A health status survey of clams, *Mya arenaria* and *Ensis siliqua*, in the Irish Sea

Cross, M.E., Lynch, S., O'Riordan, R.M., Culloty, S.C.

Aquaculture and Fisheries Development Centre, School of Biological, Earth and Environmental Sciences, University College Cork, Ireland.

Abstract

The soft shell clam, *Mya arenaria*, and the razor clam, *Ensis siliqua*, are widely distributed in Irish waters. Though the reproductive biology and other aspects of the physiology of these species has been previously investigated, little or no data are currently available on their health status. As this knowledge is essential for correct management of a species, *M. arenaria* and *E. siliqua* were examined to assess their current health status using histological and molecular methods, over a period of sixteen months. No pathogens or disease were observed in *M. arenaria*, and low incidences of Prokaryote inclusions, trematode parasites, *Nematopsis* spp. and inflammatory pathologies were recorded in razor clams for the first time in Northern European waters.

Key words: *Ensis siliqua*, *Mya arenaria*, Irish Sea, *Nematopsis*, *trematode*.

Introduction

The soft-shell clam, *Mya arenaria*, is widely distributed in coastal and intertidal soft substrates in European waters, including the Irish and British coasts (Eno *et al*., 1997; O'Riordan *et al*., 2002). This clam is currently fished on North American shores (Beal, 2002; Connell *et al*., 2007), and is considered a species of interest for future commercial exploitation in the Irish Sea. *Ensis siliqua*, the razor clam, is a native species in Europe, and is distributed along the European Atlantic coast from the Norwegian Sea south to the Mediterranean and along the Atlantic coast of Morocco (DaCosta *et al*., 2010). It is abundant in the British Isles, and widely distributed along the east coast of Ireland (Fahy, 1999), where it is currently harvested by commercial fisheries.

To manage an exploited species, knowledge of its biology, including the health status and the effects of environmental irregularities on this status, is essential (Bignell *et al*., 2008; Lopez *et al*., 2011). Presently, no data are available on the pathogens that may be present in European *M. arenaria*, though several diseases and

parasites, including neoplasia, *Vibrio parahaemolyticus* and *Perkinsus* sp. protozoans, have been documented in this species on the Atlantic west coast (Brousseau & Baglivo, 1991; Dungan *et al*., 2002; Earle & Crisley, 1975). A range of parasites and pathological alterations including protozoans, ciliates, germinoma, disseminated neoplasia, basophilic inclusions, intracellular prokaryote-like organisms, viral inclusions, and bacterial proliferations, including *Vibrio,* have recently been identified in *Ensis siliqua* on the Spanish coast where *Ensis* species are intensively exploited, but to date there has been no report of these conditions present in razor clams further north in Europe (Perez, 2011; Ruiz *et al*., 2013).

Due to the lack of information currently available on the health status of *M. arenaria* and *E. siliqua* in Northern Europe, this study was undertaken to assess the current health status of these two species, concentrating on the Irish Sea area where *Ensis siliqua* is currently exploited, using both histological and molecular methods.

Materials and Methods

Soft-shell clam, *M. arenaria,* specimens (n=30) were collected in Bannow Bay, Co Wexford, Ireland, each month from March 2010 to June 2011, and 31 *M. arenaria* were collected in Flaxfort Strand, Cork, Ireland, in June 2011. Thirty live *Ensis siliqua,* which had been fished in the Skerries region of the Irish Sea were obtained monthly from June 2010 to September 2011, from a commercial shellfish wholesaler on the east coast of Ireland, and 34 *E. siliqua* were fished by hand at Oxwich Beach, Swansea, Wales, in September 2011.

A section of the body of each clam was cut out, which contained the gonad, renal gland and digestive tract, and sections of the gill and mantle. The tissue was fixed in Davidson's solution for 48 hours and stored at 4°C. Slides were prepared using standard histological techniques outlined by Porter (1974). The prepared microscope slides were screened for the presence of any parasites and abnormal conditions or pathologies.

All gill tissue samples of clams were preserved in 90% Ethanol at dissection. DNA extraction was carried out by Chelex-100 resin (Bio-Rad) extraction. The extracted DNA of 463 sampled *M. arenaria* was subjected to PCR amplification using Ostreid Herpes Virus (OSHV)-specific primers C2/C6 and OSHV-For/OSHV-

Rev (Renault & Arzul, 2001), as the clams were obtained from an area (Bannow Bay, Wexford, Ireland) where this virus is present in Pacific Oysters, *Crassostrea gigas*. Both *Mya arenaria* (n=463) and *E. siliqua* (n=484) samples were screened for the presence of bacterial DNA using the generic forward primer 18Scom-F and the reverse primer 18Scom-R (Zhang & Lin, 2005), and for detection of haplosporidian species using the forward primer (Hap-F1) and reverse primer (Hap-R3) (Renault *et al*., 2000).

Results

Screening of the histological slides of the 494 *Mya arenaria* revealed no parasites or pathological indications of disease in any of the individuals sampled in Bannow Bay or Flaxfort Strand, and no pathogens were identified in the 34 razor clams sampled in Oxwich Bay, Swansea. Of the 484 *Ensis siliqua* screened in the current study, only 6 individuals were identified with pathogenic states, all of which were sampled in the Skerries region of the Irish Sea. In the 6 individuals, Prokaryote inclusions (n=2), Trematode metacercaria (n=1), *Nematopsis* spp. oocysts or sporozoites (n=3), and eosinophilic bodies (n=2) were observed.

Polymerase chain reaction (PCR) amplification of *M. arenaria* samples using primers specific to Ostreid Herpes Virus (OSHV) and of *M. arenaria* and *E. siliqua* samples using the generic bacterial 18Scom and Haplosporidian species SSU rDNA primers resulted in either no amplification, or the amplification of clam DNA only.

Discussion

To date, little or no disease or pathogens have been reported in *Mya arenaria* on European shores (Strasser *et al.,* 1999), in contrast to the number of diseases and parasites being present in the more intensively cultured *M. arenaria* populations of North America. No parasites, pathogenic states or disease were found in any of the soft shell clams sampled in either of the two sites in the current study, despite a heavy prevalence of trematodes, neoplasia and *Nematopsis* spp. in *Cerastoderma edule* within the same area (Morgan *et al.*, 2012), and Ostreid Herpes Virus previously identified in *Crassostrea gigas* in Bannow Bay (Lynch *et al.*, 2012). While varying environmental temperatures would seem to have affected the timing of gametogenesis in this population (Cross *et al*., 2012), neither energy used for spawning, nor the stress of oscillating environmental temperatures resulted in a

disease state, as has been recorded in *M. arenaria* from other areas (Brousseau & Baglivo, 1991) and other bivalve species (Gagnaire *et al*., 2006; Harvell *et al*., 1999; Malham *et al*., 2009).

In the present study no pathogens were found in *E. siliqua* sampled in Oxwich, Swansea, and only 1.2% of *E. siliqua* clams screened from the Skerries showed the presence of parasites or pathologies, and the majority of the symbionts and conditions observed did not cause host damage. While *Nematopsis* was previously recorded in *E. arcuatus* clams sampled in Cill Chiarain Bay, Co. Galway, Western Ireland (Fahy *et al.*, 2002), prokaryote inclusions, trematode metacercaria, *Nematopsis*, and eosinophilic bodies, are observed in north European *E. siliqua* for the first time in the current study.

The lack of any disease or parasites found in the sampled *M. arenaria* would indicate that this species is very healthy in its European range, in contrast to North American populations. A low level of parasites and pathogens were observed in *E. siliqua* samples from the Irish Sea, leading us to conclude that razor clam populations are also healthy in this area. The intensive culture of *M. arenaria* in North America (Wallace, 1997), and *E. siliqua* in the Galicia region of Spain (DaCosta *et al*., 2010) may have resulted in these clam populations being under stress due to over-crowding, which could make them more susceptible to infections and disease (Renault & Novoa, 2004). Future exploitation in the Irish Sea with increased pressures on stock may influence the health status of bivalve species. However, data from the present study indicates healthy populations of *M. arenaria* and *E. siliqua* in the Irish Sea, with little or no prevalence of disease.

References

Beal, B.F. 2002. Adding value to live, commercial size soft-shell clams (*Mya arenaria* L.) in Maine, USA: results from repeated, small-scale, field impoundment trials. Aquaculture 210(1-4): 119-135.

Bignell, J.P., Dodge, M.J., Feist, S.W., Lyons, B., Martin, P.D., Taylor, N.G.H., Stone, D., Travalent, L., Stentiford, G.D. 2008. Mussel histopathology: effects of season, disease and species. Aquatic Biology 2: 1-15.

Brousseau, D. J. & Baglivo, J. A. 1991. Disease progression and mortality in neoplastic *Mya arenaria* in the field. Marine Biology 110(2): 249-252.

Connell, L.B., MacQuarrie, S.P., Twarog, B.M., Iszard, M., Bricelj, V.M. 2007. Population differences in nerve resistance to paralytic shellfish toxins in softshell clam, *Mya arenaria*, associated with sodium channel mutations. Marine Biology 150 (6):1227-1236.

Cross, M.E., Lynch, S., Whitaker, A., O'Riordan, R.M., Culloty, S.C. 2012. The reproductive biology of the softshell clam, *Mya arenaria*, in Ireland, and the possible impacts of climate variability. Journal of Marine Biology.

DaCosta, F.D., Martínez-Patiño, D., Ojea, J., Nóvoa, S. 2010. Larval rearing and spat production of the razor clam *Ensis siliqua* (Bivalvia: Pharidae). Journal of Shellfish Research 29(2): 347-351.

Dungan, C.F., Hamilton, R.M., Hudson, K.L., McCollough, C.B., Reece, K.S. 2002. Two epizootic diseases in Chesapeake Bay commercial clams, *Mya arenaria* and *Tagelus plebeius.* Diseases of aquatic organisms 50(1): 67-78.

Earle, P. M. & Crisley, F. D. 1975. Isolation and characterization of *Vibrio parahaemolyticus* from Cape Cod soft-shell clams (*Mya arenaria*). Applied and Environmental Microbiology 29(5): 635.

Eno, N.C., Clark, R.A., Sanderson, W.G. 1997. Non-native marine species in British waters: a review and directory, Published by JNCC, Peterborough.

Fahy, E. 1999. Biology and Exploitation of a razor clam bed at Gormanstown, Co. Meath, Ireland. Third International Conference on Shellfish Restoration, Cork, September 1999.

Fahy, E., Lyons Alcantara, M., Norman, M., Browne, R., Roantree, V., Pfeiffer, N. 2002. Mortalities in *Ensis arcuatus* (Jeffreys) (Solenacea) in western Ireland. Journal of Shellfish Research 21(1): 29-32.

Gagnaire, B., Frouin, H., Moreau, K., Thomas-Guyon, H., Renault, T. 2006. Effects of temperature and salinity on haemocyte activities of the Pacific oyster, *Crassostrea gigas* (Thunberg). Fish & Shellfish Immunology 20(4): 536-547.

Harvell, C.D., Kim, K., Burkholder, J.M., Colwell, R.R., Epstein, P.R., Grimes, D.J., Hofmann, E.E., Lipp, E.K., Osterhaus, A., Overstreet, R.M. 1999. Emerging marine diseases--climate links and anthropogenic factors. Science 285(5433): 1505.

Lopez, C, Darriba, S, Iglesias, D, Ruiz, M, Rodriguez, R. 2011. Pathology of sword razor shell (*Ensis arcuatus*) and grooved razor shell (*Solen marginatus*). In: Guerra, A., Lodeiros, C., Baptista, M., da Costa, F. (Eds.). Razor Clams: Biology, Aquaculture and Fisheries. Conselleria do Mar, Xunta de Galicia: 131-168.

Lynch, S.A., Carlsson, J., Reilly, A.O., Cotter, E., Culloty, S.C. 2012. A previously undescribed ostreid herpes virus 1 (OsHV-1) genotype detected in the pacific oyster, *Crassostrea gigas*, in Ireland. Parasitology 1(1): 1-7.

Malham, S.K., Cotter, E., O'Keeffe, S., Lynch, S., Culloty, S.C., King, J.W., Latchford, J.W., Beaumont, A.R. 2009. Summer mortality of the Pacific oyster, *Crassostrea gigas*, in the Irish Sea: The influence of temperature and nutrients on health and survival. Aquaculture 287(1-2): 128-138.

Morgan, E., O'Riordan, R.M., Kelly, T.C., Culloty, S.C. 2012. Influence of disseminated neoplasia, trematode infections and gametogenesis on surfacing and mortality in the cockle *Cerastoderma edule*. Diseases of aquatic organisms 98(1): 73.

O'Riordan, R. 2002. The accidental introduction of marine animals into Ireland. In: C. Moriarty and D.A. Murray (eds) Biological Invaders: the impact of exotic species. Royal Irish Academy, Dublin: 95-106.

Perez, J. 2011. Pathologies in pod razor clam (*Ensis siliqua*). In: Guerra, A., Lodeiros, C., Baptista, M., da Costa, F. (Eds.). Razor Clams: Biology, Aquaculture and Fisheries. Conselleria do Mar, Xunta de Galicia: 119-130.

Porter, R. 1974. Reproductive cycle of the soft-shell clam, *Mya arenaria*, at Skagit Bay, Washington. Fishery Bulletin 72(3): 648-656.

Renault, T. & Arzul, I. 2001. Herpes-like virus infections in hatchery-reared bivalve larvae in Europe: specific viral DNA detection by PCR. Journal of Fish Diseases 24(3): 161-168.

Renault, T. & Novoa, B. 2004. Viruses infecting bivalve molluscs. Aquating Living Resources 17(4): 397-409.

Renault, T., Stokes, N., Chollet, B., Cochennec, N., Berthe, F., Gérard, A., Burreson, E. 2000. Haplosporidiosis in the Pacific oyster *Crassostrea gigas* from the French Atlantic coast. Diseases of aquatic organisms 42(3): 207-214.

Ruiz, M., Darriba, S., Rodríguez, R., López, C. 2013. Histological survey of symbionts and other conditions of pod razor clam *Ensis siliqua* (Linnaeus, 1758) in Galicia (NW Spain). Journal of Invertebrate Pathology 112(1): 74-82.

Strasser, M., Walensky, M., Reise, K. 1999. Juvenile-adult distribution of the bivalve *Mya arenaria* on intertidal flats in the Wadden Sea: why are there so few year classes? Helgoland Marine Research 53(1): 45-55.

Wallace, D.E. 1997. The molluscan fisheries of Maine. In: MacKenzie, C.L., Burrell, V.G., Rosenfield, A., Hobart, W.L. (Eds.), The History, Present Condition, and Future of the Molluscan Fisheries of North and Central America and Europe. Atlantic and Gulf coast, US Dept. Comm.,Washington, D.C. NOAA Tech. Rep. 127. 1: 63-86.

Zhang, H. & Lin, S. 2005. Phylogeny of dinoflagellates based on mitochondrial cytochrome b and nuclear small subunit rDNA sequence comparisons. Journal of Phycology 41, 411–420.