montagui* (intercept) and *Austrominius modestus* (AM) from removal plots at high (1/intercept), mid (2) and low (3) shore at four field sites in the Algarve in August 2014, February and August 2015 and February 2016. Species, site and shore height were used as predictors for abundance of recruits. Significant p values (< 0.05) are highlighted in bold.

	estimate	**± SE**	**z value**	**p**
Intercept	4.24	0.256	16.5	**< 0.001**
Species AM	-3.96	0.165	-23.9	**< 0.001**
Quatro Águas	-0.52	0.279	-1.8	0.060
Praia da Faro	-1.22	0.282	-4.3	**< 0.001**
Farol	0.32	0.275	1.1	0.240
Height 2	0.88	0.213	4.1	**< 0.001**
Height 3	1.63	0.193	8.4	**< 0.001**

Recruits of *C. montagui* also dominated removal plots that were re-cleared in February 2015 and August 2015. Highest abundances of this species were recorded at Farol in both August 2015 (166.0 ± 55.94 individuals per 25cm^2) and February 2016 (114.7 ± 11.34 individuals per 25cm^2) (Table 10). Recruits of *A. modestus* were recorded at all four sites in plots that were re-cleared during both time periods (Table 10). As in cumulative removal plots, this species was present at much lower densities than the native *C. montagui.* The highest abundance of *A. modestus* recruits in August 2015 was 2.7 (± 1.08) individuals per 25cm^2 at Farol, but recruits of *A. modestus* were absent from re-cleared plots at this site in February 2016. Highest abundances of *A. modestus* at this time interval were recorded at Quatro Águas (1.1 ± 0.45 individuals per 25cm^2) and Praia da Faro (1.1 ± 0.47 individuals per 25cm^2). *A. amphitrite* was only recorded at one site, Praia de Faro at very low abundances in both August 2015 (0.3 ± 0.25 individuals per 25cm^2) and February 2016 (0.8 ± 0.43 individuals per 25cm^2) (Table 10).

Table 10. Mean (± SE) abundance of recruits of three barnacle species *Chthamalus montagui* (CM), *Austrominius modestus* (AM) and *Amphibalanus amphitrite* (AA) per 25cm^2 in removal plots (n=6 per site) at four sites in the Algarve, Ihla da Armona, Quatro Águas, Praia da Faro and Farol in August 2015 and February 2016. Plots were cleared in February 2015 and August 2015.

	Aug-15			Feb-16		
	CM	**AM**	**AA**	**CM**	**AM**	**AA**
Ihla da Armona	124.3 ± 84.11	0.8 ± 0.75	0.0 ± 0.00	88.3 ± 29.94	0.3 ± 0.33	0.0 ± 0.00
Quatro Águas	78.8 ± 15.65	0.2 ± 0.16	0.0 ± 0.00	69.3 ± 15.13	1.1 ± 0.45	0.0 ± 0.00
Praia da Faro	70.6 ± 28.74	2.0 ± 1.05	0.3 ± 0.25	26.9 11.13	1.1 ± 0.48	0.8 ± 0.43
Farol	166.0 ± 55.94	2.7 ± 1.08	0.0 ± 0.00	114.7 ± 11.34	0.0 ± 0.00	0.0 ± 0.00

Northern limits

Recruits of *S. balanoides* dominated removal plots at all four sites in both September 2014 and March 2015 (Figures 20-23) and were significantly more abundant than recruits of *A. modestus* ($p < 0.001$; Table 11). Highest densities of *S. balanoides* recruits were recorded at Watersports Slipway in September 2014 (179.6 ± 12.33 individuals per 25cm^2) (Figure 21), while lowest abundances of recruits of this species was recorded at Kames Bay (104.6 ± 18.62 individuals per 25cm^2) (Figure 23) at this time period (Figure 15). By March 2015, the abundance of *S. balanoides* recruits had decreased across all sites. Highest abundances were again recorded at Watersports Slipway (123.2 ± 6.54 individuals per 25cm^2) (Figure 21), with lowest abundances of *S. balanoides* recruits recorded at Millport Harbour (72.3 ± 14.19) at this time (Figure 20).

A. modestus recruits were recorded at all sites in September 2014 and March 2015 (Figures 20-23). Abundances ranged from 0.4 (± 0.24) individuals per 25cm^2 at Harbour Wall (Figure 20) to 2.5 (± 0.60) individuals per 25cm^2 at Watersports Slipway (Figure 21). The abundance of *A. modestus* recruits in removal plots increased between September 2014 and March 2015 at all four sites. Highest abundances in March 2015 were recorded at Harbour Wall (3.6 ± 1.25 individuals per 25cm^2) (Figure 20) while lowest abundances were found at Kames Bay (1.1 ± 0.50 individuals per 25cm^2) ($p < 0.05$; Table 11; Figure 23). There was no significant difference in the abundance of *A. modestus* recruits with shore height or between the two sampling dates (Table 11).

In September 2014, *C. montagui* recruits were recorded at all sites with the exception of Watersports Slipway (Figures 20-23). Abundances ranged from 0.2 (± 0.15) individuals per 25cm^2 at Butterlump (Figure 22) to 2.8 (± 1.09) individuals per 25cm^2 at Harbour Wall (Figure 20). *C. montagui* recruits increased in abundance at three sites between September 2014 and March 2015 with highest abundances recorded at Kames Bay (76.0 ± 16.42 individuals per 25cm^2) in March 2015 (Figure 23). Abundances at the remaining sites ranged from 0.2 (± 0.22) individuals per 25cm^2 at Watersports Slip (Figure 21) to 5.8 (± 3.90) individuals per 25cm^2 at Butterlump (Figure 22).

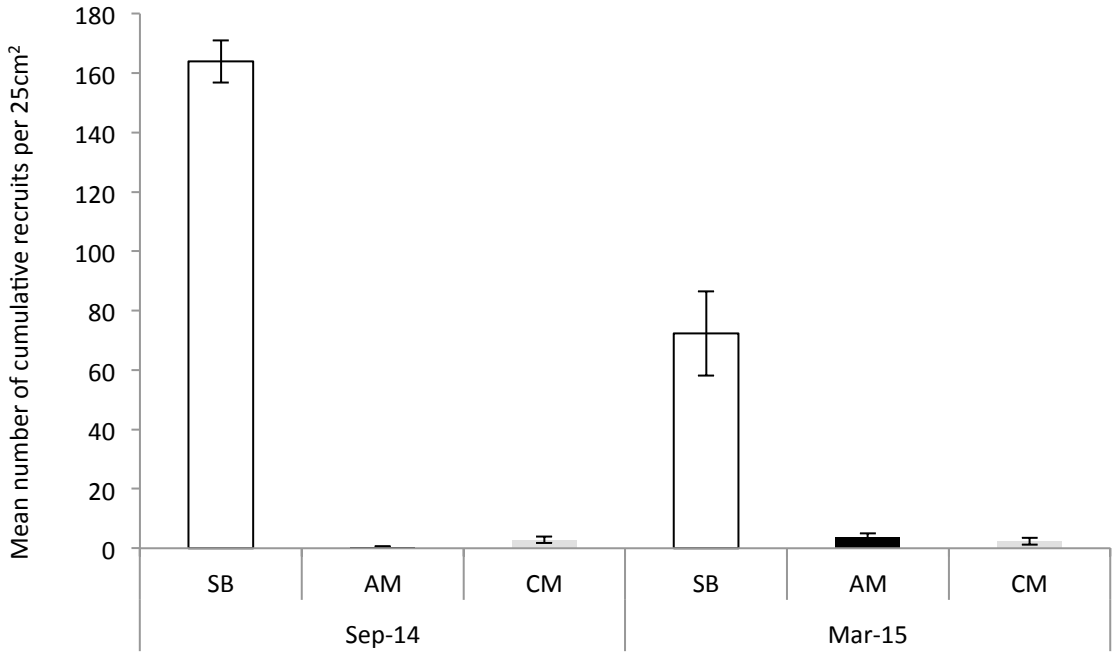

Figure 20. Mean (± SE) abundance of recruits of three barnacle species *Semibalanus balanoides* (SB; white), *Austrominius modestus* (AM; black) and *Chthamalus montagui* (CM; dark grey) per 25cm^2 in removal plots (n=9 per site) at Harbour Wall, Isle of Cumbrae, Scotland in September 2014 and March 2015. Plots had been initially cleared in March 2014.

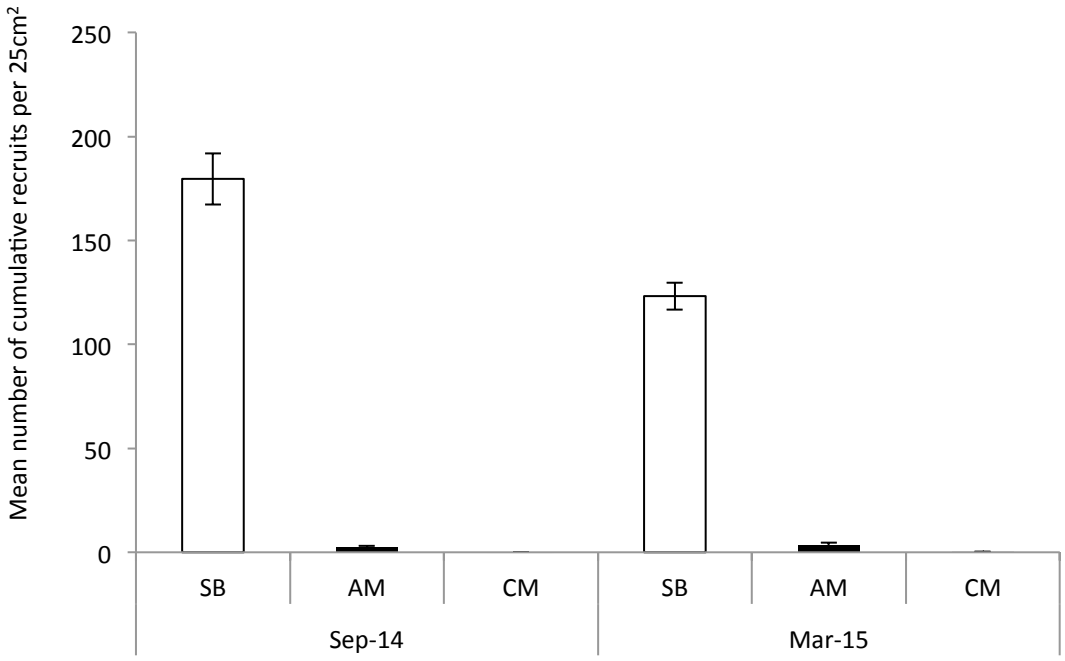

Figure 21. Mean (± SE) abundance of recruits of three barnacle species *Semibalanus balanoides* (SB; white), *Austrominius modestus* (AM; black) and *Chthamalus montagui* (CM; dark grey) per 25cm^2 in removal plots (n=9 per site) at Watersports Slipway, Isle of Cumbrae, Scotland in September 2014 and March 2015. Plots had been initially cleared in March 2014.

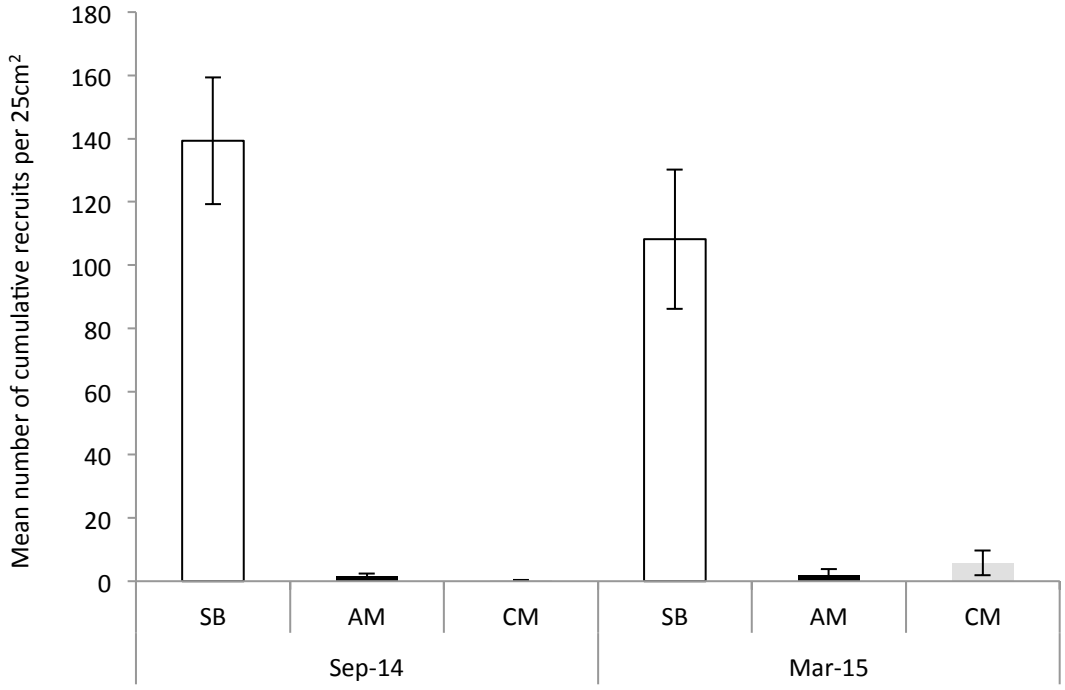

Figure 22. Mean (± SE) abundance of recruits of three barnacle species *Semibalanus balanoides* (SB; white), *Austrominius modestus* (AM; black) and *Chthamalus montagui* (CM; dark grey) per 25cm^2 in removal plots (n=9 per site) at Butterlump, Isle of Cumbrae, Scotland in September 2014 and March 2015. Plots had been initially cleared in March 2014.

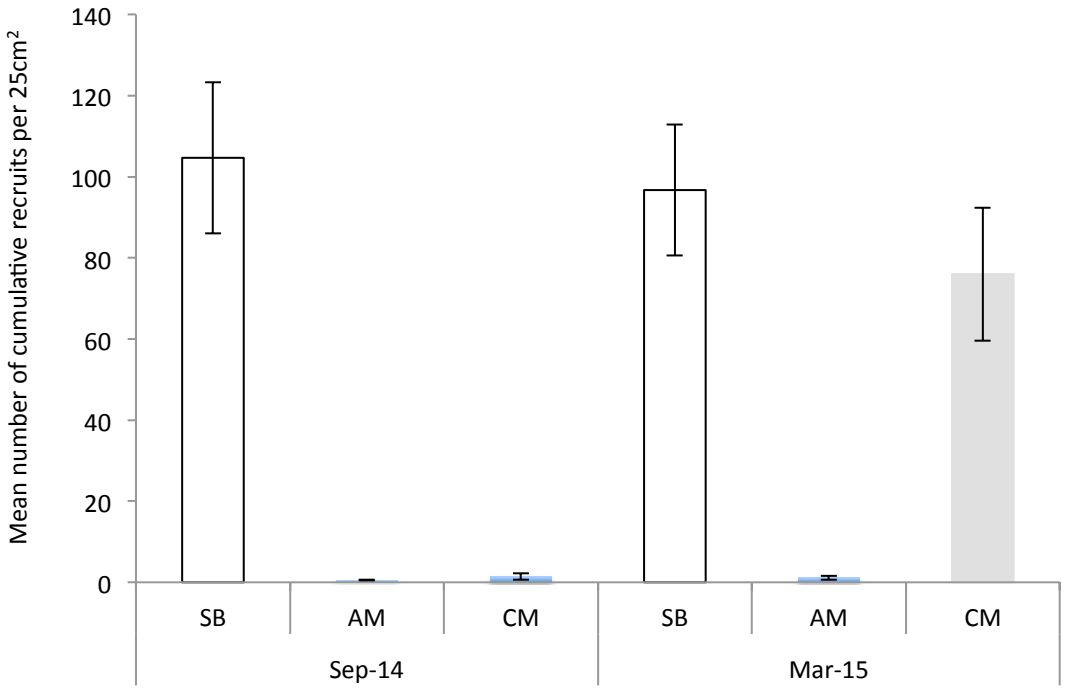

Figure 23. Mean (± SE) abundance of recruits of three barnacle species *Semibalanus balanoides* (SB; white), *Austrominius modestus* (AM; black) and *Chthamalus montagui* (CM; dark grey) per 25cm^2 in removal plots (n=9 per site) at Kames Bay, Isle of Cumbrae, Scotland in September 2014 and March 2015. Plots had been initially cleared in March 2014.

Table 11. Negative binomial regression statistics for abundance data of recruits of three barnacle species, *Austrominius modestus* (intercept), *Chthamalus montagui* (CM) and *Semibalanus balanoides* (SB) from removal plots at high (1/intercept), mid (2) and low (3) shore at four field sites on the Isle of Cumbrae in September 2014 (intercept) and March 2015. Species, shore height, site and time period were used as predictors for abundance of recruits. Significant p values (< 0.05) are highlighted in bold.

	estimate	**± SE**	**z value**	**p**
Intercept	0.81	0.247	3.3	**< 0.001**
Species CM	-0.40	0.225	-1.8	0.070
Species SB	4.42	0.197	22.4	**< 0.001**
Height 2	-0.16	0.194	-1.3	0.180
Height 3	-0.18	0.218	-0.8	0.380
Watersports Slip	-0.20	0.241	-0.8	0.380
Butterlump	-0.21	0.248	-0.8	0.380
Kames Bay	-0.51	0.252	-2.0	**< 0.050**
March 2015	0.29	0.188	1.5	0.120

Following clearance in March 2015, a different pattern of recruitment was observed in September 2015 and March 2016 in comparison to the previous year. *S. balanoides* recruits were again recorded at all sites at both time intervals, but at much lower abundances than the previous year (Figures 24-27). In September 2015 a maximum abundance of 10.1 (± 1.93) individuals per 25cm^2 was recorded at Harbour Wall (Figure 24) while the lowest abundance of *S. balanoides* recruits was recorded at Watersports Slipway (1.8 ± 0.38 individuals per 25cm^2) (Figure 25). The abundance of *S. balanoides* recruits increased at three sites between September 2015 and March 2016. Despite having the highest abundance of recruits of this species in September 2015, Harbour Wall was the only site where the abundance of *S. balanoides* recruits had decreased by March 2016 (7.3 ± 1.46 individuals per 25cm^2) (Figure 24). Butterlump had the highest abundance of recruits of this species in September 2016 (14.2 ± 6.66 individuals per 25cm^2) (Figure 26) while the lowest abundance was recorded at Watersports Slipway (6.4 ± 2.55 individuals per 25cm^2) (Figure 25).

A. modestus recruits were significantly more abundant than those of *S. balanoides* in September 2015 ($p < 0.001$; Table 12). Recruits of *A. modestus* were recorded at all four sites at both sampling periods (Figures 24-27), and there was no significant difference in the abundance of recruits of this species in September 2015 and March 2016 (Table 12). Recruits of this species were least abundant at the high shore and significantly more abundant at the mid ($p < 0.001$) and low shore ($p < 0.001$; Table 12). Highest abundances were recorded at Butterlump in both September 2015 (30.3 ± 8.23 individuals per 25cm^2) and March 2016 (65.6 ± 20.14 individuals per 25cm^2) (Figure 26). Lowest abundances were recorded at Harbour Wall in September 2015 (7.7 ± 2.08 individuals per 25cm^2) (Figure 24) and at Watersports Slipway in March 2016 (7.3 ± 2.14 individuals per 25cm^2) (Figure 25), the only site where a decrease in the abundance of *A. modestus* recruits was recorded between September 2015 and March 2016.

C. montagui recruits were significantly more abundant than those of *A. modestus* in September 2015 ($p < 0.001$; Table 12). Highest abundances were recorded at Kames

Bay for this species in both September 2015 (133.5 ± 41.85 individuals per 25cm^2) and March 2016 (229.6 ± 63.29 individuals per 25cm^2) (Figure 27), which was the highest density recorded for any species at any site on the Isle of Cumbrae during this study. However, recruits of *C. montagui* were not found at all four sites and were absent from Watersports Slipway in both September 2015 and March 2016 (Figures 25).

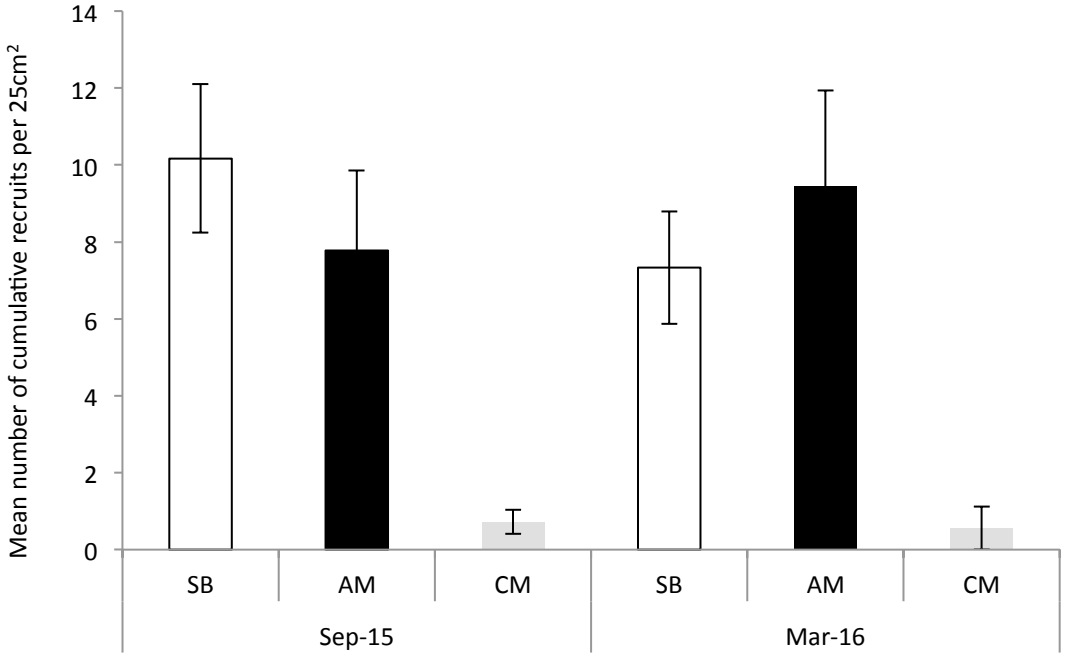

Figure 24. Mean (± SE) abundance of recruits of three barnacle species *Semibalanus balanoides* (SB; white), *Austrominius modestus* (AM; black) and *Chthamalus montagui* (CM; dark grey) per 25cm^2 in removal plots (n=9 per site) at Harbour Wall, Isle of Cumbrae, Scotland in September 2015 and March 2016. Plots had been initially cleared in March 2015.

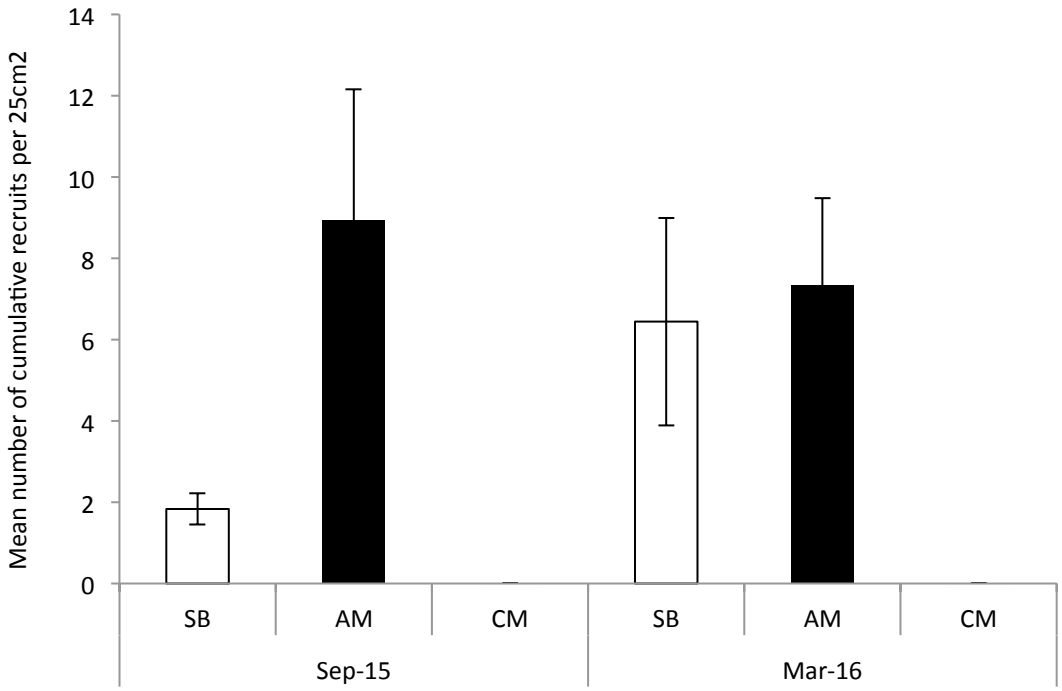

Figure 25. Mean (± SE) abundance of recruits of three barnacle species *Semibalanus balanoides* (SB; white), *Austrominius modestus* (AM; black) and *Chthamalus montagui* (CM; dark grey) per 25cm^2 in removal plots (n=9 per site) at Watersports Slipway, Isle of Cumbrae, Scotland in September 2015 and March 2016. Plots had been initially cleared in March 2015.

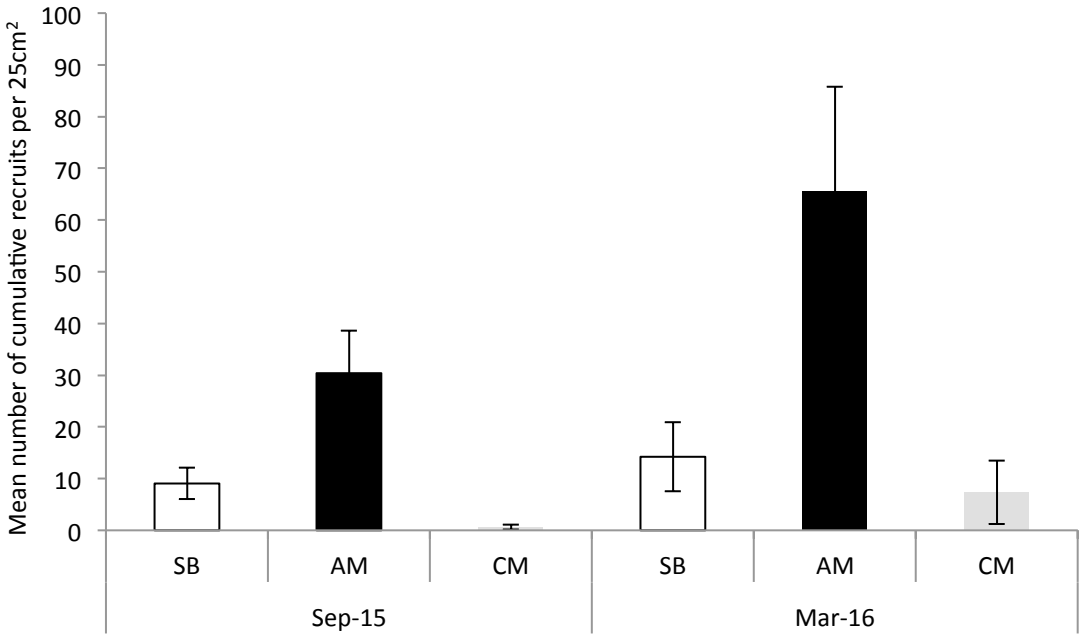

Figure 26. Mean (± SE) abundance of recruits of three barnacle species *Semibalanus balanoides* (SB; white), *Austrominius modestus* (AM; black) and *Chthamalus montagui* (CM; dark grey) per 25cm^2 in removal plots (n=9 per site) at Butterlump, Isle of Cumbrae, Scotland in September 2015 and March 2016. Plots had been initially cleared in March 2015.

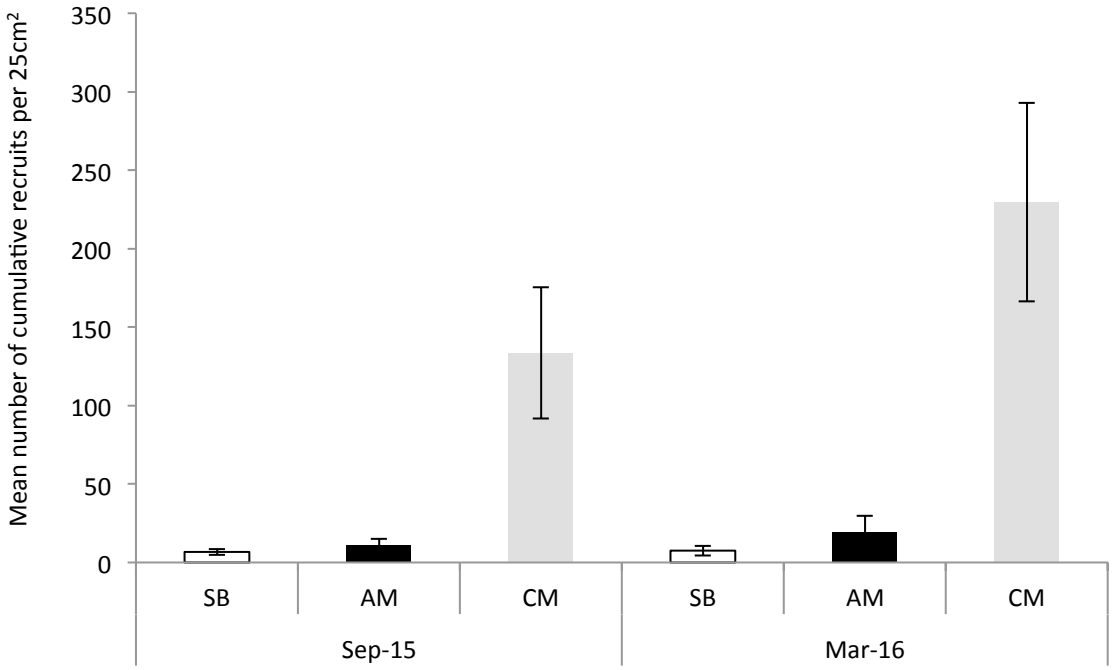

Figure 27. Mean (± SE) abundance of recruits of three barnacle species *Semibalanus balanoides* (SB; white), *Austrominius modestus* (AM; black) and *Chthamalus montagui* (CM; dark grey) per 25cm^2 in removal plots (n=9 per site) at Kames Bay, Isle of Cumbrae, Scotland in September 2015 and March 2016. Plots had been initially cleared in March 2015.

Table 12. Negative binomial regression statistics for abundance data of recruits of three barnacle species, *Austrominius modestus* (intercept), *Chthamalus montagui* (CM) and *Semibalanus balanoides* (SB) from removal plots at high (1/intercept), mid (2) and low (3) shore at four field sites on the Isle of Cumbrae in September 2015 (intercept) and March 2016. Species, shore height and time period were used as predictors for abundance of recruits. Significant p values (< 0.05) are highlighted in bold.

	estimate	**± SE**	**z value**	**p**
Intercept	1.79	0.231	7.7	**< 0.001**
Species CM	1.25	0.261	4.8	**< 0.001**
Species SB	-0.78	0.234	-3.3	**< 0.001**
Height 2	1.34	0.248	5.4	**< 0.001**
Height 3	1.01	0.240	4.0	**< 0.001**
March 2016	0.34	0.210	1.6	0.090

The species with the highest abundance of recruits recorded in plots that were re-cleared was *C. montagui* in both March 2015 and March 2016. Densities of 9.6 (± 2.95) individuals per 25cm^2 were recorded for this species at Kames Bay in March 2015 and 49.1 (± 35.86) individuals per 25cm^2 at the same site in March 2016. *C.*

montagui recruits were absent from re-cleared plots at Watersports Slipway on both occasions and also from Harbour Wall in March 2015. Although *C. montagui* recruits were recorded in re-cleared plots at this site in March 2016, it was at a lower density (0.6 ± 0.44 individuals per 25cm^2) than the other sites where this species was present (Table 13).

S. balanoides was absent from all re-cleared plots in March 2015 and were only recorded at one site, Harbour Wall in March 2016 (2.3 ± 1.14 individuals per 25cm^2). Recruits of *A. modestus* were recorded in re-cleared plots at all sites on both occasions, with the exception of Kames Bay in March 2015. Maximum densities of *A. modestus* recruits in re-cleared plots were recorded at Butterlump (7.6 ± 3.02 individuals per 25cm^2) in March 2015 and at Harbour Wall (7.2 ± 2.08 individuals per 25cm^2) in March 2016 (Table 13).

Table 13. Mean (± SE) abundance of recruits of three barnacle species *Semibalanus balanoides* (SB), *Austrominius modestus* (AM) and *Chthamalus montagui* (CM) per 25cm^2 in removal plots (n=9 per site) at four sites on the Isle of Cumbrae, Harbour Wall, Watersports Slipway, Butterlump and Kames Bay in March 2015 and March 2016. Plots were cleared in September 2014 and September 2015.

	Mar-15			Mar-16		
	SB	**AM**	**CM**	**SB**	**AM**	**CM**
Harbour Wall	0.0 ± 0.00	0.3 ± 0.18	0.0 ± 0.00	2.3 ± 1.14	7.2 ± 2.08	0.6 ± 0.44
Watersports Slipway	0.0 ± 0.00	0.1 ± 0.11	0.0 ± 0.00	0.0 ± 0.00	2.4 ± 0.79	0.0 ± 0.00
Butterlump	0.0 ± 0.00	7.6 ± 3.02	3.2 ± 2.25	0.0 ± 0.00	5.0 ± 2.33	5.0 ± 4.79
Kames Bay	0.0 ± 0.00	0.0 ± 0.00	9.6 ± 2.95	0.0 ± 0.00	3.0 ± 2.64	49.1 ± 35.86

6.4.4 Seawater Temperature

Mean seawater temperatures in the Algarve, Portugal ranged from 22.1 ^{o}C in August 2015 to 16.0 ^{o}C in March 2014. Temperatures recorded for the Isle of Cumbrae, Scotland were much lower, ranging from 7.0 ^{o}C (± 0.01 ^{o}C) in March 2015 to 14.4 ^{o}C (± 0.02 ^{o}C) in July 2014. Temperature trends were the same for both locations, with lowest temperatures recorded between January and May and highest temperatures recorded from July to September (Figure 28).

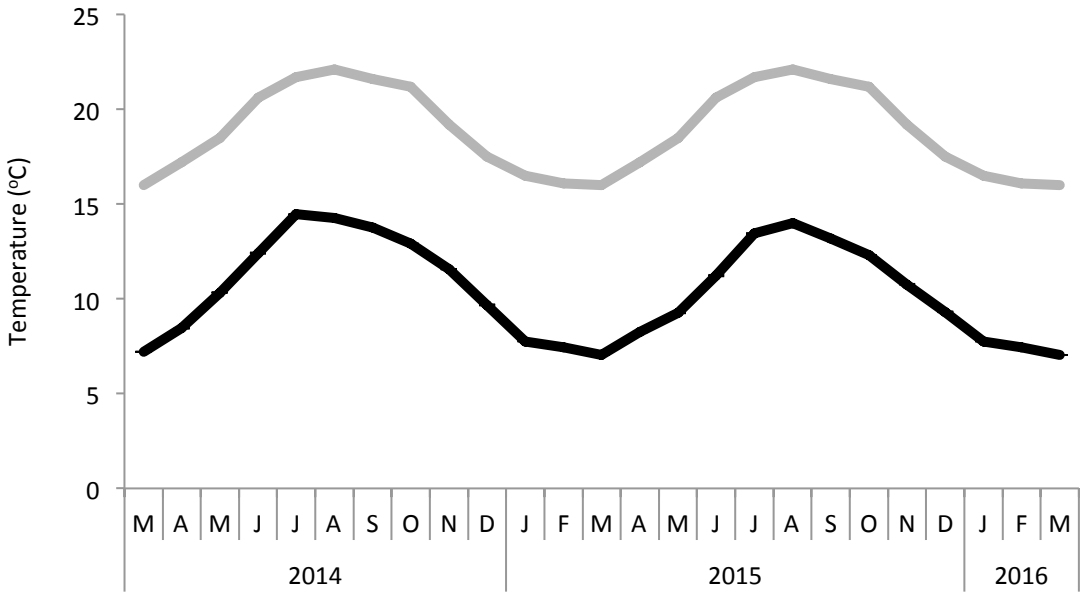

Figure 28. Mean (± SE) monthly seawater temperatures (^{o}C) for the Algarve, Portugal (grey) and the Isle of Cumbrae, Scotland (black).

6.5 Discussion

Despite being widespread on the Isle of Cumbrae and in the Algarve, *Austrominius modestus* remains less abundant than native barnacle species at both of these locations. *A. modestus* was more abundant at sites on the Isle of Cumbrae than in the Algarve, despite being a warm water adapted species. The non-native species was recorded with embryos, at a range of developmental stages, on all sampling dates in both the Algarve and the Isle of Cumbrae, over a temperature range of 7^{o}C – 22^{o}C, which includes the warmest and coldest parts of the year at both locations. Recruits of *A. modestus* were recorded in removal plots at field sites at both locations, but at lower abundances than the native species. On the Isle of Cumbrae, removal plots were dominated by the native *S. balanoides* in September 2014 and March 2015, and by *C. montagui* and *A. modestus* the following year. Recruitment at sites in the Algarve was more consistent, with *C. montagui* recruits dominant on all occasions. Recruits of *A. modestus* were recorded at all sites, but always at much lower densities than the native species.

Several traits have been attributed to the success of *A. modestus* in Europe, including eurythermal larvae and adult stages which can cope with large temperature fluctuations and desiccation stress (Harms 1999). While this study has confirmed the ability of *A. modestus* to produce well-developed embryos at its European range limits, across a wide range of temperatures, this did not always ensure successful recruitment. For example, despite a higher proportion of *A. modestus* recorded with embryos that were ready to hatch, in comparison to the native *C. montagui* in the Algarve, recruits of the native species by far outnumbered *A. modestus* in removal plots at this location, and were even observed to settle on top of adult *A. modestus* at some locations. On the Isle of Cumbrae, higher levels of *A. modestus* recruitment were observed in September 2015 and March 2016 in comparison to the previous year, although there was little difference in the percentage of individuals with embryos between the two time periods.

Apart from climate (Davies *et al.* 1998; Helmuth *et al.* 2006), competition and interspecific interactions can also play an important role (Wethey 2002) in determining the range limits of a species. The same factors are often not responsible for controlling the range limits of a species at different locations. For example, at one range boundary environmental factors may limit the abundance of a species while at an other boundary biotic factors may be controlling abundance and distribution (Brown *et al.* 1996). In the Algarve, *A. modestus* failed to recruit at high densities at any site throughout the entire study, despite having a higher proportion of individuals with well-developed embryos than the native *C. montagui* on most occasions. The fact that adult *A. modestus* are by far outnumbered by *C. montagui* may produce a situation where *A. modestus* nauplii are diluted by high densities of *C. montagui* nauplii in the plankton, even if a lower proportion of *C. montagui* are found to have embryos that are ready to hatch. In addition to this, the high level of water exchange within the Ria Formosa and currents within the lagoon may limit the amount of *A. modestus* reaching substrates suitable for settlement. Low abundances of recruits could also be due to high levels of mortality during the planktonic phase, or strong competitive pressure from high densities of *C. montagui* settlers. It is known that *A. modestus* cyprids have a lower tolerance to desiccation than *C.*

montagui (Foster 1971), and so desiccation stress at the settlement stage may cause high levels of mortality. This could explain the extremely low levels of *A. modestus* recruitment observed during July and August 2014 at the time of warmest sea and air temperatures.

The difference in recruitment success of *A. modestus* on the Isle of Cumbrae in 2014-2015, in comparison to 2015-2016, seems to indicate that temperature alone is not responsible for determining the abundance of this species at this location. During 2014-2015, *S. balanoides* recruits were the most abundant recruit in removal plots at all sites. In the following year, however, they were present at much lower densities, despite little difference in recorded seawater temperatures. In the absence of *S. balanoides* recruits, *A. modestus* recruits were recorded at higher abundances than the previous year and were the dominant species at one site, Butterlump. The low levels of *S. balanoides* recruitment coincided with a much lower proportion of individuals recorded with embryos than the previous year, however there was little difference between the proportion of *A. modestus* recorded with embryos. Variations in recruitment between years as observed for *S. balanoides,* are common for barnacle species (Connell 1961; Hawkins and Hartnoll 1982; Jenkins *et al.* 2000; Kent *et al.* 2003; Burrows *et al.* 2010). Differences can be due to water column processes, such as currents (Kendall *et al.* 1985) or differences in the timing of larval release and the phytoplankton bloom (e.g. Connell 1961; Hawkins and Hartnoll 1982). The absence of embryos recorded at sites on the Isle of Cumbrae in March 2015, in comparison to March 2014, may be due to a difference in the timing of the phytoplankton bloom between years at this location.

At northerly locations such as the Isle of Cumbrae, cold water adapted species like *S. balanoides* may have a competitive advantage over southern species, allowing them to persist despite climatic warming (Connell 1961; Hawkins *et al.* 2008; Poloczanska *et al.* 2008; Hawkins *et al.* 2009). Additionally the occurrence of occasional mass recruitment events as described by Hansson *et al.* (2003) continue to ensure the persistence of native species. As observed in the present study, in years where *S. balanoides* recruits successfully, strong competitive pressure from *S. balanoides*

recruits appears to play an important role in limiting the abundance of *A. modestus* on the Isle of Cumbrae. Temperature is also known to play an important role in recruitment processes, with cold winters having a negative impact on *A. modestus* recruitment (den Hartog 1953; Crisp 1958; Crisp and Southward 1959; Crisp 1964; Harms and Anger 1989; Buschbaum 2001), which may require more than one year for populations to recover (Harms and Anger 1989), while favouring *S. balanoides* populations. Such periodic adverse years play an important role in determining the abundance of invasive populations at their range limits (Sexton *et al.* 2009).

Sea surface temperatures have been increasing since the 1980s in the North East Atlantic (Hiscock *et al.* 2004) and continued warming is predicted for the next century (IPCC 2014). *A. modestus* is warm water adapted and it has been suggested that this species is an ecological sleeper (Witte *et al.* 2010), with recent increases in its abundance at certain locations being attributed to increasing seawater temperatures (Lawson *et al.* 2004; Witte *et al.* 2010). *A. modestus* may have an advantage over native species in the face of climatic warming as it is eurythermal and also has a shorter generation time than *S. balanoides* and *C. montagui* (Harms 1999), giving it greater potential to adapt, producing greater thermal tolerances (Zerebecki and Sorte 2011). In addition to *A. modestus*, the natives *C. montagui* and *C. stellatus* are also warm water adapted species. However, *S. balanoides* is a cold water adapted arctic-boreal species, and so will be affected differently by increasing temperatures. *S. balanoides* has been identified as a "northern retreater", which are species predicted to decline in abundance and retreat northwards over the next fifty years. *Chthamalus* spp. and *A. modestus* are classified as "southern rapid extenders", which are expected to increase in abundance at current locations and to extend their range northwards, assuming there are no barriers to dispersal (Hiscock *et al.* 2004). In the case of *C. montagui*, a higher frequency of successful recruitment is predicted to increase the abundance of this species as *S. balanoides* declines (Svensson *et al*. 2006). A similar mechanism could increase the abundance of *A. modestus*.

S. balanoides has already exhibited a northward range shift at its southern geographical limits (Wethey and Woodin 2008) as predicted for cold water adapted

species (Hiscock *et al.* 2004; Simkanin *et al.* 2005; Hawkins *et al.* 2008), however, it remains abundant at more northern locations. Burrows *et al.* (2010) found that *S. balanoides* accounted for 95% of individuals surveyed at over 250 sites in Scotland between 2001 and 2006. This species is still regarded as the most abundant intertidal barnacle species in the British Isles (Burrows *et al.* 2010; Gomes-Filho *et al*. 2010) and also remains the dominant species on the Isle of Cumbrae. Given the northern location of the Isle of Cumbrae, it seems probable that *S. balanoides* will continue to be the dominant barnacle in the intertidal zone at this location for some time, despite its increased vulnerability to warmer water temperatures compared with the other *C. montagui* and *A. modestus* (Southward 1991; Jenkins *et al.* 2001; Pineda *et al.* 2002), in particular there may be an increase in the frequency of recruitment failure years for *S. balanoides* in the future (Svensson *et al.* 2005). In addition to the overall warming trend, there is also predicted to be an increase in extreme weather events, such as cold winters, which will interrupt long term warming (Wang *et al.* 2010; Wethey *et al.* 2011), and likely facilitate the persistence of *S. balanoides.*

Connell (1961) showed that *S. balanoides* exhibits a strong competitive force on *Chthamalus*, restricting its distribution to the high shore level, and Poloczanska *et al.* (2008) predicted that the decline of *S. balanoides* as water temperatures increase will release *C. montagui* from such competition. However, as the competitive interactions between *C. montagui* and *A. modestus* are unknown, it remains to be seen whether one of these species could outcompete the other or if they would coexist. In the current study, *C. montagui* was commonly found at the high shore level at survey sites on the Isle of Cumbrae, while *A. modestus* was recorded along with *S. balanoides* at the mid and low shore levels. *A. modestus* is capable of occupying higher parts of the shore than *S. balanoides* (Lawson *et al.* 2004; Witte *et al.* 2010) and high densities of *C. montagui* may currently be limiting the upper distribution of *A. modestus* on the shore at its northern limits. Although not examined in this study it is known that *A. modestus* can occur subtidally, though Watson *et al.* (2005) reported that *A. modestus* that settled in the subtidal zone at Lough Hyne had low survival rates in comparison to other species.

Data from the Isle of Cumbrae also revealed that *A. modestus* and *C. montagui* seem to have complementary distributions, with *A. modestus* present in low abundances at sites where *C. montagui* is abundant and vice versa, possibly indicating that the abundance of one species controls that of the other, or that the two species show different tolerances to environmental factors, such as exposure, with *C. montagui* dominating at more exposed shores. It has previously been reported that the patchy distribution of *A. modestus* on the east coast of Scotland may be due to the lack of sheltered shores on a coast that is open and exposed (Nall *et al*. 2015). The presence of such exposed locations, which are unfavourable to *A. modestus* (Moore 1944; Southward 1955; Crisp 1958), ensures that, despite increases in water temperature, certain locations will remain dominated by native barnacles, with a better tolerance of exposed conditions.

The low abundances of *A. modestus* recorded in the Algarve would indicate that increases in water temperature here might not necessarily result in increases in the abundance of *A. modestus.* The native *C. montagui* was found to be the dominant barnacle species at all sites in the Algarve where abundance estimates were made, which is in keeping with previous surveys in the intertidal zone on the Portuguese coast (Range and Paula 2001; Boaventura *et al.* 2002), and was also the dominant recruit in removal plots. It would be worthwhile investigating whether *A. modestus* is present at more southerly locations, in Spain or Africa, and, if so, at what densities. This would determine whether conditions limiting the abundance of this species are unique to the Algarve or common at southern range of its European distribution, therefore helping to predict its future abundance at such locations. The current climatic situation in Portugal may provide a good insight into the potential conditions that will be experienced at more northerly locations in the future. However, as *A. modestus* is already established at more northerly locations, for example in Ireland, it may be able to attain higher levels of abundance at these locations in the future in comparison to those currently seen in the Algarve. Regardless of the relative abundances of the two species, what is of importance is the ability of one or both of these species to act as a substitute for *S. balanoides.*

The presence of non-native species in many ecosystems has caused an irreversible change in community composition, producing a more homogenised global assemblage (Witte *et al.* 2010). An important question is whether these new assemblage will have similar ecosystem functioning, despite shifts in species composition, by warm-water adapted species replacing cold-water adapted congeners (Somero 2010). These homogenised ecosystems are likely to be more resilient to future change, as native species become increasingly poorly adapted to cope with new environmental conditions. The presence of non-native species could even be viewed as necessary to ensure continuity of local ecosystem function and service provision (Walther *et al.* 2009). However, this is dependent on non-native species playing a similar functional role to the species they are replacing. As highlighted by Svensson *et al.* (2006) it remains unknown whether *C. montagui* can successfully replace *S. balanoides* as a food source for other animals and how differences in larval release could impact the ecosystem; the same is true for *A. modestus.* In the most general sense it would appear that the three barnacle species do play a very similar functional role (Olenin *et al.* 2007), however small differences in feeding (Southward 1955) and reproduction could potentially alter ecosystem functioning. Given that in the future intertidal barnacle communities are likely to be made up of *C. montagui* and *A. modestus* and perhaps other non-native barnacle species, the impact that this could have on ecosystem function is an area that should warrant further investigation.

6.6 References

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Chapter 7

Does enemy release play a role in the invasion ecology of *Austrominius modestus* within its invaded range?

This chapter, in revised format, is being prepared for submission to the journal *Journal of Experimental Marine Biology and Ecology*

7.1 Abstract

Within their new ranges, invasive species often escape the effects of predators and pathogens, which are present in their native distribution. This is termed the Enemy Release Hypothesis (ERH) and is one of the factors that has been attributed to the success of invasive species at new locations. These successful invaders pose a major threat to native biodiversity and ecosystem structure and functioning. Although ERH has been well documented, there have been few studies examining the role it plays in the invasion process. During this study, levels of parasitism of the non-native barnacle species *Austrominius modestus* and a native barnacle species (*Semibalanus balanoides*/*Chthamalus montagui*) by trematode cysts and a parasitic isopod were compared at the northern, western and southern limits of the invaded range of this species (Scotland, Ireland and Portugal), over an eighteen month period. Sampling was carried out on a monthly basis in Ireland and biannually in Scotland and Portugal. There were fluctuations in the intensity and prevalence of infection throughout the study period, but, overall, the local barnacle species had a higher level of infection than *A. modestus*. Highest levels of infection were recorded in Scotland and no infection was recorded in Portugal. Infection did not seem to negatively impact reproduction of any of the barnacle species examined. Interestingly, at the northern and southern sampling locations, *A. modestus* remained significantly less abundant than native barnacle species, despite being free of parasites, or experiencing lower levels of parasitism than native species. This indicates that though it may play a role in the invasion of *A. modestus,* enemy release is not a key factor in the successful establishment of this species. However, *A. modestus* may play an important role in the transmission of trematode parasites in the marine environment by filtering cercariae at a different rate to natives or by diverting some of the parasite load of the native species.

KEYWORDS: Enemy Release Hypothesis, barnacle, *Austrominius modestus*, invasion ecology, parasitism

7.2 Introduction

Within their new distributions, invasive species often escape the effects of predators and pathogens that they would encounter in their native ranges. This is termed the Enemy Release Hypothesis (ERH), and success of invasive species in new ranges has been attributed to this, amongst other factors, due to increased size and competitive ability (Williamson and Fitter 1996; Hudson and Greenman 1998; Torchin *et al.* 2001; Torchin *et al.* 2003). Despite being widely accepted, empirical evidence is often lacking to support ERH in the case of successful invaders (Maron and Vilà 2001; Colautti *et al*. 2004). Evidence to the contrary has even been reported, with non-native plants found to be more susceptible to herbivory by native grazers than congeneric native plant species (Agrawal and Kotanen 2003). Such cases may be due to reduced genetic diversity of invasive species and their status as a novel host for native pathogens, potentially making them more susceptible than natives to infection (Coluatti *et al.* 2004). While ERH may not apply in all situations, and may not be solely responsible for the success of non-natives, there are also data in support of ERH (Keane and Crawley 2002). In a review of marine invasive species, Torchin *et al.* (2002) reported that introduced species do experience some level of enemy release when compared to their native range. For invasive plant species, those that experience high levels of enemy release, have been found to be more "problematic" invaders (Mitchell and Power 2003). In addition to reduced parasitism in comparison to their native ranges, non-native species can have lower levels of parasitism than their native counterparts (Torchin *et al.* 2003).

Parasites are ubiquitous in the marine environment (Torchin *et al.* 2002) and can alter their host's physiological functions, impact population dynamics and community structure, potentially altering ecosystem functioning (Hudson *et al.* 2006; Poulin and George-Nascimento 2007; Kuris *et al.* 2008; O'Connell-Milne *et al.* 2016), and so may play an important role in the invasion process (Prenter *et al.* 2004). However, there have been few studies examining the role of parasites in the invasion process (Mitchell and Power 2003; Torchin *et al.* 2003). In particular, investigating whether parasites affect native and invasive species in different ways, and whether any such differences can actually play a role in the invasion success of a given species

(Torchin *et al.* 2003). Although many parasites are host-specific, it can still be possible for invasive species to interfere with native host-parasite interactions by creating a "dilution effect" (Torchin et al. 2003). For example, in laboratory mesocosm experiments, two invasive species, *Crassostrea gigas* and *Crepidula fornicata* reduced the parasitic load in native mussels by interfering with transmission of the parasite and thus inhibiting its life cycle. Despite filtering the parasite, neither invasive species was adversely affected, as they are not ideal hosts for that species (Thieltges *et al.* 2009).

The non-native barnacle species *Austrominius modestus* is widespread on Atlantic European coasts (Tøttrup *et al.* 2010). This species co-occurs with the native arctic-boreal barnacle species *Semibalanus balanoides* in the middle and northern parts of the range it has invaded and the warm water adapted *Chthamalus montagui* and *C. stellatus* throughout its entire invaded range. Adult barnacles are sessile suspension feeders (Rainbow and Wang 2001) and have the ability to consume a wide size range of suspended particles (Crisp and Southward 1961).

Cysts of the digenean trematode *Maitrema arenaria* Hadley and Castle 1940 and adults of the parasitic isopod *Hemioniscus balani* Buchholtz 1866 are both known to infect barnacles (Hadley and Castle 1940; Blower and Roughgarden 1989; Carrol *et al.* 1990; Irwin *et al.* 1990; Sari and Malek 2000). In the case of *M. arenaria*, barnacles act as an intermediate host in the life cycle of the trematode. While feeding, barnacles consume cercariae (trematode larval stage), which were produced in the previous host, generally a gastropod. Within the barnacle, the cercariae invade the gut wall and encyst to form metacercariae. In the final host, usually a shore bird, the metacercariae develop into adult trematodes. These adult trematodes reproduce sexually and eggs are shed with the hosts faeces into the water. Ciliated miracidia, which are a free-living form, hatch from the eggs in the water column and infect the first intermediate host, allowing the cycle to continue (Carrol *et al.* 1990; Sari and Malek 2000). Turnstones are one of the few bird species known to consume barnacles, thus limiting the transmission of *M. arenaria*. However, it is known that dog whelks, which predate on barnacles and are

consumed by bird species such as rock pipits, oystercatchers, purple sandpipers and whimbrels, can act as paratenic host for *M. arenaria* (McCarthy *et al.* 1999).

Hemioniscus balani is known to infect at least fourteen different species of barnacle and has a global distribution (Blower and Roughgarden 1989). Following an ectoparasitic stage on calanoid copepods, male cryptoniscid stages are produced. These enter the mantle cavity of barnacles and attach to an ovary. The male then metamorphoses into a female stage, which is sessile (Arnott 2001). The female stage consumes ovarian fluid from its host and precludes egg production by the host. This castration, temporarily turns hermaphroditic barnacles into males, which can have a large impact on host population dynamics, in particular in the case of obligatory cross-fertilisers (Blower and Roughgarden 1989) such as *A. modestus* and *S. balanoides* (Barnes and Crisp 1956). For reproduction to occur, a free-living male isopod mates with the sessile female, before moving to an unparasitised barnacle host where it then metamorphoses into a female. Larvae develop within the female, which eventually bursts, releasing mature planktonic larvae into the water (Blower and Roughgarden 1989).

Since there is no published information on the levels of parasitism experienced by *A. modestus* in its native range, it is not possible to make comparisons with its European range. Therefore, the objectives of this study were to compare the levels of parasitism of trematode cysts and parasitic isopods in *A. modestus* and a native barnacle species at the northern, western and southern limits of its invaded range, to determine whether this species experiences some level of enemy release in comparison to natives. Additionally, by sampling at multiple locations within its invaded range, it was possible to investigate whether there was any relationship between levels of parasitism and latitudinal variation and whether or not parasites can play a role in controlling the distribution of *A. modestus.*

7.3 Materials and Methods

7.3.1 Study sites

Three sampling locations, representing the northern (Scotland), southern (Portugal) and western (Ireland) limits of the invaded range of *Austrominius modestus* were selected for this study (Figure 1). Fintry Bay (N55°46.014' W004°56.521') (Figure 2) is located on the Isle of Cumbrae, Firth of Clyde, Scotland (Figure 1), which is close to the recorded northern geographic limit of *A. modestus* in Europe (Moore and Howson 2014; Nall *et al.* 2015). Fintry Bay is a fairly sheltered site on the west coast of Cumbrae island, consisting of a rocky shore comprised of bedrock and with loose stones present from the mid to low shore. Bullens Bay (N51°38.578' W008°33.092') (Figure 3) is located on the south coast of Ireland (Figure 1). This location represents the western limit of this species in Europe and is also the biogeographic boundary of northern and southern intertidal species (Simkanin *et al.* 2005; Herbert *et al.* 2007). Bullens Bay has both rocky outcrops and small stones and boulders. It is a sheltered site and lies in close proximity to a mobile home and camping site, which may be sources of pollution. Farol (N 36°58.665' W 007°51.963') (Figure 4) is located on Ihla de Culatra, on the south coast of Portugal (Figure 1), which is the southern-most published limit of *A. modestus* in Europe (O'Riordan and Ramsay 2013). This site is sheltered and is subject to high levels of boating activity from ferries, water taxis and leisure craft, which could have an impact on water quality. Sampling sites in Scotland and Ireland were selected due to previous sampling and associated datasets for these particular locations, which could be used for comparison with data collected during the present study. The field site in Portugal, Farol, was chosen due to its location within the Ria Formosa Natural Park, which is an area of ecological importance (Newton and Mudge 2003; Gamito and Erzini 2005), a high abundance of *A. modestus* in comparison to other locations nearby and the availability of small stones with barnacles growing on them which could easily be transported to the laboratory.

Figure 1. The location of sampling sites on the Isle of Cumbrae, Scotland (1), Cork, Ireland (2) and the Algarve, Portugal (3). Map crated using ArcGIS.

Figure 2. Photograph of sampling site at Fintry Bay, Isle of Cumbrae, Scotland (Source: author's own).

Figure 3. Photograph of sampling site at Bullens Bay, Cork, Ireland (Source: author's own).

Figure 4. Photograph of sampling site at Farol, Ihla de Culatra, Algarve, Portugal (Source: author's own).

7.3.2 Sample collection and screening

Samples of *A. modestus* and a native barnacle species (*Semibalanus balanoides* in Scotland and Ireland and *Chthamalus montagui* in Portugal) were collected from field sites on a monthly (Ireland) or biannual (spring and autumn) basis (Scotland and Portugal) between 2014 and 2016. Spring and autumn were chosen for sampling, as it has been found that infection of *Maritrema arenaria* can peak in March and September (Sari and Malek 2000). Additionally these time periods represented times of highest and lowest seawater temperatures during the year.

Although *C. montagui* occurs at all the sampling areas in this study, it was not chosen as the native barnacle species for comparison across all sites, as it was not commonly found on small stones or limpets which could be collected and transported to the laboratory at field sites in Ireland and Scotland. Additionally, previous data collected at Bullens Bay and on the Isle of Cumbrae had screened *S.*

balanoides and *A. modestus* (O'Riordan and Culloty unpublished data), so using the same species during this study allowed for direct comparison. Small stones or limpets with barnacles growing on them were brought from the field sites to the laboratory.

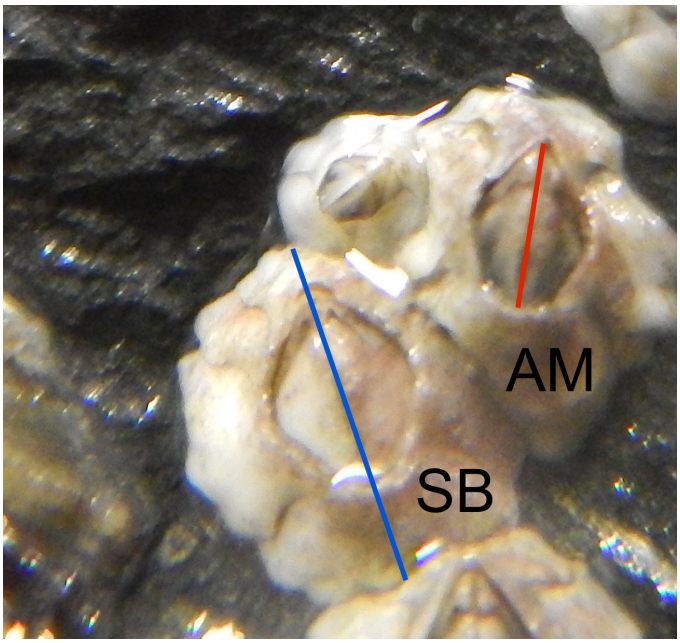

Figure 5. The invasive barnacle species *Austrominius modestus* (AM) and a native barnacle species *Semibalanus balanoides* (SB) used for parasite screening. The red line represents the measurement taken for the opercular diameter of each individual, and the blue line represents the rostro-carinal diameter measurement (Source: author's own).

Samples were kept in tanks with aerated seawater until they were examined under the microscope (Nikon SM 2645 (Ireland), Zeiss Stemi 2000-c (Portugal) and Olympus BH-2 (Scotland); X 5 magnification), never more than 48 hours after collection. Sixty individuals of each barnacle species were screened for parasites on each sampling occasion. For screening, an individual barnacle was removed from its substrate using a scalpel and placed on the edge of a well slide. The rostro-carinal diameter (RCD) and the opercular diameter (OD) (Figure 5) of each individual were measured using digital vernier calipers (Silver Line Tools, digital vernier calipers). The body of the barnacle was extracted from the shell with fine forceps and placed in the well of the slide with 70% ethanol. The shell cavity was scraped out using a pair of fine forceps to ensure all parasites were transferred to the well of the slide. The slide was then examined under a binocular microscope, where using fine forceps and a pointer, the

body mass was squashed and manipulated in order to identify any parasites present. When present, trematode cysts (Figure 6) and parasitic isopods (Figure 7) were counted, measured (using a calibrated eye piece graticule) and, in the case of parasitic isopods, the sex was also determined. Additionally, the reproductive state of each screened barnacle was recorded following O'Riordan and Murphy (2000) for *A. modestus*, O'Riordan *et al.* (1995) for *C. montagui* and Barnes and Barnes (1974) for *S. balanoides*.

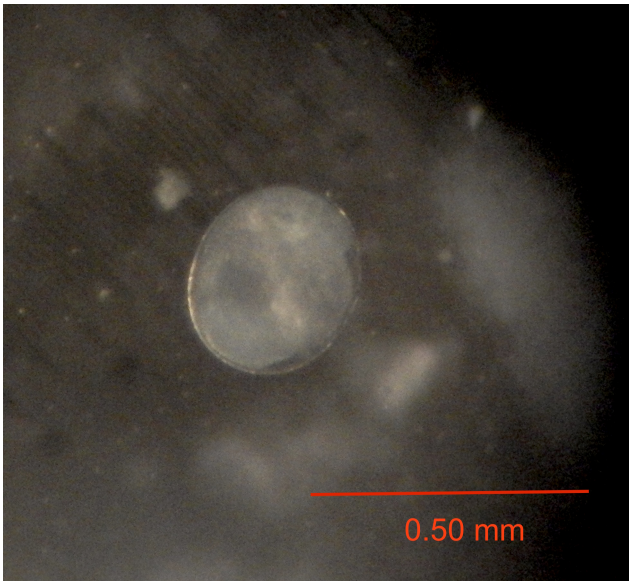

Figure 6. Trematode cyst (*Maritrema arenaria*) found in a sample collected from Bullens Bay, Ireland (Source: author's own).

Figure 7. Female parasitic isopod, *Hemioniscus balani*, found in a sample collected from Bullens Bay, Ireland (Source: author's own).

7.3.3 Temperature data

Seawater temperatures were obtained for each sampling location for the duration of the study. Seawater temperatures for the Isle of Cumbrae were obtained from Marine Scotland. Hourly seawater temperature data recorded by buoys M3 and M5 off the south coast of Ireland was obtained from Met Éireann (The Irish Meteorological Service). Mean (± SE) values were calculated for these locations. Mean seawater temperature values for Faro on the coast of the Algarve, Portugal were obtained from the global seawater temperature website (www1).

7.3.4 Statistical analysis

Data analysis was carried out using RStudio software version 3.3.0 (R Core Team 2016). Two-way ANOVA and TukeyHSD post-hoc tests were used to examine any differences in host size and cyst size with species and or sampling location. Linear regression was used in order to examine whether there was a relationship between opercular diameter and rostro-carinal diameter. Negative binomial regression ("MASS" package; Venables and Ripley 2002) was used to examine the relationship between number of cysts and host size and number of cysts and seawater temperature. This test was used as number of cysts is count data, and in this case there were many zeros and low values with fewer high values. Data were not transformed following O'Hara and Kotze (2010), however by using negative binomial regression, the skewed nature of the data was accounted for. Proportional data for prevalence of infection and proportion of infected and uninfected individuals without embryos were analysed using the "prop.test" function in R. Sequential Holm-Bonferroni corrections were applied to the p values in order to reduce the probability of Type 1 error (Holm 1979; Getano 2013) using a Holm-Bonferroni sequential correction EXCEL calculator (Gaetano 2013).

7.4 Results

7.4.1 Host size

There were significant differences in RCD between species ($F(2,656)=222.42$, $p < 0.001$) and location ($F(2,3594)=81.61$, $p < 0.001$). There was also a significant interaction between species and location ($F(1,205)=139.36$, $p < 0.001$). TukeyHSD post-hoc tests revealed that *Semibalanus balanoides* had a larger mean RCD in Scotland (6.3 mm ± 0.11 mm) compared with Ireland (4.5 mm ± 0.04mm) (Figure 8). *Austrominius modestus* from Scotland had the largest mean RCD (5.00 mm ± 0.08 mm), followed by Portugal (4.6 mm ± 0.05 mm), with those from Ireland being the smallest (4.5 mm ± 0.03 mm) (Figure 8). *Chthamalus montagui* had the smallest mean rostro-carinal diameter (4.0 mm ± 0.05 mm) of the three species (Figure 8).

There were also significant differences in OD between species ($F(2,3474)=98.93$, $p < 0.001$) and location ($F(2,3474)=356.25$, $p < 0.001$). There was a significant interaction between species and location ($F(1,3474)=144.41$, $p < 0.001$). *Semibalanus balanoides* had a larger mean opercular diameter in Scotland (2.9 mm ± 0.05 mm) compared with Ireland (1.9 mm ± 0.02 mm) (Figure 8). Largest mean opercular diameter of *A. modestus* was recorded for specimens from Portugal (2.5 mm ± 0.03mm), followed by Scotland (2.4 mm ± 0.05 mm), while individuals collected at the Irish sampling site had the smallest mean opercular diameter (2.1 mm ± 0.02 mm) (Figure 8). *Chthamalus montagui* had the smallest mean opercular diameter of the three species (1.8 mm ± 0.02 mm) (Figure 8).

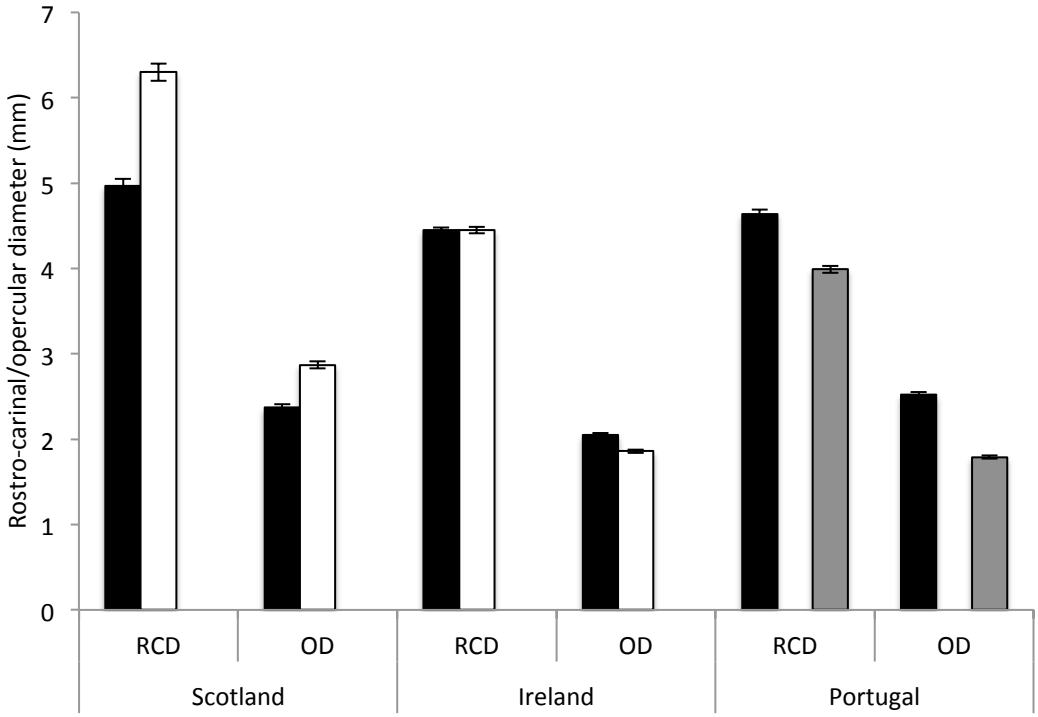

Figure 8. Mean rostro-carinal diameter (RCD) and opercular diameter (OD) in mm (± SE) of three barnacle species, *Austrominius modestus* (black), *Semibalanus balanoides* (white) and *Chthamalus montagui* (grey) collected from sampling sites in Scotland, Ireland and Portugal between 2014 and 2016.

7.4.2 Infection by Maritrema arenaria

Cysts of *Maritrema arenaria* were recorded in both *A. modestus* and the native barnacle species *S. balanoides* at sampling sites in Scotland and Ireland during this study. No trematode cysts were recorded from the sampling site in Portugal in either *A. modestus* or *C. montagui*. At Bullens Bay, Ireland, *S. balanoides* had a significantly higher prevalence of infection than *A. modestus* (χ^2 = 17.715, 1df, p < 0.001) overall. A higher proportion of *S. balanoides* were found to contain cysts in comparison to *A. modestus* on almost all sampling occasions, with the exception of June and August 2014 and October 2015 (Figure 9). However, July 2014 was the only month where there was a significant difference in the prevalence of *M. arenaria* cysts in *A. modestus* (7%) and *S. balanoides* (32%) (χ^2 = 11.718, 1df, p < 0.001) (Figure 9), and was also the highest proportion of *S. balanoides* recorded to contain cysts at this location during this study. The highest proportion of *A. modestus* infected by *M. arenaria* (18%) was recorded in the previous month, June 2014. Lowest levels of infection for *S. balanoides* were recorded in August 2014 (0% of individuals infected),

March 2015 (2% of individuals infected) and June 2015 (2% of individuals infected) (Figure 9). Screened individuals of *A. modestus* were found to contain no cysts in November 2014 and January, March, June and November 2015 (Figure 9). When cysts were present, lowest levels of infection recorded were in July and August 2015 with 2% of individuals found to contain cysts on both occasions (Figure 9).

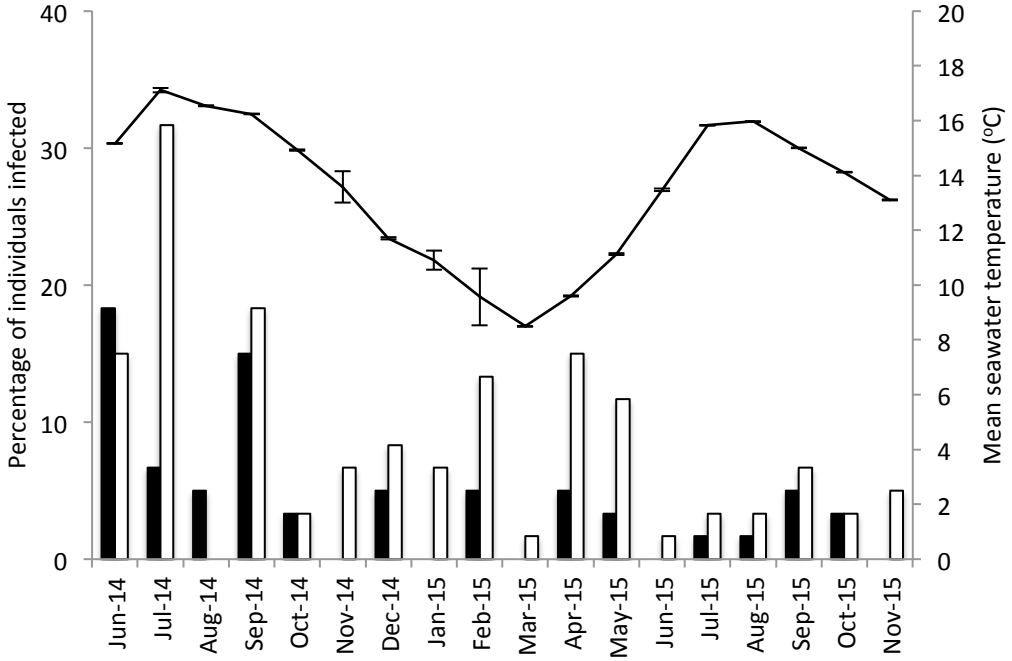

Figure 9. Percentage of individuals of *Austrominius modestus* (black bars) (n=60) and *Semibalanus balanoides* (white bars) (n=60) infected with trematode (*Maritrema arenaria*) cysts at monthly intervals from June 2014 to November 2015 at Bullens Bay, Ireland. Mean seawater temperature (± SE) is represented by the line.

At Fintry Bay, Scotland, *S. balanoides* also had a higher prevalence of infection compared with *A. modestus* overall (χ^2 = 99.09, df = 1, $p < 0.001$), with a higher percentage of *S. balanoides* individuals found to contain cysts than individuals of *A. modestus* at each sampling period at this location (Figure 10). These differences were found to be significant for each time period ($p < 0.01$).

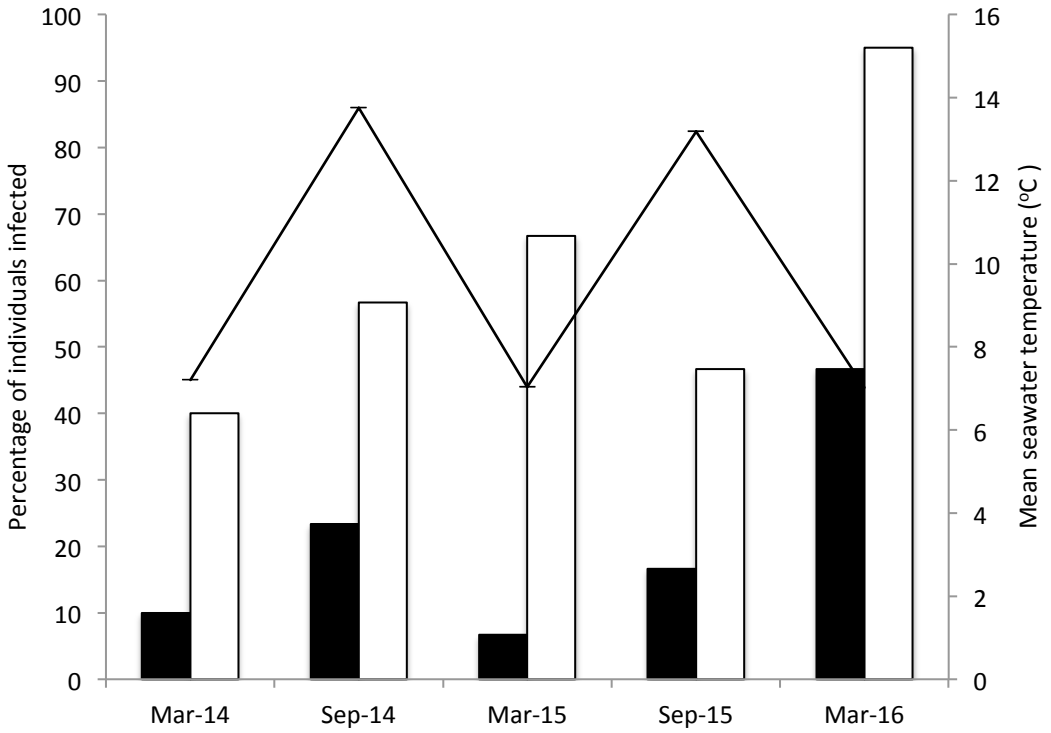

Figure 10. Percentage of individuals of *Austrominius modestus* (black bars) (n=60) and *Semibalanus balanoides* (white bars) (n=60) infected with trematode (*Maritrema arenaria*) cysts on five sampling occasions between March 2014 and March 2016 at Fintry Bay on the Isle of Cumbrae, Scotland. Mean seawater temperature (± SE) is represented by the line.

Highest prevalence of infection for both *A. modestus* (47%) and *S. balanoides* (95%) from Fintry Bay, Scotland was recorded in March 2016. Both species were found to have a significantly higher percentage of individuals containing cysts at the end of the study in March 2016 compared to the start in March 2014 (AM: $\chi^2 = 16.13$, df = 1, $p < 0.01$; SB: $\chi^2 = 37.00$, df = 1, $p < 0.01$). Despite fluctuating temperatures between March (approximately 7 °C) and September (approximately 13 °C) there was a trend towards an increasing proportion of individuals found with cysts, which did not directly follow any changes in seawater temperature (Figure 10). There was a significantly higher prevalence of infection of both *A. modestus* and *S. balanoides* in Fintry Bay (Figure 10) compared to Bullens Bay (Figure 9) (AM: $\chi^2 = 89.90$, df = 1, $p < 0.01$; SB: $\chi^2 = 400.99$, df = 1, $p < 0.01$). Fintry Bay also had lower seawater temperatures compared to Bullens Bay (Figures 9 and 10).

At Bullens Bay, the intensity of infection for both barnacle species generally ranged from 1 - 1.5 cysts per individual, however there were some exceptions. During sampling between June 2014 and November 2015, highest intensities were recorded for *A. modestus* in July 2014 (90.5 cysts per individual) and September 2014 (23.66 cysts per individual). Highest intensities recorded for *S. balanoides* during this time was much lower with a maximum of 1.4 cysts per individual recorded in December 2014 (Table 1).

Table 1. Intensity of infection (mean number of cysts per infected individual) of *Maritrema arenaria* cysts in two barnacle species *Austrominius modestus* (AM) and *Semibalanus balanoides* (SB) collected from Bullens Bay, Co. Cork on a monthly basis from June 2014 to November 2015 inclusive.

	AM	**SB**
June 2014	1.3 ± 0.15	1.0 ± 0.00
July 2014	90.5 ± 86.20	1.3 ± 0.21
August 2014	0.0 ± 0.00	1.0 ± 0.00
September 2014	23.7 ± 22.5	1.0 ± 0.00
October 2014	1.5 ± 0.50	1.0 ± 0.00
November 2014	0.0 ± 0.00	1.0 ± 0.00
December 2014	1.0 ± 0.00	1.4 ± 0.40
January 2015	0.0 ± 0.00	1.0 ± 0.00
February 2015	1.0 ± 0.00	1.1 ± 0.13
March 2015	0.0 ± 0.00	1.0 ± 0.00
April 2015	1.0 ± 0.00	1.2 ± 0.15
May 2015	1.0 ± 0.00	1.0 ± 0.00
June 2015	0.0 ± 0.00	1.0 ± 0.00
July 2015	1.0 ± 0.00	1.0 ± 0.00
August 2015	1.0 ± 0.00	1.0 ± 0.00
September 2015	1.0 ± 0.00	1.0 ± 0.00
October 2015	1.0 ± 0.00	1.0 ± 0.00
November 2015	0.0 ± 0.00	1.3 ± 0.33

Intensity of infection was higher in Scotland, when compared to Ireland. While typical intensity of infection from Bullens Bay was approximately one cyst per infected individual, at Fintry Bay the lowest recorded mean intensity was 1.5 (± 0.15) cysts per infected individual, and mean intensities of 3-4 cysts per individual were recorded on multiple sampling occasions (Table 2). There was no consistent trend for either *S. balanoides* or *A. modestus* to have a higher mean intensity of infection at Fintry Bay, throughout the study. Highest intensities of infection were recorded for

both species in September 2014 (AM: 3.4 ± 0.34; SB: 4.5 ± 1.20) and March 2016 (AM: 2.1 ± 0.34; SB: 6.2 ± 0.65).

Table 2. Intensity of infection (mean number of cysts per infected individual ± SE) of *Maritrema arenaria* cysts in two barnacle species *Austrominius modestus* (AM) and *Semibalanus balanoides* (SB) collected from Fintry Bay, Isle of Cumbrae, Scotland on a biannual basis from March 2014 to March 2016 inclusive.

	AM	SB
2014		
March	1.7 ± 0.34	1.2 ± 0.48
September	3.4 ± 0.59	4.5 ± 1.20
2015		
March	1.3 ± 0.25	3.4 ± 0.56
September	1.9 ± 0.31	1.5 ± 0.15
2016		
March	2.1 ± 0.34	6.2 ± 0.65

7.4.5 Host size and infection

Overall there was a significant positive relationship between number of cysts and rostro-carinal diameter ($p < 0.001$). There was an overall significant positive relationship between rostro-carinal diameter and number of cysts at Fintry Bay, Scotland ($p < 0.001$), however this was not significant for either species individually (Figure 11). For data collected at Bullens Bay, Ireland, there was a significant ($p < 0.05$) relationship between rostro-carinal diameter and number of cysts over all, however this was not significant for either *A. modestus* or *S. balanoides* individually (Figure 12).

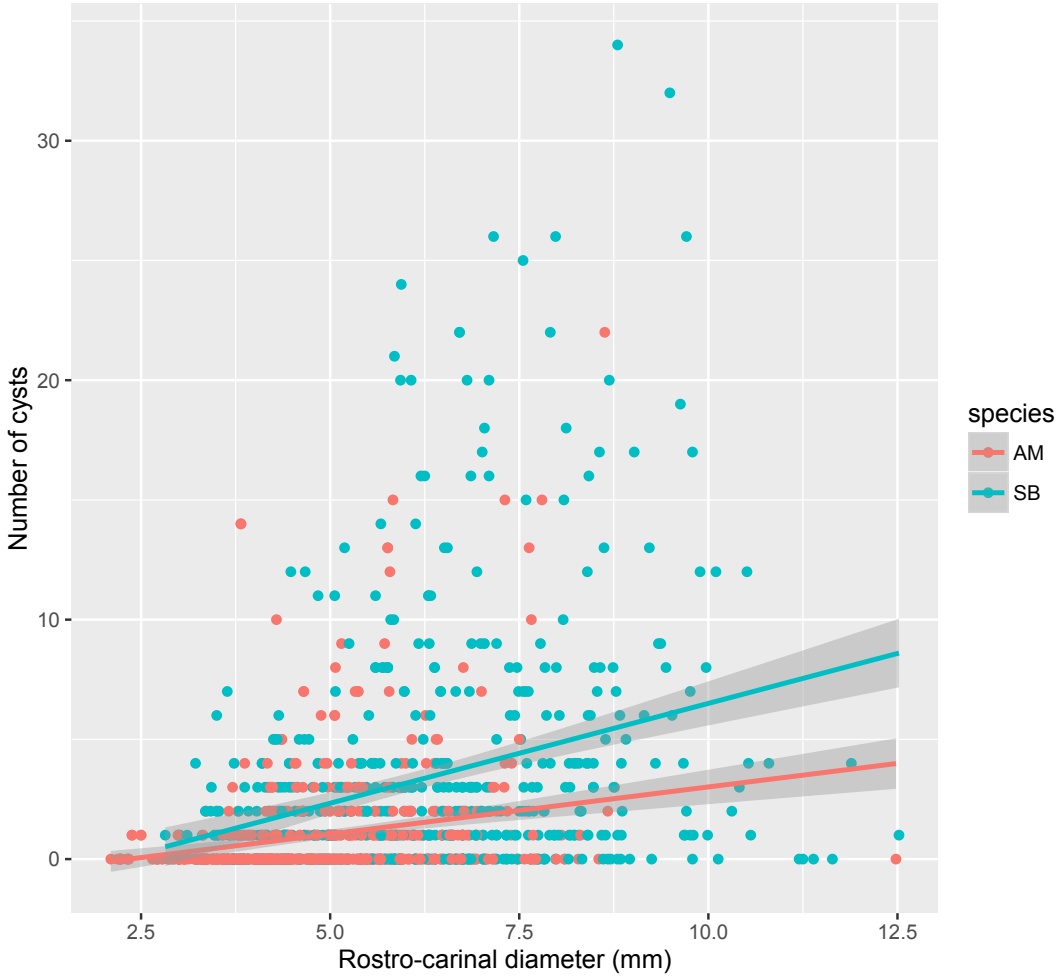

Figure 11. Rostro-carinal diameter (mm) and number of cysts recorded for individuals of two barnacle species, *Austrominius modestus* (AM; red) and *Semibalanus balanoides* (SB; blue) from Fintry Bay, Isle of Cumbrae, Scotland, during 2014 - 2016. Blue and Red lines represent the regression lines for each species with standard error represented by the grey shaded area either side of the line.

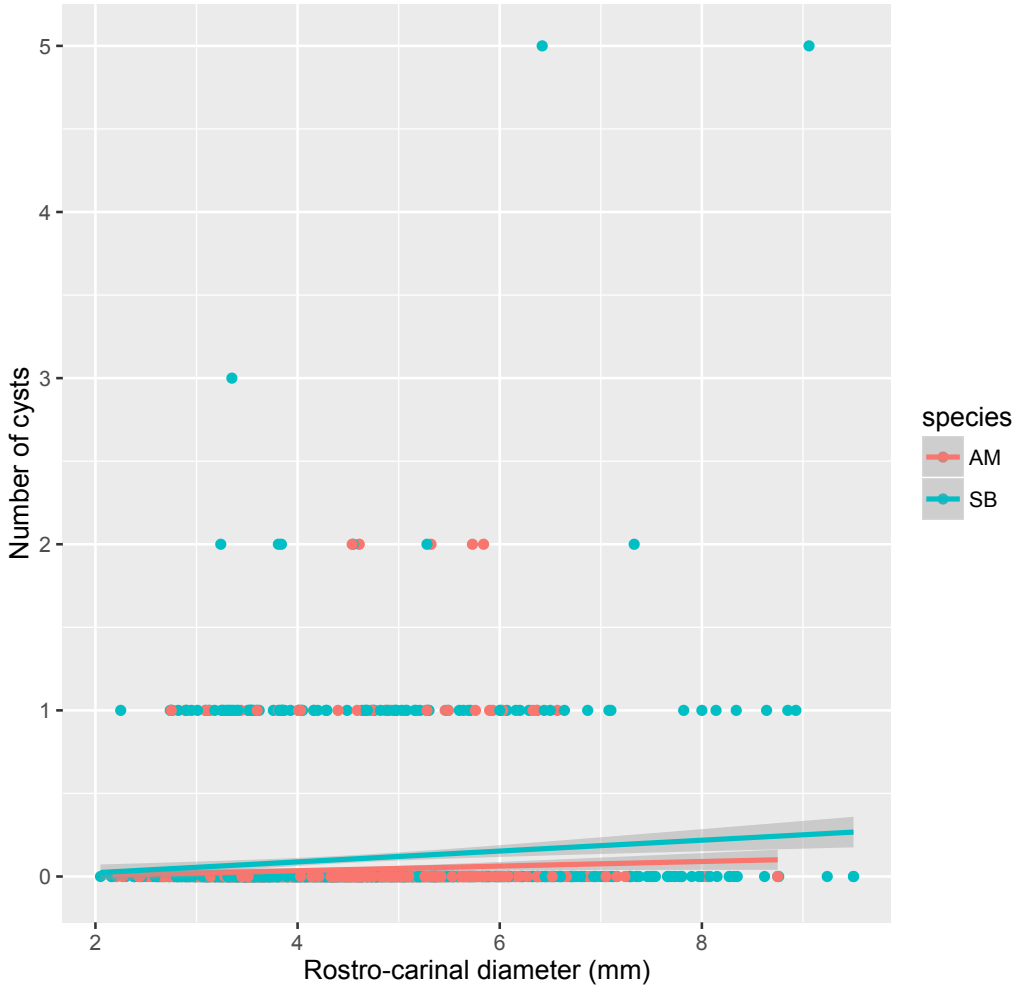

Figure 12. Rostro-carinal diameter (mm) and number of cysts recorded for individuals of two barnacle species, *Austrominius modestus* (AM; red) and *Semibalanus balanoides* (SB; blue) from Bullens Bay, Ireland, during 2014 - 2015. Blue and Red lines represent the regression lines for each species with standard error represented by the grey shaded area either side of the line.

7.4.6 Cyst size

There were significant differences between cyst size with barnacle species ($F(1,1091)=28.66$, $p < 0.001$) and location ($F(1,1091)=8.32$, $p < 0.01$), however there was no significant interaction between species and location. Mean cyst size recorded from *S. balanoides* was larger than that for *A. modestus* in both Ireland (Table 3) and Scotland (Table 4). Overall, cysts recorded from the sampling location in Scotland (0.26 mm ± 0.004 mm) were smaller than those recorded from Ireland (0.27 mm ± 0.001 mm).

Table 3. Mean (± SE) size (mm) of *Maritrema arenaria* cysts found in two barnacle species, *Austrominius modestus* (AM) and *Semibalanus balanoides* (SB) from Bullens Bay, Ireland from June 2014 to November 2015. – indicates that no cysts were recorded at this time period.

	AM	**SB**
June 2014	0.24 ± 0.014	0.31 ± 0.012
July 2014	0.18 ± 0.004	0.23 ± 0.008
August 2014	-	0.22
September 2014	0.35 ± 0.009	0.22 ± 0.024
October 2014	0.23 ± 0.025	0.28 ± 0.031
November 2014	-	0.32 ± 0.010
December 2014	0.27 ± 0.007	0.28 ± 0.024
January 2015	-	0.25 ± 0.018
February 2015	0.22 ± 0.007	0.31 ± 0.025
March 2015	-	0.25
April 2015	0.27 ± 0.012	0.29 ± 0.007
May 2015	0.33 ± 0.021	0.27 ± 0.011
June 2015	-	0.33
July 2015	0.29	0.29 ± 0.021
August 2015	0.29	0.26 ± 0.010
September 2015	0.27 ± 0.021	0.28 ± 0.013
October 2015	0.029 ± 0.082	0.32 ± 0.010
November 2015	-	0.32 ± 0.010
Overall	0.26 ± 0.007	0.27 ± 0.005

Table 4. Mean (± SE) size (mm) of *Maritrema arenaria* cysts found in two barnacle species, *Austrominius modestus* (AM) and *Semibalanus balanoides* (SB) at Fintry Bay on the Isle of Cumbrae, Scotland between March 2014 and March 2016.

	AM	**SB**
2014		
March	0.29 ± 0.015	0.30 ± 0.004
September	0.26 ± 0.002	0.26 ± 0.003
2015		
March	0.25 ± 0.022	0.27 ± 0.002
September	0.27 ± 0.005	0.28 ± 0.004
2016		
March	0.27 ± 0.002	0.29 ± 0.001
Overall	0.27 ± 0.002	0.28 ± 0.001

7.4.7 Infection by Hemioniscus balani

The parasitic isopod, *Hemioniscus balani* was not recorded on any sampling occasion from specimens collected at Farol, Portugal or Fintry Bay, Scotland. In samples collected at Bullens Bay, *H. balani* was recorded in *S. balanoides* in five out of eighteen months and eight out of eighteen months for *A. modestus.* The proportion of individuals infected was generally low for both barnacle species, ranging from 2% to 17% (Figure 13). There was no significant difference in the prevalence of infection of *A. modestus* and *S. balanoides* over the entire sampling period from June 2014-November 2015, or at any individual sampling occasion. Highest proportions of individuals infected were in June and July 2014 for *S. balanoides* (13% and 12% respectively) and in July 2014 for *A. modestus* (17%) `(Figure 13). Mean seawater temperatures in both of these months were high (June: 15.2 ^{o}C ± 0.01 ^{o}C; July 17.1 ^{o}C ± 0.08 ^{o}C), with July 2014 having the highest temperature for this location during the study period. Maximum mean temperature in 2015 was recorded in August (16.0 ^{o}C ± 0.13 ^{o}C), however prevalence of *H. balani* infection remained low during this time. Generally, a single *H. balani* was found in infected individuals, however on two occasions (June and July 2014), an individual of *S. balanoides* was found to contain two individuals of *H. balani* (Table 5), on both occasions the parasites were male and female.

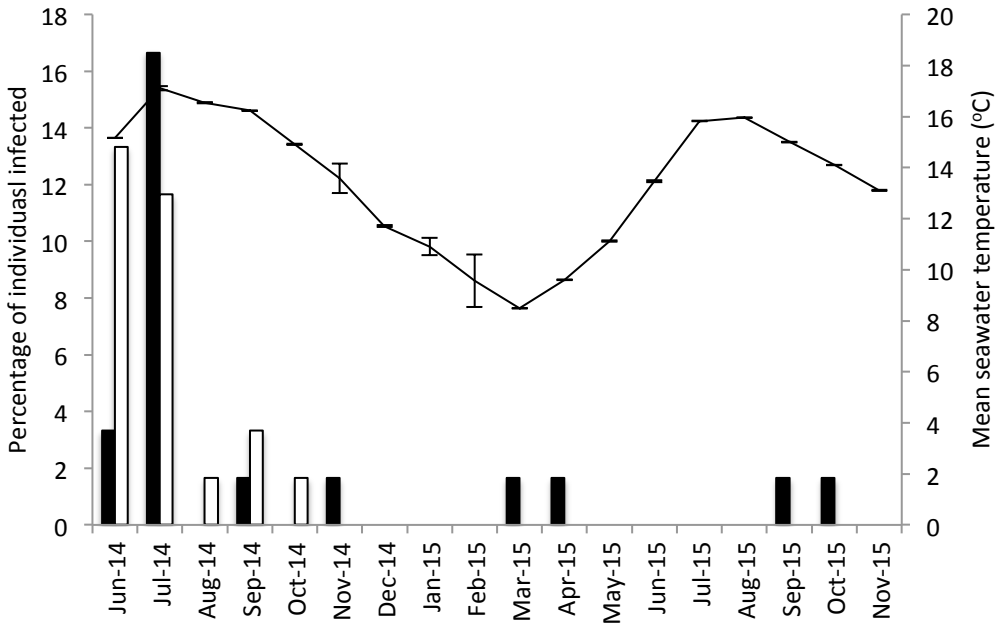

Figure 13. Percentage of individuals of *Austrominius modestus* (black bars) (n=60) and *Semibalanus balanoides* (white bars) (n=60) infected with the parasitic isopod, *Hemioniscus balani* at monthly intervals from June 2014 to November 2015 at Bullens Bay, Ireland. Mean seawater temperature (± SE) is represented by the line.

Table 5. Intensity of infection (mean number of isopods per infected individual ± SE) of *Hemioniscus balani* in two barnacle species *Austrominius modestus* (AM) and *Semibalanus balanoides* (SB) collected from Bullens Bay, Co. Cork on a monthly basis from June 2014 to November 2015.

	AM	**SB**
June 2014	1.0 ± 0.00	1.1 ± 0.13
July 2014	1.0 ± 0.00	1.2 ± 0.17
August 2014	1.0 ± 0.00	0.0 ± 0.00
September 2014	1.0 ± 0.00	1.0 ± 0.00
October 2014	0.0 ± 0.00	1.0 ± 0.00
November 2014	1.0 ± 0.00	0.0 ± 0.00
December 2014	0.0 ± 0.00	0.0 ± 0.00
January 2015	0.0 ± 0.00	0.0 ± 0.00
February 2015	0.0 ± 0.00	0.0 ± 0.00
March 2015	1.0 ± 0.00	0.0 ± 0.00
April 2015	1.0 ± 0.00	0.0 ± 0.00
May 2015	0.0 ± 0.00	0.0 ± 0.00
June 2015	0.0 ± 0.00	0.0 ± 0.00
July 2015	0.0 ± 0.00	0.0 ± 0.00
August 2015	0.0 ± 0.00	0.0 ± 0.00
September 2015	1.0 ± 0.00	0.0 ± 0.00
October 2015	1.0 ± 0.00	0.0 ± 0.00
November 2015	0.0 ± 0.00	0.0 ± 0.00

7.4.8 Infection by both Maritrema arenaria and Hemioniscus balani

Infection of a barnacle host by both *M. arenaria* and *H. balani* was rare, and only recorded on six occasions at Bullens Bay. All instances of double infection were recorded between June 2014 and September 2014. *S. balanoides* and *A. modestus* were equally affected, with each species being infected by both parasites simultaneously on three occasions.

7.4.9 Infection and reproduction

At both Bullens Bay and Fintry Bay, a changing proportion of each barnacle species would either possess embryos or not, depending on the time of year. To determine whether or not *M. arenaria* infection was associated with a higher incidence of individuals without embryos than would be expected, a comparison was made of the percentage of individuals without embryos that were infected by *M. arenaria* and those that had no embryos but also had no cysts. At Bullens Bay, 82% of infected *S. balanoides* were recorded not to posses any embryos, compared to 84% of uninfected individuals (χ^2 = 0.01, df = 1, p = 0.892). 67% of *A. modestus* that were infected by *M. arenaria* had no embryos at this location, while 67% of uninfected individuals of this species were recorded without embryos throughout the study (χ^2 = 0.00, df = 1, p = 1) (Figure 14). There was no significant difference in the proportions of infected and uninfected individuals without embryos for either barnacle species at any individual sampling period between June 2014 and November 2015.

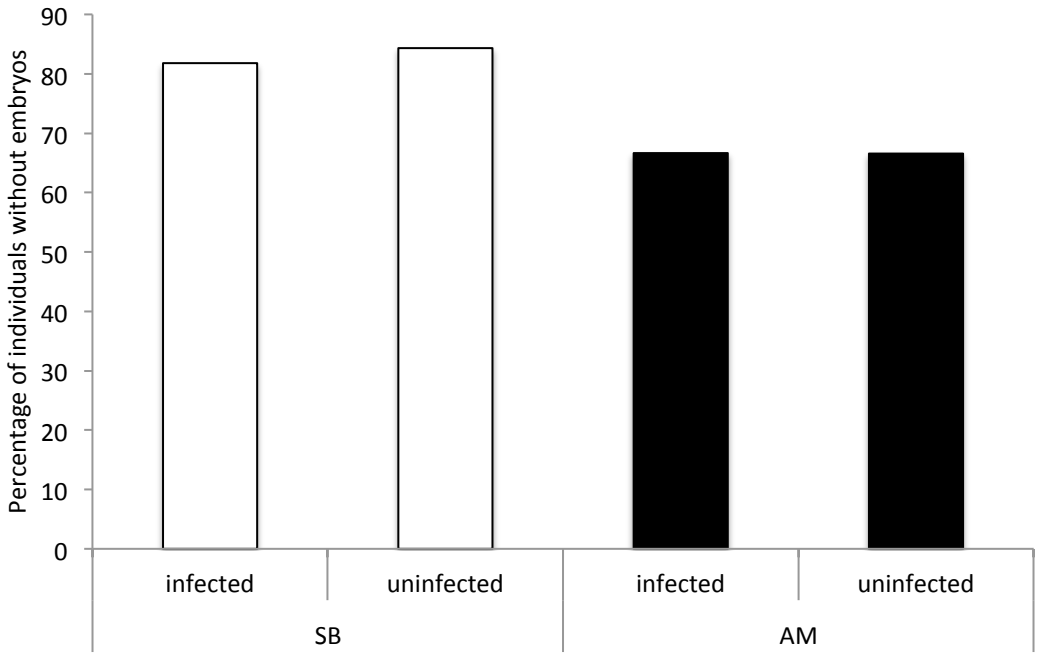

Figure 14. Percentage of two barnacle species, *Semibalanus balanoides* (SB; white) and *Austrominius modestus* (AM; black) without embryos, which were found to be infected or uninfected by trematode (*Maritrema* arenaria) cysts at Bullens Bay, Cork, Ireland from June 2014 – November 2015 inclusive.

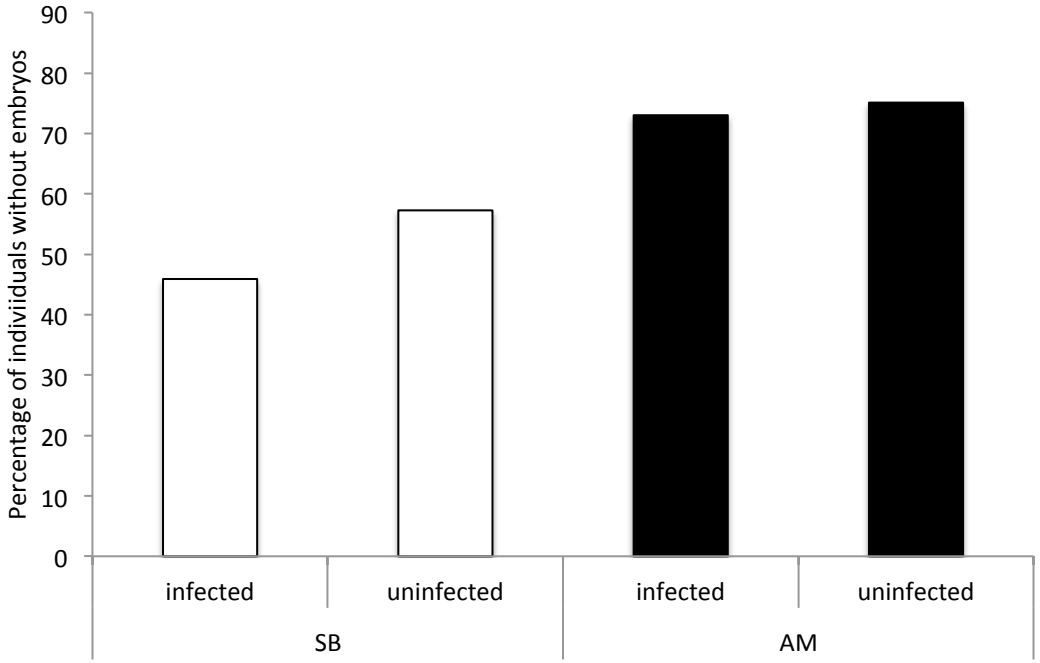

Figure 15. Percentage of two barnacle species, *Semibalanus balanoides* (SB; white) and *Austrominius modestus* (AM; black) without embryos, which were found to be infected or uninfected by trematode (*Maritrema* arenaria) cysts at Fintry Bay, Isle of Cumbrae, Scotland, on five sampling occasions from March 2014 – March 2016 inclusive.

This was also the case at Fintry Bay, where 46% of infected *S. balanoides* had no embryos, compared to 57% of uninfected individuals (χ^2 = 0.96, df = 1, p = 0.325) during the entire study. Similarly, 73% of infected *A. modestus* had no embryos, in comparison to 75% of uninfected individuals (χ^2 = 0.03, df = 1, p = 0.860) (Figure 15). There was a significant difference in September 2015 for *A. modestus* (χ^2 = 6.12, df = 1, p < 0.01), however this was due to a higher proportion of uninfected individuals without embryos (68%), than those infected (20%). There was no significant difference at any other time period. Hosts of both barnacle species infected by *M. arenaria* were found to contain well developed embryos throughout the study. This indicates that the absence of embryos in infected barnacles was not necessarily due to infection and was comparable to the reproductive state in uninfected individuals at both locations.

The majority of hosts infected by *H. balani* did not contain any embryos, however in June 2014, *S. balanoides* which were infected by this species were found to contain embryos at a range of developmental stages, including stage 4, which is very well developed. Additionally, in September 2014, a specimen of *A. modestus* infected by *H. balani* contained developing embryos.

7.4.10 Seawater temperature and infection

The highest prevalence and intensity of *M. arenaria* infection was recorded for the northern sampling site, which had the lowest range of seawater temperatures of all sampling locations (7.0 ^{o}C ± 0.01 ^{o}C – 13.8 ^{o}C ± 0.01 ^{o}C) (Figure 10), while cysts were absent from the southern field site with highest range of water temperatures (16.0 ^{o}C – 22.1^{o}C). Data from both Fintry Bay (Figure 10) and Bullens Bay (Figure 11) indicate that prevalence of infection follows a seasonal pattern as opposed to one driven solely by temperature.

7.5 Discussion

The invasive species *A. modestus* was infected by *M. arenaria* at sampling locations in Scotland and Ireland, but it did experience a lower prevalence of infection than the native barnacle species *S. balanoides*, and so can be said to be experiencing

enemy release. *A. modestus* was free of parasites at Farol, the southern field site, however so was the native *C. montagui*. Levels of infection by the parasitic isopod *H. balani* for both *A. modestus* and *S. balanoides* were generally low, and this species was only consistently recorded from one field site, Bullens Bay, though never at high abundances. O'Riordan and Murphy (2000) reported a similar occurrence of *H. balani* from *A. modestus* at another site in Cork, where *H. balani* was present in *A. modestus* in ten out of fifteen months, with a maximum of 12% of individuals infected in October 1993.

Trematodes are known to infect a wide range of species as their second intermediate host (Krakau *et al.* 2006), and so it is perhaps not surprising that the invasive *A. modestus* is infected by *M. arenaria*. Despite having a lower prevalence of infection, a higher intensity of infection was recorded overall for *A. modestus* in comparison to *S. balanoides* at Bullens Bay, Ireland. This is likely due to the occurrence of very high levels of infection of individual hosts in July and September 2014 and was not a regular occurrence. Levels of infection of *M. arenaria* recorded during this study were similar to those reported by Sari and Malek (2000) for *Perforatus perforatus* in Wales and Carrol *et al.* (1990) for *S. balanoides* in Ireland, but low in comparison to other authors, who recorded 600 – 1,000 cysts to be present in individual barnacles (*Semibalanus balanoides*) in cases of heavy infection (Hadley and Castle 1940).

It is thought that infection by *M. arenaria* causes little damage to the key organs of the barnacle host (Combes 1991; Poulin 1994), in comparison to the previous gastropod host, where the hosts' gonads are often destroyed to facilitate asexual reproduction by the parasite (Combes 1991; Poulin 1994). In the present study there was no significant difference found in the number of infected and uninfected barnacles without embryos in Scotland or Ireland. It is possible that this is due to the low intensity of infection recorded during this study. It is common for hosts to only have a partial parasite load (Poulin and George-Nascimento 2007), and a higher intensity of cysts may be required to have a negative effect on the reproductive output of the host. Cysts are usually found surrounding the gut of the host barnacle

(Hadley and Castle 1940), so it is possible that infection may interfere with the host's digestion, thus limiting growth and reproduction over time. The parasitic isopod *H. balani,* is known to inhibit reproduction of the host. The majority of hosts found to contain this parasite during this study did not contain any embryos. However, levels of infection of this parasite were generally low at Bullens Bay, Ireland, the one site where it was recorded on more than one occasion, with no significant difference in its prevalence in *A. modestus* and *S. balanoides*.

Results from this study show that larger barnacles were more likely to have more cysts, which is consistent with the findings of other authors in Ireland (Carrol *et al.* 1990) and Wales (Sari and Malek 2000), with older larger barnacles having a longer exposure time to parasites. The native *S. balanoides* was larger in size than *A. modestus* on the Isle of Cumbrae and at Bullens Bay, making it more susceptible to infection by *M. arenaria.* However, although it is smaller in size, *A. modestus* has a higher rate of cirral beat than that of native species (Southward 1955), which would suggest that it is more likely to ingest trematode cercaria than the native species. It has previously been recorded that *A. modestus* is capable of consuming high abundances of trematode larvae (Prinz *et al.* 2009), though no comparison was made with a native barnacle species. Despite the lower prevalence of infection in comparison to native species, *A. modestus* may play an important role in the transmission of trematode parasites in the marine environment by filtering cercariae at a different rate to natives (Prinz *et al*. 2009) or by diverting some of the parasite load of the native species (Krakau *et al.* 2006). If *A. modestus* is less likely to be consumed by the final hosts than native barnacle species, then *A. modestus* could represent a dead end in the life cycle of this parasite (Mackenzie 1999). It has previously been reported that *A. modestus* is less likely to be consumed by dog whelks (*Nucella lapillus*) in comparison to *S. balanoides* (Barnett 1979), however no information is available regarding predation by birds.

The relationship between host size and number of cysts could also in part explain the lack of infection of *C. montagui* in Portugal, as this species had the smallest rostro-carinal and opercular diameter of the three barnacle species examined during this

study. However, previous records of *M. arenaria* infecting *C. montagui* (O'Riordan 1992; Sari and Malek 2000) indicate that its small size does not inhibit infection, but may limit the extent of parasitism this species experiences. The absence of *M. arenaria* cysts at the sampling site in the Algarve, Portugal is more likely due to the lack of suitable intermediate hosts at this sampling location. Additionally, *M. arenaria* has been recorded as having a Northern Atlantic distribution (Carrol *et al.* 1990) and may not be found as far south as the Algarve. However, *H. balani* is known to have a global distribution, and does not require multiple intermediate hosts, but was also absent from barnacles screened at the Portuguese location during 2014 and 2015. Previous work by O'Riordan and Ramsay (2013) also noted the absence of *H. balani* from samples of *A. modestus* collected during April 2012 and 2013, at sites in the Algarve.

It is known that parasites account for a large amount of biomass and biodiversity (Kuris *et al.* 2008), playing an important role in many ecosystems (Hudson *et al.* 2006; Poulin and George-Nascimento 2007). Temperature is known to be an important factor for parasites during their life cycles and predicted increases in sea surface temperatures (IPCC 2014) are likely to alter parasite-host interactions (Marcogliese 2001). In the present study, highest prevalence of *M. arenaria* infection was recorded at the location with coolest seawater temperatures, while there was no infection recorded at the warmest and most southerly site. While this could imply that increasing temperatures may decrease the prevalence of *M. arenaria* infection in barnacles, overall there was a trend towards a higher abundance of cysts with warmer water temperatures at locations where cysts were found. Additionally, increasing temperatures may stress hosts, making them more susceptible to infection (Macrogliese 2001). The invasive *A. modestus* is warm water adapted and more likely to be able to cope with increasing seawater temperatures than the arctic-boreal species *S. balanoides* (Buckeridge 2012), possibly limiting its susceptibility to increased infection. It is also possible that differences between locations could be due to differences in the abundance of intermediate and/ or final hosts.

The fact that *A. modestus* is infected by parasites within its invaded range, despite the lower prevalence of infection, could indicate that the invasive species is not at an advantage compared to natives due to parasite release (Colautti *et al.* 2004). It is believed that enemy release may be important at the stages of initial invasion, however over time, infection by native parasites could eliminate any such advantages (Krakau *et al.* 2006). This may have been the case for *A. modestus*, although there is no information available regarding levels of parasitism for this species in the years immediately following its introduction. This species has been present in Europe for over seventy years (Bishop 1947; Crisp and Chipperfield 1948) and so has had a long exposure time to native parasites. The extent which ERH can confer an advantage to invasive species, is determined by the effect that the predator or pathogen has on its hosts (Torchin *et al.* 2002). It seems likely that unless present in very high numbers, infection by *M. arenaria* cysts will have little impact on barnacle hosts. Infection by *H. balani* is potentially much more harmful as it is known to castrate its host, however levels of infection recorded during this study were very low and not high enough to have a noticeable impact on host population dynamics. It was noted by Southward and Crisp (1954) that *A. modestus* may act as a reserve host for *H. balani,* potentially producing a high levels of infection in the native host *S. balanoides* and may warrant future monitoring.

Despite the fact that *A. modestus* was found to be free of parasites at its southern invaded range limit and to have a lower prevalence of infection than native barnacle species at its northern limits, this species is still found to be present in lower abundances than in native barnacle species at these locations (O'Riordan and Ramsay 1999; Range and Paula 2001; Boaventura *et al.* 2002; Gallagher *et al.* 2015). This indicates that infection by *M. arenaria* and *H. balani* is not exclusively responsible for controlling the abundance or distribution of *A. modestus* within its invaded range. Additionally, infection did not appear to have any impact on the ability to reproduce. It is important to consider that only two parasites were examined during this study, and that the presence or absence of other parasites, or differences in the bacterial load of *A. modestus* compared to native species

(Clements *et al*. 2013) could play a role in the invasion of this species and would be worth examining in future studies.

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Chapter 8

Long-term infection trends in two barnacle species, *Austrominius modestus* and *Semibalanus balanoides* at two locations in Europe

8.1 Abstract

Parasites account for a large amount of biomass and biodiversity and play an important role in many ecosystems. They can alter their host's physiological functions, impact population dynamics and community structure. Parasites can be used as indicators of environmental quality and anthropogenic impacts, as they reveal information about their hosts and the environment. In order to monitor changes in infection, baseline information is required, but is often not available. In the present study levels of infection of two barnacle species, the native arctic boreal *Semibalanus balanoides* and the non-native species *Austrominius modestus* were monitored in Ireland and Scotland at time scales varying from months to years. Both barnacle species were infected at both locations, however *A. modestus* had significantly lower levels of infection than the native barnacle species. In Ireland there was a decrease in infection between sampling carried out in 2006-2007 and 2014-2015. In Scotland levels of infection increased from 2008 - 2015 and there was also an increase from 2014 – 2015. It is difficult to attribute these changes to a single factor, but changing environmental conditions, host stress and abundances of other intermediate hosts are likely contributing factors. Predicted future changes in environmental conditions, such as increasing temperatures, are likely to be stressful for the native arctic boreal barnacle species, making it more susceptible to infection than *A. modestus.* Continued monitoring of these species at the study locations would provide an insight into such changes and allow for a better understanding of potential changes in ecosystem function.

KEYWORDS: barnacles, parasites, monitoring, *Austrominius modestus*, *Semibalanus balanoides*

8.2 Introduction

Parasites are ubiquitous in the marine environment (Torchin *et al.* 2002) and are known to account for a large amount of biomass, biodiversity (Kuris *et al.* 2008) and energy flow (Thieltges *et al.* 2008). They play an important role in many ecosystems, potentially altering ecosystem function, as they can change their host's physiological functions, impacting population dynamics and community structure (e.g. Hudson *et al.* 2006; Poulin and George-Nascimento 2007; Kuris *et al.* 2008; O'Connell-Milne *et al.* 2016). Parasites can be used as indicators of environmental quality and anthropogenic impacts (Lafferty 1997; Mackenzie 1999), as they reveal information about their hosts and the environment (Marcogliese 2004).

In addition to having an impact on population and community dynamics of native species, infection may play an important role in the invasion process of non-native species (Prenter *et al.* 2004), though this is not well studied (Mitchell and Power 2003; Torchin *et al.* 2003). *Austrominius modestus* is an Australasian barnacle species (Moore 1944), which is now widespread on Atlantic European coasts (Tøttrup *et al.* 2010). Non-native species often escape the effects of predators and pathogens within their new distributions that they would encounter in their native ranges. This is termed the Enemy Release Hypothesis (ERH), and has been attributed to the success of invasive species in new ranges, due to increased size and competitive ability (Williamson and Fitter 1996; Hudson and Greenman 1998; Torchin *et al.* 2001; Torchin *et al.* 2003).

Cysts of the digened trematode *Maitrema arenaria* and adults of the parasitic isopod *Hemioniscus balani* are both known to infect barnacles (Hadley and Castle 1940; Blower and Roughgarden 1989; Carrol *et al.* 1990; Irwin *et al.* 1990; Sari and Malek 2000). Infection by these parasites, in particular by *H. balani,* can impact the reproductive success of the hosts and can also have a large impact on host population dynamics (Blower and Roughgarden 1989).

In order to monitor changes in infection, baseline information is required (Lafferty *et al.* 2004), but is often lacking (Ward and Lafferty 2004). Levels of parasitism of the native barnacle species *S. balanoides* and the Australasian barnacle species *A. modestus* were monitored in Ireland and Scotland at multiple time intervals during this study, providing information on levels of infection at different temporal and spatial scales. Additionally, it was possible to investigate whether there was a difference in the level of infection observed in the native *S. balanoides* and the non-native *A. modestus* over time at different locations.

8.3 Materials and Methods

8.3.1 Study sites

Sampling locations were located in Bullens Bay, Ireland and on the Isle of Cumbrae, Firth of Clyde, Scotland. These locations are representative of the western and northern limits (respectively) of *Austrominius modestus* in Europe. A single sampling site was located at Bullens Bay (N51°38.578' W008°33.092') where monthly sampling was carried out from June 2006 – November 2007 and from June 2014 – November 2015. This site is in close proximity to a caravan site, and some residential houses, which may be a potential source of pollution. Multiple field sites were located on the Isle of Cumbrae: Watersports Slipway (55°46.58'N 4°53.55'W), Butterlump (55°45.55'N 4°56.42'W), Millport Harbour (N55°45.08' W004°55.13'), West Bay (55°44.95'N 4°56.10'W) and Fintry Bay (N55°46.01' W004°56.52'). Watersports Slipway and Millport Harbour were selected as they are subject to higher levels of boating activity than other potential sites, potentially impacting water quality at these locations. Samples were collected from Fintry Bay and Millport Harbour in March and September 2014, March and September 2015 and March 2016. The three additional sites, West Bay, Butterlump and Watersports Slipway, were sampled in September 2008, March 2015 and September 2015.

Figure 1. Location of sampling sites on the Isle of Cumbrae, Scotland (1) and at Bullens Bay, Cork, Ireland (2). Map created using ArcGis.

8.3.2 Sample collection and screening

Samples of *A. modestus* and the native barnacle species, *Semibalanus balanoides*, were collected from field sites on each visit. Sixty individuals of each species were screened for parasites on each sampling occasion, with the exception of West Bay, Butterlump and Watersports Slipway, where thirty individuals of each species were screened. Sampling during March and September at the northern field site was chosen as it has previously been reported that infection of *M. arenaria* peaks during March and September (Sari and Malek 2000). Additionally these time periods also correspond with times of highest and lowest seawater temperatures during the year.

Samples were kept in tanks with aerated seawater until they were examined under the microscope (Nikon SM 2645 (Ireland) and Olympus BH-2 (Scotland); X 5 magnification), and were always analysed within 48 hours of collection. For screening, an individual barnacle was removed from its substrate using a scalpel and placed on the edge of a well slide. The rostro-carinal diameter and the opercular diameter of each individual were measured using a digital vernier calipers (Silver Line Tools, digital vernier calipers). Using forceps, the body of the barnacle was extracted from the shell and placed in the well of the slide with 70% ethanol. The shell cavity was scraped out using a pair of fine forceps to ensure all parasites were transferred to the well of a cavity slide. The slide was then examined under a binocular microscope and using fine forceps and a pointer the body mass was squashed and manipulated in order to identify any parasites present. When present, trematode cysts and parasitic isopods were counted, measured (using a calibrated eye piece graticule) and in the case of parasitic isopods the sex was also determined.

8.3.3 Seawater Temperature

Seawater temperature data were obtained for each sampling location for the duration of the study. Seawater temperatures for the Isle of Cumbrae were obtained from Marine Scotland. Seawater temperature data recorded by buoys M3 and M5 off the south coast of Ireland was obtained from Met Éireann (The Irish Meteorological Service). Mean monthly (±SE) values were calculated for each location.

8.3.4 Statistical analysis

Data analysis was carried out using RStudio software version 3.0.3 (R Core Team 2016). Proportional data for prevalence of infection of both barnacle species at various sampling sites and times were compared using the "prop.test" function in R. Sequential Holm-Bonferroni corrections were applied to the p values in order to reduce the probability of Type 1 error (Holm 1979; Getano 2013) using a Holm-Bonferroni sequential correction EXCEL calculator (Gaetano 2013).

8.4 Results

8.4.1 Infection by Maritrema arenaria

Bullens Bay

Cysts of *M. arenaria* were recorded in both *Austrominius modestus* and the native barnacle species *Semibalanus balanoides* at all sampling sites during this study. At Bullens Bay, *S. balanoides* had a significantly higher prevalence of infection than *A. modestus* in 2006-2007 (χ^2 = 86.211, 1df, $p < 0.001$) (Figure 2) and 2014-2015 (χ^2 = 17.715, 1df, $p < 0.001$) (Figure 3). A higher proportion of *S. balanoides* were found to contain cysts in comparison to *A. modestus* on almost all sampling occasions, with the exception of one month in 2006-2007 (Figure 2) and two months in 2014-2015 (Figure 3).

Maximum prevalence of infection for *S. balanoides* was 42% during the 2006-2007 sampling period (Figure 2) and 32% during 2014-2015 (Figure 3). Maximum prevalence of infection for *A. modestus* during 2006-2007 (17%) (Figure 2) was very similar to that recorded during 2014-2015 (18%) (Figure 3). Mean prevalence of *M. arenaria* cyst infection was significantly higher during 2006-2007 for *A. modestus* (11% ± 2.9%) (χ^2 = 20.972, 1 df, $p < 0.001$) and *S. balanoides* (23% ± 2.4%) (χ^2 = 88.84, 1 df, $p < 0.001$) compared to 2014-2015 (SB: 8% ± 1.8%; AM: 4% ± 1.1%) (Figures 2 and 3). Seawater temperature values were very similar for both sampling periods, however minimum and maximum recorded mean values were lower and higher, respectively in 2014-2015 (8.5 °C ± 0.01 °C – 17.1 °C ± 0.08 °C) (Figure 3) in comparison to 2006-2008 (10.0 °C ± 0.02 °C – 16.4 °C ± 0.03 °C) (Figure 2).

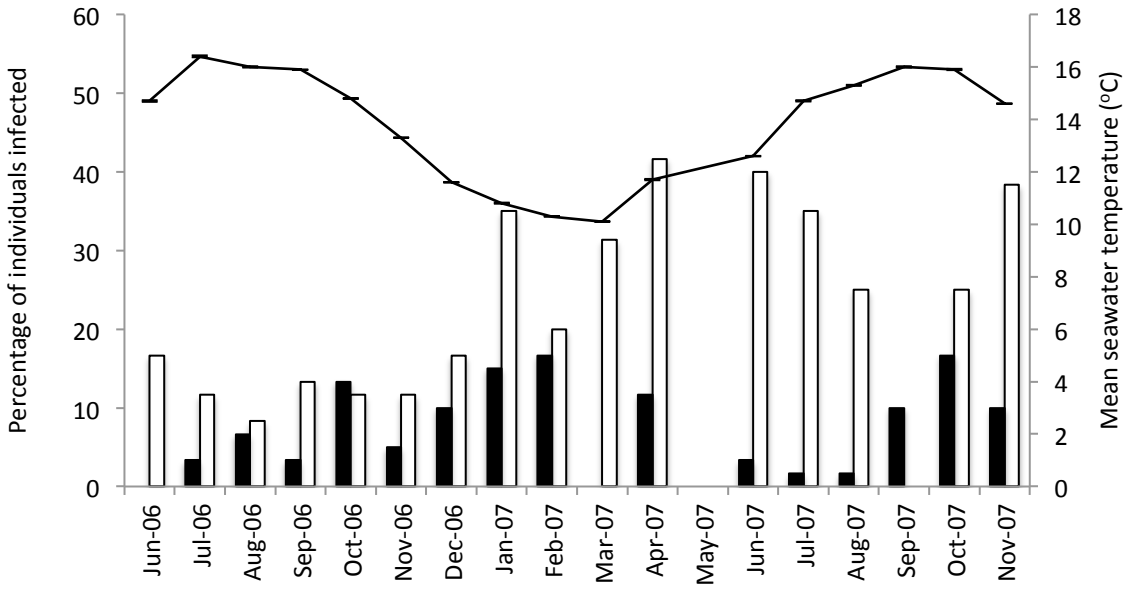

Figure 2. Percentage of individuals of *Austrominius modestus* (black bars) (n=60) and *Semibalanus balanoides* (white bars) (n=60) infected with trematode cysts (*Maritrema arenaria*) at monthly intervals from May 2006 to February 2008 at Bullens Bay, Ireland. Mean (± SE) seawater temperatures are represented by the line. No data are available for either species for May 2007 and for *S. balanoides* in September 2007.

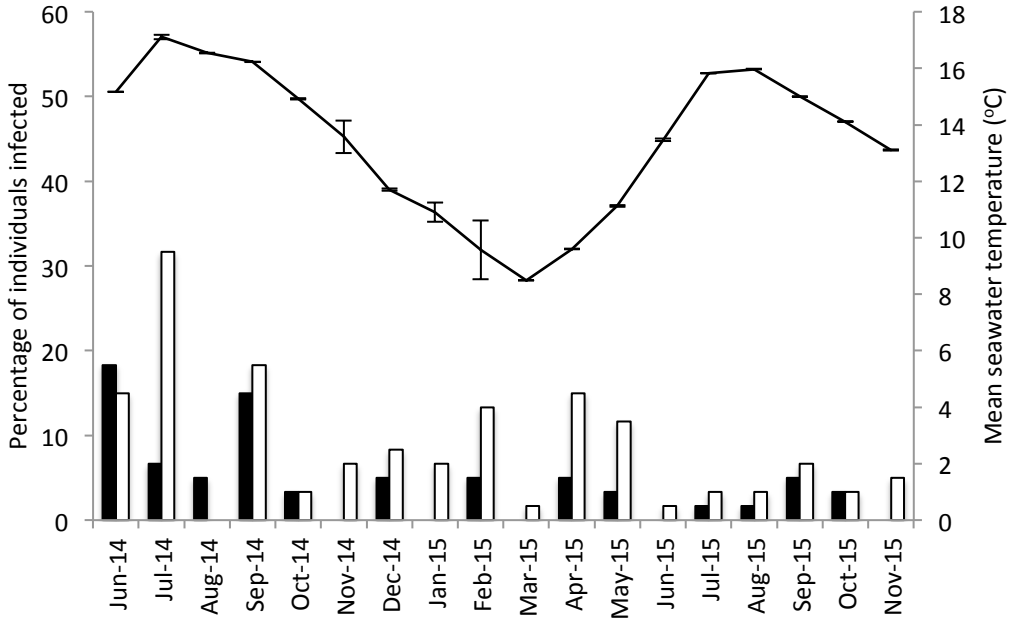

Figure 3. Percentage of individuals of *Austrominius modestus* (black bars) (n=60) and *Semibalanus balanoides* (white bars) (n=60) infected with trematode (*Maritrema arenaria*) cysts at monthly intervals from June 2014 to November 2015 at Bullens Bay, Ireland. Mean (± SE) seawater temperature data are represented by the line.

In samples screened between June 2006 and November 2007, intensity of infection generally ranged from 1.0 – 1.5 cysts per individual (Table 1). Highest intensity during this time period was recorded for *S. balanoides* in October 2007, with 3.9 (± 1.30 SE) cysts per individual, higher than the maximum intensity recorded for this species in 2014-2015 (1.4 ± 0.40 cysts per infected individual, December 2014) (Table 1). Highest intensity for *A. modestus* was 3.0 cysts (± 0.00) per individual in August 2007, which was lower than that recorded during 2014-2015, where intensities of 90.5 (± 86.20) cysts per infected individual and 23.7 (± 22.5) cysts per infected individual were recorded in July and September 2014 respectively (Table 1).

Table 1. Intensity of infection (mean number of cysts per infected individual ± SE) of *Maritrema arenaria* cysts in two barnacle species *Austrominius modestus* (AM) and *Semibalanus balanoides* (SB) collected from Bullens Bay, Co. Cork on a monthly basis from June 2006 to November 2007 and June 2014 to November 2015. n/a indicates that there are no data available for that time period.

	2006 - 2007		**2014-2015**	
	AM	*SB*	*AM*	*SB*
June	0.0 ± 0.00	1.4 ± 0.31	1.3 ± 0.15	1.0 ± 0.00
July	1.0 ± 0.00	1.1 ± 0.14	90.5 ± 86.20	1.3 ± 0.21
August	1.0 ± 0.00	1.2 ± 0.20	0.0 ± 0.00	1.0 ± 0.00
September	1.0 ± 0.00	1.4 ± 0.18	23.7 ± 22.5	1.0 ± 0.00
October	1.1 ± 0.14	1.0 ± 0.00	1.5 ± 0.50	1.0 ± 0.00
November	1.0 ± 0.00	1.4 ± 0.20	0.0 ± 0.00	1.0 ± 0.00
December	1.3 ± 0.33	1.5 ± 0.24	1.0 ± 0.00	1.4 ± 0.40
January	1.3 ± 0.23	1.3 ± 0.12	0.0 ± 0.00	1.0 ± 0.00
February	1.2 ± 0.13	1.7 ± 0.36	1.0 ± 0.00	1.1 ± 0.13
March	0.0 ± 0.00	1.2 ± 0.09	0.0 ± 0.00	1.0 ± 0.00
April	1.3 ± 0.25	1.6 ± 0.19	1.0 ± 0.00	1.2 ± 0.15
May	n/a	n/a	1.0 ± 0.00	1.0 ± 0.00
June	1.0 ± 0.00	1.2 ± 0.08	0.0 ± 0.00	1.0 ± 0.00
July	1.0 ± 0.00	1.2 ± 0.11	1.0 ± 0.00	1.0 ± 0.00
August	3.0 ± 0.00	2.1 ± 0.48	1.0 ± 0.00	1.0 ± 0.00
September	1.0 ± 0.00	n/a	1.0 ± 0.00	1.0 ± 0.00
October	1.2 ± 0.13	3.9 ± 1.30	1.0 ± 0.00	1.0 ± 0.00
November	1.4 ± 0.24	1.3 ± 0.12	0.0 ± 0.00	1.3 ± 0.33

Isle of Cumbrae

Overall, *S. balanoides* had a significantly higher prevalence of infection than *A. modestus* (χ^2 = 103.57, df = 1, p < 0.001) at both Fintry Bay (Figure 4) and Millport Harbour. At Fintry Bay, *S. balanoides* had a significantly higher prevalence of infection than *A. modestus* at each time period (p = 0.005) (Table 2). At Millport Harbour, a higher proportion of individuals of *A. modestus* were infected by *M. arenaria* (82%) compared with *S. balanoides* (68%) in September 2014 (Figure 5), however, this was not found to be significant. *S. balanoides* had a higher prevalence of infection at all other time periods (Figure 5), but the difference was only significant in September 2015 (χ^2 = 35.20, df = 1, p < 0.01) and March 2016 (χ^2 = 20.26, df = 1, p < 0.01).

Table 2. "Prop. test" results with Holm-Bonferroni sequential correction (Holm 1979; Gaetano 2013), comparing the prevalence of infection of the trematode *Maritrema arenaria* in the barnacle *Semibalanus balanoides* in comparison to *Austrominius modestus* in March and September 2014, March and September 2015 and March 2016 at Fintry Bay on the Isle of Cumbrae, Scotland. Significant p values (p < 0.05) are highlighted in bold.

	χ^2	df	p value
Mar-14	10.7	1	**0.005**
Sep-14	12.53	1	**0.005**
Mar-15	43.95	1	**0.005**
Sep-15	11.12	1	**0.005**
Mar-16	31.62	1	**0.005**

Higher prevalence of infection was recorded at Millport Harbour compared to Fintry Bay overall (χ^2 = 103.57, df = 1, p < 0.01) for both *S. balanoides* (χ^2 = 24.32, df = 1, p < 0.01) and *A. modestus* (χ^2 = 97.33, df = 1, p < 0.01) (Figures 4 and 5). These differences were significant for *A. modestus* in March and September 2014 and March 2015 (p = 0.005) (Table 3). However, they were only significant for two time periods for *S. balanoides* (March 2014: χ^2 = 15.31, df = 1, p < 0.01; September 2015: χ^2 = 26.41, df = 1, p < 0.01).

Table 3. "Prop. test" results with Holm-Bonferroni sequential correction (Holm 1979; Gaetano 2013), comparing the prevalence of infection of the trematode *Maritrema arenaria* in the barnacle *Austrominius modestus* at Fintry Bay in comparison to Millport Harbour on the Isle of Cumbrae, Scotland in March and September 2014, March and September 2015 and March 2016. Significant p values ($p < 0.05$) are highlighted in bold.

	χ^2	df	p value
Mar-14	46.09	1	**0.005**
Sep-14	38.62	1	**0.005**
Mar-15	18.18	1	**0.005**
Sep-15	6.01	1	0.08
Mar-16	4.91	1	0.08

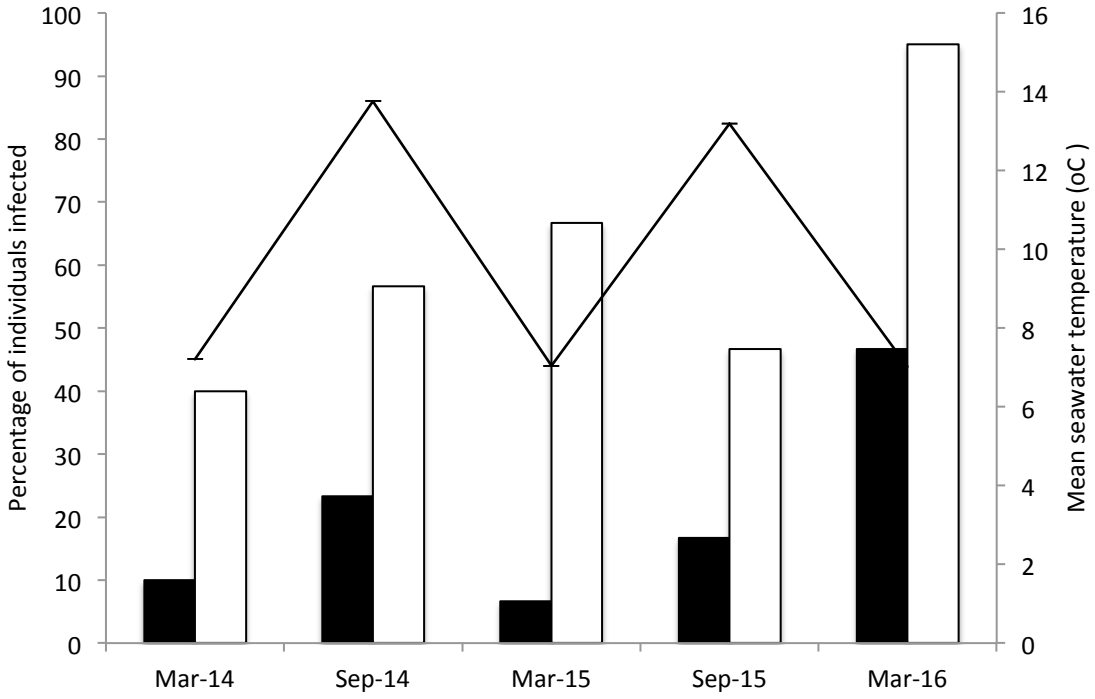

Figure 4. Percentage of individuals of *Austrominius modestus* (AM; black) (n=60) and *Semibalanus balanoides* (SB; white) (n=60) infected with cysts of *Maritrema arenaria* from Fintry Bay, Isle of Cumbrae, Scotland in March and September 2014 and 2015. Mean seawater temperature (± SE) is represented by the line.

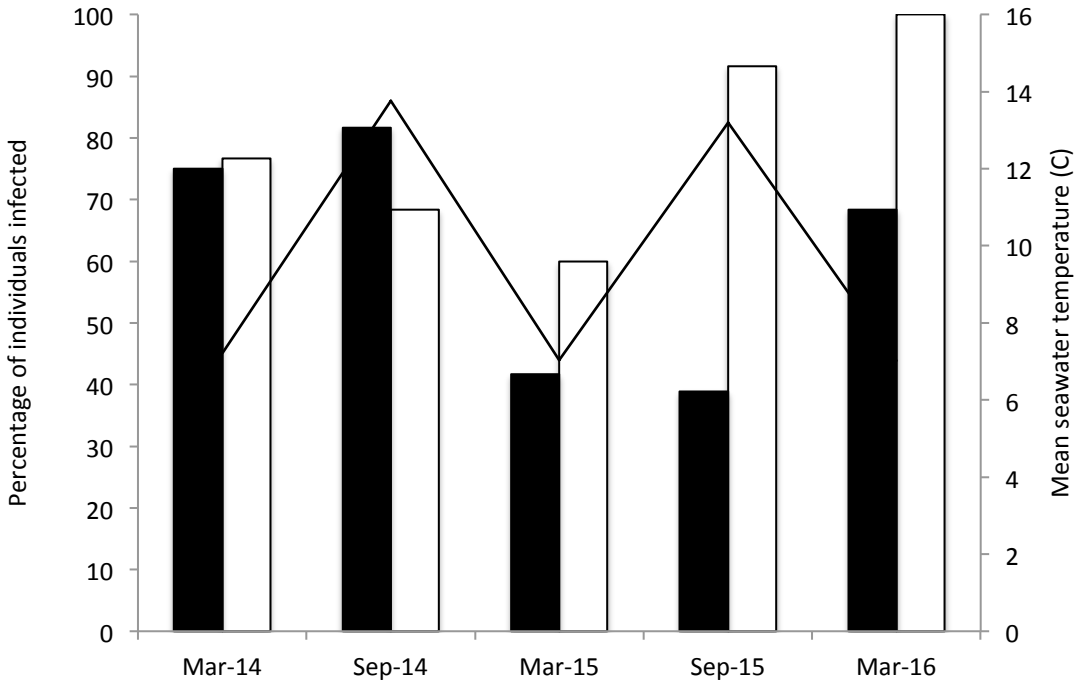

Figure 5. Percentage of individuals of *Austrominius modestus* (AM; black) (n=60) and *Semibalanus balanoides* (SB; white) (n=60) infected with cysts of *Maritrema arenaria* from Millport Harbour, Isle of Cumbrae, Scotland in March and September 2014 and 2015. Mean seawater temperature (± SE) is represented by the line.

Mean seawater temperatures in March 2014 were 7.2°C (± 0.01 °C) and 7.0 °C (± 0.01 °C) in March 2015. Similarly, temepratures in September 2014 (13.76 °C ± 0.008 °C) were very similar to those recorded in September 2015 (13.2 °C ± 0.01 °C). Despite the consistency in temperature fluctuations, both *A. modestus* and *S. balanoides* showed an increase in prevalence of infection over the study period at Fintry Bay (Figure 4), while at Millport Harbour, both species showed a decrease in prevalence of infection, before increasing again in September 2015 and March 2016 (Figure 5). By March 2016, 100% of individuals were infected at Millport Harbour (Figure 5), and 95% of individuals at Fintry Bay at this time (Figure 4).

The intensity of *M. arenaria* infection fluctuated for both barnacle species at both sites, between March 2014 and March 2016. One species or one site did not consistently have a higher intensity of infection. Maximum intensity of infection for *S. balanoides* was 10.1 (± 1.03) cysts per infected individual at Millport Harbour in March 2016. This was higher than the maximum intensity recorded for *A. modestus*

of 6.7 (± 0.99) cysts per infected individual at Millport Harbour in March 2014 (Table 4).

Table 4. Intensity of infection (mean number of cysts per infected individual ± SE) of *Maritrema arenaria* cysts from two barnacle species *Austrominius modestus* (AM) and *Semibalanus balanoides* (SB) from Millport Harbour and Fintry Bay in March and September 2014, 2015 and March 2016

	Millport Harbour		*Fintry Bay*	
	AM	**SB**	**AM**	**SB**
2014				
March	6.7 ± 0.99	5.9 ± 0.87	1.7 ± 0.34	1.2 ± 0.48
September	2.8 ± 0.43	3.6 ± 0.52	3.4 ± 0.59	4.5 ± 1.20
2015				
March	1.8 ± 0.19	2.1 ± 0.19	1.3 ± 0.25	3.4 ± 0.56
September	2.5 ± 0.47	6.8 ± 0.80	1.9 ± 0.31	1.5 ± 0.15
2016				
March	3.2 ± 0.53	10.1 ± 1.03	2.1 ± 0.34	6.2 ± 0.65

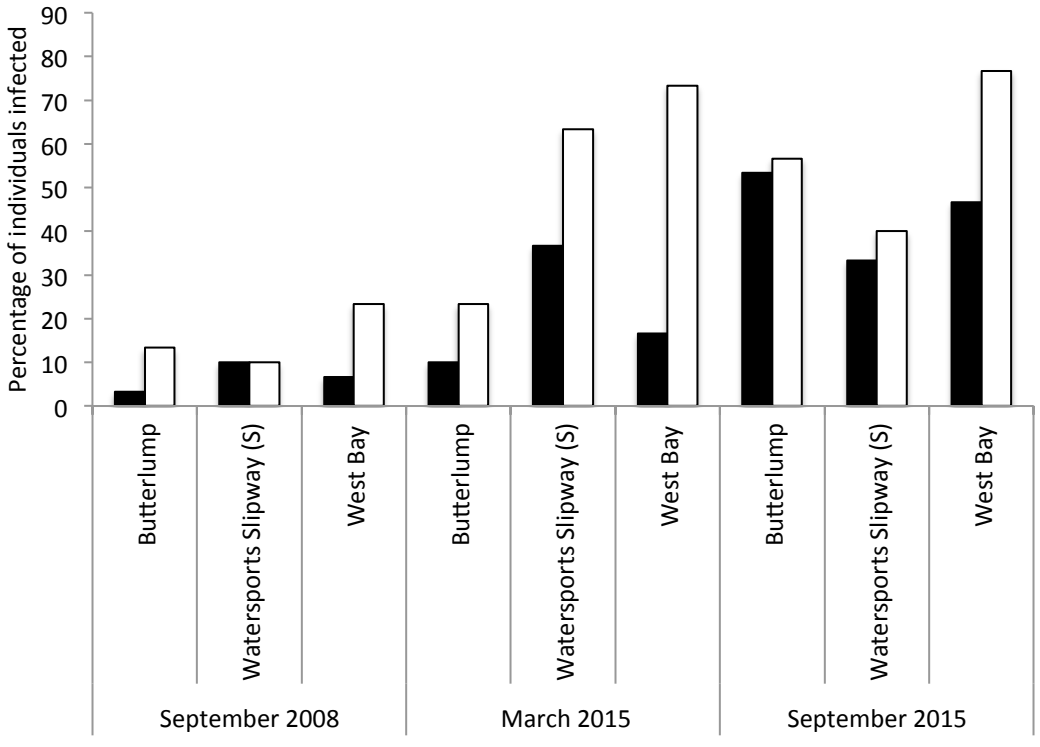

Figure 6. Percentage of individuals of *Austrominius modestus* (black) (n=30) and *Semibalanus balanoides* (white) (n=30) infected with cysts of *Maritrema arenaria* from Butterlump, Watersports slipway (south) and West Bay, on the Isle of Cumbrae, Scotland in September 2008, March 2015 and September 2015.

At West Bay, Butterlump and Watersports Slipway (S), *S. balanoides* had a higher prevalence of infection at each site at each time interval, with the exception of Watersports Slipway in September 2008, when both *S. balanoides* and *A. modestus* had an equal proportion of individuals (10%) containing cysts (Figure 6). There was no significant difference in the prevalence of infection of *S. balanoides* and *A. modestus* at Butterlump or Watersports Slipway, however *S. balanoides* had a significantly higher prevalence of infection overall at West Bay (χ^2 = 20.74, 1 df, p < 0.01).

Overall, prevalence of infection increased between September 2008 and September 2015 for both *A. modestus* (χ^2 = 19.190, 1 df, p < 0.01) and *S. balanoides* (χ^2 = 45.306, 1 df, p < 0.01), with a higher proportion of individuals of both species recorded to contain cysts at all sites in September 2015 in comparison to September 2008 (Figure 5). Seawater temperatures in September 2008 were higher (14.0^{o}C ± 0.01 ^{o}C) than in September 2015 (13.2 ^{o}C ± 0.01 ^{o}C). Highest prevalence of infection for *A. modestus* was recorded at Butterlump (53%) in March 2015, however there was also a high prevalence of infection at West Bay (47%) (Figure 6). Highest prevalence of infection for *S. balanoides* was recorded at West Bay (73%) in March 2015. A high proportion of *S. balanoides* individuals were also found to contain cysts at Watersports Slipway (63%), while Butterlump had the lowest prevalence of infection at this time, with only 23% of *S. balanoides* individuals screened found to contain cysts (Figure 6).

Table 5. Intensity of infection (mean number of cysts per infected individual ± SE) of *Maritrema arenaria* cysts from two barnacle species *Austrominius modestus* (AM) (n=30) and *Semibalanus balanoides* (SB) (n=30) from Butterlump, Watersports Slipway (South) and West Bay in September 2008, March 2015 and September 2016.

	Butterlump		*Watersports Slipway (S)*		*West Bay*	
	AM	**SB**	**AM**	**SB**	**AM**	**SB**
September 2008	1.0 ± 0.00	3.3 ± 2.25	1.3 ± 0.33	1.0 ± 0.00	5.0 ± 3.00	1.4 ± 0.30
March 2015	1.4 ± 0.30	1.0 ± 0.00	1.6 ± 0.27	3.8 ± 0.81	5.4 ± 3.67	20.2 ± 5.75
September 2015	2.9 ± 0.73	2.9 ± 0.96	1.2 ± 1.33	1.8 ± 0.30	4.9 ± 1.75	4.6 ± 1.10

Highest intensities of infection were recorded at West Bay for both barnacle species at the majority of time periods. Maximum intensity of infection across all three sites was recorded for both *S. balanoides* (20.2 ± 5.75 cysts per infected individual) and *A. modestus* (5.4 ± 3.67 cysts per infected individual) in March 2015. There was no consistent trend in terms of intensity of infection for species or time. Generally *S. balanoides* had a higher mean intensity of infection than *A. modestus*, but mean intensities fluctuated for both species between time periods (Table 5).

8.4.2 Infection by Hemioniscus balani

Bullens Bay

Between June 2006 and November 2007, low levels of *H. balani* infection were recorded, with the percentage of individuals infected not exceeding 5% (Figure 7). There was no significant difference in the prevalence of infection of *A. modestus* or *S. balanoides* overall or at any specific time interval. The highest proportion of individuals infected by the isopod was 5%, recorded for *S. balanoides* in February 2007 and *A. modestus* in March 2007 (Figure 7). During this eighteen-month sampling period, individuals of both *A. modestus* were infected during nine months and *S. balanoides* were infected in ten of the months, though only simultaneously in six of these months (Figure 7). In each case of infection, just one individual *H. balani* was recorded from each barnacle (Table 6).

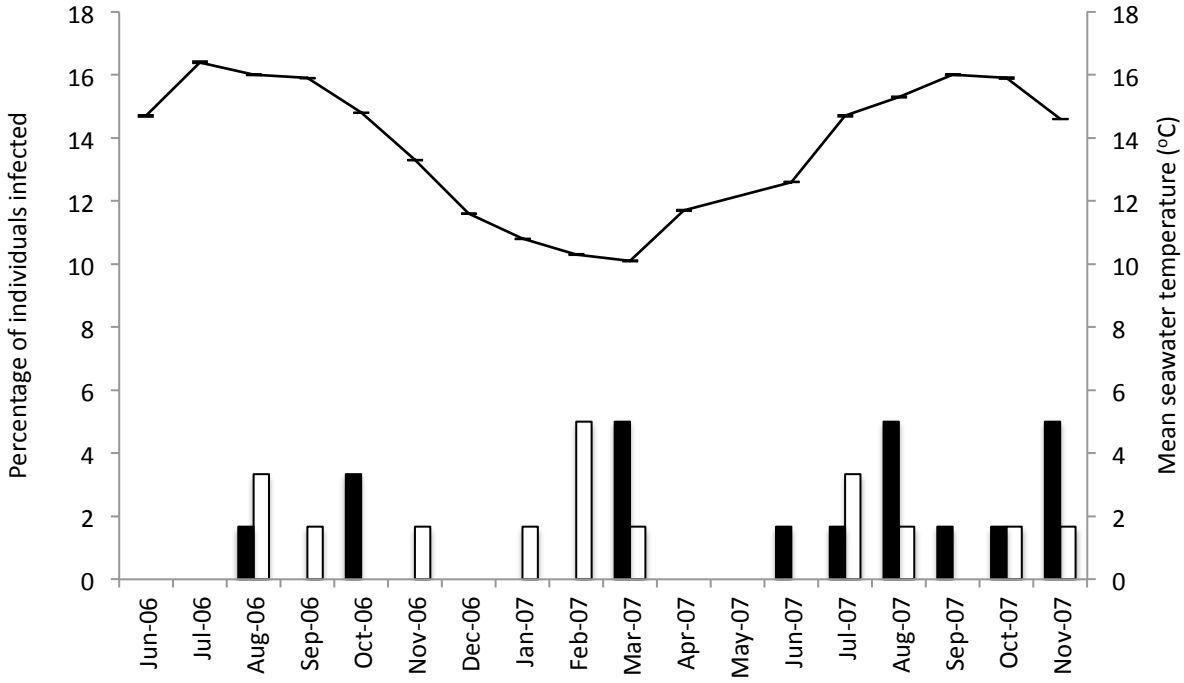

Figure 7. Percentage of individuals of *Austrominius modestus* (black bars) (n=60) and *Semibalanus balanoides* (white bars) (n=60) infected with the parasitic isopod, *Hemioniscus balani* at monthly intervals from May 2006 to February 2008 at Bullens Bay, Ireland. Mean (± SE) seawater temperature is represented by the line. No data are available for either species for May 2007 or for *S. balanoides* in September 2007.

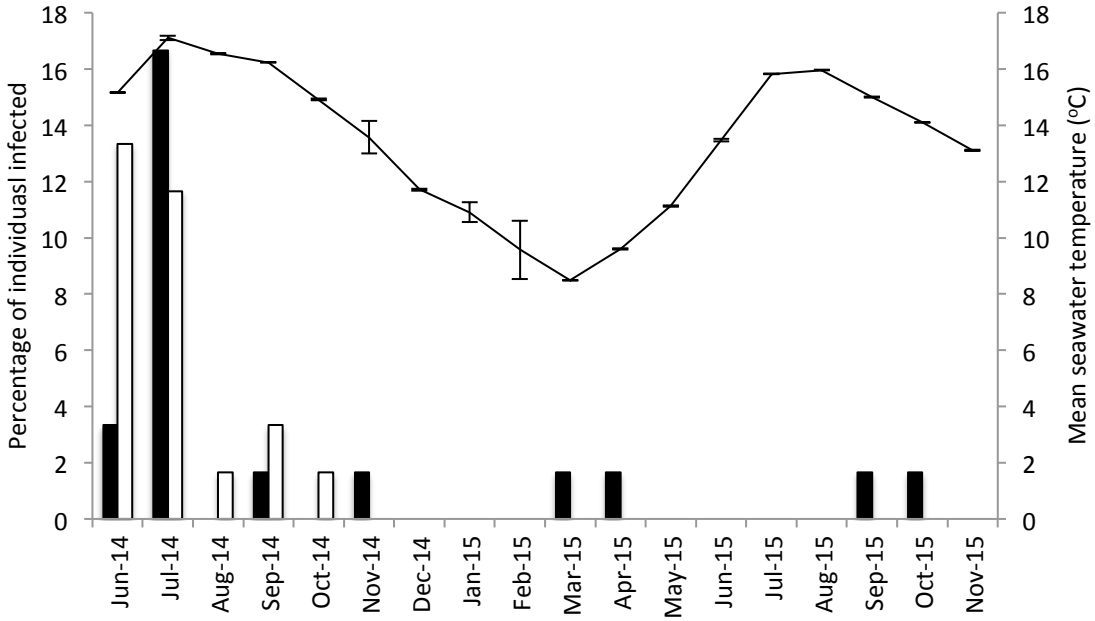

Figure 8. Percentage of individuals of *Austrominius modestus* (black bars) (n=60) and *Semibalanus balanoides* (white bars) (n=60) infected with the parasitic isopod, *Hemioniscus balani* at monthly intervals from June 2014 to November 2015 at Bullens Bay, Ireland. Mean (± SE) seawater temperature is represented by the line.

During 2014-2015, the proportion of individuals infected was again generally low for both barnacle species, ranging from 2% to 17% (Figure 8). There was no significant difference in the prevalence of infection of *A. modestus* and *S. balanoides* over the entire sampling period from June 2014-November 2015, or on any individual sampling occasion. Highest proportions of individuals infected were in June and July 2014 for *S. balanoides* (13% and 12% respectively) and in July 2014 for *A. modestus* (16.66%) (Figure 8). Generally, a single *H. balani* was found in infected individuals, however on two occasions (June and July 2014), an individual of *S. balanoides* was found to contain two individuals of *H. balani* (Table 6), on both occasions the parasites were male and female. There was no significant difference in the prevalence of infection of *A. modestus* or *S. balanoides* by *H. balani* in 2006 - 2007 and 2014 - 2015.

Table 6. Intensity of infection (mean number of isopods per infected individual ± SE) of *Hemioniscus balani* in two barnacle species *Austrominius modestus* (AM) and *Semibalanus balanoides* (SB) collected from Bullens Bay, Co. Cork on a monthly basis from June 2006 to November 2007 and June 2014 to November 2015. n/a indicates that there are no data available for that time period.

	2006 - 2007		**2014-2015**	
	AM	*SB*	*AM*	*SB*
June	0.0 ± 0.00	0.0 ± 0.00	1.0 ± 0.00	1.1 ± 0.13
July	0.0 ± 0.00	0.0 ± 0.00	1.0 ± 0.00	1.2 ± 0.17
August	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00	0.0 ± 0.00
September	0.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00
October	1.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	1.0 ± 0.00
November	0.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00	0.0 ± 0.00
December	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
January	0.0 ± 0.00	1.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
February	0.0 ± 0.00	1.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
March	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00	0.0 ± 0.00
April	0.0 ± 0.00	0.0 ± 0.00	1.0 ± 0.00	0.0 ± 0.00
May	n/a	n/a	0.0 ± 0.00	0.0 ± 0.00
June	1.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
July	1.0 ± 0.00	1.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
August	1.0 ± 0.00	1.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
September	1.0 ± 0.00	n/a	1.0 ± 0.00	0.0 ± 0.00
October	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00	0.0 ± 0.00
November	1.0 ± 0.00	1.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

Isle of Cumbrae

A single male *H. balani* was recorded from *S. balanoides* from Millport Harbour in September 2015.

8.4.3 Infection by both Maritrema arenaria and Hemioniscus balani

Infection of a barnacle host by both *M. arenaria* and *H. balani* was rare. Eight occasions of double infection were recorded at Bullens Bay during 2006-2007 and on six occasions here during 2014-2015. During 2006-2007 *A. modestus* was affected on five occasions and *S. balanoides* on three, while during 2014-2015 both species were equally affected, with each species being infected by both parasites simultaneously on three occasions.

On the one occasion *H. balani* was recorded at the northern sampling location, in Millport Harbour, the host (*S. balanoides*) was also found to contain cysts of *M. arenaria*.

8.5 Discussion

Both the non-native species *Austrominius modestus* and the native *Semibalanus balanoides* were infected by *Maritrema arenaria* at sampling locations in Scotland and Ireland during this study. *A. modestus* experienced a lower prevalence of infection than *S. balanoides* at both locations. The difference in prevalence of infection between the native *S. balanoides* and *A. modestus* was not always significant at Millport Harbour the site with highest infection, in comparison to other sampling sites. This would indicate that in areas where cysts are abundant, the non-native species does not experience as much enemy release as sites with lower levels of infection. Both barnacle species were also infected by the parasitic isopod *Hemioniscus balani* in Ireland, but levels of infection were low during both time periods. This species was only recorded once at a single sampling site on the Isle of Cumbrae throughout the study. There was no significant difference in the prevalence of *H. balani* infection in *A. modestus* and *S. balanoides*.

Comparing data collected in 2006-2007 and 2014-2015 at Bullens Bay, there was an overall decrease in the prevalence of infection of *M. arenaria* cysts for both barnacle species, though there was little difference in intensity of infection. Two occasions of high intensity of infection recorded during 2014-2015 for *A. modestus* were due to high numbers of cysts recorded in a single individual, and was not an indication of increased intensity of infection overall. Although prevalence of infection decreased for both species between 2006-2007 and 2014-2015, the native, arctic boreal *S. balanoides* exhibited a higher prevalence of infection than *A. modestus* during both periods. In comparison, on the Isle of Cumbrae, there was an overall trend towards increasing prevalence of infection for both barnacle species at all sampling sites between 2008-2015 and also from 2014-2016. Highest prevalence of infection for both *A. modestus* and *S. balanoides* were recorded in March 2016, with 100% of *S. balanoides* individuals screened from Millport Harbour found to contain cysts.

There are multiple factors that influence spatial and temporal changes in parasite populations, making it difficult to attribute these changes to a single environmental factor (Lafferty 1997). Species with multiple life stages, such as trematodes, can decrease in abundance with increasing pollution (Mackenzie 1999). However, it has also been reported that areas that can be considered more polluted, such as fishing ports and areas in close proximity to sewage outfall plants can have higher abundances of *M. arenaria* cysts (Carrol *et al.* 1990). Kinsale Harbour, a site used for water quality monitoring close to Bullens Bay, was classified as “unpolluted” in 2010 (EPA 2010) an improvement from “intermediate” in 2006 (EPA 2006). This improvement in water quality may have been a factor contributing to the decreasing prevalence of infection at this site.

On the Isle of Cumbrae, Millport Harbour consistently had a higher proportion of hosts infected by *M. arenaria* than Fintry Bay, which could be considered a “cleaner” site, as it has less boating activity and is further from residential areas than the harbour. In addition to differences in water quality, the environmental conditions at Millport Harbour may be more stressful for the barnacle hosts, thus making them more susceptible to infection (Lafferty 1997). The overall increase in *M. arenaria*

infection in barnacle hosts on the Isle of Cumbrae during the study cannot be linked with any known changes in water quality but could be associated with changes in the abundance of other hosts in the life cycle of this parasite (Carrol *et al.* 1990; Lafferty 1997; Mackenzie 1999). For example, increased abundances of the final hosts, shore birds, have been linked to higher abundance of trematodes in Californian coastal wetlands (Hechinger and Lafferty 2005).

Despite finding very high proportions of individuals containing cysts on the Isle of Cumbrae, levels of infection were still quite low in comparison cases of heavy infection recorded by Hadley and Castle (1940), with 600 – 1,000 cysts recorded in individual barnacles (*Semibalanus balanoides*), but in keeping with the findings of by Sari and Malek (2000) for *Perforatus perforatus* in Wales and Carrol *et al.* (1990) for *S. balanoides* in Ireland. However, it is common for hosts to only have a partial parasite load (Poulin and George-Nascimento 2007). It is thought that infection by *M. arenaria* causes little damage to the key organs of the barnacle host (Combes 1991; Poulin 1994), in comparison to the previous gastropod host, where the hosts' gonads are often destroyed to facilitate asexual reproduction by the parasite (Combes 1991; Poulin 1994). However, cysts are usually found surrounding the gut of the host barnacle (Hadley and Castle 1940), so it is possible that infection may interfere with the host's digestion, thus limiting growth and reproduction over time.

Temperature is known to be an important factor for parasites during their life cycles (Marcogliese 2001). Predicted increases in sea surface temperatures (IPCC 2014) are thought to increase levels of infection (Harvell *et al.* 2002) and alter parasite-host interactions (Marcogliese 2001), though this is dependent on the sensitivity of both host and parasite to temperature changes (Lafferty *et al.* 2004). In the present study, higher prevalence of *M. arenaria* infection was recorded on the Isle of Cumbrae, which has colder seawater temperatures than Bullens Bay, however this difference may be due to other environmental factors and not necessarily temperature. Trends at each location recorded in the present study do not seem to be directly linked with any changes on seawater temperature. Future temperature changes, which stress hosts, could make them more vulnerable to infection (Marcogliese 2001). The non-

native *A. modestus* is warm water adapted and more likely to be able to cope with increasing seawater temperatures than the arctic boreal species *S. balanoides* (Buckeridge 2012), possibly increasing the susceptibility of the native species to infection.

A. modestus showed lower levels of infection than the native barnacle species in both Ireland and Scotland during this study. The extent to which this enemy release can confer an advantage to the invasive species is determined by the effect that the predator or pathogen has on its host (Torchin *et al.* 2002). It seems likely that, unless present in very high numbers, infection by *M. arenaria* cysts will have little impact on barnacle hosts. Infection by *H. balani* is potentially much more harmful as it is known to castrate its host, however there was no significant difference in the level of infection of the native barnacle species and *A. modestus* found during this study. Overall levels of infection of *H. balani* recorded during this study were very low and not high enough to have a noticeable impact on host population dynamics.

Changes in the prevalence of infection found at the study sites during this study were exhibited by both barnacle species. This indicates that although *A. modestus* experiences release from enemies, currently it is affected in the same way as the native species by factors controlling levels of parasitism. Given the complex life cycle of *M. arenaria*, it is difficult to link changes in prevalence to specific environmental factors, though host stress, environmental conditions and abundances of other intermediate hosts are the most likely drivers of change. However, it seems likely that at current intensities of infection, these particular parasites are not having a detrimental impact on the barnacle hosts. Continued monitoring of infection is important in order to conserve ecosystems in the face of environmental change (Ward and Lafferty 2004). Future monitoring of these species is recommended, in particular with predicted warming seawater temperatures, which could increase the susceptibility of native hosts to infection, thus altering both the dynamics of the host and parasite populations.

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Chapter 9

Do older artificial structures support higher abundances of native barnacle species?

This chapter is in preparation for submission to the journal *Marine Ecology Progress Series*

9.1 Abstract

Artificial structures are now a common feature of coastlines globally. The communities present on these structures have been found to differ from those on the surrounding natural substrate and are often dominated by non-native species. Over time, as the structures themselves become weathered and eroded and the community present undergoes succession, it is possible for a community similar to that found on the natural substrate to form. The aim of this study was to test the hypothesis that older structures would have higher abundances of native barnacle species and conversely that new structures would be dominated by the non-native species *Austrominius modestus*. *A. modestus* was present on all nine structures surveyed and was the dominant species at eight of the survey sites. The oldest structure surveyed had the highest abundance of both native barnacle species and overall there was a significant positive relationship with the abundance of native species and age of the structure. The dominance of *A. modestus* on structures ranging from 10-107 years old, indicated that regardless of the age of the structure, this non-native species has the ability to dominate artificial structures, however it is possible that exposure and disturbance may facilitate the dominance of *A. modestus*. Artificial structures often create sheltered conditions on wave-exposed areas of coastline. *A. modestus* is known to favour sheltered conditions, and its dominance on these structures along areas of exposed coast may play an important role in the invasion of this species, allowing it to survive in areas where it otherwise would not become established.

KEYWORDS: artificial structures, non-native species, barnacles, *Austrominius modestus*, urbanisation, novel ecosystems

9.2 Introduction

Coastal zones are becoming increasingly urbanised and artificial structures are now a common feature of many coastlines (Glasby and Connell 1999; Bulleri 2006; Bulleri and Chapman 2010). Increasing populations in coastal locations, along with high levels of tourism, the need to protect coastal areas from rising sea levels and storm surges all necessitate the construction of novel structures such as pier pilings, breakwaters and sea walls (Bacchiocchi and Airoldi 2003; Moschella *et al.* 2005; Bulleri and Chapman 2010; Chapman and Underwood 2011; Mineur *et al.* 2012). The communities which form on these structures have been shown to differ from those present on surrounding natural substrates (Glasby and Connell 1999; Connell 2001; Chapman 2003; Bulleri and Chapman 2004; Bulleri 2005a; Bulleri 2005b; Bulleri *et al.* 2005; Vaselli *et al.* 2008; Lam *et al.* 2009) and are often dominated by non-native species (Bulleri and Airoldi 2005; Wasson *et al.* 2005; Glasby *et al.* 2007; Tyrrell and Byers 2007; Cordell *et al.* 2013).

The differences in the communities present on natural and artificial substrates have been attributed to multiple factors including differences in recruitment (Chapman 2003; Knott *et al.* 2004), post settlement mortality (Chapman 2003; Bulleri 2005b; Coombes *et al.* 2015), substrate (Bulleri 2005a; Coombes *et al.* 2015), competition (Tyrrell and Byers 2007), predation (Dumont *et al.* 2011), availability of microhabitats (Chapman 2003; Browne and Chapman 2011) and aspect (Knott *et al.* 2004). Community composition can also be influenced by changes in water flow and depositional processes brought about by the presence of the structure itself (Martin *et al*. 2005; Martins *et al.* 2009).

Age of structure has been noted to affect the community present on them (Chapman and Bulleri 2003). Given their age, communities on novel substrates have not had the chance to undergo succession (Firth *et al.* 2014) or weathering (Moschella *et al.* 2005) as seen on natural rocky shores and these factors can also contribute to differences in community composition. Over time, a community more resembling that of the natural shore community may form on artificial substrates, for example the Plymouth breakwater built in the 1800s supports a very similar community to

that found on a natural rocky shore (Southward and Orton 1954; Hawkins *et al.* 1983; Firth *et al.* 2014). However, this is not always the case and it has also been found that regardless of age, artificial structures differ in community composition to the surrounding community (Bulleri and Chapman 2010).

Barnacles are one of the main space occupiers in the intertidal zone (Rainbow 1984) and are capable of colonizing a wide range of substrates, both natural and artificial (Buckeridge 2010). In the case of novel substrates, the non-native barnacle species *Austrominius modestus* is more likely to successfully colonise a new structure as it produces multiple broods throughout the entire year (Moore 1944; Moyse 1960; Patel and Crisp 1960; Moyse and Nelson-Smith 1963). The more restricted breeding cycles of the native species (Burrows *et al.* 1992; Anderson 1994), limit their chances to settle on the new substrate. However, unless all available space is colonised by the non-native species, it should be possible for natives to settle on this substrate during subsequent breeding cycles (Crisp and Davies 1955; Hui and Moyse 1987), possibly increasing in abundance with time.

It has previously been reported that *A. modestus* is capable of rapidly colonising artificial structures placed in the marine environment (Bracewell *et al.* 2012; Bracewell *et al.* 2013). However, it has also been found that native species are capable of colonising and surviving on artificial structures in addition to *A. modestus*, though often at low abundances (Gallagher *et al.* 2016). This study examined the relationship between the age of artificial structures and the abundance of the non-native barnacle species *A. modestus* and the native barnacle species *Semibalanus balanoides* and *Chthamalus montagui* in south-west Ireland. It tested the hypothesis that older structures would have higher abundances of native barnacle species and conversely that newer structures would be dominated by the non-native *A. modestus*.

9.3 Materials and Methods

9.3.1 Species abundance

Surveys of the abundance of three barnacle species *Austrominius modestus*,

Semibalanus balanoides and *Chthamalus montagui* were carried out on nine artificial structures (all piers or slipways) of different ages in south-west Ireland (Table 1), between January and July 2016. At each site, three line transects were carried out along the vertical side of each structure, starting where barnacles first appeared and ending either at the low tide mark or at the bottom of the pier or slipway itself. Stations were located along each transect at 40cm drops in vertical height. At each station ten 10cm x 10cm quadrats were sampled haphazardly for each of the three barnacle species separately (i.e. 30 quadrats per station). Spat was not recorded in order to produce comparable estimates for surveys carried out at any time during the period from January to July.

Table 1. Site name, location (GPS coordinates), substrate type, year built and age of nine artificial structures in south-west Ireland. The age of each structure was either obtained from locals or from Cork County Council records.

Site name	GPS	Substrate	Year built (age)
Ahakista Slipway	N 51°35.978' W 009°37.879'	Smooth concrete	2006 (10)
Durrus Pier	N 51°37.074' W 009°32.434'	Rough conglomerate	2004 (12)
Abbey Slipway, Bantry	N 51°40.644' W 009°28.273'	Smooth concrete	2001 (15)
New Slipway Bantry	N 51°40.706' W 009°28.373'	Smooth concrete	2000 (16)
Cunnamore Pier	N 51°30.240' W 009°25.460'	Smooth concrete	2000 (16)
Schull Harbour Slipway	N 51°31.491' W 009°32.670'	Smooth concrete	1985 (31)
Crookhaven Pier	N 51°28.132' W 009°43.554'	Large stones, concrete	1973 (43)
Railway Pier, Bantry	N 51°40.908' W 009°27.632'	Rough conglomerate	1909 (107)
Zetland Pier	N 51°41.717' W 009°35.860'	Rough conglomerate	1891 (125)

9.3.2 Statistical Analysis

All statistical analysis was carried out using R Studio Software 3.3.0 (R Core Team 2016). Negative binomial regression ("MASS" package; Venables and Ripley 2002) was used to examine the relationship between species abundance and age of structure and the relationship between species abundance and shore height. Negative binomial regression was chosen as species abundance is in the form of count data, while a Poisson regression could also have been used, the negative binomial regression model was found to have a lower AIC value, indicating that it was a better fit for the data.

9.4 Results

9.4.1 Abundance

The non-native *Austrominius modestus* was the most abundant barnacle species on all artificial structures surveyed with the exception of Zetland Pier, where *Chthamalus montagui* dominated. At the remaining eight sites, *A. modestus* was at least five times more abundant than the native barnacle species. Highest mean abundances of *A. modestus* were recorded at Schull Harbour Slipway with 228.2 (± 10.01) ind. 100cm^{-2} (Figure 1). This species was also present at similar mean abundances at the New Slipway Bantry (220.3 ± 13.29 ind. 100cm^{-2}), Abbey Slipway Bantry (196.2 ± 7.53 ind. 100cm^{-2}), Cunnamore Pier (179.9 ± 5.97 ind. 100cm^{-2}) and the Old Railway Pier Bantry (166.1 ± 9.48 ind. 100cm^{-2}) (Figure 1). The lowest recorded mean abundance of *A. modestus* was 39.4 (± 1.70) ind. 100cm^{-2} at Ahakista Slipway, however it still outnumbered native barnacle species at this site (Figure 1).

Highest abundances of *C. montagui* were recorded at Zetland Pier, with a mean abundance of 161.1 (± 11.24) ind. 100cm^{-2} (Figure 1). This species was not recorded at Abbey Slipway, Bantry and was present at very low abundances at Schull Harbour Slipway (0.1 ± 0.04 ind. 100cm^{-2}) and Cunnamore Pier (0.2 ± 0.07 ind. 100cm^{-2}) (Figure 1). Apart from the Old Railway Pier in Bantry, where *C. montagui* was recorded at a density of 58.3 (± 5.83) ind. 100cm^{-2}, it was not recorded at densities exceeding 7 ind. 100cm^{-2} at any of the remaining sites (Figure 1). *Semibalanus balanoides* was recorded at all survey sites, though it was not the dominant species at any location. Highest abundances of this species were also recorded at Zetland Pier (32.5 ± 2.59 ind. 100cm^{-2}) (Figure 1). This species was not recorded to exceed an abundance of 16 ind. 100cm^{-2} at any other location (Figure 1).

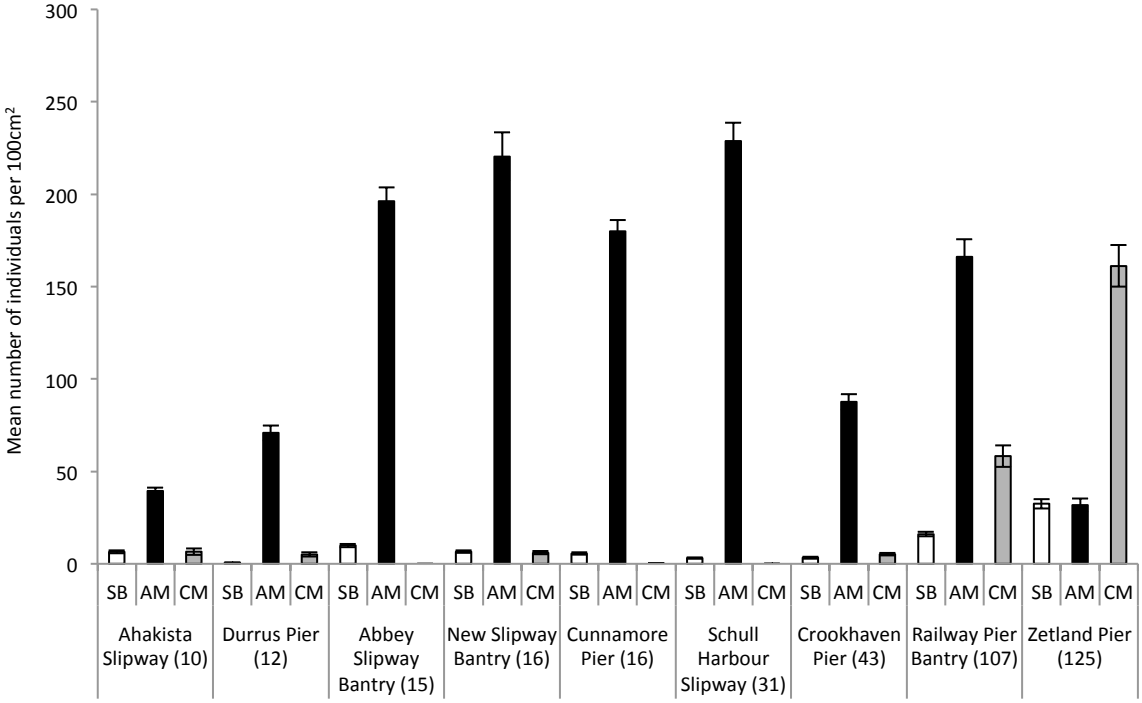

Figure 1. Mean number of individuals per 100cm^2 of the barnacles *Semibalanus balanoides* (SB; white bars), *Austrominius modestus* (AM; black bars) and *Chthamalus montagui* (CM; grey bars) on nine artificial structures in south-west Ireland. Numbers in brackets following site names represent the age of the structure. Error bars represent standard error.

9.4.2 Abundance and Structure Age

Both native species displayed a significant positive relationship with structure age (SB: estimate = 0.01 (± 0.001), z value = 16.34, $p < 0.001$; CM: estimate = 0.03 (± 0.001), z value = 22.08, $p < 0.001$) (Figures 2 and 3), being more abundant on older structures. In contrast, *A. modestus* had a significant negative relationship with increasing structure age (estimate = -0.00 (± 0.00), z value= -8.56, $p < 0.001$), displaying lower abundances on older structures (Figure 4).

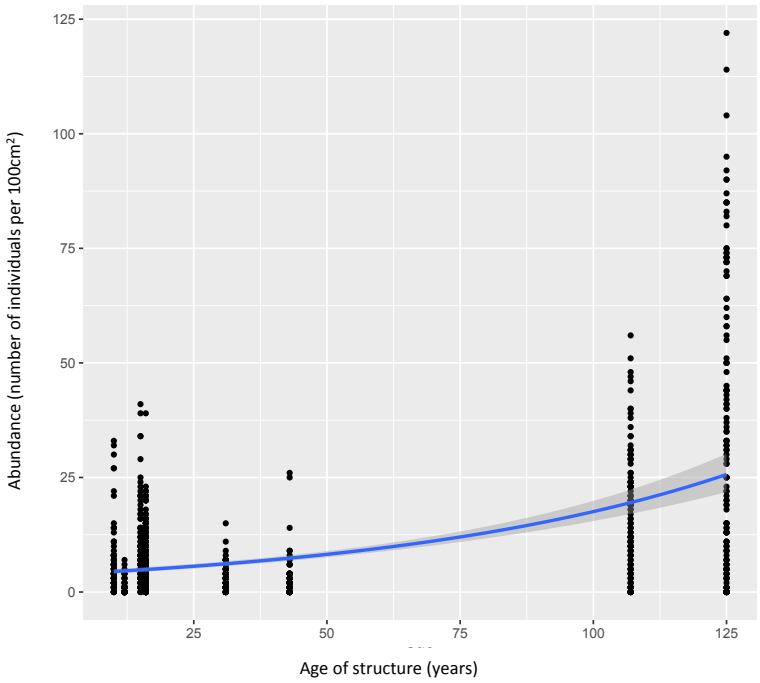

Figure 2. Abundance of *Semibalanus balanoides* recorded on artificial structures of different ages. The blue line represents negative binomial regression with grey areas indicating standard error values.

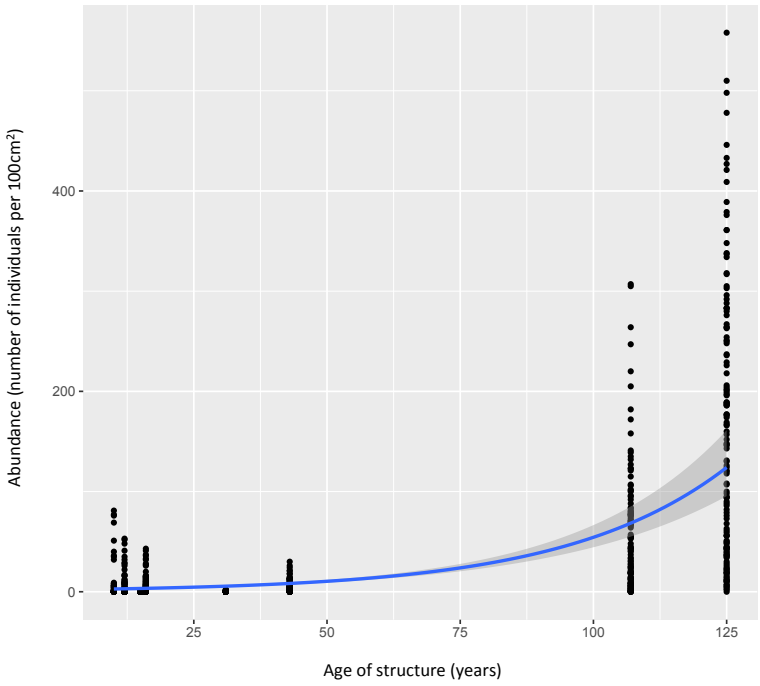

Figure 3. Abundance of *Chthamalus montagui* recorded on artificial structures of different ages. The blue line represents negative binomial regression with grey areas indicating standard error values.

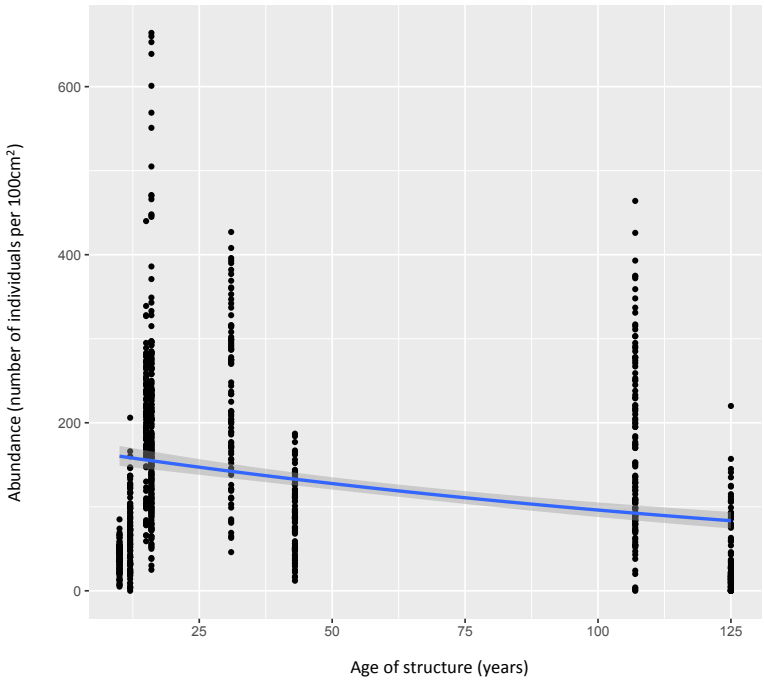

Figure 4. Abundance of *Austrominius modestus* recorded on artificial structures of different ages. The blue line represents negative binomial regression with grey areas indicating standard error values.

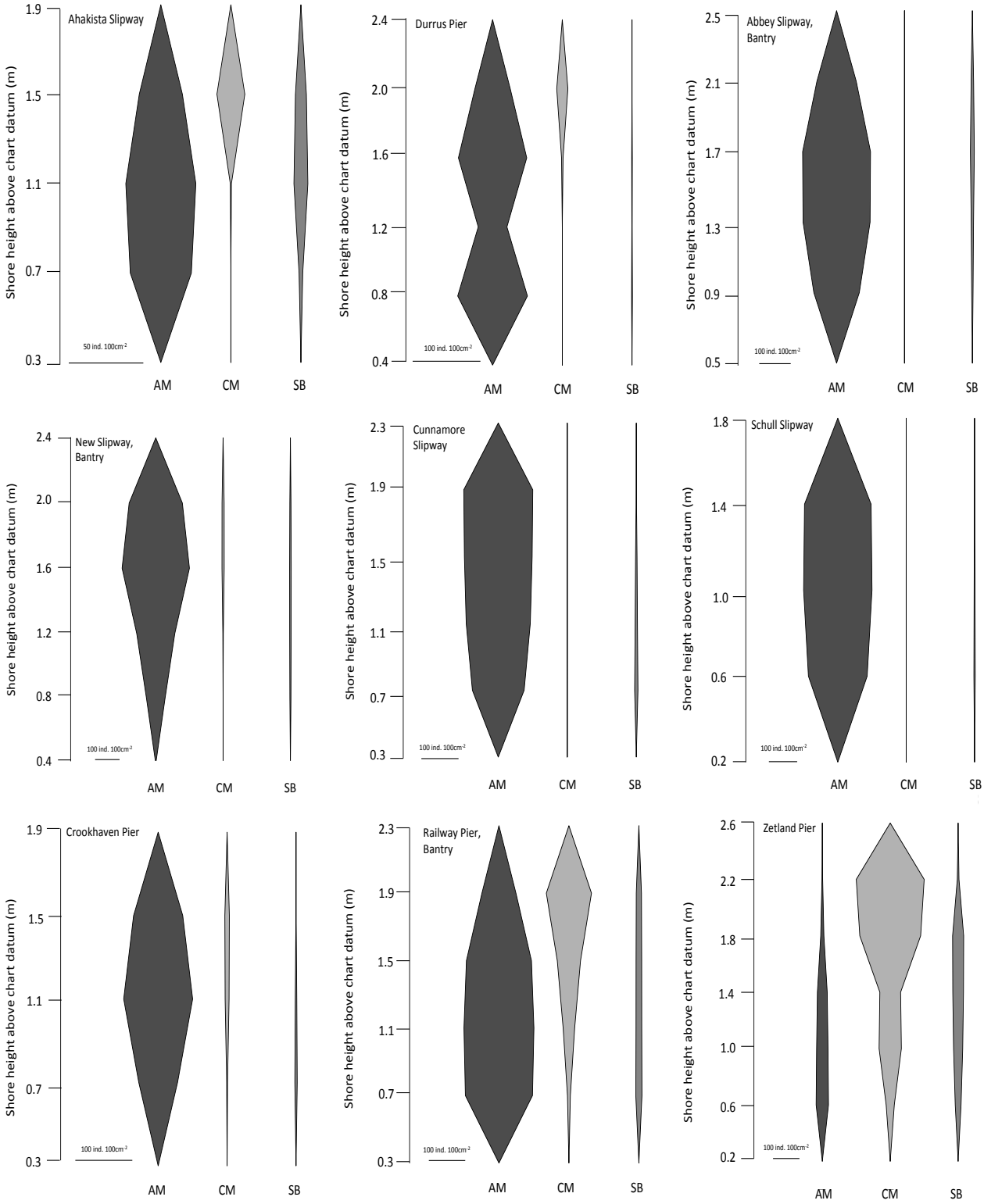

Figure 5. Vertical distribution of *Austrominius modestus* (AM), *Semibalanus balanoides* (SB) and *Chthamalus montagui* (CM) on nine artificial structures in south-west Ireland. Values represented are mean abundances from three transects at each site. Note that y-axis scales and abundance scales differ for each site.

9.4.3 Abundance and Shore Height

Both *A. modestus* and *C. montagui* were found to be more abundant with increasing shore height, however this relationship was only significant in the case of *C. montagui* (estimate = 1.8 (± 0.18), z value = 9.99, $p < 0.001$). *S. balanoides* decreased in abundance with increasing shore height, but this was not found to be statistically significant. While *C. montagui* was most abundant at the high shore level, both *A. modestus* and *S. balanoides* displayed broader patterns of zonation, being present at similar abundances across a range of shore heights at any one site (Figure 5). In the case of *A. modestus*, this species was most abundant at the mid shore levels, with the exception of Durrus Pier (Figure 5). *S. balanoides* was also more abundant at the mid and low shore levels in comparison to the high shore (Figure 5).

9.5 Discussion

The non-native species *Austrominius modestus* was present on all artificial structures surveyed and was the dominant species at all sites, with the exception of Zetland Pier. This was the site with highest recorded abundances of both native barnacle species and was also the oldest artificial structure surveyed. The native species *Semibalanus balanoides* and *Chthamalus montagui* were recorded at all survey sites, with the exception of *C. montagui,* which was absent from Abbey Slipway, Bantry. Both native species displayed a positive relationship with structure age, with higher abundances on older structures.

The findings of this study agree with those of Tyrrell and Byers (2007), whereby native species are found on artificial substrates, however in comparison to natural shores relative abundances differ, with non-indigenous species dominating on artificial structures. There was a significant negative relationship between *A. modestus* abundance and structure age, although highest recorded mean abundances of *A. modestus* were on structures of intermediate age (15-31 years old). This indicates that despite either being built before the introduction of *A. modestus*, in which case native species must have initially dominated the structure, or having had sufficient time for natives to become established over multiple

breeding seasons, *A. modestus* is most likely to be the dominant barnacle species on artificial structures regardless of age.

Artificial structures dominated by *A. modestus* could play an important role in the invasion of *A. modestus.* The presence of these structures often provides a sheltered habitat along wave-exposed areas of coastline (Vaselli *et al.* 2008; Bulleri and Chapman 2010) and can act as stepping-stones for invasive species (Glasby and Connell 1999; Bulleri and Airoldi 2005; Vaselli *et al.* 2008). High abundances of non-native species on artificial structures can also infiltrate the surrounding community on natural substrata (Simkanin *et al.* 2012). The south-west coast of Ireland has many areas of very exposed shoreline which are not suitable for the establishment of *A. modestus,* as discussed in Chapter 2. The presence of artificial structures at various points along the coast may facilitate the spread of this species in locations where it otherwise would not become established. In fact the low abundance of *A. modestus* at Zetland Pier, may be due to exposure as opposed to structure age. It is possible that the negative relationship between structure age and *A. modestus* abundance was skewed by low abundances of this species on the two older structures, which are also more exposed than the other structures (Gallagher pers. obs.). The effect of exposure on species abundance was not examined in this study, though it is possible that is plays an important role in determining the species composition of artificial structures.

Surface roughness also plays an important role in determining the community composition on these structures (Moschella *et al.* 2005). Barnacle larvae are known to show a preference for rough as opposed to smooth surfaces and often settle in depressions or grooves on the substrate (Crisp and Barnes 1954). The majority of newer piers and slipways surveyed were constructed from smooth concrete with few cracks or crevices, though over time erosion of the substrate may produce a more heterogeneous surface. Older structures comprised of a rough conglomerate mixture or large stones, both of which have many cracks and crevices, which may be a factor promoting the recruitment and survival of native species. In particular, Chthamalid recruitment and survival has been shown to be higher on rougher

surfaces (Coombes *et al.* 2015) and *S. balanoides* settlement has been found to be higher on rough as opposed to smooth surfaces (Knight-Jones 1953; Crsip and Barnes 1954; Hills and Thomason 1998).

Patterns of zonation observed on the structures surveyed were similar to those commonly observed on natural rocky shores, where *C. montagui* dominates at the high shore level, while *S. balanoides* is abundant at the mid and low shore. This zonation is brought about by competitive pressure from *S. balanoides* forcing *C. montagui* into the high shore (Connell 1961). At survey sites where *C. montagui* was present, it was the dominant species at the high shore level. Where *C. montagui* was absent, or only recorded at very low abundances, *A. modestus* was dominant at the high shore level, while *S. balanoides* remained abundant at the mid and low shore levels. *S. balanoides* may be better able to compete with *A. modestus* further down the shore and may be able to persist on artificial structures at the mid and low shore level, while *A. modestus* replaces *C. montagui* at the high shore.

On many of the structures surveyed, in particular those surveyed during the summer months, large areas of the substrate were covered in ephemeral algae, which persisted due to a lack of grazing by limpets (Hawkins *et al.* 1983). These algae were observed to be growing over adult barnacles, potentially smothering them and their presence was also inhibiting the settlement of new spat. If this is an annual event it would have a negative effect on the native species, in particular *S. balanoides,* as the presence of the algae coincides with the settlement period of this species. By the time *C. montagui* is settling the algae will likely have died away and there may even be additional space for settlement available if adult barnacles were smothered. The presence of these algae may also coincide with the peak of *A. modestus* settlement, however, the continuous breeding of this species will facilitate the establishment of settlers on the substrate once the algae are gone. The presence of this algae is also important in terms of the management regimes in place at the different sites. It is known that at some of the sites surveyed in this study they are treated with bleach or other chemicals to remove algae growing on the surface. This disturbance has the potential to cause mortality of barnacles present on the sides of the structure and

could promote the abundance of *A. modestus* as opposed to native barnacle species. The frequency of these treatments is not known, nor is it known at how many of the sites surveyed such management regimes are in place, but where they are present it is likely to play a role in increasing the abundance of *A. modestus* as opposed to native species, regardless of structure age.

Although *A. modestus* was the dominant barnacle on the majority of structures surveyed, the populations on these artificial substrates differ from those on a natural shore in terms of population structure and reproductive capacity. For example, Moreira *et al.* (2006) found that younger and smaller limpets (*Siphonaria denticulata*) were present on artificial seawalls, while larger individuals were only found on natural substrates. Additionally, individuals on seawalls were found to produce fewer and smaller egg masses than those on the natural shore, raising questions as to the long-term sustainability of populations on artificial structures, which may rely on the import of propagules from other locations to persist.

In both terrestrial and marine ecosystems, urbanised areas can result in novel ecosystems with species combinations and relative abundances that have not previously occurred (Hobbs *et al.* 2006). Though not compared directly in this study, the communities present on the structures surveyed differ from those generally found in natural communities in south-west Ireland (see Chapter 2). Given the distinct dominance of the non-native *A. modestus* on artificial structures, it seems appropriate to consider them novel ecosystems. Consideration of how these ecosystems function and their impact on the surrounding community is of upmost importance. Apart from facilitating the survival and spread of *A. modestus* on areas of a coastline where it otherwise may not occur, high levels of boating activity around such structures could further promote the movement of this species.

The presence of native barnacle species on the structures surveyed, despite their low abundances, illustrate that it is possible for these species to colonise and survive on artificial structures. In order to maintain biodiversity, design of future artificial structures in the marine environment could incorporate various features to try and

promote the abundance and persistence of native barnacle species, such as increasing surface texture and rugosity (Moschella *et al.* 2005; Firth *et al.* 2014; Coombes *et al*. 2015), coinciding the completion of construction with the time of native species settlement, treating the substrate to enhance settlement of native species and management of disturbance caused by maintenance regimes (Airoldi and Bulleri 2011). Additionally, given the continued proliferation of artificial substrates in coastal zones it would be of interest to investigate whether these novel ecosystems differ in function to those on the natural substrate.

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Chapter 10

Conclusions

10.1 Summary of main findings

Since its introduction to Europe in the 1940s *Austrominius modestus* has become widespread on European Atlantic shorelines (Harms 1999; Tøttrup *et al.* 2010). Initially its spread was well documented (see Harms 1999 for review), however little has been published on this species since the 1980s. Recent reports describing increases in the abundance of *A. modestus* with warming sea surface temperatures (Lawson *et al.* 2004; Witte *et al.* 2010), have suggested that *A. modestus* could be an "ecological sleeper" (Witte *et al.* 2010), potentially becoming more abundant with predicted climate change. With the exception of some anecdotal accounts of *A. modestus* posing a competitive threat to native barnacle species (Knight Jones 1948; Crisp 1960; Barnes and Barnes 1962; Bennell 1981; Kochmann and Crowe 2014), very little was known about the potential impacts of this species and how it interacts with native barnacle species. This study aimed to gain a better understanding of the competitive interactions between *A. modestus* and native species, the factors which contribute to the invasion success of this non-native species in Ireland and towards the northern and southern invaded limits of this species in Europe.

Long-term monitoring of the recruitment of *A. modestus* and native barnacle species at six field sites in south-west Ireland revealed that *A. modestus* became the dominant recruit in available space, in both removal and control plots, at the majority of sites, with the exception of wave-exposed locations. Recruits of native barnacle species also colonised and survived at all field sites, co-existing with *A. modestus*. The provision of free space did not appear to promote the abundance of *A. modestus* recruits relative to the native barnacle species. A comparison of the growth and survival of the native barnacle *Semibalanus balanoides,* which occupies a similar niche to *A. modestus* in the intertidal zone on sheltered shores, suggested that the fast initial growth rate of *S. balanoides* recruits allows them to utilise a temporal niche before recruits of the non-native species become abundant. Survival rates of *S. balanoides* recruits in the presence of *A. modestus* and in the absence of the non-native species were very similar, indicating that the presence of the non-native species may not always pose a competitive threat to *S. balanoides.*

Native barnacle species were also found to persist in Lough Hyne Marine Nature Reserve, a location where *A. modestus* has been recorded as the dominant intertidal barnacle species in past decades (Lawson *et al.* 2004). The provision of free space did not promote the abundance of recruits of either native species or *A. modestus*. Continued dominance of *A. modestus* at this location is facilitated by the ability of *A. modestus* to breed throughout the entire year via continuous broods (Moore 1944; Crisp and Davies 1955; Moyse 1960; Patel and Crisp 1960; Moyse 1963, also see Chapter 5). The slow overall flushing rate of the Lough (Johnson *et al*. 1995), in addition to the separation from adjacent coastal waters via the rapids (Jessopp 2007; Jessopp and McAllen 2007), facilitates the accumulation of *A. modestus* larvae at this location and its continued abundance.

The production of continuous broods by *A. modestus* is one of the key factors contributing to its success. As seen during recruitment monitoring, while *S. balanoides* recruits, the product of a single annual brood, undergo post settlement mortality, continued recruitment of *A. modestus* allows overall numbers to remain stable or even increase before native species breed again the following year. A comparison of the stage of embryo development of *A. modestus* and *S. balanoides* at a single location over eighteen months, indicates that the inter-annual variability of *S. balanoides* recruitment (Hawkins and Hartnoll 1982; Jenkins *et al.* 2000; Hills and Thomason 2003) facilitates *A. modestus* abundance increases during years of poor *S. balanoides* recruitment. Even if some *A. modestus* broods are not successful, the likelihood is that subsequent broods will be. While *A. modestus* does experience enemy release in comparison to native barnacle species, this was not found to promote reproductive success or abundance of the non-native species.

The role of inter-annual variation in recruitment success of the native barnacle species in controlling *A. modestus* abundance was also illustrated at field sites on the Isle of Cumbrae, Scotland, near the northern limit of this species. Higher abundances of *A. modestus* recruits were recorded in the absence of *S. balanoides* recruits on the Isle of Cumbrae in September 2014 and March 2015, in comparison to the previous year when *S. balanoides* had high levels of recruitment. Competitive pressure from *S.*

balanoides at the recruit stage is likely limiting the abundance of *A. modestus* at this location, where native barnacle species remain dominant. At the southern European limit of *A. modestus,* native barnacle species are also found to be dominant, however it seems that desiccation stress or the inability of larvae to reach suitable substrates for settlement are the controlling factors at this location, but these need to be investigated further.

In contrast to natural rocky shores in south-west Ireland, *A. modestus* by far outnumbered native barnacle species on the majority of artificial structures surveyed. This may be due to the sheltered conditions created by the artificial structures themselves, which would promote the abundance of *A. modestus*, or it may be due to the ability of *A. modestus* to settle on smooth substrata while natives prefer rough surfaces (Hills and Thomason 1998; Coombes *et al.* 2015). Of particular concern are the low abundances of chthamalid barnacles recorded on artificial structures. The predicted decline in *S. balanoides* (Hiscock 2004; Simkanin *et al.* 2005; Svensson *et al.* 2005; Hawkins *et al.* 2008; Poloczanska *et al.* 2008; Wethey *et al.* 2011; Mieszkowska *et al.* 2014) in addition to the increasing abundance of artificial structures (Glasby and Connell 1999; Bulleri 2006; Bulleri and Chapman 2010), could result in these structures becoming solely colonised by *A. modestus.* On natural rocky shores at exposed locations chthamalids will continue to dominate as conditions are not suitable for the non-native species, however at more sheltered locations it is likely that *C. montagui* will persist at the high shore level while *A. modestus* will replace *S. balanoides* at the mid and low shore at sheltered locations.

10.2 Synthesis

A. modestus has many of the typical characteristics of an invasive species, having a high reproductive output, short time to maturity, opportunistic nature, ability to breed throughout the entire year and over a range of temperatures. However, *A. modestus* is not typical of the perceived idea of invasive species in that it does not currently seem to be capable of outcompeting native barnacle species, causing any loss in biodiversity. Native species were found to coexist with *A. modestus* in the

intertidal zone on natural rocky shores and even on artificial structures, although *A. modestus* was usually the dominant species on these structures.

There are some key differences in the niche occupied by *A. modestus* and native barnacle species, which also play an important role in facilitating coexistence. *A. modestus* is most often found in sheltered, estuarine areas with highest abundances at the mid and low shore levels. This species can persist in areas that are subject to high levels of disturbance, due to its opportunistic nature, which is typical of invasive species. Although the native species *S. balanoides* is also found at the mid to low shore, this species is more tolerant of wave exposed conditions and less tolerant of estuarine conditions in comparison to *A. modestus. C. montagui* dominates at the high shore level, generally at more wave exposed locations and does not have a high tolerance of estuarine conditions. Both native species have a lower tolerance of disturbance, facilitating high abundances of *A. modestus* in harbours and ports, on both artificial and natural substrates.

Within the field of invasion biology, there are many theories relating to invasive species. This study has given an insight into the importance of site-specific conditions, which can either promote or impact negatively on the abundance of *A. modestus.* In addition to this, the impacts of *A. modestus* can also differ between locations. Although invasive species are recognised as a threat on a global scale, the results of this study highlight the importance of understanding the factors controlling the abundance of invasive species and differences in their potential effects at a local scale.

10.3 Future research

Future research should focus on gaining a better understanding of the factors controlling the abundance of this species in the Algarve, Portugal, the current documented southern limit of *A. modestus* in Europe (see Chapter 6), in particular to determine whether or not desiccation stress is having a limiting impact on recruitment. Understanding the factors acting at the southern range limit are particularly important as increasing sea surface temperatures will produce similar

conditions at more northern locations over time. Given the importance of the year round breeding capacity of *A. modestus,* determining its total fecundity throughout its range would also be of interest in order to gain a better understanding of its invasive potential. Currently it would appear that sheltered locations with high retention times are particularly susceptible to high abundances of *A. modestus.* It is unlikely that *A. modestus* will entirely displace native barnacle species solely through competitive interactions, however, it will replace native barnacle species if they decline, either due to climate and/or poor recruitment success. With the predicted decline of *S. balanoides,* European shores will be colonised by warm water species including chthamalid barnacles and *A. modestus*. How these ecosystems will differ to our current ecosystems or those solely dominated by chthamalids warrants further investigation.

The role of predation in the invasion ecology of *A. modestus* also warrants future work. There has only been one published study examining differences in predation of *A. modestus* and *S. balanoides* by dog whelks (Barnett 1979) and further research on this topic is required. This data would be important not just in terms of understanding whether or not *A. modestus* experiences enemy release, in comparison to native barnacles in terms of predation, but also to link this with infection by the trematode cysts *Maritrema arenaria,* as predation of infected barnacles by dog whelks is likely to play an important role in the transmission of this parasite to the final host.

Although invasive species generally have negative connotations, it is not always the case in reality. Davis (2003) noted that there have been very few recorded extinctions due to competition from invasive species and, where extinctions occur, it is generally between trophic levels e.g. predator and prey. A review by Katsanevakis *et al.* (2014) noted that many invasive species have both positive and negative impacts and that the positive impacts are largely under estimated. Of particular importance in determining the ecosystem level impact of *A. modestus* is the functional role that this species plays in comparison to the native barnacle species. In the general sense it would seem that *A. modestus* plays a similar functional role to

natives (Olenin *et al.* 2007), however, it is possible that differences in rate of cirral beat (Southward 1955), potential differences in diet (Stone 1989) and contribution of larvae to the plankton community could mean that *A. modestus* plays a different role to native barnacle species, but this is currently speculative.

If *A. modestus* differs in its functional role to native barnacle species, the question remains, does this matter? Again this cannot be answered until differences and thus impacts are identified. If the presence of *A. modestus* does not have a negative impact on ecosystem structure and function its presence could potentially be seen as positive. Currently, its presence is resulting in increased biodiversity while it coexists with native species and this species could potentially act as a replacement for *S. balanoides* under future climatic situations. As previously stated by Witte *et al.* (2010) the presence of generalist non-native species may contribute to ecosystems that are better able to cope with future environmental change. In this particular case, if *A. modestus* was not present, only chthamalid barnacles would persist following the decline of *S. balanoides*. Overall the presence of generalist non-native species and the absence of specialist native species is not an ideal situation nor could it be perceived as positive by many people. In this specific case it is important to consider the potential positive effects (e.g. the ability to carry out functional role of native barnacle species if they decline) of the presence of *A. modestus,* but to also be aware that this species could have increased negative effects (e.g. outcompeting native species, negative impacts on ecosystem functioning) impacts at certain locations in the future.

10.4 References

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Acknowledgements

Firstly I would like to thank my three supervisors Dr Ruth Ramsay, Dr Rob McAllen and Prof. Sarah Culloty for all their time, support and encouragement during this research. I would especially like to thank Ruth, for encouraging me to apply for funding in the first place and for your constant enthusiasm and positivity.

This research would not have been possible without funding from the National University of Ireland Travelling Studentship, University College Cork Strategic Research Fund, European Co-operation in Science and Technology Short Term Scientific Mission funding and the School of BEES travelling fund.

I would like to thank the technical staff in the School of BEES for their assistance at various points throughout this work, but in particular I would like to thank Luke Harman for his help and company while carrying out rasta rock art, I mean field work, at Lough Hyne. Thanks to Declan O'Donnell from the National Parks and Wildlife Service for giving permission to carry out field work in Lough Hyne and also providing helpful information on the dates of construction of piers and slipways in west Cork. I would also like to thank Dr. Mark Jessopp and Dr. Tom Reed for their statistical advice during this project.

Thanks to my office mates and the other PhD students in BEES for their support and friendship but in particular to Greg, I will miss our weekly tea meetings but I think we did a pretty good job of keeping each other on track. Thanks for being my PhD buddy!

I would also like to thank Prof. Teresa Borges at the University of the Algarve for hosting me during my field work in Portugal, organising the permit for me to carry out my field work in the Ria Formosa, helping me to identify potential field sites and mostly for making me feel so welcome – muito obrigada! Thanks also to the staff and students in CCMAR for welcoming me into their laboratory. Thanks also to technical

staff at the Marine Biology Station at Millport for facilitating my laboratory work there.

A big thank you to my parents for all your love and support. Also thanks to my dad for keeping me company on lots of my field visits, no matter what the weather, and for always helping in any way.

Finally to Matty, thank you for always being there.