UNIVERSITY OF SOUTHAMPTON

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES
SCHOOL OF OCEAN AND EARTH SCIENCE

Restoration of the European flat oyster *Ostrea edulis* using elevated broodstock reefs

by

Amonsak Sawusdee

Thesis for the degree of Doctor of Philosophy

November 2015

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES

Ocean and Earth Science

Thesis for the degree of Doctor of Philosophy

RESTORATION OF THE EUROPEAN FLAT OYSTER *OSTREA EDULIS* USING ELEVATED BROODSTOCK REEFS

Amonsak Sawusdee

The precarious status of flat oyster *Ostrea edulis* stocks in Europe is widely acknowledged. To build a scientific basis for oyster restoration, an elevated experimental reef stocked with *O. edulis* was established within Poole Bay (Dorset, UK). Oysters were planted out on twenty four oyster reef modules (80cm above sea bed) and compared with oysters held on the sea bed close to each reef module to test the hypothesis that a reef habitat enhances physiological performance of *O. edulis* and improves local biodiversity. The environmental data indicated that there was no significant difference in temperature or salinity between the elevated reefs and sea bed. Whilst total suspended solids were significantly higher at the sea bed than at 80 cm above the sea bed at every sampling interval. The filtration rates of oysters varied with elevation (reef/sea bed) and months. Filtration rates of reef oysters were significantly higher than sea bed oysters. Respiration rates varied among months but were not significantly affected by elevation. Elevation and month also affected the total number of haemocytes and the granulocyte population; reef oysters had significantly higher numbers of haemocytes than sea bed oysters. Althought geographical variation in filtration rate was also observed, this study suggested that the improved physiology of reef oysters in summer was still notable when comparing between different populations. An increase in filtration rate for reef oysters may, however, increase the likelihood of uptake of the protozoan parasite, *Bonamia* sp.. However, haemocyte and haemolymph protein data suggested that there was no differential impact on physiology between reef and sea bed oysters as a result of *Bonamia* sp. infection. Fifty-four epifaunal species including oyster spat were found on oyster valves in reef boxes whilst only 23 species with no oyster spat were found on oyster valves in oyster cages laid on sea bed. The epifaunal community on oyster valves on reefs was significantly different from those sea bed valves but also had some species in common. Sixty-five mobile species were observed in reef boxes, whilst 47 species were recorded in oyster cages. These differences in faunal communities observed on the elevated reefs and sea bed implied that the presence of elevated reef habitats created by *O. edulis* valves can enhance or accelerate local diversity in Poole Bay. As current stocks of European flat oysters (*O. edulis*) in Europe have declined in both abundance and distribution, the results of this pilot study suggest that the culture of oysters on elevated reef structure represents at least a partial solution to improve *O. edulis* physiology for restoration in Europe.

Table of Contents

List of tables

List of figures

DECLARATION OF AUTHORSHIP

I, Amonsak Sawusdee

declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

Restoration of European flat oyster *Ostrea edulis* using elevated broodstock reefs

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Parts of this work have been published as:

Sawusdee, A., Jensen, A.C., Collins, K.J., Hauton, C. 2015., Improvement in the physiological performance of European flat oysters *Ostrea edulis* (Linnaeus, 1758) cultured on elevated reef strctures: Implications for oyster restoration. Aquaculture, 444, 41-48.

Signed:.....

Date:.....

Acknowledgements

The success of this doctoral thesis would not have been achieved without the kind contributions of a number of admirable people. Above all, I would like to acknowledge my heartfelt gratitude to my supervisors, Dr. Antony C. Jensen and Dr. Chris Hauton for their excellent supervision and guidance on my PhD research. It was a great time to learn by doing research with them. Also, I would like to express my gratitude to Dr. Lawrence E. Hawkins for constructive comments and opinions in every PhD panel meeting. I would like to extend my gratitude to Dr. Ken Collins and his scuba diving team at NOCS for their help on constructing and recovering oyster reefs. My grateful thanks to Jenny Mallinson who spent her valuable time in retirement to teach me how to identify epifaunal and mobile species. I am very grateful to Prof. Dr. Tuantong Jutagate, for guiding and inspiring me to study in the UK. I wish to thank Royal Thai Government, National Science and Technology Development Agency (NSTDA) under Ministry of Science and Technology for a PhD scholarship. My grateful thanks are also given to Natural England for a partial funding support to my PhD project.

I also gratefully thank the crew of RV *Bill Conway*, Stephanie Deane and Chengqian Chen for their help on field work. Grateful thanks also expressed to Dr. Wanwiwa Tumnoi, Dr. Lisa Kamphausen, Dr.Hanna Schuster, Nicola Pratt, Matt O'Shaughnessy and Jiraporn Charoenvattanaporn who always supported and guided my laboratory works. Special thanks are extended to Dr Benjamin J. Ciotti for his considerable contribution to my PhD research, especially his advice on statistical analysis. I would also like to thank Dr. Lauren Hall, Dr. James Morris and Dr. Anouska Panton for their help to correct the English in my thesis.

I would also like to thank friends in Marine Invertebrate Physiology and Immunology research group (MIPI lab) at National Oceanography Centre (NOCS), University of Southampton, and my colleagues at Walailak University, Thailand for their friendship and support. Finally, my special thanks go to my beloved family, my father and mother, my wife and younger brother and sister. Without their endless love, belief, encouragement, and support, none of this success would have been possible.

Chapter 1

General introduction

Oysters have formed a part of the human diet for many centuries (Orton, 1937) and today many species of oyster are exploited commercially (Kamphausen et al., 2011; Laing et al., 2006; Smith et al., 2006). Globally, oysters are keystone species in some marine ecosystems, providing substantial ecological services, especially where their reefs serve as a nursery and spawning ground for marine organisms (Beaumont et al., 2007). Oysters, as filter feeders, remove phytoplankton, suspended solids, and organic particles from the water column and therefore help to control eutrophication (Fulford et al., 2007; Fulford et al., 2010). Many species of oysters such as the European flat oyster *Ostrea edulis* and the eastern oyster *Crassostrea virginica* (Lenihan, 1999; Micu et al., 2008) are also known to form biogenic reefs, providing habitat for a wide range of other marine algae, plants and animals that benefit shoreline stabilization (Meyer et al., 1997; Scyphers et al., 2011). It can be concluded that, when allowed to flourish, oysters are significant as ecosystem engineers and environmental regulators in estuarine and marine ecosystems.

Currently, the World's oyster populations have declined in both abundance and distribution (Wilberg et al., 2011). Eighty-five percent of oyster reefs have been lost and periodic decline in the productivity of oyster beds is a world-wide feature (Beck, 2011). These periodic declines in oyster stocks present serious problems, not only because they provide protein sources and support the fishing industry, but also because of their role in the provision of ecosystem services in the coastal zone. The causes of collapse in oyster stocks have been attributed to overfishing, parasites/diseases and failure of adequate spatfall to balance and sustain further the parental stocks (Kamphausen et al., 2011). As such, this considerable decline of oyster stocks has been indirectly and directly affecting the world's human population in terms of both food security and quality of the marine environment.

There are many existing strategies that have been applied to restore and maintain oyster fisheries, such as developing broodstock sanctuaries, supplementing hard substrata on the estuarine bottom, relocating stocks, shell planting, seed planting, oyster gardening and occasionally supplementing natural populations with hatchery reared stocks (Coen et al., 2007; Laing et al., 2006). Among the methods that have been used for the purpose of oyster recovery, artificial oyster reefs have become popular in many of the world's coastal zones, especially in the USA. In a number of coastal zones in the USA artificial reef strategies have been employed in an attempt to promote the recovery of the Eastern oyster (*C. virginica*), for the purpose of increasing oyster stocks and improving the marine environment through the maintenance of ecosystem services. Often, the common goal of artificial oyster reef construction is not only for habitat restoration but also for fisheries enhancement and recovery of the biodiversity. However, to date there has been no study evaluating the merits of building artificial reefs or broodstock reefs on which to restore communities of the native flat oyster (*O. edulis*) in Europe. Furthermore, explicit evidence for the benefits of constructing artificial oyster reefs is generally lacking.

In light of the paucity of available data it is highly desirable that research and assessment of the recovery potential of *O. edulis* through intervention is validated at an experimental pilot-scale prior to the large scale introduction of this technique as a habitat restoration and management strategy.

1.1 Ecosystem services of oyster reefs

Ecosystem services, as a concept for understanding the way in which nature directly or indirectly benefits humans, has led to a suite of approaches that are increasingly being used to support sustainable management of biodiversity and ecosystems (Coen et al., 2007; Grabowski and Peterson 2007; Grabowski et al., 2012). There are several different definitions and classifications of ecosystem services depending on what kinds of environmental resources that have been managed using this concept (Brumbaugh and Toropova, 2008; Grabowski et al., 2012). Similar to the Millennium Ecosystem Assessment (MEA), the UK National Ecosystem Assessment (UK NEA) classifies services

along functional frameworks into four broad categories (i.e. provisioning, regulating, supporting and cultural services). Within the four categories, supporting services (e.g. primary production, cycling of water and nutrients, and provisioning of foraging, spawning and nursery habitats) are those that are necessary for the production of all other three categories (Brumbaugh and Toropova, 2008; Harrison et al., 2014; Millennium Ecosystem Assessment, 2005). Oyster reefs are one of four major natural habitats (others include seagrass habitat, coral reef habitat and mangroves) that have been widely reported to provide a number of valuable ecosystem services that can be impacted by local, regional, and global influences (Coen and Luckenbach, 2000; Grabowski et al., 2012). Grabowski and Peterson (2007) identified seven ecosystem services that are delivered by oysters: food production; water filtration and concentration of biodeposits; provision of habitats for marine vertebrates and invertebrates; sequestration of carbon; augmentation of fishery resources; stabilization of benthic or intertidal habitats (e.g. marsh) and increase of landscape diversity.

Oyster reef structures are known to make a valuable contribution to shallow water biodiversity by providing hard settlement surfaces and by increasing habitat complexity (Coen and Luckenbach, 2000; Luckenbach et al., 2005). Oysters themselves also provide suitable and complex substrates for a variety of epifaunal species (Bartol and Mann, 1997; Korringa, 1952; Laing et al., 2006). Several studies have revealed that many of the epifaunal species are often observed on elevated oyster reefs (Korringa, 1951; Summerhayes et al., 2009). Harrison et al. (2014) suggested that the ecosystem services of water quality regulation, water flow regulation, mass flow regulation and landscape aesthetics were improved by enlarging the community and habitat area. On oyster reef structures, the large community of suspension feeders, encrusting or fouling organisms (e.g., tunicates, bryozoans, sponges, barnacles, polychaetes, oysters and other associated epifaunal species), performs supporting and regulating services to the ecosystem by the way of water and nutrient cycling through their suspension feeding activity (Brumbaugh et al., 2006). The suspension feeders contribute to a decrease in concentrations of microscopic phytoplankton, sediments, and suspended inorganic particles in the water column, thereby controlling the overall abundance of phytoplankton growing in the overlying waters (Brumbaugh et al., 2006). The reduction of

sediments in the water column can further improve light penetration (Newell and Koch, 2004) and thus increase primary productivity and also support photosynthesis and fertilization of submerged aquatic plants (Carroll et al., 2008; Newell and Koch, 2004; Thayer et al., 1978; Wall et al., 2008). Destruction of oyster beds and their filtering and grazing performance can lead to excess nutrient levels and more intense phytoplankton or algae blooms with an increased risk of anoxic events, as reported in Chesapeake Bay after the damage of *Crassostrea* reefs (Dame, 1996). Moreover, oyster reefs help counteract the overbalance in anthropogenic nitrogen loading in coastal areas by promoting bacterially mediated denitrification induced by concentrated bottom biodeposits of faeces and pseudofaeces (Newell et al., 2002). Furthermore, Shumway et al. (2003) reported that filter feeding shellfish, including oysters, not only remove nitrogen from the water column but they also incorporate a high quantity of it into their tissues. Shellfish are approximately 1.4 percent nitrogen and 0.14 percent phosphate by weight. Therefore, when shellfish are harvested, that nitrogen is simultaneously removed from the system (Shumway et al., 2003). It is clear that the improvement of water conditions through filtration activity of oysters and other suspension feeders on elevated reef structures positively influences ecosystem services (supporting services, e.g. improving habitats and environmental conditions) in coastal and marine zones. These supporting and regulating services provided by oysters and elevated reef structures can directly and indirectly enhance marine biodiversity and subsequently support ecosystem goods and services (Newell, 1988; Rothschild et al., 1994).

Biogenic reefs created by oysters (e.g. *C. virginica*) provide the base of the food chain in coastal and marine ecosystems (Harding and Mann, 1999; Lenihan et al., 2001; Peterson et al., 2003). Reef habitats provide suitable spaces for several epifaunal species which are important prey for higher trophic organisms (Harding and Mann, 1999). Additionally, the abundance of epifauna on elevated reef structures will attract other mobile fauna (e.g. gastropods, crustaceans and other resident invertebrates) (Bahr and Lanier, 1981; Rothschild et al., 1994; Wells, 1961). In turn these predators will be consumed by juvenile and adult fish that use oyster reefs for foraging and refuge from predators, which leads directly and indirectly through the provision of forage species to an enhanced production of economically

important fishery stocks (Breitburg et al., 2000; Harding and Mann, 2001; Peterson et al., 2003, Tolley and Volety, 2005). It is clear that oyster reefs promote an increased transfer of energy among trophic levels from the primary producers to the higher orders of consumers or predators (Baird et al., 2004).

The idea that natural and artificial oyster reefs constructed in the USA can enhance local biodiversity have widely been accepted (Coen et al., 2007; Harding and Mann, 1999; Lenihan et al., 2001). Our understanding of the potential effects of biodiversity loss on the delivery of ecosystem services is improving (Balvanera et al., 2013; Cardinale et al., 2012). Recently, a systematic review by Harrison et al. (2014) confirmed that biodiversity indicators (e.g. species richness, diversity and abundance of individuals) very positively correlate with ecosystem services and they play a major role in the generation of ecosystem services. Therefore, it can be concluded that the greater the improvement of habitat biodiversity, the greater the potential to increase ecosystem services.

It is clear that previous studies have suggested that the most important direct driver of biodiversity loss and ecosystem service failure is habitat damage (Cardinale et al., 2012). From a conservation point of view, the most valuable ecosystem service of an oyster reef is the increase of biodiversity through provision of a richly-textured biogenic habitat that provides a living space for many invertebrates and fish (Hooper et al., 2012; Millennium Ecosystem Assessment, 2005). As previously discussed, oysters themselves and their elevated reef structures in USA can enhance biodiversity and stimulate ecosystem services through the improvement of surrounding environmental conditions that are beneficial for marine organisms. In the UK, the benefits of bivalve shells, laid on the sea bed, providing suitable substrates for epifaunal settlement has been previously published (Korringa, 1951; Smyth and Roberts, 2010). However, to date there has been no study evaluating the merits of building artificial reefs on which to restore an *O. edulis* population. Furthermore, explicit evidence for the benefits of constructing artificial *O. edulis* reefs in terms of enhancing local biodiversity and consequently influencing ecosystem services is generally lacking. Therefore, research is necessary to establish the beneficial effects of elevated reef construction on both oyster restoration and the maintenance of biodiversity.

1.2 Status and the evidence of reef forming of *Ostrea edulis*

Ostrea edulis has been cultivated and exploited in Europe since the time of the Roman empire (Gunther, 1897). This species has been amongst the most commercially important marine resources in European waters (Orton, 1937). Prior to industrialisation of the *O. edulis* fishery, oysters formed a staple part of the diet of many poorer coastal communities (Edwards, 1997). However, *O. edulis* has a long history of exploitation and stocks have declined throughout their entire geographical range (Edwards, 1997; Mackenzie et al., 1997).The decline of this species has been principally due to over exploitation (Edwards, 1997; Mackenzie et al., 1997), low rates of recruitment, destruction of natural oyster beds, the effects of the extremely cold winters in the 1960s and 1970s, technological improvements in fishing, declining water quality due to industrial and municipal effluents (Tubbs, 1999) and a lack of effective management (University Marine Biological Station Millport, 2007). In the past forty years, production of flat oysters has also been negatively affected by two parasites (*Bonamia ostreae* and *Marteilia refringens*) (Baud et al., 1997). Mass mortalities of *O. edulis* populations throughout Europe resulting from the disease bonamiasis caused by *B. ostreae* have been reported since the 1960s (Lallias, 2008). This protozoan is an intracellular parasite and belongs to the order of Haplosporidae and the phylum of cercozoan (Cochennec et al., 2000). In Europe, *B. ostreae* was first detected in France in the late 1970s (Lallias, 2008). The complete life cycle of this parasite remains uncertain. However, it appears that transmission can occur directly from oyster to oyster and that even when an area has been left fallow for a number of years the parasite persists, infecting flat oysters after they are reintroduced to the area (van Banning, 1985). Additional factors contributing to the decline of *O. edulis* fisheries included predators (e.g. the tingle (*Ocenebra erinacea*) and American oyster drill (*Urosalpinx cinerea*)) and competitors e.g. slipper limpet (*Crepidula fornicata*) (Harding, 1996; Korringa, 1952; MacKenzie, 1970; Utting and Spencer, 1992).

Oysters are vulnerable to overfishing because they have a relatively long lifespan and sporadic reproduction (Laing et al., 2006; Orton, 1937; Sparck, 1951). Landings of native oysters in Europe started to decline in the early 18th

century (Grizel and Heral, 1991). More recently in England, annual landings of native oysters fell from more than 2000 tonnes in the 1920s to a few hundred tonnes by the early 1990s (Edwards, 1997; Laing et al., 2006). Currently, the main British stocks of *O. edulis* are situated on the Scottish west coast, the south-east and Thames estuary, the Solent and the River Fal (Jackson and Wilding, 2007) and are considered to be very low in comparison with the stocks of the late 1800s (Laing et al., 2005). In the Firth of Forth, Scotland, where there were historically significant fisheries (Smith et al., 2006), *O. edulis* has disappeared although a few individuals were recently reported (Ashton, 2010). The Solent, one of the main European flat oyster beds in the 1970s and 80s, contained Europe's largest self-sustaining *O. edulis* oyster fishery, supporting 450 boats and over 700 fishermen at its peak. However, the oyster population has been in continual decline since the 1980s, with an extreme decline observed in 2007. Recently, Kamphausen et al. (2011) reported that reproductively active parental stocks are rare and that sex ratios are biased in some locations; i.e. the number of males greatly exceeding that of females. This could be a reason to explain the observation that no spat settlement was found from 2008 to 2011 (Kamphausen et al., 2012) in the Solent fishery. The precarious status of the Solent stock led to the closure of the Solent fishery in 2012. It is clear that the decline of *O. edulis* represents a significant problem to European coastal economies not only in terms of food security (Vanstaen and Palmer, 2010), but also adversely affecting the quality of the marine environment (Fulford et al., 2007; Fulford et al., 2010)

The current poor status of *O. edulis* stocks has not gone unnoticed and concern for the native oyster is long standing. Within the UK, the native oyster Biodiversity Action Plan (BAP) (http://www.ukbap.org.uk/ukplans.aspx?ID=495) was launched in 1996 as a direct outcome of the Rio Convention (BRIG, 2007). The UK BAP (Clarke, 2001; Laing et al., 2005; The UK Biodiversity Action Plan, 1999) for the oyster *O. edulis* has the following targets: 1) to maintain the existing geographical distribution of the native oyster within UK inshore waters, 2) to expand the existing geographical distribution of the native oyster within UK inshore waters where biologically feasible, 3) to maintain the existing abundance of the native oyster within UK inshore waters, and 4) to increase the abundance of the native oyster within UK inshore waters, where biologically feasible. However, since its launch in 1996 progress with the native

oyster BAP has been hampered by a lack of fundamental research with which to revise management practice and restore or enhance ecosystem biodiversity.

An understanding of the marine biodiversity and ecological functions of *O. edulis* is essential for attaining the UK BAP targets, as stated above, for the species. Lenihan and Peterson (1998) referred to oysters as ecosystem engineers that create biogenic habitat important to estuarine biodiversity, benthic-pelagic coupling and fishery production. Oysters are also recognised as keystone species which can influence community structure (Raj, 2008). However, the establishment of biogenic oyster 'reefs' could only happen in undisturbed *Ostrea* populations, something that rarely exists in Europe. The concept is a challenging one; conventional wisdom suggests that species such as *C. virginica* develop substantial biogenic reefs along the eastern seaboard of the USA if left undisturbed (Lenihan, 1999) but because of the long established practice of stock relaying, native European oysters have been regarded as individuals rather than assemblages.

Modern literature on the topic is scarce, but extensive *O. edulis* reefs have been recorded by Micu et al., (2008) along the Bulgarian coast of the Black Sea. The largest biogenic structures described were 7m high, 30-35m long and 10m wide and built entirely of *Ostre*a valves. No living *Ostrea* were found during the survey but anecdotal evidence suggested that live oysters had been collected from the site as recently as 2-3 years before the survey (2004-5). 'Clocsk' (local name for clusters of oysters) of up to seven *Ostrea* are found sublittorally in unfished areas of Strangford Lough (Roberts, pers.com.). In the 'less fished' areas of the Solent groupings of up to three oysters have also been found (Jensen, pers. comm.)

The current distribution and abundance of *O. edulis* is very different from that inferred from historical records. Extensive reefs appear to have existed as early as 1866 when the Fishery Commission report of that date describes collection of "large and coarse" oysters taken from banks at depth of 27-44 m in the English Channel (Olsen, 1883). Similarly, the Piscatorial Atlas of the North Sea, English and St George's Channels of 1883 highlights an area 24,000 km^2 as "oysters" (Houziaux, 2008). Roberts (2007), referring to this map, describes: "these oyster grounds consisted of reefs built of oysters, knitted and interlaced with countless other invertebrates. The bottom of the North Sea was hardened

by a living crust, something that many scientists today find hard to believe". Interestingly, Korringa (1951) refers to the fact that the preferred surface for native oyster spat settlement is on the new growth of adult oysters and expressed concern then for the fishery since the harvesting of adults meant loss of the new recruits.

More recent experimental evidence for the reef-forming tendencies of *O. edulis* can be found. Kennedy and Roberts (1999) showed that the preferred settlement site was live oysters and Pascual and Zampatti (1995) identified that for *Ostrea puelchana* the preferred settlement on live oysters of the same species was chemically mediated. The link between the availability of bivalve adults and settlement behaviours of oyster larvae has been established several times in the literature. Three hypotheses have been advanced concerning the origin of the chemical inducers: one indicates conspecifics as the source (Bayne, 1969; Crisp, 1965; Keck et al., 1971), the second points to the biofilms on oyster shells (Bonar et al., 1986; Fitt et al., 1990; Weiner et al., 1989) and the third identifies both live oysters and biofilms as producing inducers that act synergistically (Tamburri et al., 1996). While reefs may have been lost, this knowledge is retained since many oyster farmers, fishers and managers consider live and dead oyster valves as the most effective oyster settlement surface (e.g. Hayles (Calshot Several Order chairperson), pers. comm.); for example - 100 spat on one live adult oyster has been recorded from Loch Ryan (Hugh-Jones, 2003). Even in exploited areas oyster spat has been seen to settle and grow on live oysters, providing the start of an 'oyster mini-reef'. Under current fishery practice these naturally-developing aggregations would be physically separated.

By forming aggregations or reefs *O. edulis* will provide a settlement substrate for a variety of epifaunal organisms (Figure 1.1), including its own larvae (Figure 1.2). Korringa (1951) listed over 250 species of epibiota on the shells of *O. edulis* situated in the Oosterschelde and there are records of species associated with *O. edulis* beds from Scottish waters (Millar, 1961). Quantification of this biodiversity (Gristina et al., 1996; Scott 2009) shows that oysters can be considered as a biogenic habitat provider as well as a commercial species. Tyler-Walters (2001) describes how oysters can build up "layer upon layer" in the absence of fishing pressure and refers to a diverse epifauna associated with oyster beds.

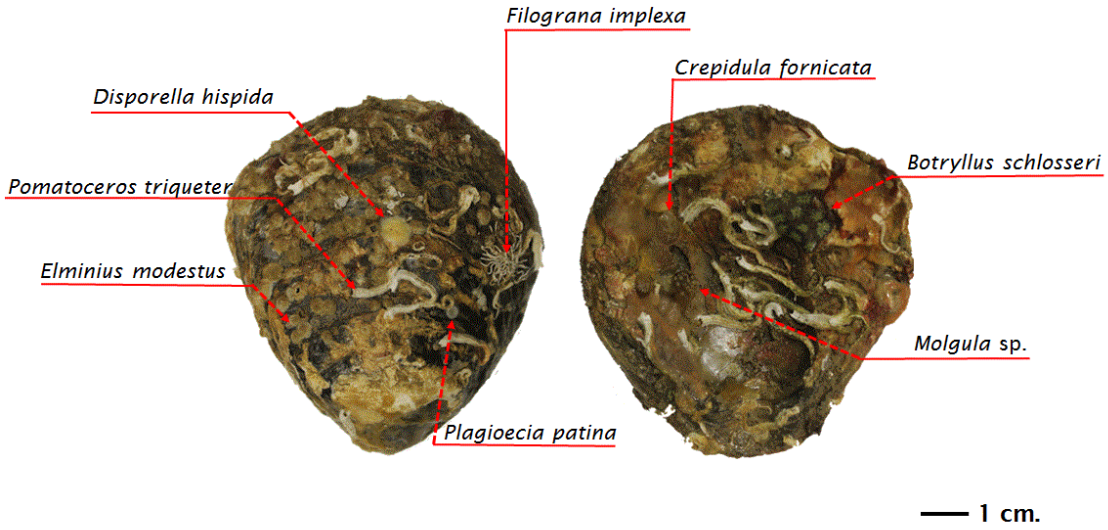

Figure 1.1 Oyster valves support settlement habitats for several epifaunal organisms. Valves collected from experimental elvated reefs in Poole Bay.

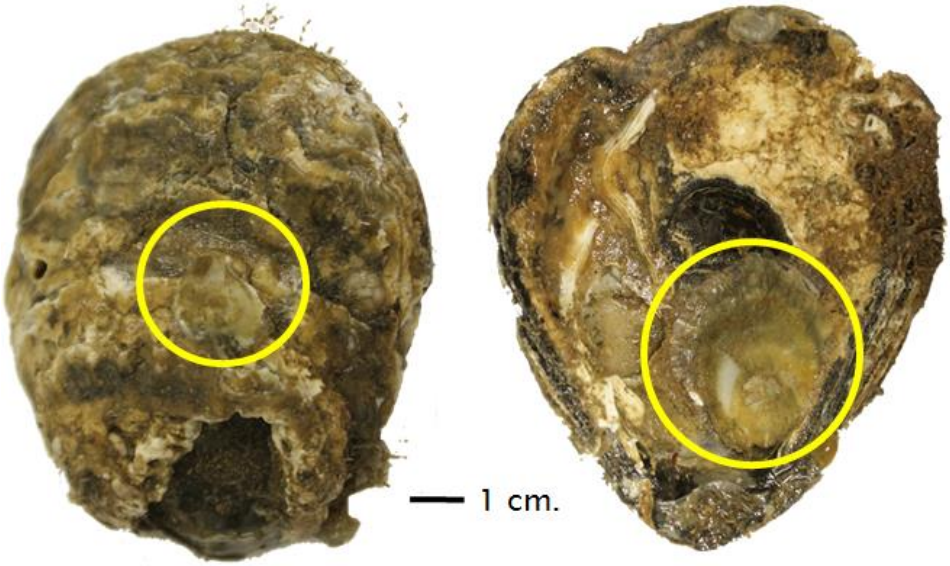

Figure 1.2 Oyster spat (yellow circle) found on dead oyster valves situated on elevated reefs at Poole Bay in April 2013. Centimetre ruler shown for scale.

In summary, it is obvious that restoring *O. edulis* populations would be beneficial to both the species and to the environment. Flat oyster populations have declined significantly in both terms of abundance and distribution. Therefore, to enhance and restore the flat oyster populations, new management approaches need to be identified and implemented. Oyster restoration using elevated oyster reefs, as have been widely used for the eastern oyster *C. virginica* in the USA, is one potential solution by creating subtidal broodstock populations which are raised off the sea bed.

1.3 Biology and ecology of *Ostrea edulis*

To restore *O. edulis* populations, an understanding of the biology and ecology of this species is initially required. *O. edulis*, a bivalve species of family Ostreidae, is found from the low intertidal down to the sublittoral zone throughout the Atlantic and Mediterranean coasts of Europe, where it is recorded in greater abundance, especially in association with highly productive estuarine areas (Airoldi and Beck, 2007; Laing et al., 2005; Lallias et al., 2007; Sobolewska and Beaumont, 2005). *O. edulis* is the native species naturally distributed around the UK and Ireland (Figure 1.3) (Jackson and Wilding, 2007)

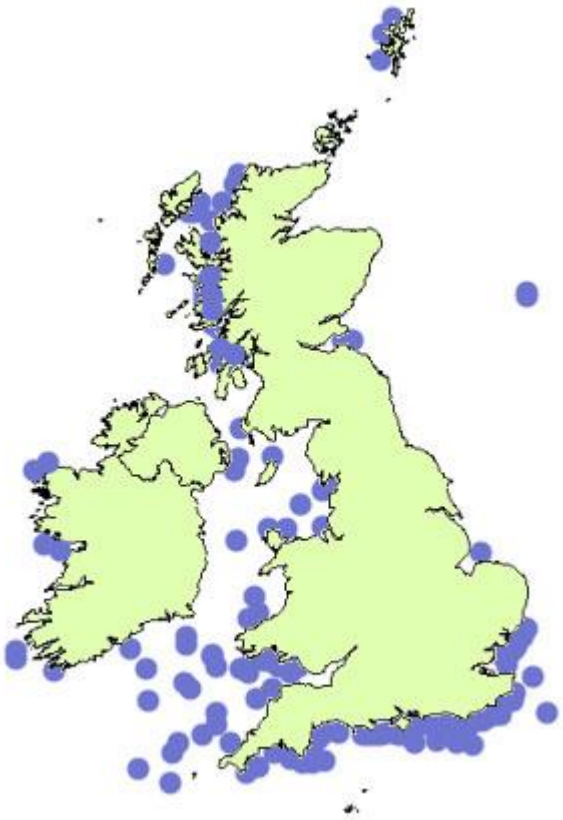

Figure 1.3 The natural distribution of *Ostrea edulis* in United Kingdom and Ireland (Jackson and Wilding, 2007)

O. edulis, a protandrous alternating hermaphrodite species, is generally assumed to reach sexual maturity in the third summer after settlement in the temperate UK waters (Kamphausen et al., 2011; Korringa, 1952). In adults, genders can be classified during the reproductive period from the presence of gametes. *O. edulis* usually first undergo gametosis as a male and, when older, the oyster then alternates between female and male functions (Laing et al., 2005). Generally, mature oysters in British waters reproduce as male or female once a year (Korringa, 1952). Exceptionally, in favourable conditions, *O. edulis* sometimes has been reported to spawn twice in one season (Korringa, 1957). Dodd et al. (1937) also supported the view that *O. edulis* becomes a functional female at maturity following an exceptional summer period because it needs a large quantity of energy to produce ovaries. Recent studies identified that there were biased sex ratios in some main oyster beds in the UK, including in the Solent (Kamphausen et al., 2011) and Chichester Harbour (Eagling, 2012). This could exacerbate the decline of *O. edulis* because of the limited parental

stocks in natural waters and a bias in sex ratio leading to the reduction of fertilization success. Evidence for the factors which drive the alteration and spawning mechanisms after ripening are still unclear (Korringa, 1952; Wilson and Simons, 1985), but for UK populations a minimum temperature of 15–16 ^{0}C is required to attain ripeness (Korringa, 1957). Mature ripe males release clumps of sperm in a steady flow into their exhalant siphon and into the environment. When most spermatozoans are released their testes are simultaneously depleted. Ripe females detect and retain sperm in feeding water taken up with the inhalant current. Then, their eggs are consequently released into the suprabranchial chamber and branchial chamber by a series of contractions before fertilization takes place using the stored sperm. Fertilized eggs and larvae are brooded within the mantle cavity for a further 6 - 15 days (Hedgecock et al., 2007; Newkirk and Haley, 1982), until the larvae have a fully formed shell of about 0.170 mm (Andrews, 1979; Orton, 1927; Walne, 1974) in length, as shown in Figure 1.4. The larvae are then released to the sea.

O. edulis usually spawns in the summer between late June and mid-September (Hedgecock et al., 2007; Kennedy and Roberts, 1999), whilst it is in a resting stage during winter and forming their eggs or sperm in spring season. Within 2-3 weeks after spawning, new oysters attain the larval stage (Laing et al., 2005). Berghahn and Ruth (2005) reported that *O. edulis* larvae have been found to passively drift in the water column up to 10 km or further, during which time phytoplankton provide crucial nutrients for this feeding stage (Laing et al., 2005). After drifting in the plankton for approximately two weeks, the larvae then develop the foot, enabling them to find and permanently settle on preferable substrates for their further metamorphosis and growth (Laing et al., 2005; Sobolewska and Beaumont, 2005). Although *O. edulis* prefer to settle on hard surfaces including hard silt, muddy gravel with shells, sand and rocks (Airoldi and Beck, 2007; Laing et al., 2005), research on the attachment preferences of *O. edulis* with different kinds of substrates, confirmed that greater spat attachment takes place on oyster shells (Korringa, 1946; Laing et al., 2005). The use of bivalve shells, or cultch, has been used as an effective mechanism to increase spat settlement in the wild (Kennedy and Roberts, 1999) Similarly, Hugh-Jones (2003) suggested new recruits preferred to settle on oyster shells, and Korringa (1946) reported that the best substrate for spat was the adult oyster, especially at the growth rings. However, Korringa (1951)

found that muddy substrates were not suitable for *O. edulis* settlement. It has been argued that post larvae prefer attaching to existing oyster valves in response to the production of semiochemicals released from live oysters (Cole and Knight-Johnes, 1949). Therefore, it is possible that using broodstock oysters as attracting mediators would be an optimal strategy for restoring oyster populations. To provide suitable substratum for the larvae to attach and colonize, quantities of oyster shells need to be planted at various locations in the sea bed (Kennedy and Roberts, 1999). To ensure that there are sufficient numbers of oysters recruiting to the adult population, the creation of protected oyster broodstock should be a major focus for any proposed restoration programme (Fariñas-Franco et al., 2013). Broodstocks of target reef-forming bivalves are often added to the reefs (Brumbaugh and Coen, 2009) in order to maximize recruitment of keystone species and accelerate reef diversity (Bartol and Mann, 1997). Recently, Fariñas-Franco et al. (2013) reported that the presence of horse mussels (*Modiolus modiolus)* and their clustering behaviour apparently favoured rapid development of a diverse community, i.e. spat settlement within artificial reefs with translocated adults was significantly higher than the reefs without translocation or natural reefs.

Figure 1.4 *Ostrea edulis* larvae, that have a fully formed shell before they were released to the sea, were observed in this study.

The recruitment pattern of *O. edulis* is sporadic and dependent on a variety of environmental and physiological factors, so that populations pass through natural cycles of expansion and contraction (Anon, 2007). Evidence for successful recruitment events in different populations have been estimated, ranging from yearly in Loch Ryan, Scotland, to intervals of two to three years and only every six to eight years in Lough Foyle (Anon, 2007). The uncertainty in recruitment success of *O. edulis* populations has meant that this species is vulnerable to overexploitation, and a succession of strong recruitment events is essential to ensure that populations recover after depletion, as has been observed widely in recent years. Sparck (1951) reported that the Danish fishery in Limfjord required closure for a period of 20 years to allow the population to recover and Laing et al. (2005) have argued that an interval of 25 years may be required. Many commercially fished species exhibit dispensation, where low population densities result in disproportionally low recruitment (e.g. Gascoigne and Lipcius, 2004) and in Allee effects, which reduce fitness of individuals due

to the decreased mixing of genes at low population densities (Allee et al., 1949). It can be predicted that *O. edulis* populations will suffer in the same manner impacts which must be considered in any attempt to sustainably manage the fishery for the future. It is clear that the decline of adult stocks directly impacts on the production of spat, which may not be successful enough to offset the normal losses associated with a planktonic recruitment phase (Korringa, 1952). Therefore, relaying broodstock in locations that support *O. edulis* biology represents a potential management solution to the twin problems of sustainability and recruitment.

Environmental factors including temperature, salinity, food availability, substrate type, current exposure and oyster density have been shown to influence the various ecotypes and ecomorphic forms of *O. edulis*, whose variability is further increased by the plastic response of shell growth to microhabitats and various epibiotic and symbiotic species (Andrews, 1979). Temperature is broadly accepted as a main factor stimulating oysters to commence their reproductive cycle (Korringa, 1957; Loosanoff, 1962; Mann, 1979; Nelson, 1928; Orton, 1920; Walne, 1974; Wilson and Simons, 1985). Beaumont and Gowland (2002) reported that low initial growth rates recorded in Orkney (Scotland) oysters were attributed to low temperatures rather than genetic variation. Laing et al. (2005) also suggested that in the UK *O. edulis* requires 8–9˚C to start their growth while Korringa (1952) noted that shell deposition would occur so long as the water temperature remained above 10˚C. Similarly, Davis and Calabrese (1969) reported that at 10˚C very little growth was recorded in *O. edulis* spat. *Ostrea edulis* is able to survive in a wide range of salinity (18-40, Laing et al., 2005). Chanley (1958) reported that a reduction of salinity from 35 to 15 over a short period of time (35 days) did not significantly impact the feeding ability of *O. edulis*. However, other studies reported that prolonged low salinities inhibit feeding ability (Korringa, 1952). The oyster takes in food items by pumping water through a filter in the gill chamber to remove suspended organic particles in the water. Cano et al. (1997) suggested that food availability is another parameter that controlled spawning season, resulting in delays or variation in spawning duration from year to year. Algae or organic matter are important nutritional sources (Grant et al., 1990) and growth rates of *O. edulis* are faster in sheltered habitats than exposed areas; however, this is thought to be attributed to the seston volume

rather than flow speed or food availability (Valero, 2006). However, high volumes of inorganic particles and sediment in water column results in clogging and damaging of the feeding structures of oysters, and leads to a reduction of pumping rates and feeding efficiency. This condition can lead to increased organism stress and oyster mortality, especially in extreme cold winters when oysters have low energy reserves on which to rely (Hutchinson and Hawkins, 1992; Loosanoff and Engle, 1947; Moore, 1977; Verma et al., 2013). It is clear that surrounding environmental conditions influence the physiological and reproductive potential of *O. edulis.* Therefore, new restoration techniques should not aim only to increase oyster density but also consider the ways to improve environmental conditions supporting *O. edulis* physiology and reproduction. This would increase the possibility of success in oyster restoration in the long term.

1.4 Current restoration efforts to enhance *Ostrea edulis* production

Historically, European restoration projects aimed at increasing natural stocks and preventing the depletion or extinction of commercially fished *O. edulis* populations have been mainly based on relaying of parental stocks (Korringa, 1952; Yonge, 1966). However, this method has not been successful in solving the decline of *O. edulis* populations. Of concern, this has also resulted in the depletion of the donor stocks, as has happened in the Essex beds and in the Firth of Forth (Key and Davidson, 1981).

In France, restoration efforts were initiated by the formation of 'oyster parks', which are constructed from a large placement of shell cultch including some adult oysters on the sea bed. This approach has proved useful (Yonge, 1966), although it has been argued that high sedimentation on the sea bed may cause decreased spat settlement and survival of new recruits (Anon, 2007). At the same time, it is unclear how many broodstock oysters are required to be placed in a single location to sustain population recruitment. Oyster cultivation and relocation have also been developed in France in order to increase survival rates of new recruits and sustain oyster supply for the fishery (Yonge, 1966). Oyster spat was collected on tiles covered with a brittle sand-lime cement from which the juvenile oysters could be removed easily, and positioned in sheltered

cages until they were adults and then relocated to suitable areas (Yonge, 1966). As direct transmission of *B. ostreae* occurs and uncertainties exist related to its life cycle, legislation (EU Directive 91/67) has been implemented to restrict the movement of oysters within the European Union particularly from areas known to contain the disease (Culloty et al., 1999)

In Norway, 'polls' or 'pools' have been adopted. These polls are used for breeding, spat collecting and growing oysters using natural seawater channelled through narrow inlets (Matthiessen, 2001). In April the cages with breeding oysters or cultch for spat collection are suspended in mid-water, at a depth of between 2-3 metres to avoid very turbid conditions on the sea bed (Anon, 2007). Oysters use the polls not only for a shelter but also use them as spawning and nursery habitats. Despite the high latitude along the Norwegian coast, in summer the water column is sufficiently warm to permit oysters to reproduce (Matthiessen, 2001). Between June and July, the larvae are collected on bunches of twigs that hang at the same depth. These spat are then relocated to grow on suitable habitats. Yonge (1966) argued that the translocation of half-grown oysters in areas with fewer disease problems should be considered but Berthe et al. (2004) has reported that the export of small oyster seed into areas with pre-existing disease problems is not sustainable.

In Ireland, the collection of spat on cultch for seeding on other suitable areas, called bed rotation, has been attempted in some areas e.g. in Tralee Bay (in 1980s) (OSPAR, 2009). Similarly, work has been carried out in Clew Bay, Cork Harbour, Lough Swilly and Lough Foyle. For example, in 1991, 250 bags of spatted native oyster cultch (*O. edulis*) from Tralee Bay were transplanted to Lough Swilly and ongrown on trestles for over a year. There were approximately 1,000 native oyster spat per bag of cultch. These oysters were subsequently seeded onto the sea bed in 1993 and the larvae were reared to approximately 40 mm. These projects were quite successful as they did increase the catch rates in the following years (OSPAR, 2009). However, the limited spat supply and the challenge of relocating oysters whilst minimising the spread of incurable diseases remain significant issues. Re-laying broodstock has previously been undertaken in Northern Ireland as well (Kennedy and Roberts, 2001). For example, in 1997, the EU funded a project aimed at re-establishing oyster beds in Strangford Lough, Northern Ireland

(Kennedy and Roberts, 2001). This project involved the participation of the local fishing community (Laing et al., 2005). The method included the laying of cultch, seeds and adult oysters at nine selected sites (Kennedy and Roberts, 1999; Laing et al., 2005). At the same time, a baseline survey established the status of *O. edulis* stock, in addition to monitoring the production of larvae and spatfall (Kennedy and Roberts, 2001; Laing et al., 2005). This restoration was estimated to increase oyster population from 100,000 individuals in 1998 to 1.2 million individuals in 2003 (Laing et al., 2005). However, the abundance of oysters was not sustained as the influence of unregulated harvesting and *B. ostreae* in this area lead to the population decline to 650,000 individuals by 2005 (Smyth et al., 2009).

In South Wales, the relaying of broodstock oysters has recently been applied (Gravestock et al., 2014; Woolmer et al., 2011). In October, 2013, between 40,000 and 50,000 individual adult oysters obtained from the Loch Ryan wild fishery, were planted by the Mumbles Oyster Company off Mumbles Pier in Swansea Bay at densities of 10 individuals/m^{-2} in a total area of two hectares, where no fishing occurred (Gravestock et al., 2014). The site was selected on the basis of evidence of historic oyster beds, the presence of existing oysters and substrates for larval settlement (Gravestock et al., 2014). In spring of 2014, approximately 4 to 5 tonnes of cultch materials, in the form of cockle shells, were additionally laid onto the sea bed close to the previous restoration site to provide substrates for new recruits in the following summer. Currently, the bed is monitored on a monthly basis to quantify oyster mortality and the appearance of predators. The growth rate of re-laid oysters is also calculated. The project has future plans to conduct spat settlement surveys, which will allow quantification of new recruits from this project. Future restoration plans will entail the relaying of approximately 10,000 juvenile oysters (5 to 15mm) directly onto the sea bed (Woolmer pers. comm.). The selected area, Mumbles Pier in Swansea Bay, is currently a *Bonamia*-free zone and no instances of bonamiasis have, to date, been reported. This restoration programme is at an early stage and the long term success of this programme remains to be determined. Previous experience suggests that adult oysters directly planted on the sea bed frequently die after they have spawned as there is insufficient energy resources for them to survive through the following winter (Kamphausen et al., 2011). Therefore, the detailed benefits of this current

relaying project will not be fully identified until at least one spawning season has taken place.

In England, the feasibility of *O. edulis* restoration has previously been conducted in some areas, including The Solent (Key and Davidson, 1981) and more recently in Chichester Harbour (Vause, 2010; Eagling, 2012). Similar to other efforts, these restoration projects piloted in England were also mainly based on laying of parental stocks (Korringa, 1952; Yonge, 1966). However, although restoration *O. edulis* using re-laying adult oysters has been previously attempted, the stock of *O. edulis* in England has continued to decline. Nevertheless, as no other successful strategy to restore this species has been approved, the relaying of adult broodstock is still a recommended practice today (Kennedy and Roberts, 1999; Laing et al., 2005).

In the Solent, several attempts for flat oyster restoration have been carried out in the northern part of Stanswood Bay in order to increase the larval supply to surrounding areas using re-laying of cultch and deposition of broodstock oysters on the sea bed (Woolmer et al., 2011). For example, in 1973 the Stanswood Bay Fishermen's Cooperative planted 375 tonnes of multiple types of mollusc shells (e.g. cockle, mussel and oyster shells) on the sea bed (Key and Davidson, 1981). The idea of this effort was to increase chances of larvae settlement by providing substrate surfaces (Key and Davidson, 1981). It was estimated that the minimum benefit of this project to oyster fishery was 10.5 tonnes of saleable oysters (Key and Davidson, 1981). As this first attempt represented at least some success, a second restoration programme using the same approach was conducted again in the same location in 1999 (Laing et al., 2005). The cultch, consisting of scallop and whelk shells, was planted in the area. Moreover, in 2003 and 2004, a limited quantity of seed oysters were added in fine sacks and were additionally placed in this area. The growth rates were assessed, showing that the seed oysters grew from 5 mm to almost 7cm in 6 months (Laing et al., 2005). Unfortunately no literature exists on the success of the latter attempt.

In 2010 the Chichester Harbour Oyster Partnership Initiative (CHOPI) was established through the cooperation between Sussex and Southern IFCA, Chichester Harbour Conservancy, Natural England and the local fishering community (Vause, 2010). The purpose of this Partnership was to survey and

manage the decline of *O.edulis* stock in Chichester Harbour with academic support from the Centre for Environment Fisheries and Aquaculture Sciences (CEFAS) in Weymouth and the National Oceanography Centre (NOCS), University of Southampton (Eagling, 2012). This group identified specific areas that were closed to fishing under a voluntary fishers's agreement and relayed 2,298 kg of broodstock oysters caught by local fishermen directly on the sea bed at a density of 40 m^{-2} (Eagling, 2012). This deployment aimed to create high density areas of breeding stock in order to increase the chance of fertilisation success (Eagling, 2012). In 2011, the reproductive status of these relocated oysters was assessed by a study at the University of Southampton (Eagling, 2012). No disruption to the gametogenic cycle and brooding larvae were found, indicating successful reproduction up until the spawning of larvae (Eagling, 2012). However, the sex ratio (male: female) of the broodstock was 3:1, differing significantly to what was naturally expected (1:1). Two years after re-laying, an increased mortality of the relayed oysters was also reported, especially after the spawning season (Jensen pers. comn). Comparisons of grab samples recovered in July (pre-spawning) and November (post-spawning) identified that one third of the sampled population had died post spawning (Jensen pers. comm.).

It is clear that the relaying of broodstock oysters can achieve partial success in increasing the density of the resident parental stocks, thereby increasing chance of fertilization success. However, from previous experiences, this method alone is insufficient to solve the problem of the decline of oyster stocks and sustain long-term *O. edulis* production. It has been confirmed that planting adult oysters directly on the sea bed in some areas (e.g. Chichester harbour) results in mortality especially after spawning. It can be hypothesised that the environmental conditions at the sea bed might negatively affect oyster physiology, reducing growth and leading to increased mortality. From these previous studies it is clear that, despite several attempts to restore *O. edulis* populations across Europe the species remains in a precarious state (Kamphausen et al., 2011). Therefore, new approaches to improve *O. edulis* stock in Europe are essentially warranted.

1.5 Knowledge gaps for oyster restoration

Improvement in the physiological performance of target species and indirect benefits to enhance biodiversity have been proposed as a performance measure of restoration effectiveness (Volety and Tolley, 2010). However, no previous reports have provided fundamental understanding of changes in oyster physiology, especially *O. edulis*, when reared on elevated artificial reef habitats. Whilst there is evidence to support the use of elevated broodstock reefs for enhancing oyster recruitment and ecosystem biodiversity with *C. virginica* in USA, there is no pilot study to investigate the benefits of this strategy for oyster physiology and local biodiversity in the UK. Anecdotal evidence of British oyster 'reefs / clusters' suggests that small structures comprised of fused oyster valves have been seen by scientific divers in Poole Bay (Burton, pers.com.). Publications describing *Ostrea* valve epibiota are limited and are not up to date (e.g. Korringa, 1951 (Holland); Gristina et al., 1996 (Italy)) although there are descriptions of the overall *Ostrea* bed communities (Mistakidis, 1951(Essex); Millar, 1961 (Scotland)). Marine communities settled on (and within) habitat created by *O. edulis* valves do not appear to have been fully assessed in the published literature within the context of UK marine biodiversity. As discussed in the previous section, the current restoration efforts re-laying adult oysters and shell cultch directly on the sea bed are insufficient to solve the precarious status of *O. edulis*. Therefore, to enhance and restore the flat oyster populations, new management approaches need to be identified and implemented. It can be hypothesized that *O. edulis* restoration using elevated broodstock reefs; such as has been as have been widely used for the eastern oyster *C. virginica* in the USA; will:

a) enhance the physiological performance and reproductive potential of *O. edulis* through the improvement of water conditions at the height of elevated reefs and reduce competition for space and resources with other benthic species (e.g. *Crepidula fornicata*)

b) improve spat recruitment to the oysters within the elevated broodstock population

c) enhance habitat biodiversity by creating niche space and surfaces for colonisation by other epibiota and mobile species.

1.6 Aims and objectives

The aim of this project is to undertake an assessment of the potential physiological and ecological benefits which may arise from siting an aggregation of oysters in an elevated structure on the sea bed. The results of this assessment will contribute to the identification of a successful management strategy to recover native oyster habitats in support of the UK Biodiversity Action Plan for *O. edulis*.

The specific objectives of this project were to:

1) establish the physiological performance of the European flat oyster (*O. edulis*) held off the sea bed on elevated reef structures within the subtidal zone. Comparisons were made to single oysters held on the sea bed. Respiration rates, filtration rates, condition index and whole haemolymph proteins were established as the indicators of physiological health. In addition, to quantify immune function of European flat oyster (*O. edulis*) on the elevated reefs and sea bed, haemolymph cell counts and occurrence of the parasite (*B. ostreae*) were compared among reef oysters, sea bed oysters (Poole Bay) and natural Poole Harbour oysters by using a nested PCR assay.

2) investigate the geographical effects on filtration and respiration rates of the European flat oyster (*O. edulis*). Oysters were collected from three locations (Poole harbour, The Solent and Chichester harbour) and assessments made of filtration and respiration rates in the laboratory.

3) assess the reproductive status of the European flat oyster (*O. edulis*) on the elevated reefs and sea bed by using gonadal maturity, gonadal index and vitellogenin-like protein concentration.

4) quantify the benefit of raised broodstock populations in terms of ecosystem biodiversity enhancement. In part, this was assessed by quantifying the colonization of live oysters and oyster valve cultch on elevated reef habitat by epibiota. The abundance, diversity and species richness per valve was measured. Comparison was made to live oysters and oyster valve cultch re-laid

on the sea bed. The number of oyster spat settled on oyster valves situated at the height of elevated reefs and sea bed were also quantified in order to access local recruitment success. This study also illustrates and compares the structure of epifaunal communities on *O. edulis* valves between the valves situated on elevated reefs and the valves re-laid on the sea bed.

5) investigate the response of mobile faunal community structure to elevated oyster valve reef habitat. Temporal variation of mobile fauna in reef boxes and sea bed cages were separately presented.

1.7 Thesis framework

To achieve the main goals of this thesis, twenty-four elevated oyster reef modules were built and deployed in Poole Bay at the University of Southampton artificial reef experimental site run by Ocean and Earth Science (OES). At every sampling interval, water samples at the height of elevated reefs and adjacent to the sea bed were collected and analysed for total suspended solids, bacterial abundance and chlorophyll *a* concentration. The information about how elevated reef modules were constructed and environmental data are presented in Chapter 2. The hypothesis that elevated reef structures improve oyster physiology (i.e. filtration and respiration rates, haemolymph proteins, haemocyte counts and condition index) though the improvement of environmental conditions were tested and the results were presented in Chapter 3. Large scale spatial variation, i.e. between populations, in physiological performance was considered. Three surveys by collecting oyster samples from Poole Harbour, Chichester Harbour and the Solent were undertaken between May to August 2014 to test how physiological indicators (respiration and filtration rates and condition index) varied with location and sampling months (Chapter 4). As the reproductive potential is related to the success of the fishery enhancement programme, the effects of elevated reef habitats on the variation in gonadal development and vitellogenin-like protein concentration between reef and sea bed oysters are examined and discussed in Chapter 5. The installation of elevated oyster reefs is not only beneficial for oysters but also possibly enhances ecosystem services through the increase of local biodiversity. The impact of elevated reef construction on local biodiversity

was investigated. The effect of elevated reef structures created by *O. edulis* valves on epifaunal colonisation on valve cultch and live oyster valves was observed and is presented in Chapter 6. The utilisation of elevated reef habitat by mobile fauna and nekton was also investigated and is discussed in Chapter 7. Finally, the results obtained from this Research, the limitations of this study and recommendations for future research are discussed in Chapter 8. An overview of the thesis framework and a link between Chapters is shown in Figure 1.5.

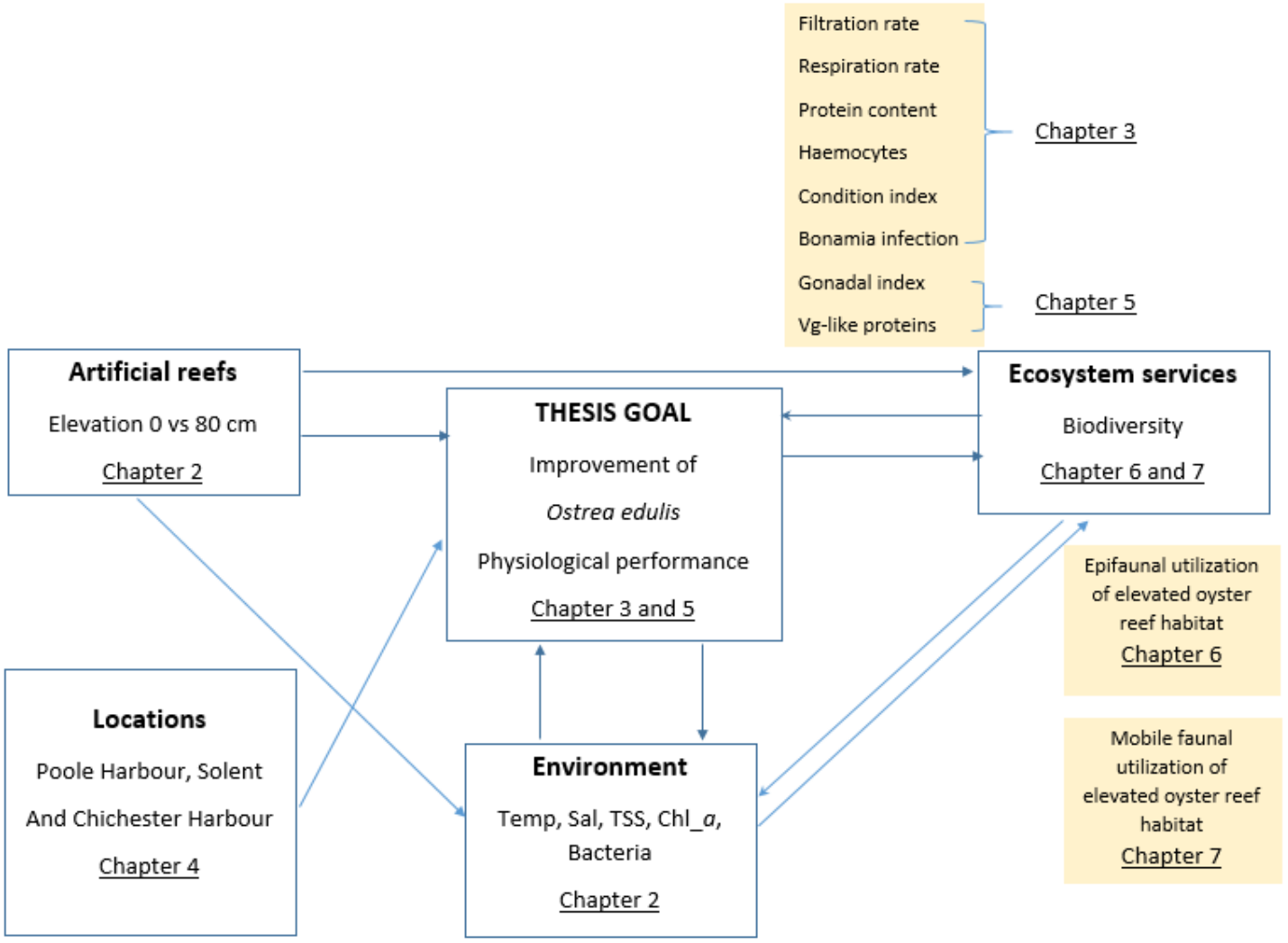

Figure 1.5 An overview of the thesis framework and a link between Chapters

Chapter 2

Elevated reef construction, sampling programmes and environmental conditions in Poole Bay

2.1 Introduction

Artificial reef structures, whether created from man-made or natural materials, have a recognised potential to protect, enhance or restore components of ecosystems and the productivity of commercial species (Bohnsack et al., 1991; Collins and Mallinson, 1984; Collins et al., 1991). The origins of artificial reef technology are traced to places as diverse as Japan and Greece. Modern deployment of artificial reefs has the longest history and has been most widespread in Eastern Asia, Australia, Southern Europe, and North America (Lindberg and Seaman, 2011). As discussed in the previous chapter, eighty-five percent of natural oyster reefs or oyster beds have been damaged (Beck, 2011). This led to the decline of oyster and relevant fishery production which are both important protein sources (Orton, 1937). To solve this issue, artificial oyster reefs have been regarded as potentially useful tools for recovering oyster population and ecosystem services (Beck, 2011). Several oyster restoration efforts have been implemented especially in the USA (Coen and Luckenbach, 2000). In theory, ecological structure and function, vertical relief, and irregular surfaces of artificial reefs vary according to biology of target species, location, construction, and degree to which they mimic natural habitats (Lindberg and Seaman, 2011). From literature reviews, there is no previous suggestion of how to construct artificial reefs for restoring *Ostrea edulis* populations, especially in the context of the UK. Therefore, this chapter reviews the artificial oyster reefs designed for previous bivalve restoration practice, especially for *Crassostrea virginica* in the USA. Those relevant methods from previous experience with other bivalves are adapted and applied to elevated reef construction, in this study, in order to pilot the influence of

elevated reef habitat on *O. edulis* physiology and investigate the improvement of local biodiversity associated with the presence of the artificial reef habitat.

The features of artificial reefs depend on the specific objectives of reef construction. In Chesapeake Bay several reef projects have been implemented responding to severe habitat loss from fishing activities (Victor and Linda, 1983) and, more recently, exposure to disease (i.e. the protozoan parasites *Minchinia nelsoni* and *Perkinsus marinus*) (Brown et al., 2005). Mostly, the primary goals of restoration projects in Chesapeake Bay were to restore the oyster (*C. virginica*) stocks, as well as the environment by filtering out nitrogen and other pollutants from the water (Brumbaugh et al., 2000). To achieve these objectives, the method that has been used predominantly has been placing high-relief cultch in the sub-tidal zone (Schulte et al., 2009). This method was also used in other areas in the USA, e.g. the Carolinas, Georgia, and Florida with the same objectives (Baggett et al., 2014). However, other kinds of artificial reefs were specifically designed for enhancing shoreline protection. These include Reef Balls and OysterBreak (Risinger, 2012; Schulte et al., 2009). Both are created from concrete designed to reduce current speeds. Additionally, Reef Balls are hollow concrete mounds with several holes that provide attachment points for oyster recruitment (Risinger, 2012; Schulte et al., 2009). OysterBreak is a system of large stackable concrete rings which are capable of being stacked to various heights. Like Reef Balls, OysterBreak is designed for both estuaries and deeper waters with higher wave energy (Risinger, 2012). In this study, artificial reefs were constructed with expectations to increase *O. edulis* production and local biodiversity. The application of using high-relief cultch to restore an oyster population is likely most appropriate as this method has been widely used for restoring *C. virginica* in the USA. Moreover, previous practices suggested that elevated reef structures enhanced recruitment and survival presumably by providing oysters with higher flow velocity and consistently higher levels of dissolved oxygen (Knights and Walters, 2010; Lenihan, 1999). Increased water flow supplies a higher quality of suspended food materials, and increases resistance to disease and parasites, thereby resulting in higher recruitment and growth (Lenihan, 1999; Lenihan et al., 1999).

Jordan-Cooley et al. (2011) argued that reef structures should be designed based on consideration of the biology of the target species, reef height and the

level of sedimentation. Similarly, Lenihan et al. (1999) suggested that reef structures should be designed to account for hydrographic conditions such as current velocities, sedimentation rates, temperature and stratification, food availability and oxygen levels. Schulte et al. (2009) stated that initial reef height is a key feature of reef architecture, which determines the eventual persistence of constructed oyster reefs, and also suggested that an elevation of approximately 30 cm off the sea bed is initially required. A high-relief substrate that mimics a natural oyster reef is essential for maximizing oyster (*C. virginica*) growth and survival rates (Schulte et al., 2009). Reef elevation must be high enough to keep the oysters above the sediment and increase chance of spat settlement (*C. virginica*) (Baggett et al., 2014; Schulte et al., 2009). Based on the information above, it can be concluded that elevated reef structures are recommended, rather than re-laying substrates directly on the sea bed without elevation. However, although elevated reef structures have been reported to enhance fishery production and local biodiversity, the information related to reef features (especially reef height) and creational success are limited. Furlong (2007) reviewed the artificial oyster reef projects in the Northern Gulf of Mexico and found that only 9 percent of 422 reef projects established the details of reef features created. The lack of documentation and dissemination of even the most basic facts of reef structure has restricted the continued development of optimal reef designs for restoration (Furlong, 2007).

Jordan-Cooley et al. (2011) assessed the success of oyster reef restoration in the Great Wicomico River, a western shore tributary of lower Chesapeake Bay, USA, where different reef heights were constructed by the Army Corps of Engineers. The field experiment featured high elevated reefs built at an average of 25–42cm in height while low elevated reefs were constructed at 8–12 cm. The result of the project showed that mean oyster density was four times higher on the high elevated reefs, about 1000 oysters per m^2, compared with those on the low elevated reefs. Schulte et al. (2009) explained that it is possibly a consequence of heavy sedimentation on the low elevated reefs, thereby resulting in reduced abundance on low elevated reefs. van Rijn (1986) also demonstrated that oysters encounter a greater volume of sediment in the water column when placed closer to the sea bed. Thomsen and McGlathery (2006) investigated the effect of artificial oyster reefs in Hog Island Bay,

Virginia, USA, at 0.5–1 m height above the sea bed and observed that they provided suitable substrates for marine organisms leading to an increase in ecosystem biodiversity. Lenihan et al. (1999) suggested that artificial reefs should be 1-2 m height in order to minimize exposure to anoxic/hypoxic conditions, enhancing exposure to faster water flow, greater food availability and reducing disease incidence and intensity. Whilst Zalmon et al. (2014) extended the suggestion that both reef height and length have to be considered. They investigated the impact of artificial reefs on the infaunal community using 0.5 m high artificial reefs with variation of reef length located on the continental shelf, north of Rio de Janeiro State, Brazil. The results showed that at the same reef height (0.5 m), the shortest length artificial reef (0.5 m length) had lower predator species than the longer reefs (10 and 15 m length). It is clear from the above examples that possibly 0.5-1 meters elevation is initially required to investigate the effects of elevation on oyster physiology and local biodiversity.

The most appropriate materials to use to form artificial oyster reefs remains a debate (Coen and Luckenbach, 2000). Previously, manipulative experiments suggested that the success of oyster (*C. virginica*) enhancement might be correlated with substrate type, including shell, concrete, rock and coal ash (Coen and Luckenbach, 2000; Lipcius and Burke, 2006). Greene and Grizzle (2005) suggested that concrete or rock is a better choice for *C. virginica* larvae settlement in comparison to dead shells. Similarly, Lipcius and Burke (2006) reported that an artificial concrete reef would be heavily colonized by a higher density of the Eastern oyster (*C. virginica*) and mussel (*Ischadium recurvum*) due to greater vertical complexity, reef stability and surface area compared with artificial reefs constructed using dead shells. In contrast, several studies argued that, compared with other materials, a higher density of oysters often recruited to and survived on oyster shell reefs (Bergquist et al., 2006; Brumbaugh et al., 2006; Coen and Luckenbach, 2000). Brumbaugh et al. (2006) revealed that dead oyster shells as reef material can also serve as a refuge that protects newly settled *C. virginica* from predators. Katayama et al. (2004) developed an artificial reef called ‘shell nursery’ made from dead shells. The results from their research demonstrated that, compared with concrete test pieces, shell nurseries had a higher density of seaweed, polychaetes, amphipods and decapods, all of which represented appropriate prey items for

small fish. In addition, the density of fish around the reef area was observed to increase, suggesting that the artificial reefs constructed of dead shells were effective in enhancing biodiversity and abundance. This study also supported several practices of using dead shells as the basis of new oyster reefs (Luckenbach et al., 2005; Meyer and Townsend, 2000). From previous studies discussed above, it is likely that both the advantage of concrete in terms of offering reef stability and the advantage of oyster shells in terms of creating reef complexity and attracting oyster settlements are necessary to be considered to make artificial reefs of sufficient stability and complexity.

Although evidence suggests that *O. edulis* can be found on a variety of hard substrates including hard silt, muddy gravel with shells, sand and rocks (Airoldi and Beck, 2007; Laing et al., 2005), previous studies of ostreids noted that *O. edulis* larvae and other *Ostrea* species prefer to settle on their live shells rather than on other substrates (Kennedy and Roberts, 1999; Korringa, 1946; Korringa, 1951). Additionally, the organic acids and other organic chemicals from adults encourages veliger-stage larval oysters to settle on their parental stocks (Brumbaugh et al., 2006; Pascual and Zampatti, 1995). Therefore, it can be concluded that the use of live oysters within an elevated reef represents an attractive mediator to induce future spat settlement.

As *O. edulis* is a filter feeding species, the hydrodynamic environment is a significant aspect of reef design. Similarly to other bivalves, high suspended sediment load in the water column is an unfavourable condition for the physiology of this species. The outcomes from previous restoration techniques for *C. virginica* in the USA suggest that using concrete to construct artificial reefs would improve stability while using a mixture of dead shells and live oysters would prove attractive for larval settlement. In this pilot study, concrete blocks were used as the base of artificial reefs, with the aim of increasing the stability of reef structures and also to lift oyster cultch and live oysters over high level sedimentation near the sea bed.

2.2 Methods

2.2.1 Elevated reef construction

Dead *O. edulis* valves were collected using an oyster dredge on the RV *Bill Conway*, (OES research vessel), from Ryde Middle, the Solent, Southampton (50˚46′ N and 1˚14′ W) (Figure 2.1A). All valves were washed with fresh water, scrubbed of epibiota and dried with natural sunlight. This 'oyster valve cultch', was used as the filling for artificial reef construction. 'Oyster reef boxes', a 0.064 m^3 cube of cleaned oyster valve cultch, (containing about 1,200 valves) was encased within a Netlon™ mesh (Figure 2.1B), and 'oyster cages' (0.032 m^3)) used to hold live oysters on the sea bed were prepared. To mimic naturally available habitat, approximately ten oyster valves were placed in each sea bed oyster cage as a representative amount of cultch naturally available on the sea bed (Figure 2.1C). Twenty-four reef boxes and twenty-four oyster cages were built and transported to Poole Bay using IFCA research vessel FPV *Tenacity* (Figure 2.1D).

The oyster reef boxes were further raised above the sediment to prevent the shell surfaces from becoming sedimented. They were positioned 40 cm above the sea bed using double layers of breeze block. Consequently, each individual raised 'reef module' comprised a breeze block base (40 cm high) with the reef box (40 cm high) attached to the top of the base. The total height of the base plus reef box height was 80 cm from the sea bed. Reef boxes were held in place on top of the breeze block mount using 150 cm steel pins (Figure 2.2A) and oyster cages were located close to each of the reef modules (Figure 2.2B-C).

Figure 2.1 Oyster valve cultch (approximately 30,000 valves) as reef materials were collected from Ryde Middle, the Solent, Southampton (A). The reef boxes were ¾ filled with oyster valve cultch – comprising approximately 1,200 valves in each box (B). Oyster cages used to hold live oysters were filled with 10 oyster valve cultch (C). Twenty-four reefs boxes and oyster cages were transported to Poole Bay using IFCA research vessel (D).

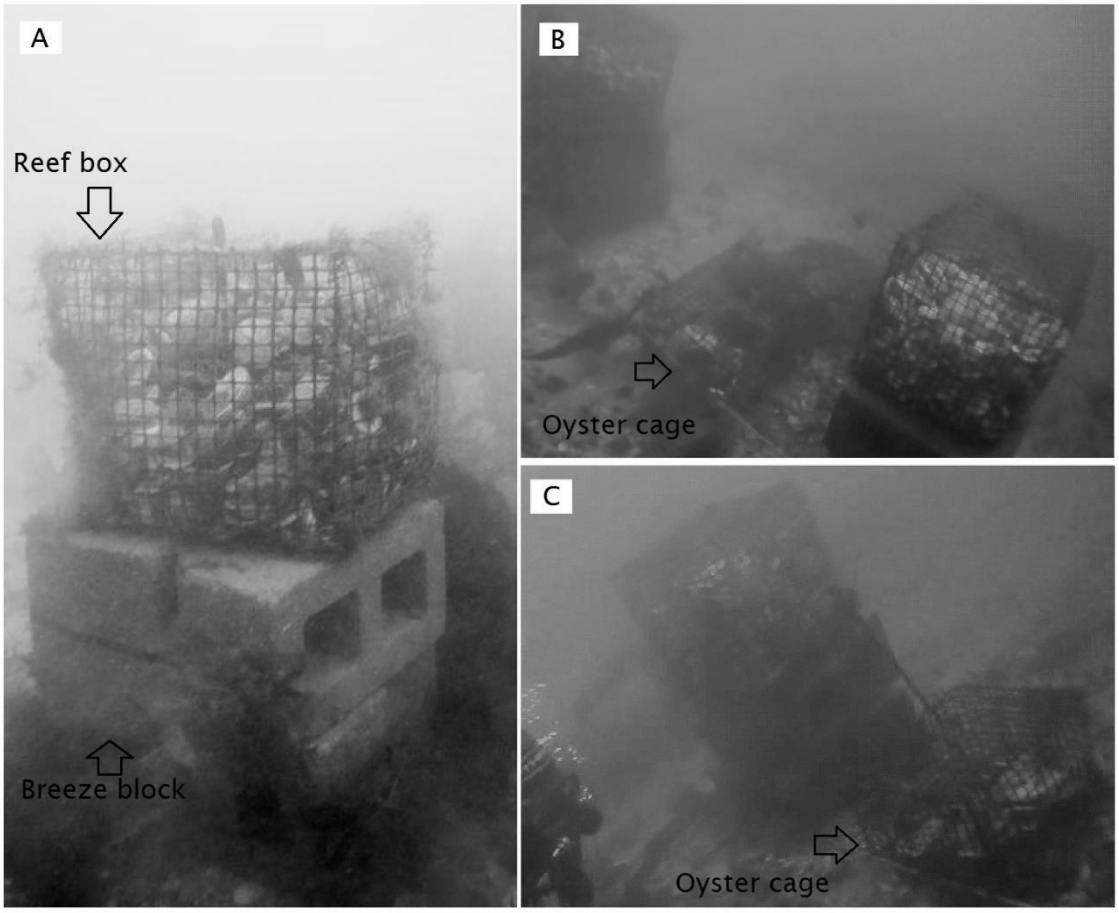

Figure 2.2 Images of the deployed reef modules and oyster cages. Each reef module comprised a 40 cm breeze block base plus a 40 cm high reef box (A), and oyster cages were located on the sea bed close to each of reef module (B-C).

In August 2012, twenty-four elevated reef modules were placed in the footprint of the artificial reef in Poole Bay, Dorset (50º 39·72´ N, 1º 54·79´ W; Figure 2.3A).The water depth in the experimental area is 10 metres below MLWS (tidal range 2 m) (Jensen et al., 2000). All elevated reef modules were placed at least 2m apart (Figure 2.3B). Twenty live oysters (Othniel Oysters Ltd., Poole), 57-99 mm in shell height, were then added as a top layer to each reef box, called 'reef oysters' and each oyster cage, called 'seabed oysters', (Figure 2.3C) by divers after they had been deployed.

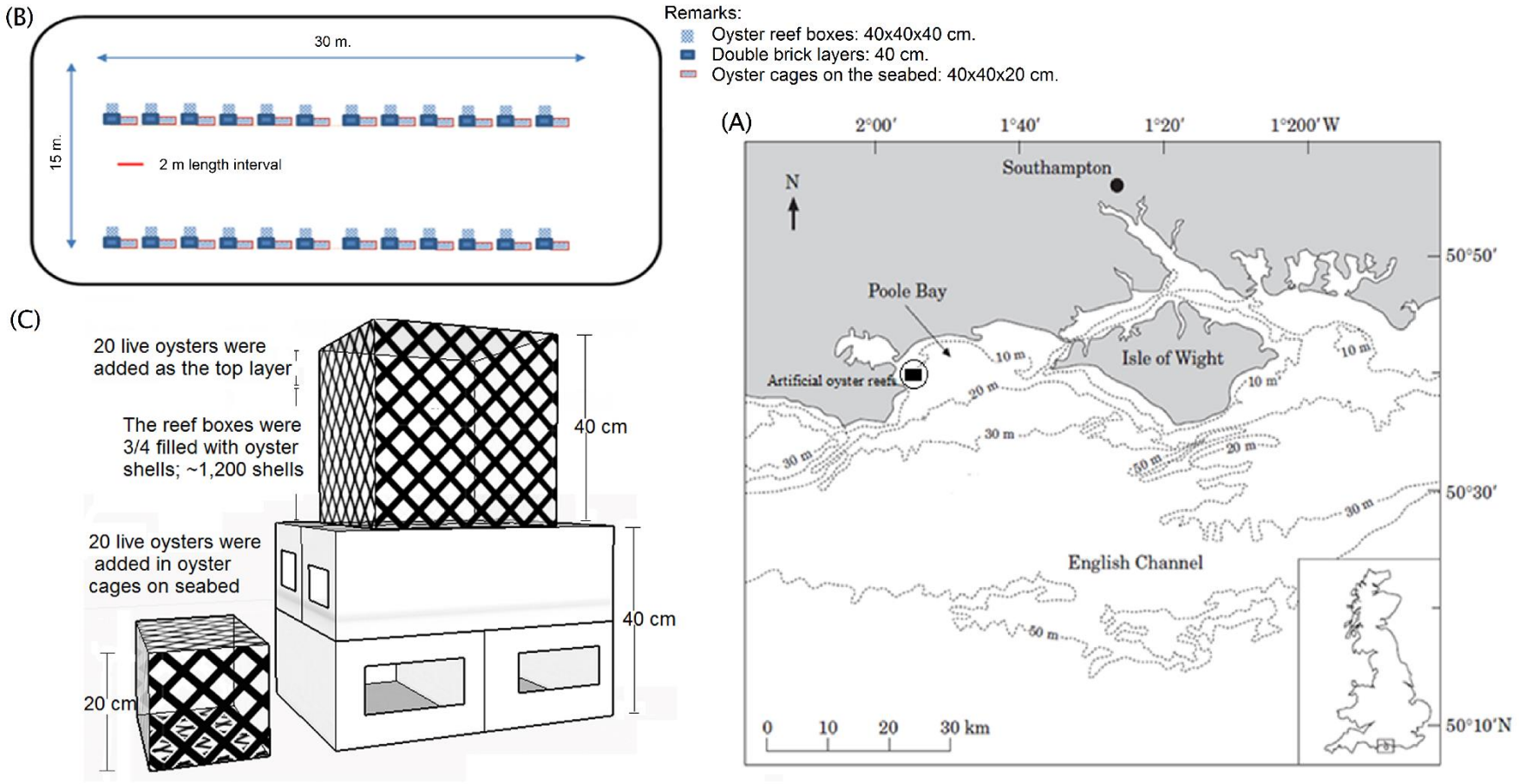

Figure 2.3 Study area and artificial oyster reef design: (A); location of Poole Bay on the south coast of the UK, (B); Schematic of the general arrangement of the 24 replicate reef modules, (C); An artificial reef module; breeze block height plus reef box height plus reef box height is 80 cm from the sea bed.

2.2.2 Sampling programmes

At each sampling interval three replicate reef boxes were recovered from the elevated reef modules as well as three replicate oyster cages from the sea bed using lifting bags operated by scuba divers. Prior to recovery, each reef box sample was wrapped in a fine mesh net to prevent the escape of animals from inside the reef box. Then, each box was covered by a large mesh net for the purpose of lifting it on board (Figure 2.4). Epibiotic colonization and oyster physiology of these deployed units were investigated four times (September 2012, April 2013, August 2013 and November 2013) during a 15 month field experiment. After recovery of the reef boxes and oyster cages by divers, five oysters from each box were removed for physiological and reproductive assessment (n = 15 from the reef boxes and the oyster cages per sampling interval). Live oysters from reef boxes and sea bed cages were kept in a cool box to prevent them from drying out and immediately returned to the NOCS aquarium in sea water taken from Poole Bay at the time of collection. After two days of acclimation, the live oyster samples from both positions (reef and sea bed) were assessed in terms of physiological performance as presented in the schematic diagram (Figure 2.5). Reef boxes and oyster cages were also transported to NOCS wrapped in fine mesh net and maintained for a week in the recirculating aquarium system. This facilitated live identification of epifaunal species.

Figure 2.4 Each reef box was wrapped in a fine mesh net (white net) to prevent the escape of mobile fauna and was secured in a second mesh net (green cargo net) for the purpose of lifting it on board.

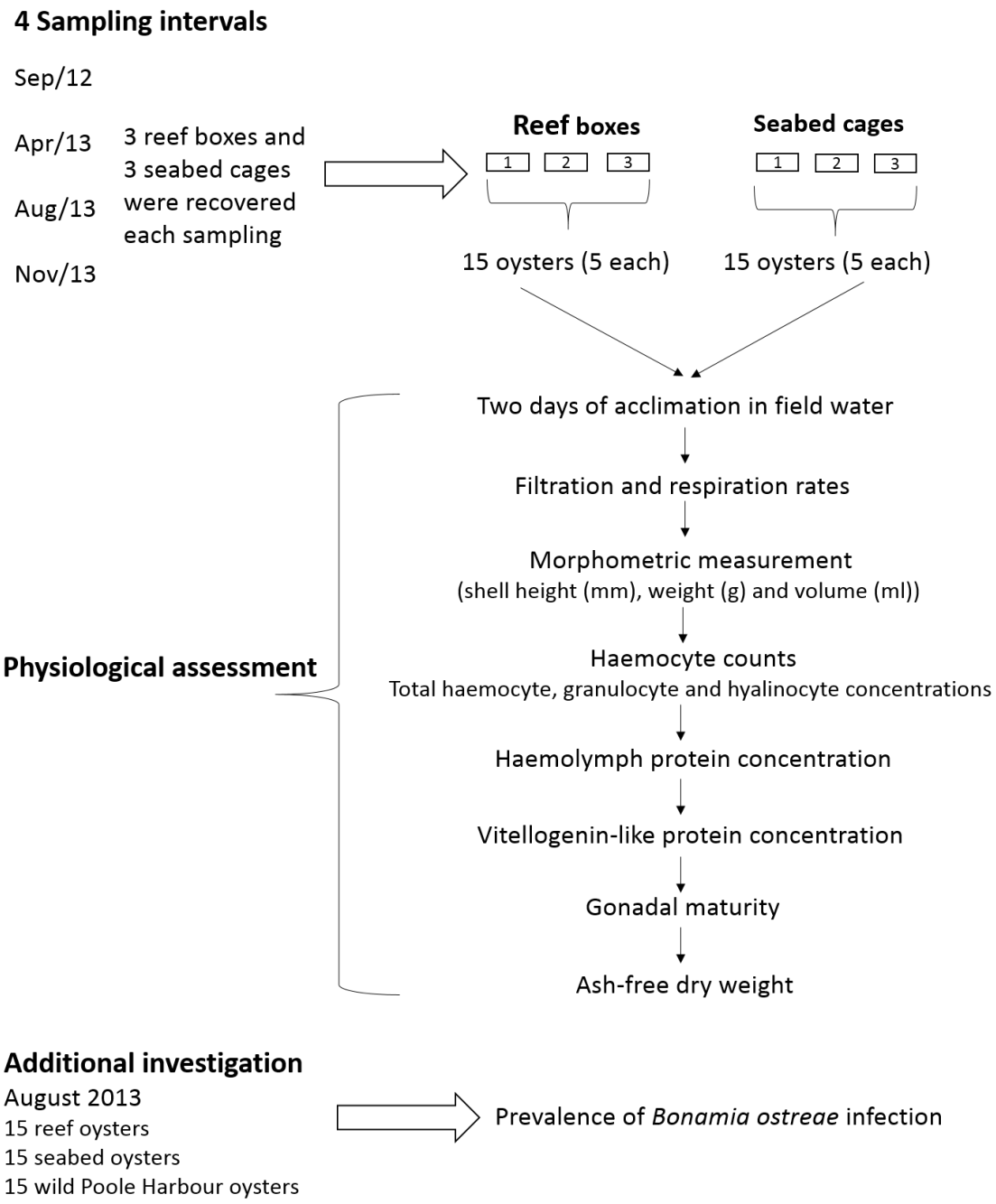

Figure 2.5 A schematic diagram presents the sequence of sampling intervals and the steps of physiological assessments of oysters collected from the elevated reefs (n=15) and the sea bed (n=15) in each sampling interval.

2.2.3 The measurement of environmental conditions

2.2.3.1 Temperature, salinity and water collections

At each sampling interval *in-situ* salinity and water temperature at the height of the elevated oyster reefs and at the sea bed were measured using a YSI probe (EXO2, Xylem Inc., USA). On each sampling occasion four replicate 1,000 ml samples of sea water were also collected by divers at a height of 80 cm above the sea bed and at the surface of the sea bed. These sea water samples were analysed for chlorophyll *a* concentration, total suspended solids and total bacterial abundance (sections 2.2.3.2 to 2.2.3.4).

2.2.3.2 Chlorophyll *a* concentration

On return to the laboratory, 100 ml of each water sample were immediately filtered onto individual 25mm Fisherbrand™ GF/F filters. The filtered samples, containing phytoplankton, were covered with aluminium foil, placed in a sealable plastic bag, labelled and stored in a -80˚C freezer until further processing. For analysis, chlorophyll *a* (Chl_*a*) was extracted by placing the filter papers in a vial, adding 6 ml of 90 % acetone and storing overnight at 4˚C. The filters were then centrifuged at 3000 rpm for 10 min at 4˚C. The fluorescence was measured by using a Turner 10-AU Fluorometer (Parsons et al., 1984). The concentration of chlorophyll *a* (µg l^{-1}) was calculated according to the equation (from Lorenzen, 1966) below:

$$\text{Chlorophyll } a \text{ concentration}(\mu\text{g } l^{-1}) = \frac{Fm}{(Fm-1)} X \frac{kv(Fo-Fa)}{Vf}$$

where

Fo = fluorescence before acidification

Fa = fluorescence after acidification

Fm = maximum acid ratio which can be expected in the absence of Pheopigments

k = calibration factor relating amount of pigment to fluorescence intensity: [(µg Chl_*a*/ml solvent)/instrument fluorescence unit]

v = volume of acetone used for extraction (ml)

Vf = volume of seawater filtered (l)

2.2.3.3 Total suspended solids (TSS)

Glass microfiber filters (Fisherbrand™; GF/C, 47 mm) were prepared by first soaking them in distilled water and then drying for at least 1 hour at 105˚C in an oven. Filter weight was then recorded (A). Next, the filters were placed onto a vacuum filter apparatus for filtration. 500 ml of the sea water from each water sample bottle was filtered. Filters were dried at 105˚C to constant weight (B) to give the weight of total suspended solids (TSS) from the volume of filtered sea water (Degen and Nussberger, 1956). TSS was determined by using the equation below.

$$Total\ suspended\ solids\ (mg\ dry\ weight\ l^{-1}) = \frac{(B - A) \times 1000}{sample\ volume\ (ml)}$$

where:

A = weight of filter (mg)

B = weight of filter plus dried residue (mg)

2.2.3.4 Total bacterial abundance

Eight 20 µl replicates of each sea water sample from reef and sea bed positions were plated on sterile culture plates with marine agar as growth media, which was prepared according to the manufacturer's recommendations (Zobell 2216E, DifcoTM, France). They were incubated at room temperature for two days before bacterial abundance was counted and expressed as colony forming units (CFU ml^{-1})

2.3 Results

2.3.1 Temperature and salinity

The field temperatures and salinities at the experimental site were 12 ˚C and 35 (September 2012), 7 ˚C and 33 (April 2013), 18 ˚C and 34 (August 2013) and 15 ˚C and 34 (November 2013), respectively. Vertical profiles of temperature and salinity indicated a fully mixed water column at each sampling event; there were no significant differences in temperature or salinity between the elevated reefs and sea bed.

2.3.2 Chlorophyll *a* concentration

Chlorophyll *a* concentrations, as an indicator of food availability in the water column, were analysed from the water samples collected on the experimental site at each sampling interval. In November 2013, the lowest chlorophyll *a* concentrations (mean ± SD) in the water column at the height of the elevated reef modules and at the sea bed were 1.21±0.03 µg l^{-1} and 1.04±0.08 µg l^{-1}, respectively. The highest chlorophyll *a* concentrations in the water column at both positions were recorded in September 2012 (9.00±1.79 µg $l^{-1}$and 7.44±1.30 µg l^{-1}, respectively) (Figure2.5). No statistically significant difference in chlorophyll *a* concentration between the height of the elevated reef modules and at the seabed was identified at any sampling interval (Mann-Whitney U, $P>0.05$).

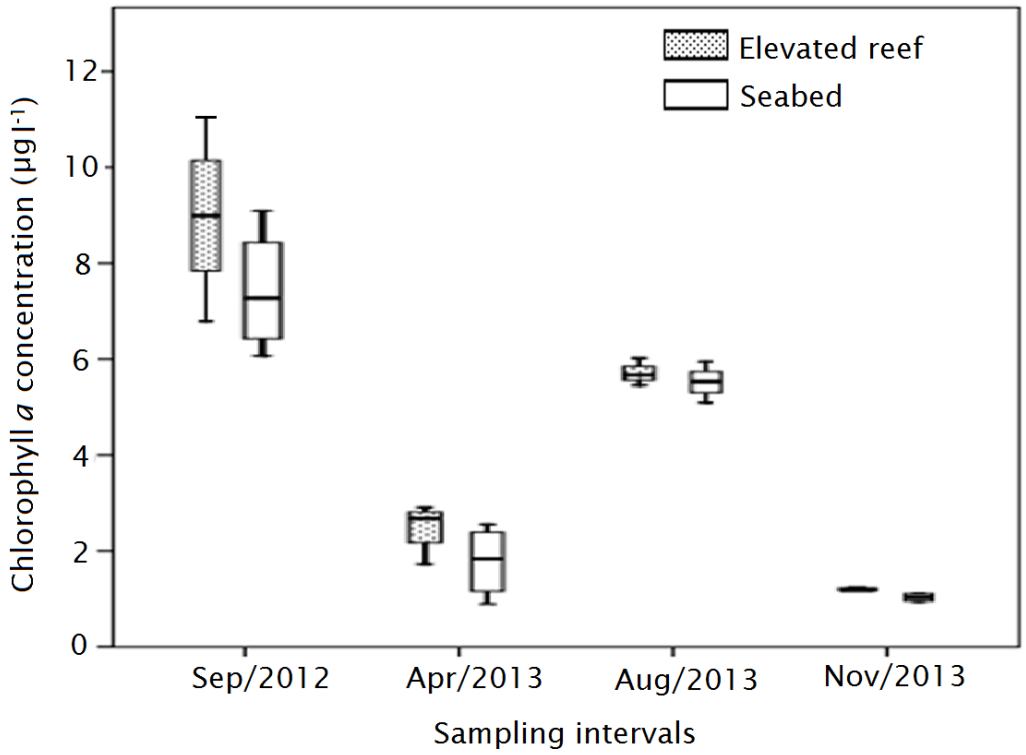

Figure 2.5 Changes in the chlorophyll *a* concentration with time and elevation at the experimental reef site showing the median, interquartiles and minimum and maximum values (range). No statistically significant difference in chlorophyll *a* concentration between the height of the elevated reef modules and at the sea bed was identified for any sampling interval (Mann-Whitney U, P>0.05).

2.3.3 Total suspended solids (TSS)

The concentration of total suspended solids in the water column around the reefs and above the sea bed at each sampling interval is shown in Figure 2.6. The TSS in the water column at the height of the elevated reefs (mean ± SD) varied between 32.2±1.54 mg dry weight l^{-1} (August 2013) and 47.2±6.16 mg dry weight l^{-1} (November 2013) while the TSS in the water column above the sea bed varied between 50.2±9.96 mg dry weight l^{-1} (August 2013) and 56.7±6.97 mg dry weight l^{-1} (November 2013). The TSS at the height of the elevated reef modules were significantly lower than that at the seabed at every sampling interval (Mann-Whitney U, P<0.05).

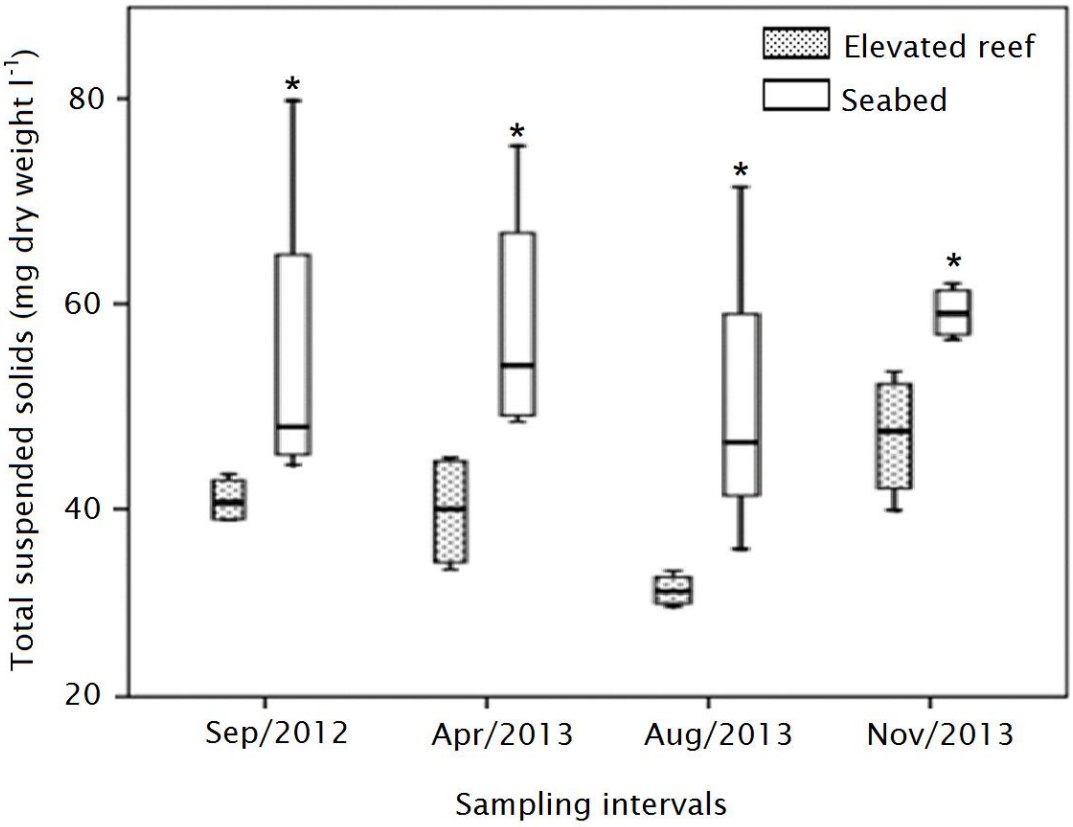

Figure 2.6 Changes in the total suspended solids with time and elevation at the experimental reef site showing the median, interquartiles and minimum and maximum values (range). Significant differences in TSS between sea bed values and values at an elevation of 80 cm are denoted by an asterisk for each sampling event (Mann–Whitney U, $P<0.05$).

2.3.4 Total bacterial abundance

Bacterial abundance in the water column at the height of the elevated reefs and above the sea bed at each sampling interval are shown in Figure 2.7. The bacterial abundance in the water column at the height of the elevated reefs (mean ± SD) varied between 662.5 ± 412.2 CFU ml^{-1} (April 2013) and 8,475.5 ± 2,225.8 CFU ml^{-1} (August 2013), while the bacterial abundance in the water column above the sea bed varied between 1,062.5 ± 996.2 CFU ml^{-1} (April 2013) and 21,787.5 ± 1,798.8 CFU ml^{-1} (August 2013). The bacterial abundance (CFU ml^{-1}) in the water column at the height of the elevated reefs was significantly lower than that at the sea bed (Mann-Whitney U, $P<0.05$) in August (18 ˚C) and November (15 ˚C) 2013.

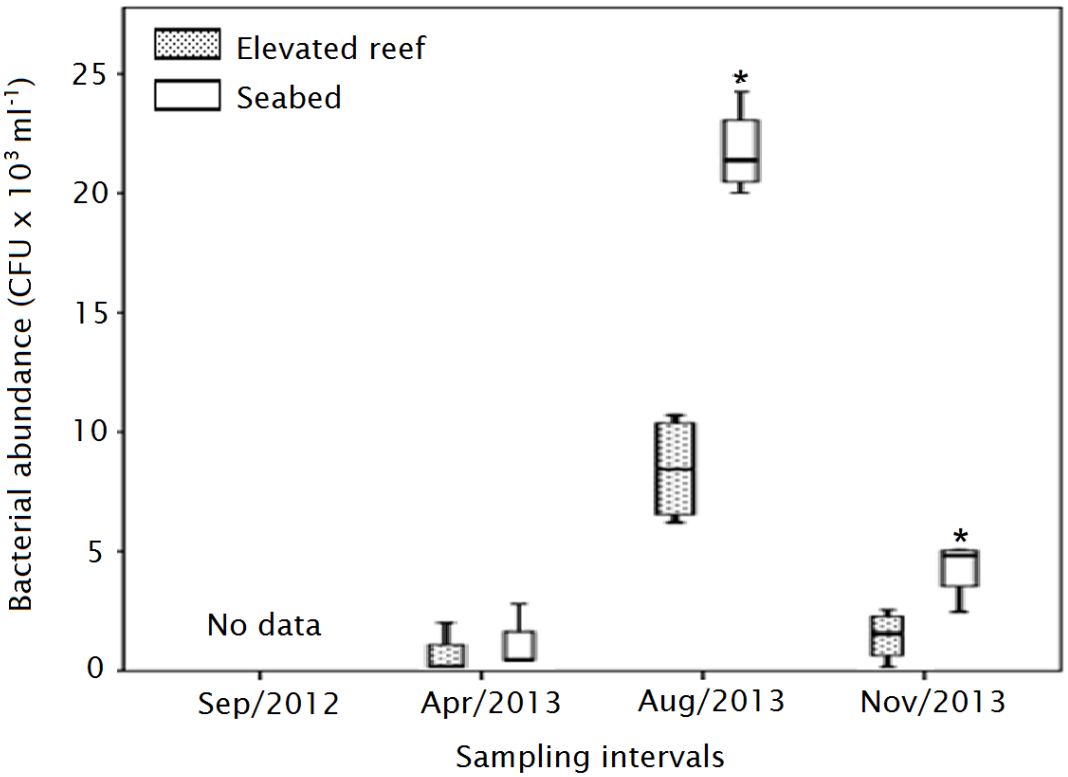

Figure 2.7 Changes in the total bacterial abundance with time and elevation at the experimental reef site showing the median, interquartiles and minimum and maximum values (range).Significant differences in bacterial abundance (Mann–Whitney U, $P<0.05$) between sea bed values and values at an elevation of 80 cm are denoted by an asterisk for each sampling event.

2.4 Discussion and conclusion

After elevated reefs were deployed in August 2012, four sampling intervals were carried out during 15 months of deployment. The reef boxes and oyster cages were recovered in September 2012 (Autumn, 12˚C), April 2013 (Spring, 7˚C), August 2013 (Summer, 18˚C) and November 2013 (Autumn, 15˚C). Unfortunately, sampling was dictated by the prevailing weather conditions. This was particularly true during the winter months, whereby it was not possible due to safety reasons, to dive to sample the reefs. Previous studies have suggested that physiological activities of *O. edulis* are reduced in winter due to lower water temperatures and lower food abundance in the water column (Bayne, 1998; Beiras et al., 1995; Buxton et al., 1981; Haure et al.,

1998; Hutchinson and Hawkins, 1992; Korringa, 1952; Korringa, 1957; Sytniki and Zolotnitskiy, 2014). Therefore, as there is less available energy to support metabolism, energy expenditure is reduced and this, in turn, has been argued to facilitate survival during the winter months (Hutchinson and Hawkins, 1992; Korringa, 1952; Korringa, 1957). As identified, water temperature exhibited no stratification in Poole Bay through the experiment and, as a consequence, it is considered likely that, at winter times, there would be minimal differences in the performance of oysters on the sea bed and those on the elevated reef modules.

The original plan of this study was to investigate the influence of artificial reef habitat on oyster physiology and local biodiversity for 24 months from experimental reef deployment. Unfortunately, after 15 months of reef deployment, elevated artificial reefs were extensively damaged. The SCUBA divers surveyed the experimental site in April 2014 and found that there were no reef boxes or oyster cages left available to collect. This issue was possibly caused by local whelk fishing reported in this protected experimental site and winter storms between January and February 2014. The limitation and problems were consistent with previous surveys in other areas. For example, an artificial coral reef in southeast Florida was significantly damaged and moved from the original position by hurricanes or long-duration tropical storms (Fikes, 2013; Lindberg and Seaman, 2011). In Mosquito Lagoon within the Canaveral National Seashore, Florida, some intertidal artificial oyster reef adjacent to major navigation channels were severely damaged by boat anchors and propellers, causing oyster mortality (Grizzle et al., 2002). Moreover, several researchers indicated that some oyster harvest practices (e.g. dredging) can also damage subtidal artificial reefs (Lenihan and Peterson, 1998; Lenihan et al., 1999). Therefore, this pilot study suggests that identifying suitable locations that do not interfere with, and are not impacted by, fishing activities such as trawling and other bottom-disturbing fishing practices is required. Moreover, appropriate reef design and location that will survive with the UK hydrodynamic conditions are also the major area where further work is required.

Previous studies suggested that *O. edulis* had maximum growth rates during the summer period (Brooks, 1994; Pogoda et al., 2011). Therefore, it is likely the greatest differences in oyster physiological performance would be seen in

the warmer summer months. Swartz (1978) has suggested that baseline studies of biological conditions should continue for at least one annual cycle. This study encompassed measurements of oyster physiology and epifaunal or mobile faunal colonisation during a 15 month experiment. Although the duration of this experiment was shorter than planned as discussed above, it is thought to be of sufficient duration to capture differences in physiological performance between reef and sea bed oysters as well as to capture the initial reef colonisation by epifauna and mobile species.

The environmental data identified no stratification and indicated that the water column was vertically fully mixed; there was no significant difference in temperature or salinity between the elevated reefs and sea bed. Water temperatures at the date of sampling varied between 7-18˚C while salinities were between 33-35. The purpose of elevated reef construction was to place oysters in conditions where water quality was improved e.g. reducing the amount of sediment and increasing food availability. In this study, the environmental data showed that the total suspended solids (TSS) in the water column at the height of the elevated reefs were significantly lower than those adjacent to the sea bed at every sampling interval while bacterial abundance in the water column at the height of the elevated reefs was significantly lower than adjacent to the sea bed in August and November 2013 when water temperatures were 18˚C and 15˚C, respectively. Previous studies revealed that turbidity or total suspended solids can be a negative factor on animal health (e.g. filtration and respiration rates) and also reduce species diversity because the sediment can smother spawning beds and substrates (Gosling, 2003; Moore, 1977). Therefore, based on the fact that *O. edulis* prefers conditions of reduced sediment load (Korringa, 1952; Yonge, 1966) the experimental reef created in Poole Bay was deemed to be sufficient to test the hypothesis that water conditions at the height of elevated reef modules 80 cm above seabed could improve physiological performances of *O. edulis*.

However, the effects of reef habitats on improving the physiological performance of *O. edulis* and enhancing ecological diversity have not been established. Therefore, this project sought to establish the effect of reef habitat on physiological performance (Chapter 3) and gonad maturation (Chapter 5) of *O. edulis* in comparison with sea bed oysters. Secondarily, the

benefits of elevated reefs in terms of enhancing epifaunal settlement and mobile fauna abundance are also discussed in Chapter 6 and 7, respectively.

Chapter 3

Effects of elevated reef habitat on the physiology and immune performance of *Ostrea edulis* and prevalence of *Bonamia ostreae* infection[1]

3.1 Introduction

Studies of *Ostrea edulis* physiology have mainly focused on environmental effects in the context of temperature (Bayne et al., 1999; Beiras et al., 1995; Buxton et al., 1981; Haure et al., 1998; Hutchinson and Hawkins, 1992; Korringa, 1952; Korringa, 1957; Sytniki and Zolotnitskiy, 2014), salinity (Korringa, 1952; Fisher, 1987; Hauton et al., 2000; Hutchinson and Hawkins, 1992), total suspended solids (Grant et al., 1990) and food availability (Coughlan, 1969; Gonzalez-Araya et al., 2012; Grant et al., 1990; Millican and Helm, 1994). However, there has been no study evaluating the merits of building artificial reefs with which to improve the physiological performance of *O. edulis*. In particular, the explicit evidence that artificial oyster reefs in the European region improve the physiological potential of *O. edulis* is lacking.

Experimental reef studies have reported that reef elevation improved the physiological performance of other bivalve species. Lenihan et al. (1999) conducted a large-scale field experiment on intertidal oyster reefs in North Carolina, USA. This revealed growth and survival rates as well as resistance to disease caused by the protozoan parasite *Perkinsus marinus* increased in *Crassostrea virginica* cultured in habitats elevated above the sea bed. This was particularly evident during the period of summer hypoxia. The improvement in physiological performance might be the result of improved environmental conditions. Additionally, the physical structures of oyster reefs affect the

[1] Parts of this chapter were published as Sawusdee et al. (2015), see Appendix 12.

surrounding environment for oysters, including flow speed, sediment deposition and accumulation, and oxygen concentration. In turn, these hydrodynamic factors influence the recruitment, growth, physiological conditions, and survival of *C. virginica* (Lenihan and Peterson, 1998). Frechette et al. (1989) identified that complexity and elevation of mussel beds in North America, Greenland and Canada, enhanced the vertical diffusive turbulent water flow past the mussels (*Mylitus edulis)*. This provided enriched nutrient water to the mussels thus improving growth. For this study it is essential to understand how reef habitat impacts the physiological performance of *O. edulis*.

The assessment of oyster physiological performance has been suggested as an indicator of restoration effectiveness (Volety and Tolley, 2010). Previous research reports that filtration (anabolism) and respiration (catabolism) rates are key variables that control physiological conditions such as the growth of marine bivalves (Barber et al., 1991; Brooks, 1994; Goulletquer et al., 1999; Huang and Newell, 2002; Hutchinson and Hawkins, 1992; Moore, 1977; Winter et al., 1984). For that reason, the comparison of both physiological variables i.e. filtration and respiration rates, between reef and sea bed oysters can identify the success of restoration efforts.

Previous studies have also suggested that other physiological factors e.g. condition index and haemolymph proteins are also useful indicators to detect physiological and reproductive status of invertebrates situated in different environments (Li et al., 2010; Lorenzon et al., 2011; Rameshkumar et al., 2009). Condition index is used to summarize the physiological activity of the animals (e.g. growth, reproduction) under natural and laboratory conditions (Lucas and Beninger, 1985). Haemolymph proteins of marine invertebrates are unique in composition, as they do not contain immunoglobulin or albumin-like proteins and the protein composition varies in relation to the physiological and functional state of the animal (Rameshkumar et al., 2009). Haemolymph proteins themselves have also been considered as a tool for monitoring the physiological condition in wild or cultured marine species exposed to different environmental conditions including oysters (Li et al., 2010) and marine crustaceans such as shrimps, lobsters, and crabs, (Lorenzon et al., 2007; Moore et al., 2000; Rosas et al., 2004). Therefore, the use of these factors as

indicators to monitor the health status of oysters positioned on the elevated reefs and sea bed is also useful.

In bivalves, haemocytes represent the backbone of the immune system (Allam abd Raftos, 2015). Poor immune competence can lead to increased parasitic infection in bivalve species (Allam and Raftos, 2015). The key function of haemocytes is their ability to perform chemotaxis towards parasites (Cheng and Howland, 1979; Howland and Cheng, 1982). Chemotactic movement of haemocytes represents the first step in phagocytosis. This is followed by the attachment of haemocytes to the particles recognized as non-self, preceding phagocytosis. Phagocytosis can be undertaken by a range of haemocyte types. Granulocytes, however, are generally the most potently phagocytic in bivalves (Kuchel et al., 2010). There are several studies establishing variation in haemocyte counts and composition in response to infection and contradicting results within and between studies were often highlighted as a likely result of physiological differences between animals from various environmental backgrounds or at different stages/level of infection (Allam and Raftos, 2015; Da Silva et al., 2008; Kuchel et al., 2010). Ford et al. (1993) showed that resistance to *Haplosporidium nelsoni* in *C. virginica* is linked to a circulating sub-population of granulocytes. Da Silva et al. (2008) suggested a better resistance against *B. ostreae* in oysters displaying high percentage of granulocytes. Allam et al. (2000) reported that resistance and susceptibility to brown ring disease in Manila clams, caused by *Vibrio tapetis*, is associated with the relative frequencies of granular haemocytes. Butt and Raftos (2008) also found that haemocytes from QX disease resistant oysters were larger than those from wild-type oysters. As haemocytes have been linked to resistance to parasitic infection, the comparison of haemocyte counts between reef and sea bed oysters is also needed in order to indicate if oyster reefs can improve the immune performance of oysters to resist or survive from the parasitic infection.

Bonamia ostreae is a protozoan parasite that infects the European flat oyster *O. edulis* (Grizel et al., 1983). A significant protozoan pathogen, *B. ostreae,* has been linked to the decline of *O. edulis* production in the European region (Carnegie and Laureau, 2004; Lallias, 2008). Urawa (1995) suggested that environmental conditions often have a strong influence on parasite-host interactions because environmental factors influence the physiological

condition, reproduction, and survival of both the parasite and their hosts. When physiological performance of the host is weak, caused by poor environmental conditions, its susceptibility to parasitism and associated disease may increase, whilst its ability to survive infection decreases (Gustaffson et al., 1994). Therefore, in order to assess the success, or otherwise, of elevated habitat, the prevalence of *B. ostreae* infection in reef and sea bed oysters should be determined.

In light of the paucity of initial physiological data discussed above associated with reef habitats, it is interesting to test the hypothesis that a reef habitat enhances physiological performance. The aim of the research reported in this chapter was to compare the physiological performance between sub-tidal *O. edulis* maintained in elevated structures off the sea bed and oysters held directly on the sea bed. Key indicators of physiological health included filtration and respiration rates, condition index, haemolymph total protein, total and differential haemocyte counts and *B. ostreae* infection status.

3.2 Methods

3.2.1 Oyster samples, morphometric measurement and ash-free dry weight

At each sampling interval (described in Chapter 2), a total of 30 individuals, 15 oysters from the sea bed and 15 oysters from elevated reefs, were recovered from the reef installation. These were returned to the experimental aquarium at the National Oceanography Centre Southampton within 4 hours of collection. Oysters were returned under damp towels in cool boxes. On arrival at the aquarium the oysters were carefully scrubbed to remove epifaunal species on oyster shells. The total length (mm) and weight (g) of each individual was measured. Volume was determined by displacement of water (ml). The oyster samples were acclimated for two days (Jo et al., 2008) in sea water collected from Poole Bay. The intention of this was to measure the physiological performance in the field rather than the acclimated physiological performance in the lab. Therefore, the two days was intended to allow them to recover from the handling and travel. Oxygen was provided using aquarium air pumps and temperature was maintained as per field conditions; 7˚C, 12˚C, 18 and 15˚C in September 2012, April 2013, August 2013 and November 2013,

respectively. After physiological performance had been quantified (see sections 3.2.2 and 3.2.3), the oysters were dried for 48 hours at 80˚C (DW), then ashed for 6 hours at 500˚C (Rodhouse, 1977) and weighed again for ash weight (AW). The resulting ash free-dry weight (AFDW) of each individual oyster was determined by the difference between the dry weight and ash weight (AFDW =DW-AW).

3.2.2 Oyster filtration rate measurement

Filtration rate was determined by measuring the consumption of a fixed ration of the prymnesiophyte *Isochrysis galbana*. Concentrated algae were diluted to 2 x 10^6 cells ml^{-1} using GF/C-filtered seawater collected from Poole Bay and maintained at temperatures consistent with those in the field during collection. Clearance rates were determined in chambers fitted with stir bars in the base and filled with 300ml of water in each chamber (Figure 3.1A). Oysters were left undisturbed in each chamber within the water baths and covered with an opaque sheet to minimize disturbance to the oysters until they had opened their valves (Figure 3.1B). At this point sufficient *Isochrysis galbana* was added to produce a final concentration of 25,000 cells ml^{-1}. 20 ml water samples from control chamber were collected at the start of the experiment. After one hour duration (end-point) a further 20 ml of seawater was collected from the control and experimental chambers. The collected water samples were fixed with 0.3 ml of 10% acid Lugol's solution for 24 h in the dark before enumeration. Algal cell numbers in each water sample were counted using a 0.1 mm deep Neubauer haemocytometer (Marienfeld; Germany), viewed under bright field optics (Olympus, 40x). The clearance rates were calculated using the following equation (Coughlan, 1969):

$$CR(l\ h^{-1}g^{-1}AFDW) = V \times \frac{(\ln C1 - \ln C2)}{T}$$

where

CR = clearance rate

V = water volume in the chamber (0.3 l)

C1 = initial algae concentration

C2= the final algae concentration

T= the time (h)

Finally, filtration rates were corrected to ash free dry weight.

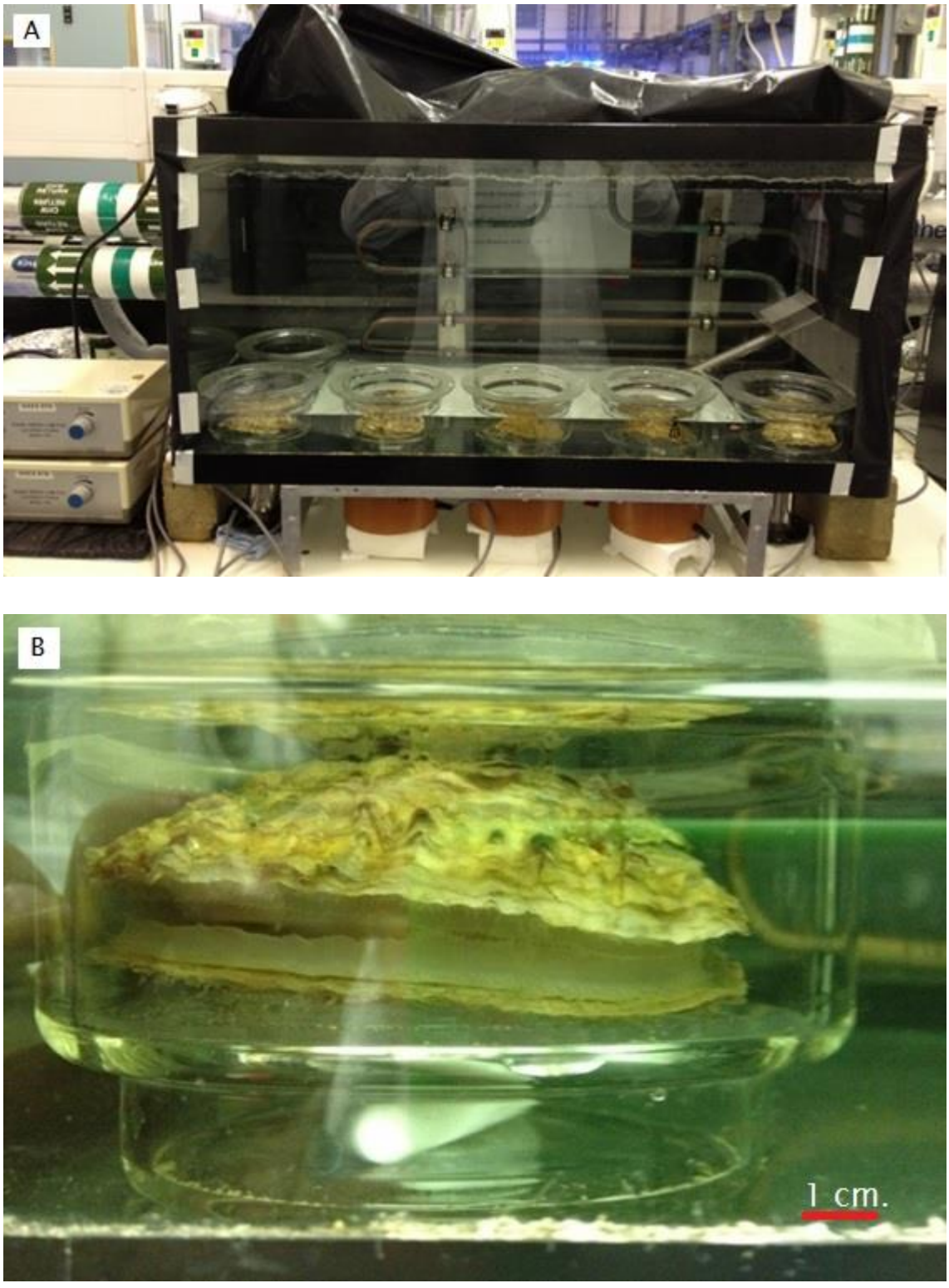

Figure 3.1 Experimental set up for oyster filtration rate measurement; (A): Each glass chamber was fitted with a stir bar in the base and temperatures were maintained as per field conditions (7˚C, 12˚C, 18 and 15˚C), (B); Oysters with valves open indicating feeding.

3.2.3 Oyster respiration rate measurement

The respirometry was carried out after oyster samples were left in the water baths for two days. The respiration rate was measured in a 1000 ml flow-through respirometer filled with GF/C-filtered surface water collected in Poole Bay. Oxygen was measured using a Fibox 3™ fibre-optic oxygen meter (PreSens; Germany) consisting of four parts: a planar oxygen-sensitive foil (sensor spot), a glass fibreoptic cable, a temperature sensor and the detector (Fibox). The sensor spot was glued to the inside of the measuring chamber (Figure 3.2A). Prior to measurement, the respirometer was calibrated for 100% and 0% oxygen saturation. This was achieved by firstly bubbling compressed air through the seawater to produce 100% oxygen saturation and then adding sodium thiosulphite in excess to the chamber to reduce the oxygen concentration to 0%. The calibration was carried out every 3 days of measurement. For each measurement, the fibre optic cable was connected to the Fibox on one side and placed directly above the sensor spot. The sensor foil is excited using emitted light. Light radiation is then emitted back from the sensor foil. The quantity of emitted light has an inverse relationship to the oxygen saturation in the environment (Klimant and Wolfbeis, 1995). Therefore, this acts as a proxy for oxygen saturation in the seawater.

Oyster respirometry measurements were made using water samples that had been collected from the surface waters of Poole Bay at each sampling interval. These surface water samples were filtered through 47 mm Fisherbrand (GF/C) filters. Oxygen-saturated seawater was then prepared by bubbling compressed air through the water vigorously for at least 20 minutes, before being used to fill the flow-through respirometers. Five respirometers, i.e. four experimental chambers with one oyster in each and one control chamber containing no oyster in order to account for bacterial respiration, were prepared. This was repeated until all the 30 oyster replicates were processed. Each animal was placed and sealed inside the experimental chamber and all air bubbles were excluded before any measurements were taken. Gas tight tubing (Masterflex® 06409-18 Tygon®, Saint-Gobain, Barrington, IL) was used to connect the chambers to a peristaltic pump to maintain a continuous flow of water through the respirometer. The experimental chambers were placed in the water bath and maintained at the same temperature as that in the field at the time of collection and were kept in the dark (Figure 3.2B).

The beginning of the measurement, the oyster samples were acclimated to the respirometer for three hours (see details in section 3.2.3.1). Oxygen saturation in each chamber was measured and recorded hourly for three hours after acclimation. The oyster samples were then removed from their chambers. The oxygen values were corrected against a control respirometer to correct for bacterial respiration. The respiration rates were calculated using the following equation (Bayne, 1998):

$$RR = (Ct_0 - Ct_1) \times \frac{(V_c - V_a)}{1000} \times \frac{60}{t_1 - t_0}$$

where:

RR = respiration rate (ml O_2 h^{-1} g^{-1} AFDW)

Ct_o = oxygen saturation (ml l^{-1}) at t_0

Ct_1 = oxygen saturation (ml l^{-1}) at t_1

V_c = volume of chamber (ml)

V_a = volume of animal (ml: measured by displacement of water)

t_0 = starting point (minute)

t_1 = ending point (minute)

t_1-t_o = time between each reading (60 minutes)

Finally, respiration rates were corrected to AFDW.

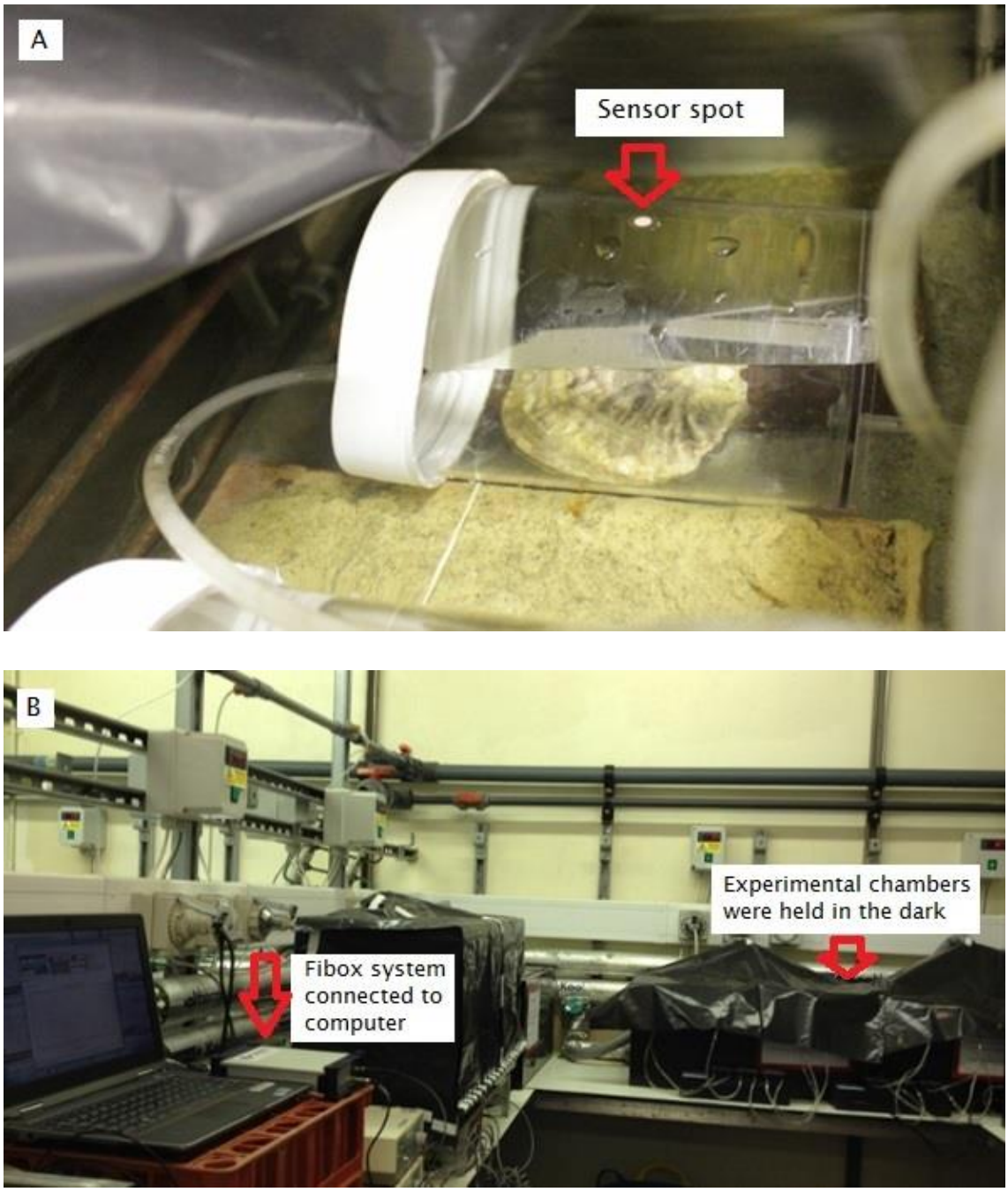

Figure 3.2 Experimental set up for respiration measurement; (A): the sensor spot was glued to the inside of the measuring chamber, (B): Fibox system was connected to a computer in order to record the oxygen saturation hourly. The tanks were covered with a dark sheet to minimize disturbance to the oysters.

3.2.3.1 Method development: acclimation time

In initial experiments, the respiration response of eight animals were measured at 15 ˚C and a salinity of 33. These initial observations showed that oysters generally had to be left for three hours to acclimate to the respirometer before reliable measurements could be made (Figure 3.3).

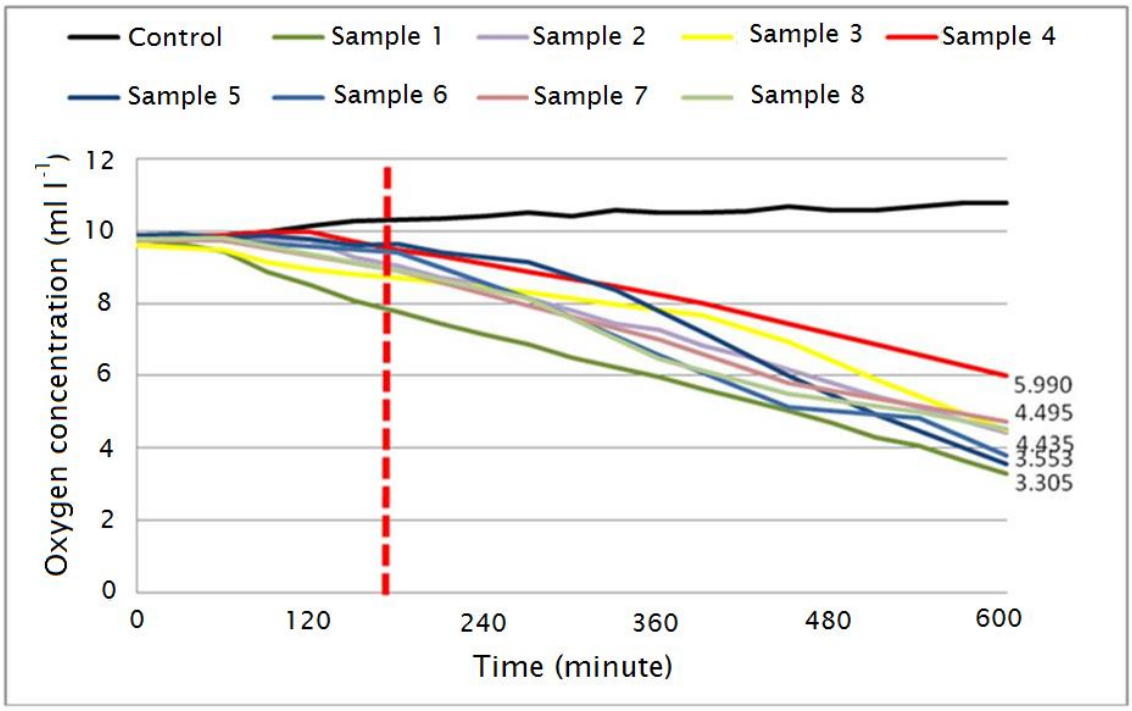

Figure 3.3 Oxygen consumption of individual oysters showing an initial three-hour interval where oxygen readings were erratic, suggesting that the oysters were still acclimating to the system (red dotted line). On this basis all experiments with oysters were left for a three hour acclimation period before measurements commenced.

3.2.4 Condition index

A body condition index was calculated for all animals to indicate physiological condition. Soft tissues and cleaned shells of oyster samples were oven-dried at 80°C on pre-dried foil dishes for 48 hours. Condition index was then calculated using the equation of Lucas and Beninger (1985):

$$\frac{DTW \times 100}{DSW}$$

where

DTW = Dry tissue weight (g)

DSW = Dry shell weight (g)

3.2.5 Haemolymph protein concentration

Haemolymph protein was determined in thirty oysters collected from the reef and sea bed at each sampling interval. Measurements of haemolymph protein would also give an indication of the balance of anabolic and catabolic processes at the time of sampling. Haemolymph protein was measured using the Bicinchoninic Acid assay kit (BCA) (Sigma-Aldrich™, Dorset UK). Briefly, BCA working reagent was prepared by mixing 50 parts of bicinchoninic acid solution (Sigma-Aldrich™, B9643) with 1 part of copper (II) sulphate solution (Sigma-Aldrich™, USA, C2284). A standard curve was prepared as described in the manufacturer's instructions using 2mg ml^{-1} BCA protein standard diluted to the concentration range of 0 – 1000 µg ml^{-1}.

200 µl of BCA working reagent was added into a 96 well plate (Sterilin®, UK) and then left on ice. Triplicate 25 µl volumes of haemolymph from each oyster were added into the 96 well plate. The plate was covered and incubated at 37 °C for 30 minutes. The absorbance value was measured at 815 nm (Labsystems Multiskan RC, Finland) and then absorbance values were compared with the standard curve to determine the haemolymph protein concentration.

3.2.6 Haemocyte counts as a proxy for immune function

Haemocytes were counted in 30 oysters collected from the reef (n=15) and sea bed (n=15) at each sampling interval (September 2012, April 2013, August 2013 and November 2013). 10 µl of haemolymph was withdrawn from the adductor muscle sinus using a sterile hypodermic syringe and 23G needle (Terumo, 0.5x25 mm) and mixed with 10 µl of sterile marine saline (Schlieper, 1972). Total and differential haemocyte cell counts were made using a 0.1 mm deep Neubauer haemocytometer (Marienfeld; Germany) using bright-field optics on an Olympus microscope (40x). Cells were classified as hyalinocytes and granulocytes (according to Hauton et al., 2000; Hutchinson and Hawkins, 1992; Malham et al., 2009)

3.2.7 Prevalence of *Bonamia ostreae* infection

In August 2013, 12 months after reef deployment, 15 oysters from elevated reefs, 15 oysters from the sea bed and also 15 wild oysters from Poole Harbour were collected to detect the protozoan *B. ostreae*. Heart tissue, containing the ventricles and haemolymph, was excised and preserved at -20˚C before further processing. The preserved oyster heart tissues were then used for extraction of genomic DNA using a spin column purification kit (DNEasy Blood & Tissue kit, QIAGEN, West Sussex UK) following the manufacturer's tissue protocol. DNA extractions were quantified spectrophotometrically using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, USA).

Nested polymerase chain reaction (Nested PCR) was carried out to investigate the prevalence of *B. ostreae* in the sampled population. This involved two sets of primers, used in two successive rounds of PCR. Firstly, the extracted DNA was amplified using the primer 'C' set (Carnegie and Laureau, 2004) (Table 3.1). First round PCR amplifications were performed in a final volume of 25 µl containing 0.5 µM forward and reverse primers, 1 µl nucleotide mix (10 mM dNTP), 7.75 µl nuclease-free water, 4 µl Magnesium chloride ($MgCl_2$; 25nM, Promega, Hants, UK), 5 µl 5X green GoTaq® Flexi buffer (Promega), 0.25 µl GoTaq® G2 Flexi DNA Polymerase (Promega; 5u/µl) and 6 µl template DNA (30-100 ng). The PCR amplification was processed in a MyCycler thermocycler (Bio-Rad, Herts, UK) using the following steps: an initial 3-minute 94ºC denaturation

followed by 40 PCR cycles consisting of one minute denaturation at 94ºC, one minute annealing at 59ºC and extension at 72ºC for one minute. A final ten minute extension was performed at 72ºC.

The first round PCR products were diluted with nuclease-free water to a final concentration of 10%, mixing 1 µl PCR product with 9 µl nuclease-free water. The second round forward and reverse primers are noted in Table 3.1 (Eurofins Genomics). The second round PCR was performed in 25 µl containing 0.5 µM forward and reverse primers, 1 µl PCR Nucleotide mix (dNTP) at 10mM each, 12.75 µl nuclease-free water, 4 µl Magnesium chloride ($MgCl_2$; 25nM, Promega), 5 µl 5X green GoTaq® Flexi buffer (Promega), 0.25 µl GoTaq® G2 Flexi DNA Polymerase (Promega; 5u/µl) and 1 µl diluted PCR product from first round amplification. The second amplification consisted of an initial 3 minutes at 95^0C denaturation followed by 35 PCR cycles consisting of one minute denaturation at 95^0C, one minute annealing at 55^0C and extension at 72^0C for one minute. A final ten minute extension was performed at 72^0C. The nested PCR products were stored at -20^0C before further processing.

PCR products were visualised on a 2% agarose gel in 1 x Tris-acetate-EDTA buffer (TAE) with 0.5 µg/ml ethidium bromide. PCR amplified products were compared against a New England Biolabs (Ipswich, UK) 100 bp DNA ladder. Electrophoretic separation was performed at 74 V for 20 minutes. The separated DNA fragments were visualized by ultraviolet transillumination in a Bio-Rad Gel Doc™ 2000 system and analysed using the Bio-Rad software Quantity One version 4.1.1. PCR products at 150 base pairs in the nested reactions were identified as putatively positive samples based on the expected amplicon size.

Table 3.1 Primer sequences, annealing temperature (T_m), and amplicon size (in base pairs) for the nested PCR assay to detect *Bonamia ostreae* infection in *Ostrea edulis*

Round	Primer sequence	Tm (^{0}C)	Predicted amplicon size (bp)
First	C_F: CGGGGGCATAATTCAGGAAC C_R: CCATCTGCTGGAGACACAG	59.0	760
Second	$NEST_F$: AAGGAATTGACGGAAGGGCAC $NEST_R$: TAAGAACGGCCATGCACCAC	59.8	150

DNA cloning was carried out by Nicola Pratt, senior marine biology technician at NOCS. Two nested PCR amplicons, which showed an amplified product of the predicted *B. ostreae* 18s rDNA fragment product size of 150 base pairs, were cut from the gel in order to extract specific DNA using QIAquick Gel Extraction Kit Protocol (QIAGEN). Purified DNA fragments were cloned using Promega pGEM -T Easy Vector Cloning Kit (Promega) according to the manufacturer's recommendations. Positive clones were identified through blue-white screening on ampicillin selective LB agar with IPTG and X-gal. Positive clones were subjected to colony PCR using standard M13 primers to confirm they contained an insert of the correct predicted size. Positive clones were grown up overnight in 5ml of LB-broth with 100 µg ml^{-1} ampicillin. Plasmid DNA was extracted from the broth cultures using a QIAprep Spin Miniprep Kit (QIAGEN) according to the manufacturer's recommendations. Purified DNA plasmids, five samples for each positive amplicon, were sent for conventional Sanger sequencing by Source BioScience LifeSciences (Nottingham, UK). Returned sequences were aligned using CLUSTALW in BioEdit™ and the consensus sequence was subject to BLAST search (Basic Local Alignment Search Tool, http://www.genome.jp/) against the NCBI database (National Centre for Biotechnology Information) to identify the PCR amplicon sequence to a particular species.

3.2.8 Statistical analyses

Physiological performance data (i.e. respiration and filtration rates, condition index, haemolymph protein and haemocyte counts) were first tested for normality and homogeneity of variance. Two-way ANOVA was performed to test how physiological performance varied with elevation (reef/sea bed) and sampling interval. Where appropriate, *post-hoc* pairwise multiple comparisons (Tukey's HSD tests) compared the effect of sampling interval or elevation on physiological performance of *O. edulis*. Pearson's correlation was also performed to test correlation between physiological factors. In all cases, statistical significance was accepted at $\alpha = 0.05$.

3.3 Results

3.3.1 Correlation between physiological parameters

Pearson's correlation indicated that filtration rates strongly positively correlated with respiration rates ($r= 0.75$, $P<0.05$), total haemocytes ($r= 0.64$, $P<0.05$) and granulocytes ($r= 0.65$, $P<0.05$). In addition, the strength of association between filtration rates and hyalinocyte concentrations, haemolymph protein and condition index were moderately positive ($r=0.54$, $P<0.05$, $r=0.48$, $P<0.05$ and $r=0.45$, $P<0.05$, respectively). In contrast, Pearson's correlation indicated the weak of association between respiration rates and total haemocytes ($r= 0.32$, $P<0.05$), granulocytes ($r= 0.32$, $P<0.05$), hyalinocyte concentration ($r= 0.28$, $P<0.05$), haemolymph protein ($r= 0.30$, $P<0.05$) and condition index ($r= 0.21$, $P<0.05$). There was no significant correlation between haemolymph protein and total haemocyte, granulocyte and hyalinocyte concentrations, meanwhile, the strength of association between haemolymph protein and condition index was very weakly negative ($r= -0.12$, $P<0.05$) (Appendix 1).

3.3.2 Comparison between filtration rates of oysters from the elevated reef and sea bed

Oyster samples on the elevated reefs and sea bed were collected to test the hypothesis that elevated reefs enhanced the filtration performance of oysters. The results were collected within a 15 month period after the elevated reefs were deployed. Filtration rates (mean ± SE) at each sampling interval are shown in Figure 3.4. Two-way ANOVA identified a significant effect of elevation (reef/sea bed) ($P< 0.005$) and sampling interval ($P<0.001$) on the filtration rate of *O. edulis* but no significant interaction between the main two effects (Table 3.2). Overall, filtration rates of reef oysters were significantly higher compared with sea bed oysters. Tukey's HSD pairwise tests showed that filtration rates also differed significantly among all sampling intervals (Table 3.3). In April 2012, when the water temperature was 7ºC, the lowest filtration rates of both reef and sea bed oysters were observed (1.33±0.10 and 1.08±0.14 l h^{-1} g^{-1} AFDW, respectively). The highest filtration rates of both reef and sea bed oysters were observed when the water temperature rose to 18ºC in August 2013 (reaching 3.95±0.32 and 3.02± 0.20 l h^{-1} g^{-1} AFDW for reef and sea bed oysters, respectively).

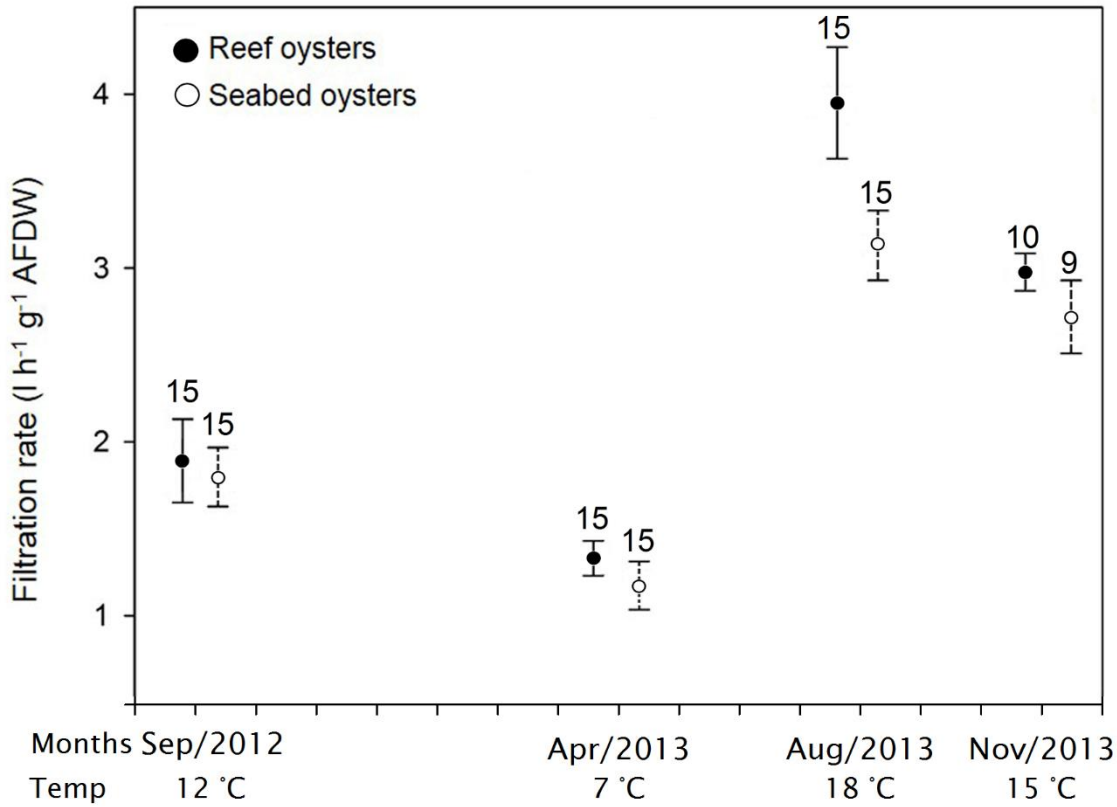

Figure 3.4 Filtration rates (l h^{-1} g^{-1} AFDW, mean ± SE) of *Ostrea edulis* collected from elevated reefs and the sea bed on each sampling occasion. The filtration rate was affected by sampling interval and elevation (two-way ANOVA, $P<0.001$ and $P<0.005$, respectively). The number of oysters analysed from each location is indicated above the error bars.

Table 3.2 Two-way ANOVA results comparing the effect of sampling interval, elevation (reef/sea bed), and the interaction between month and elevation on filtration rate (l $h^{-1}g^{-1}$AFDW). Significant differences are denoted by asterisks.

Sources of variation	df	F	P-value
Sampling interval	3	61.06	0.001*
Elevation (reef/sea bed)	1	8.35	0.005*
Sampling interval x Elevation	3	2.11	0.104

Table 3.3 *Post-hoc* (Tukey's HSD test) results comparing the effect of sampling interval or temperature on filtration rate (l h^{-1} g^{-1} AFDW).

Post-hoc test the effect of sampling interval	P value		
	Sep 2012 (12 ˚C)	Apr 2013 (7 ˚C)	Aug 2013 (18 ˚C)
Apr 2013	<0.01		
Aug 2013	<0.001	<0.001	
Nov 2013 (15 ˚C)	<0.001	<0.001	<0.05

3.3.3 Comparison between respiration rates of oysters from the elevated reef and sea bed

Respiration rates were measured to test the effect of elevated reefs on the catabolism of oysters (Figure 3.5). Two-way ANOVA identified a significant effect of sampling interval ($P< 0.001$) but no significant effect of elevation and no significant interaction between the two main effects on respiration rate were found (Table 3.4). Tukey's HSD pairwise tests showed that there was no difference in the respiration rates of oysters collected in August 2013 (18˚C), November 2013 (15˚C) and September 2012 (12˚C). However, these rates were significantly higher than oysters sampled in April 2013 when water temperature was the lowest at 7˚C (Table 3.5). Additionally, the lowest respiration rates (mean ± SE) of both reef (0.38±0.04 ml O_2 h^{-1} g^{-1} AFDW) and sea bed (0.33±0.04 ml O_2 h^{-1} g^{-1} AFDW) oysters were observed in April 2013, whilst the highest respiration rates of reef (0.82±0.08 ml O_2 h^{-1} g^{-1} AFDW) and sea bed (0.60± 0.06 ml O_2 h^{-1} g^{-1} AFDW) oysters were observed in August 2013 when the greatest water temperature was observed (18ºC).

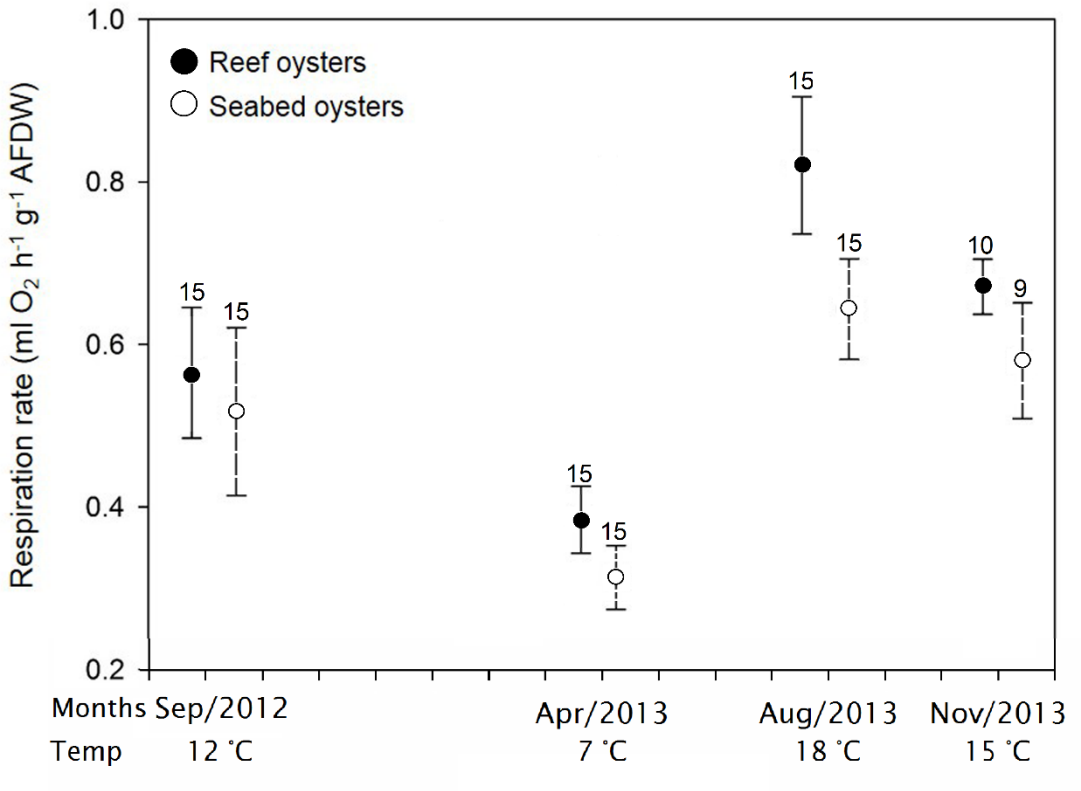

Figure 3.5 Respiration rates (ml O_2 h^{-1} g^{-1} AFDW, mean ± SE) of *Ostrea edulis* collected from elevated reefs and sea bed on each sampling interval. The respiration rate was affected by sampling month (two-way ANOVA, $P<0.001$).The number of oysters analysed from each location is indicated above the error bars.

Table 3.4 Comparison of the effect of sampling interval, elevation (reef/sea bed), and the interaction between sampling interval and elevation on respiration rate (ml $O_2h^{-1}g^{-1}$AFDW). Significant differences are denoted by asterisk.

Sources of variation	df	F	P-value
Sampling interval	3	9.16	0.001*
Elevation (reef/sea bed)	1	3.14	0.080
Sampling interval x Elevation	3	0.71	0.547

Table 3.5 *Post-hoc* (Tukey's HSD test) results comparing the effect of sampling interval or temperature on respiration rate (ml O_2 $h^{-1}g^{-1}$AFDW). "ns" represents no significant difference

Post-hoc test the effect of sampling intervals	P value		
	Sep 2012 (12 ˚C)	Apr 2013 (7 ˚C)	Aug 2013 (18 ˚C)
Apr 2013	<0.05		
Aug 2013	ns	<0.001	
Nov 2013 (15 ˚C)	ns	<0.005	ns

3.3.4 Comparison between condition index of oysters from the elevated reef and sea bed

Condition index of each oyster collected from both the elevated reef and sea bed was measured as a general physiological indicator. The mean condition index of oyster soft tissue varied among sampling intervals (Figure 3.6). Two-way ANOVA indicated that the sampling interval had a significant effect on condition index ($P < 0.001$) but that elevation did not (Table 3.6). Pairwise multiple comparisons (Tukey's HSD test) showed that the condition index of oysters collected in August 2013 was significantly higher than at the other sampling intervals. Moreover, condition index in April 2013 was significantly higher than November 2013 ($P < 0.001$) but not statistically significantly different between remaining sampling intervals (Table 3.7). In November 2013, the lowest condition index (mean ± SE) of both reef and sea bed oysters were observed (2.46±0.07 and 2.65±0.11, respectively), while the highest condition index of both reef and sea bed oysters were recorded in August 2013 (4.73±0.21 and 4.12± 0.20, respectively).

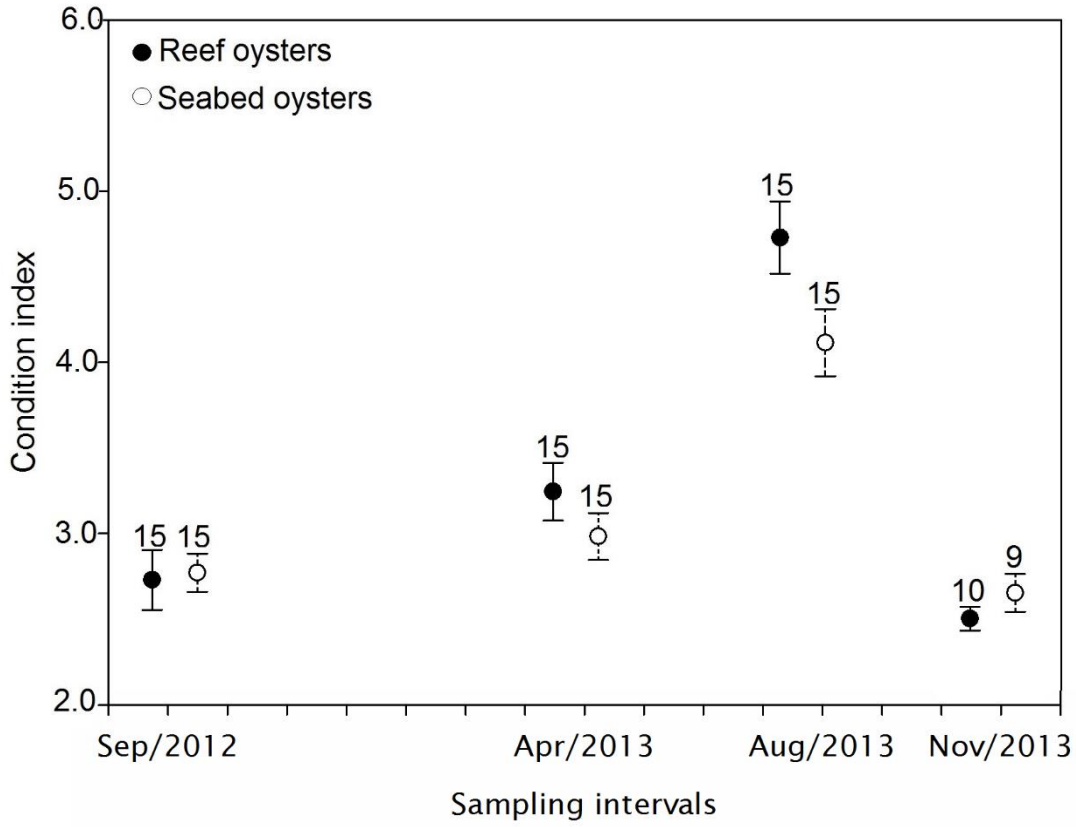

Figure 3.6 Condition index (mean ± SE) of *Ostrea edulis* collected from elevated reefs and the sea bed. Condition index was significantly affected by sampling interval (two-way ANOVA, P<0.001). The number of oysters analysed from each location is indicated on top of the error bars.

Table 3.6 Comparison of the effect of sampling interval, elevation (reef/sea bed), and the interaction between sampling interval and elevation on condition index. Significant differences are denoted by asterisks.

Sources of variation	df	F	P-value
Sampling interval	3	62.28	0.001*
Elevation (reef/sea bed)	1	1.93	0.167
Sampling interval x Elevation	3	2.23	0.090

Table 3.7 *Post-hoc* (Tukey's HSD test) results comparing the effect of sampling interval or temperature on condition index. "ns" represents no significant difference

Post-hoc test the effect of sampling intervals	P-value		
	Sep 2012 (12 ˚C)	Apr 2013 (7 ˚C)	Aug 2013 (18 ˚C)
Apr 2013	ns		
Aug 2013	<0.001	<0.001	
Nov 2013 (15 ˚C)	ns	<0.001	<0.001

3.3.5 Comparison between haemolymph protein concentration of oysters from the elevated reef and sea bed

Haemolymph protein concentrations of reef and sea bed oysters during the experiment varied between 0.49-3.28 (2.16± 0.46, mean ± SE) and 0.40-3.01 (1.94±0.34, mean ± SE) mg ml^{-1}, respectively (Figure 3.7). Two-way ANOVA indicated that haemolymph protein concentration differed significantly between sampling intervals (P<0.01) but there was no significant effect of elevation on haemolymph protein concentration (Table 3.8). Haemolymph protein concentration was highest in April 2013 and was significantly higher than August 2013 (P<0.01), whilst there was no difference in haemolymph protein concentration of oysters between other sampling intervals (Table 3.9).

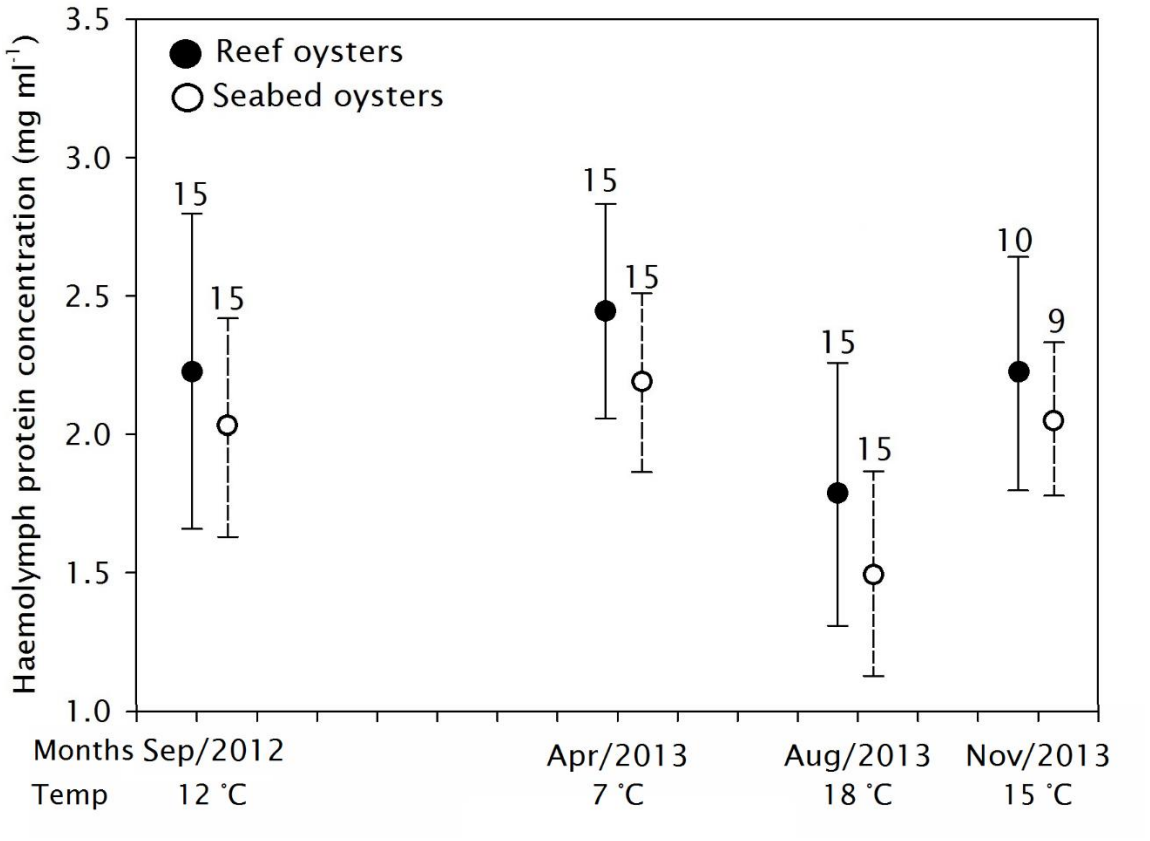

Figure 3.7 Haemolymph protein concentration (mean ± SE) of *Ostrea edulis* collected from elevated reefs and the sea bed on each sampling interval. Haemolymph protein concentration was affected by sampling interval (two-way ANOVA, $P<0.01$). The number of oysters analysed from each location is indicated above the error bars.

Table 3.8 Two-way ANOVA results comparing the effect of sampling interval, elevation (reef/sea bed), and the interaction between interval and elevation on haemolymph protein concentration (mg ml^{-1}). Significant differences are denoted by asterisks.

Sources of variation	df	F	P-value
Sampling interval	3	4.029	0.009*
Elevation (reef/sea bed)	1	2.129	0.148
Sampling interval x Elevation	3	0.052	0.984

Table 3.9 *Post-hoc* (Tukey's HSD test) results comparing the effect of sampling interval or temperature on haemolymph protein concentration (mg ml^{-1}). "ns" represents no significant difference

Post-hoc test the effect of sampling intervals	P-value		
	Sep 2012 (12 ˚C)	Apr 2013 (7 ˚C)	Aug 2013 (18 ˚C)
Apr 2013	ns		
Aug 2013	ns	<0.01	
Nov 2013 (15 ˚C)	ns	ns	ns

3.3.6 Comparison between haemocyte concentration of oysters from the elevated reef and sea bed

Total haemocyte counts differed significantly between sampling intervals (two way ANOVA, P<0.001) and with elevation (two way ANOVA, P<0.05) (Table 3.10). Total haemocyte counts (mean ± SE) of reef and sea bed oysters remained low in September 2012 (4.01± 0.25 and 4.10 ± 0.37 $x10^{-6}$ cells ml^{-1}, respectively) and April 2013 (5.06± 0.69 and 4.06 ± 0.43 $x10^{-6}$ cells ml^{-1}, respectively) but peaked in August 2013 (16.20± 1.45 and 12.71 ± 1.62 $x10^{-6}$ cells ml^{-1}, respectively) (Figure 3.8). In general, haemocyte numbers were low when *in situ* water temperatures were 12˚C and 7˚C. Additionally, two-way ANOVA indicated that hyalinocyte counts (Figure 3.9A) differed significantly between sampling intervals (P<0.001) but there was no significant effect of elevation on hyalinocytes. Granulocyte counts (Figure 3.9B) differed significantly between sampling intervals (two way ANOVA, P<0.001) and with elevation (two way ANOVA, P<0.01). Tukey's HSD pairwise tests showed that there was no difference in total haemocyte count, hyalinocytes and granulocytes in oysters collected in September 2012 and April 2013, whilst there were significant differences in the total haemocyte count of oysters between other sampling intervals (Table 3.11).

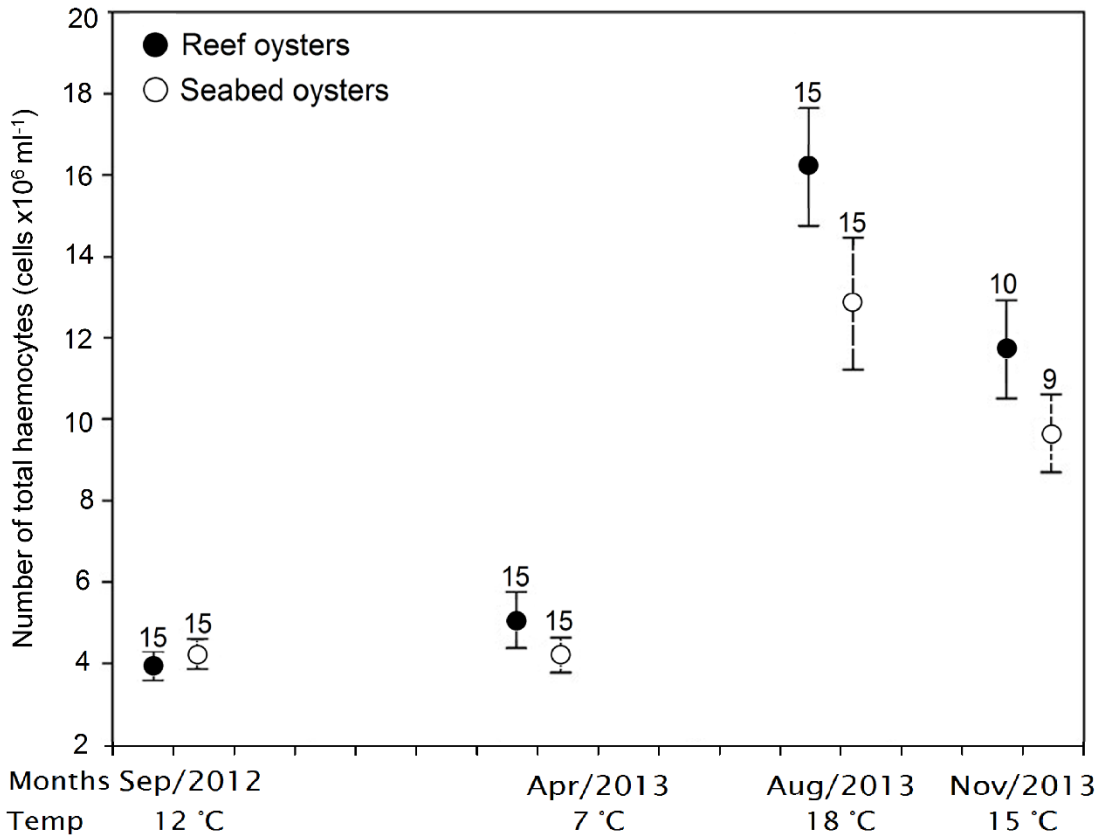

Figure 3.8 Temporal variation of total haemocyte count (x 10^6 cells ml^{-1}, mean ± SE) of *Ostrea edulis* collected from the experimental reefs and sea bed on each sampling interval. The total haemocyte counts were affected by sampling interval and elevation (two-way ANOVA, $P<0.001$ and $P<0.05$, respectively). The number of oysters analysed from each location is indicated above the error bars.

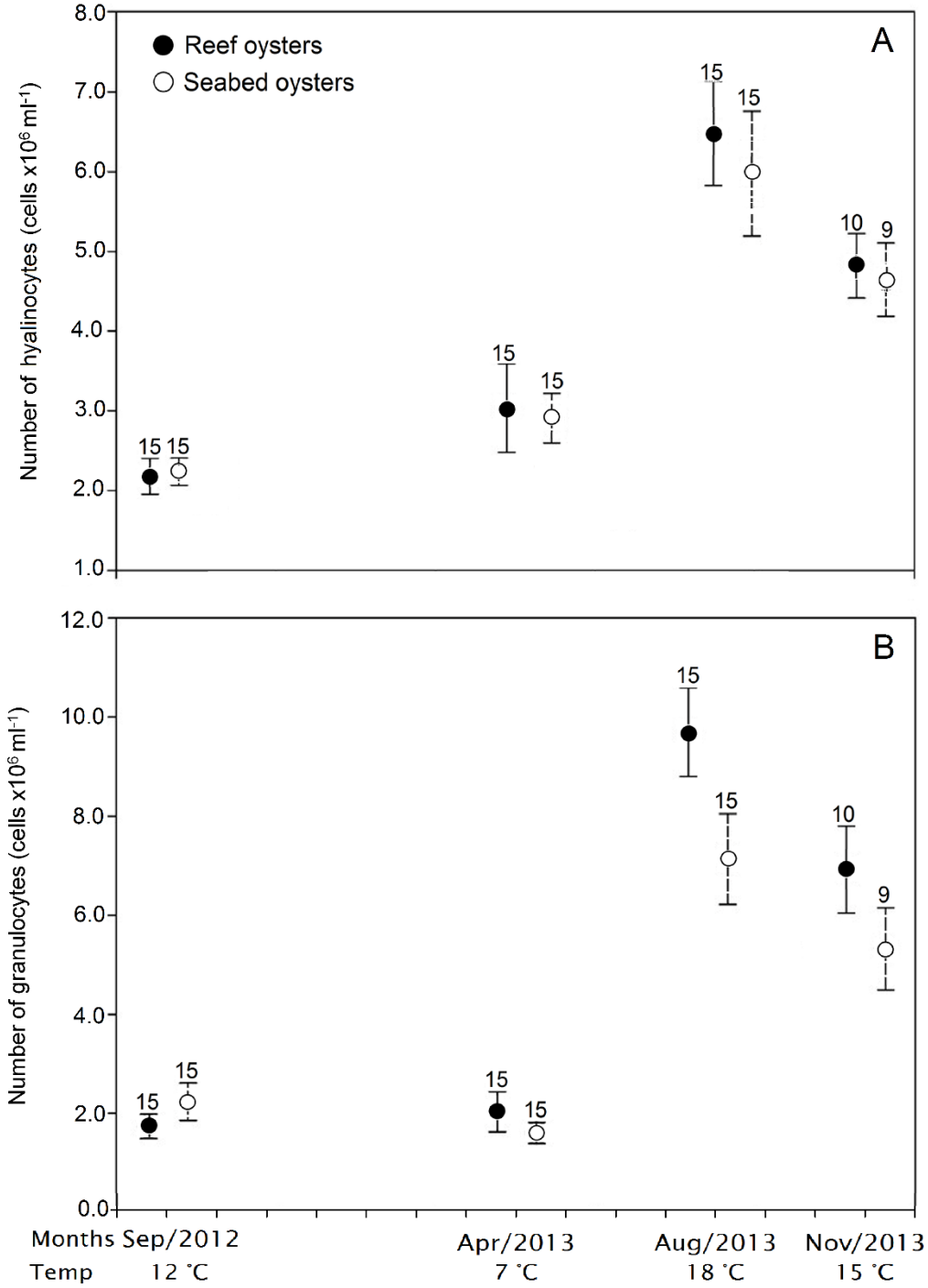

Figure 3.9 Temporal variation of hyalinocytes (A) and granulocytes (B) (x 10^6 cells ml^{-1}, mean ± SE) of *Ostrea edulis* collected from the experimental reefs and sea bed on each sampling interval. The number of hyalinocytes were significantly different between sampling intervals ($P<0.001$). The numbers of granulocytes were affected by sampling interval and elevation (two-way ANOVA, $P<0.001$ and $P< 0.01$ respectively). The number of oysters analysed from each location is indicated above the error bars.

Table 3.10 Two-way ANOVA results comparing the effect of sampling interval, elevation (reef/sea bed), and the interaction between sampling interval and elevation on haemocyte counts. Significant differences are denoted by asterisks.

Sources of variation	df	F	P-value
Total haemocytes (cells ml^{-1})			
Sampling interval	3	57.016	0.001*
Elevation (reef/sea bed)	1	5.385	0.022*
Sampling interval x Elevation	3	1.406	0.245
Hyalinocytes (cells ml^{-1})			
Sampling interval	3	29.187	0.001*
Elevation (reef/sea bed)	1	2.033	0.157
Sampling interval x Elevation	3	0.134	0.939
Granulocytes (cells ml^{-1})			
Sampling interval	3	64.598	0.001*
Elevation (reef/sea bed)	1	7.012	0.009*
Sampling interval x Elevation	3	2.774	0.045

Table 3.11 *Post-hoc* (Tukey's HSD test) results comparing the effect of sampling interval or temperature on haemocytes counts. "ns" represents no significant difference

Post-hoc test the effect of sampling interval	P-value		
	Sep 2012 (12 ˚C)	Apr 2013	Aug 2013
Total haemocytes			
Apr 2013 (7 ˚C)	ns		
Aug 2013 (18 ˚C)	<0.001	<0.001	
Nov 2013 (15 ˚C)	<0.001	<0.001	<0.01
Hyalinocytes			
Apr 2013 (7 ˚C)	ns		
Aug 2013 (18 ˚C)	<0.001	<0.001	
Nov 2013 (15 ˚C)	<0.001	<0.01	<0.05
Granulocytes			
Apr 2013 (7 ˚C)	ns		
Aug 2013 (18 ˚C)	<0.001	<0.001	
Nov 2013 (15 ˚C)	<0.001	<0.001	<0.01

3.3.7 Prevalence of *Bonamia* sp. infection in *Ostrea edulis* from the elevated reefs and sea bed

In August 2013, 45 heart specimens were collected from reef, sea bed and Poole Harbour oysters (15 oysters from each location). All samples were analysed for the prevalence of *B. ostreae* infection using nested PCR (Figure 3.10). The result of the nested PCR showed that 100% of the oysters collected from Poole Harbour were positive for *B. ostreae* (positive amplicons at 150bp), whereas 86.67 % of reef oysters and 66.67 % of sea bed oysters were infected (Table 3.12). The consensus sequence of the positive amplicons is shown in Table 3.13. BLAST searches indicated that the most related alignment consequence to consensus sequence of positive amplicons in this study was DNA of the 18S small ribosomal subunit of *Bonamia* sp. (Accession number KC578009, Table 3.14). The similarity of DNA of the 18S small ribosomal

subunit of *B. ostreae* with consensus sequence was also high (98.66 %, E-value=$6e^{-66}$). However, the closest reported sequence to the one isolated in this study was for *Bonamia* sp. (KC578009). As a result the sequences isolated from the Poole oysters are hereafter referred to as *Bonamia* sp.

Table 3.12 Prevalence of *Bonamia* sp. infection in oysters collected from three locations (elevated reef, sea bed and Poole harbour) in August 2013.

Locations	Positive		Negative		Total (n)
	n	%	n	%	
Elevated reef	13	86.67	2	13.33	15
Sea bed	10	66.67	5	33.33	15
Poole Harbour	15	100.00	0	0.00	15

Table 3.13 The consensus sequence of positive amplicons of the 18S rDNA sequence for *Bonamia* sp. detected in oysters collected from three locations (elevated reef, sea bed and Poole harbour) in August 2013.

5'-AAGGAATTGACGGAAGGGCACCACAAGATGTGGAGCCTGCGGCTTAATTTGAT TCAACACGGGAAAACTTACCAGGTCCAGACATAGTAAGGATTGACAG ATTAAAGTTCTTTCTTGATTCTATGCATGGTGGTGCATGGCCGTTCTT A-3'

Table 3.14 BLAST results showing the top 20 of Accession numbers related to the consensus sequence. The alignment of 150 base pairs of consensus sequence against nucleotide databases (GenBank, EMBL and RefSeq) confirmed the sequence identity of *Bonamia* sp.

Accession numbers	Description and location	Score (%)	E value
KC578009	*Bonamia* sp. ex *Saccostrea glomerata* 18S ribosomal RNA gene, New South Wales, Australia	100	1e-68
JX977119	*Bonamia* sp. ex *Saccostrea glomerata* 18S ribosomal RNA gene, New South Wales, Australia	100	1e-68
JF831807	*Bonamia exitiosa* clone BexSSU_*Cariakensis*_FL small subunit ribosomal RNA gene, Florida, USA	100	1e-68
JF831806	*Bonamia exitiosa* clone BexSSU_*Oauporia* small subunit ribosomal RNA gene, New Zealand	100	1e-68
JF831805	*Bonamia exitiosa* clone BexSSU_*Oconchaphila* small subunit ribosomal RNA gene, California , USA	100	1e-68
JF831804	*Bonamia* sp. ex *Ostrea edulis* small subunit ribosomal RNA gene, California, USA	100	1e-68
JF831802	*Bonamia exitiosa* clone *BexSSU_Sglomerata* small subunit ribosomal RNA gene, Australia	100	1e-68
JF831801	*Bonamia exitiosa* clone Bex_*Oequestris*_ARG small subunit ribosomal RNA gene, Argentina	100	1e-68
JF495410	*Bonamia exitiosa* small subunit ribosomal RNA gene, New Zealand	100	1e-68
JF495409	*Bonamia* sp. ex *Ostrea puelchana* small subunit ribosomal RNA gene	100	1e-68

Table 3.14 (Cont.)

Accession numbers	Description and location	Score (%)	E value
JF495408	*Bonamia* sp. ex *Ostrea angasi* small subunit ribosomal RNA gene, Australia	100	1e-68
GQ385242	*Bonamia* sp. ex *Ostrea stentina* small subunit ribosomal RNA gene, Tunisia	100	1e-68
GQ366703	*Bonamia* sp. ex *Ostrea chilensis* small subunit ribosomal RNA gene, Chile	100	1e-68
DQ312295	*Bonamia s*p. SC2006 18S ribosomal RNA gene and internal transcribed spacer, Australia	100	1e-68
AY542903	*Bonamia* sp. ex *Crassostrea ariakensis* small subunit ribosomal RNA gene, North Carolina, USA	100	1e-68
AF337563	*Bonamia* sp. 18S ribosomal RNA gene, France	100	1e-68
EU016528	*Bonamia* sp. EA-2007 small subunit ribosomal RNA gene, Galicia, Spain	98.66	5e-67
JQ936481	*Bonamia ostreae* strain SD small subunit ribosomal RNA gene, China	98.66	6e-66
JN040832	*Bonamia ostreae* from USA small subunit ribosomal RNA gene, California, USA	98.66	6e-66
JN040831	*Bonamia ostreae* from Netherlands small subunit ribosomal RNA gene, Netherlands	98.66	6e-66

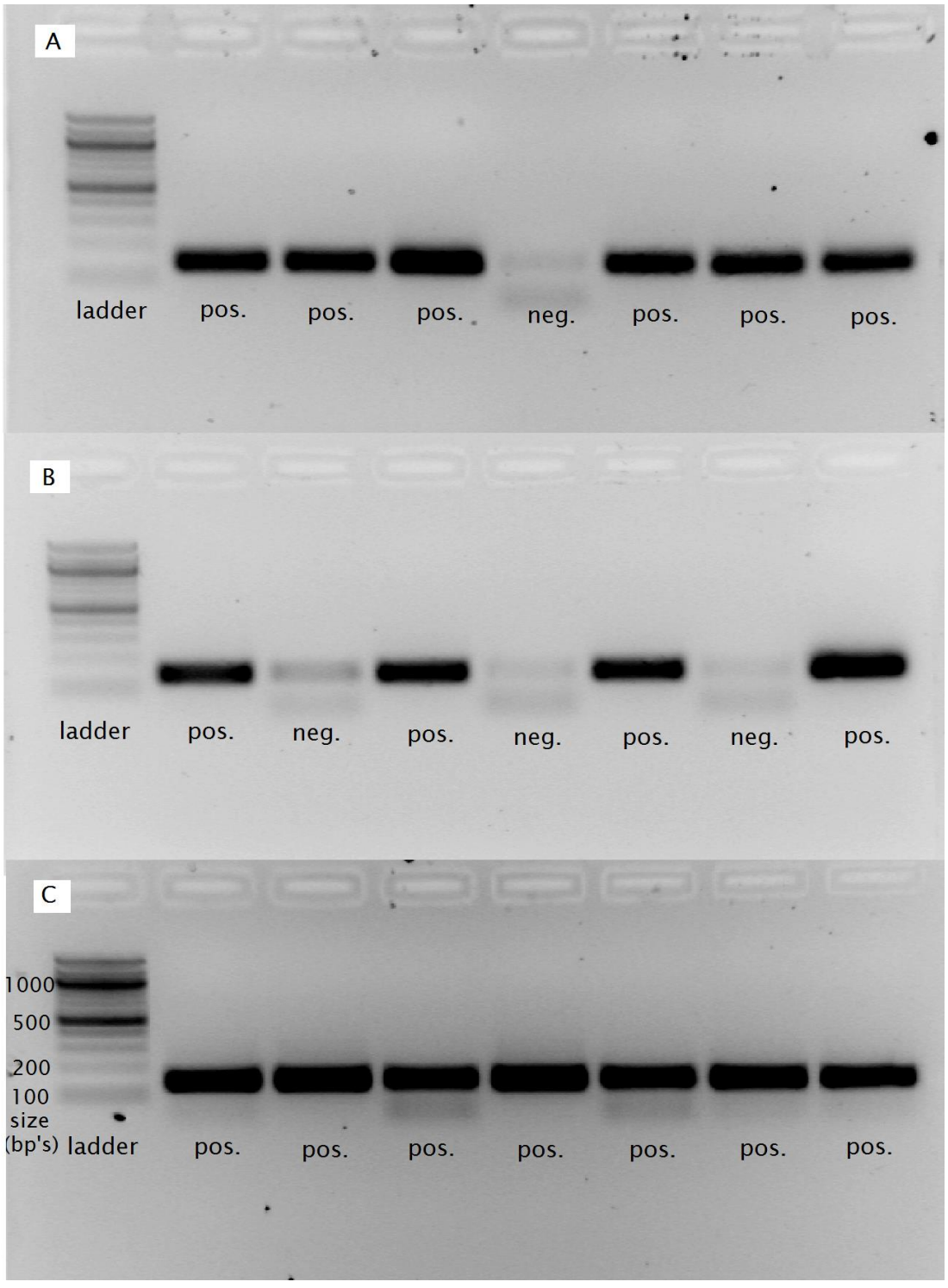

Figure 3.10 Gel showing results of nested PCR screening of oyster heart tissue for *Bonamia* sp. infection in each location (A: reef oysters, B: sea bed oysters, C: Poole Harbour oysters). The amplified fragments of 150 base pairs (pos) indicates infection and the absence of amplified fragments (neg) represents no infection.

3.4 Discussion and conclusion

Artificial oyster reefs have been created globally in order to increase the abundance of target oyster species and also restore ecosystem complexity. However, the benefits to physiological performance, especially of the native oyster *O. edulis*, have not yet been established. Previous studies have reported that environmental factors and food quality and quantity control the filter feeding activity of bivalves (Honkoop and Beukema, 1997; Marsden, 2004; Pilditch and Grant, 1999; Ward and Shumway, 2004; Yukihira et al., 2006). Temperature and salinity are the most significant abiotic factors affecting the physiological performance of *O. edulis*, e.g. filtration and respiration (Beiras et al., 1995; Haure et al., 1998; Hutchinson and Hawkins, 1992; Rodhouse, 1977; Walne, 1972). However, particle size, algal species and their surface properties have been shown also to affect food selectivity of oysters and other molluscs (Rosa et al., 2013; Shumway et al., 1985; Ward et al., 1997)

However, to our knowledge, this is the first study comparing the filtration and respiration rates of *O. edulis* at the same *in situ* temperature and salinity but at different heights above the sea bed. The outcome of this project presented the novel observation that elevation had a significant effect on the filtration rate of oysters, such that the reef oysters in this study had higher filtration rates compared with sea bed oysters. Filtration rate is a key determinant in the energy available for oysters and ultimately influences their ability to meet their energetic demands (Barber et al., 1991; Brooks, 1994; Moore, 1977; Winter et al., 1984). The fact that reef oysters showed the highest filtration rates at times of elevated temperature and chlorophyll *a* (Chapter 2) indicated that these individuals would maximise their energy intake during the important growing season. As a result, these elevated oysters would have an advantage over oysters on the sea bed. Conversely, data presented in Chapter 2 demonstrated that TSS and bacterial abundance adjacent to the sea bed were significantly higher than 80 cm above the sea bed. Therefore, we cannot exclude the possibility that these environmental conditions might have impacted the physiological performance of sea bed oysters by reducing their filtration rate. Although oysters and other bivalves can reject unwanted particles (Newell and Jordan, 1983; Shumway et al., 1985; Yonge, 1926) with occasional rapid closure of their valves to expel accumulated silt and through

pseudofaecal production (Yonge, 1926; Yonge, 1966), an increase in suspended sediment has been shown to cause a decrease in the filtration rate of *O. edulis* (Grant et al., 1990; Korringa, 1952). Hutchinson and Hawkins (1992) revealed that the clearance rate of the *O. edulis* dropped about 70% as the organic and inorganic particles rose from 1 to 7 mg l^{-1}. Indeed, oysters can sustain periods of valve closure in response to environmental perturbation (Cavanaugh, 1983; Orton, 1937; Pennec et al., 1995; Vismann, 1991). Filtration rate has been considered to be the most significant driver of scope for growth (Barber et al., 1991; Hutchinson and Hawkins, 1992). Therefore, sea bed oysters might have had a reduced growth rate during the summer months in Poole Bay. In accordance with our results, Bartol et al. (1999) reported that growth rates of *C. virginica* were greatest at sub-tidal locations and they grew faster at the surface of constructed reefs compared with greater depths. Comeau et al. (2010) explained that preferable food flux conditions in the upper water column improved *C. virginica* growth. Suspended oysters required 3 to 4 years to grow to market size but 5-8 years were usually needed to complete a crop of oysters directly laid on the sea floor (Bataller et al., 1999; Comeau, 2013). Wheat and Ruesink (2013) found that the field filtration potential of on-bottom oysters (*C. gigas*) was lower than those suspended above the bottom on long-line aquaculture. Oysters cultured on elevated artificial structures, therefore, appear to improve filtration rates over those on the sea bed.

This study also confirmed previous publications (Buxton et al., 1981; Mathers, 1974; Rodhouse, 1977) that sampling interval in context of sea water temperature significantly affected the filtration rate of *O. edulis*. The highest filtration rate was observed in August 2013 when the temperature rose to 18ºC. Similarly, Walne (1972) described the ideal temperature for optimum filtration rates was close to 20ºC. Likewise, Buxton et al. (1981) reported that the maximum filtration rate was between 15 and 20ºC.

Respiration rate has been used as a physiological indicator of bivalve catabolism (Goulletquer et al., 1999; Huang and Newell, 2002; Rodhouse, 1977) and several studies have reported that the metabolic performance of marine bivalves can respond to the seasonal change of sea water temperature (Ezgeta-Balic et al., 2011; Bayne et al., 1999; Buxton et al., 1981; Dame et al., 1992; Haure et al., 1998; Sytniki and Zolotnitskiy, 2014). The data from this

study supports those previous publications in showing that sampling interval, in the context of temperature, is also a key factor controlling oxygen consumption in oysters. Respiration rates of both sea bed and reef oysters showed significant seasonal variation that reached maximum values in August 2013 (18˚C) and the lowest values in April 2013 (7˚C). Therefore, it can be concluded that there was no significant difference in the metabolic rate, inferred from respiration rate, between reef and sea bed oysters, but that the metabolic rate of all oysters was positively influenced by increased temperature.

Condition index has been widely used as an indicator to investigate bivalve physiology, especially in aquaculture (Filgueira et al., 2013; Pogoda et al., 2011). In this study, there was no significant effect of elevation (reef/sea bed) on the condition index of oysters. The condition indices of bivalves have also been usually applied as an economic indicator of market outcomes (Orban et al., 2002). Significantly, seasonal variation in condition index was observed in this study. Similarly, previous publications confirmed that temperature generally controlled the condition index of marine bivalves (Denis et al., 1999; Sasikumar and Krishnakumar, 2011). Several studies have shown a significant relationship between condition index and chlorophyll *a* (Helson et al., 2007; Hickman et al., 1991; Perez-Camacho et al., 1995). Tissue weight of bivalves varies during the seasonal cycle, normally based on gonadal maturation of animals and therefore affects the condition index value (Boscolo et al., 2003; Li et al., 2009; Pogoda et al., 2011). In addition to these sources of variability on condition index, the relation between condition index and gonadal maturation will be discussed in Chapter 5.

Haemolymph protein levels fluctuate with changes in environmental and physiological conditions and play fundamental roles in the physiology of oysters and marine crustaceans from oxygen transport, osmoregulation, reproduction and stress responses (Hagerman, 1983; Li et al., 2010; Lorenzon et al., 2008). Data in this study confirm that the haemolymph protein concentration of *O. edulis* showed seasonal variations. The lowest level of haemolymph protein was found in August 2013 when water temperature was highest and oysters were either ripe or spent (Chapter 5). Successful reproduction is an energetically demanding process (Fisher and Newell, 1986). Therefore, a possible explanation to account for this decline in protein

concentration post spawning is the contribution made by proteins to gametogenesis (Li et al., 2009). This finding is supported by previous publications, which have identified that seasonal variations in total protein concentrations in haemolymph serum were linked to reproductive phases in a number of bivalves such as the Eastern oyster *C. virginica* (Fisher and Newell, 1986), Pacific oyster *C. gigas* (Li et al., 2009; Luna-Gonzalez et al., 2008), the blue mussel *M. edulis* (Mulvey and Feng, 1981) and the giant scallop *Plactopecten magellanicus* (Thompson, 1977). Moreover, Luna-Gonzalez et al. (2008) also reported that levels of total proteins correlated with environmental conditions i.e. protein levels remained low in summer when water temperatures were high and animals were possibly faced with parasite infection. However, in this study, there was no significant effect of elevation (reef/sea bed) on the haemolymph proteins of *O. edulis*.

Bivalve haemocytes form an important line of defence against pathogens and are also susceptible to environment changes (Comesana et al., 2012; Fisher, 1987; Hauton et al., 2000; Laureau et al., 2003). They have been used as an indicator of immune performance. da Silva et al. (2008) identified that oysters from different geographical areas with different environmental conditions have significant differences in their circulating haemocyte population. It has been widely documented that seasonal variation influences the total number, types, and behaviour of haemocytes in bivalves (Fisher, 1988; Fisher et al., 1996; Hauton et al., 1998; Oliver and Fisher, 1995; Mccormick-Ray and Howard, 1991). However, to date, no study has reported the effects of elevation (reef/sea bed) on the circulating haemocyte population of *O. edulis*. In this study, the total haemocyte and granulocyte counts were significantly affected by elevation and sampling interval. The concentration of total haemocytes was highest in August 2013 and lowest in September 2012, with elevated reef oysters exhibiting higher haemocytes especially during warm summer months. Therefore, we contend that these reef oysters would have had a favourable energy budget, which permitted higher haemocyte and specifically granulocyte densities. Granulocytes have a higher phagocytic ability in comparison to hyalinocytes (Chu, 2000; Fisher, 1988) and contain a greater abundance of lysosomes that contribute to intracytoplasmic pathogen degradation (Carballal et al., 1997; Cheng, 1981; Lopez et al., 1997) through the release of lysosomal enzymes (Cheng, 1981; Montes et al., 1995). Thus, granulocytes are believed

to be more efficient in killing microorganisms than hyalinocytes and have been demonstrated to produce more reactive oxygen species in both stimulated and non-stimulated conditions (Lambert et al., 2003; Soudant et al., 2013). Da Silva et al. (2008) suggested that high granulocyte concentrations would improve *O. edulis* immune performance and consequently would contribute to longer lifespan. Therefore, higher total haemocyte and granulocyte counts would suggest that the elevated reef habitat benefitted the immune performance of *O. edulis* and might contribute to an increased ability to endure protozoan parasite infection.

Bonamia spp. has been diagnosed in oysters collected from various ecosystems from estuaries and intertidal zones to deep coastal waters (Arzul et al., 2009). In this study, the results of nested PCR suggested that all oysters collected from Poole Harbour (100%) were infected with *Bonamia* sp. Also, the prevalence of *Bonamia* sp. infection for natural Poole Harbour oysters was higher compared with relocated oysters in the experimental site, Poole Bay (reef and sea bed oysters). The prevalence of *Bonamia* sp. infection in reef oysters (87%) was slightly higher than sea bed oysters (66%). In accordance with our results, previous studies by Bucke (1988) and Montes et al. (1994) suggested that *B. ostreae* potentially infects oysters during filtration or respiration. Therefore, it could be argued that increasing the filtration rate for reef oysters as well as the presence of intermediate hosts (Lynch et al., 2007) at elevated reefs (result in Chapter 7, e.g. *Ophiotrix fragilis*) may increase the likelihood of uptake of the protozoan parasite, *Bonamia* sp. However, as the life cycle of this protozoan parasite is still unknown, the significance of intermediate hosts on the reefs remains uncertain. Therefore, this gap of knowledge needs to be clarified and included in further research.

The method, nested PCR, used to detect *Bonamia* sp. in this study is more sensitive than other methods (e.g. light microscopy, histology, single-round PCR (OIE protocol (World Organisation for Animal Health, 2012)) that have been previously used. This could explain why the high percentage of prevalence of *Bonamia* sp. infection was observed in this study. Previous studies reported lower prevalence of *B. ostreae*. Arzul et al. (2006) used histology to investigate the prevalence of *B. ostrea*e infection in the Bay of Quiberon, South Brittany, France, an interesting site to study bonamiosis in flat oyster populations because flat oysters there have been infected since 1979. They found that the

prevalence of infection seasonally varied from 2 to 37% and the mean was only 13%. Kamphausen (2012) detected *B. ostreae* infection in Solent oysters using PCR technique (one round PCR) and found that only eight percent of oysters were infected. It appeared likely that although the prevalence of *Bonamia* infection in this study was high, it is possible that the intensity of infection was very low as the result from first round PCR showed no positive amplicons. This study suggested that, in order to confirm that oysters are uninfected, nested PCR is required since primary PCR is possibly not sensitive enough to detect *Bonamia* infection at low intensity.

To date, no publication has clearly identified the mechanisms by which *Bonamia* sp. damages *O. edulis* tissues. In this study it is not clear what the physiological impact of *Bonamia* sp. on the oysters was. Robert et al. (2009) suggested that *O. edulis* mortality correlated with a high intensity of *B. ostreae* infection. It is possible that the infection at low intensity in this study would not significantly affect oyster physiology. The link between parasite load and protein levels in bivalve haemolymph has also been documented. Ford (1986) suggested that heavily infected oysters (*C. gigas*) had about 30% of the protein levels of uninfected individuals. However, in this study, no significant differences in the concentration of haemolymph proteins (Section 3.3.4) between reef and sea bed oysters were found. Therefore, based on this finding, there would be no differential impact on physiology between reef and sea bed oysters as a result of *Bonamia* sp. infection. To confirm this argument, the direct measurement of the *Bonamia* sp. impacts on physiological performance of *O. edulis* (e.g. phagocytosis and respiratory burst) needs to be addressed in future investigations.

Although infections by *B. ostreae* can be detected throughout the year (Culloty and Mulcahy, 1996), prevalence and the intensity of infection tends to rise during the warm season with highest prevalence occurring in spring (Engelsma et al., 2010). Indeed Rowley et al. (2014) recently reported that *B. ostreae* outbreaks occurred between 12–20˚C, and may be limited by low temperatures. In contrast, a study of bonamiosis prevalence as well as haemocyte activities showed that prevalence was higher at low temperature (10˚C) compared to higher temperature (20˚C), suggesting that low temperatures may affect immunocompetence of the host and/or the ability of the parasite to infect healthy oysters (Cochennec et al., 2000). Therefore, future work should

establish the importance of *Bonamia* sp. in long-term oyster reef health as well as investigating long-term temperature studies and the reservoirs of infection.

In conclusion, this study has shown that elevation (reef/sea bed) affected the physiological performance of *O. edulis*. The European flat oyster on top of elevated reefs (80 cm above sea bed) had significantly higher filtration rates compared with oysters on the sea bed. The elevated reef oysters had significantly greater numbers of total haemocytes and granulocytes in comparison with sea bed oysters. It could be argued that increasing the filtration rate for reef oysters may increase the likelihood of uptake of the protozoan parasite, *Bonamia* sp. However, the intensity of *Bonamia* sp. infection was very low. Based on the fact that haemocytes are important in terms of immune defence in the host, and reef oysters had significantly higher numbers of total haemocytes and granulocytes than sea bed oysters, this may increase the potential of reef oysters to survive infection. The concentration of haemolymph proteins also implied that there was no significant effect of *Bonamia* infection on *O. edulis* physiology. This chapter has demonstrated that there are some benefits to oyster physiology from reef habitat, but increasing the filtration rate for reef oysters may increase the possibility of uptake of the protozoan parasite, *Bonamia* sp. However, before this management method can be advocated for widespread use, it is worth investigating the typical variation in physiological performance between different populations of *O. edulis*. Is the improved physiology of reef oysters still notable when comparing between different populations or is the variation in physiological performance between populations just as great as the difference between reef and sea bed habitats? Therefore, to answer this question, the following chapter explores the variation in physiological performance of oysters between different geographic locations.

Chapter 4

Geographic variation in filtration, respiration and condition index of *Ostrea edulis*

4.1 Introduction

Previous studies revealed that the physiological capability of several marine invertebrates varied with geographic locations (Bonsdorff and Wenne, 1989; Sommer and Pörtner, 2002). Invertebrate species such as the fiddler crab *Uca rupax*, the polychaetes *Clymenella torquata* and *Arenicola marina* and the prawn *Pandalus montagui* showed differing abilities to maintain metabolic rate with latitude. In addition, those species collected from higher latitudes had higher metabolic rates in comparison with those collected from lower lattitudes even when the metabolic rates were measured in controlled conditions at the same temperature (Fox and Wingfield, 1937; Mangum, 1963; Vernberg, 1962). In bivalves, Bonsdorff and Wenne (1989) studied the geographic variation in physiological performance of *Macoma balthica* between northern and southern Baltic Sea populations and found significant differences in the condition index resulting in slight differences in the time of onset of spawning (March-early May in south, and late April-May in the north). The variation in physiological performance of invertebrates between populations has been linked to the difference in environmental conditions and food availability between sites. Moreover, in aquaculture research, numerous studies have detected that the amount of biochemical reserves and the condition index can vary substantially among bivalves cultured in nearby sites within the same embayment such as *Mytilus galloprovincialis* (Baek et al., 2014; Fernandez-Reiriz et al., 1996) and *Austrovenus stutchburyi (*Norkko and Thrush, 2006), or in proximate habitats in a given coastal ecosystem such as *Crassostrea gigas* (Kang et al., 2000), *Mytilus edulis* (Okumus and Stirling, 1998) and *Perna viridis* (Sasikumar and

Krishnakumar, 2011), owing to spatial differences in chlorophyll *a* concentration, food availability and quality.

Hawkins et al. (1993) previously identified variation in physiological performance of *Ostrea edulis* between different locations. They investigated the variation in physiological performance of *O. edulis* collected from three different locations i.e. Loch Sween (Scotland), Conwy (north Wales), and the Solent (England). The oysters were collected from these locations and placed in the same area at an experimental site at Calamansack Creek in the river Helford, Cornwall, UK. The oyster samples were collected at 6 and 18 months after deployment. The results showed that there were significant differences in filtration rate and scope for growth between populations. Conwy oysters had significantly greater filtration rates and scope for growth than Loch Sween and the Solent oysters, while the lowest filtration rates and scope for growth were observed in Solent oysters. This study also suggested that the differences in oyster physiology between populations were probably a result of phenotypic plasticity as enzyme electrophoresis showed the three populations to be genetically very similar, with little variation. Therefore, it is possible that although it is believed that the *O. edulis* in the UK is genetically one population as oysters were moved and relocated around the UK (Laing et al., 2005), the difference in water conditions between small geographic ranges might cause the variation in oyster physiology.

Recently, Pogoda et al. (2011) investigated the growth performance of *O. edulis* and *C. gigas* exposed to offshore conditions in the North Sea, Germany. They found that although there was no significant difference in water temperature and salinity between sampling sites, water quality, e.g. natural diet concentration, varied. This affected the growth performance of both oyster species between locations. This agreed with the study of Andrews (1979) which reported that differences in environmental regimes between oyster grounds influenced their ecotypes and physiology. Therefore, the assessment of the physiological performance of oysters inhabiting different locations is essential in order to evaluate the effects of spatial variation in the context of these different environments.

The findings in Chapter 3 suggest that raised reef structures of 80 cm above the sea bed could significantly improve the filtration rate of *O. edulis* and

ultimately result in enhancement of immune performance i.e. increasing the total numbers of haemocytes, especially granulocytes. However, spatial/geographic variation in the physiological performance of *O. edulis*, in this study, has not yet been addressed. In order to investigate physiological performance of *O. edulis* between different geographic locations, replicates were collected at the same time from three different wild populations of *O. edulis* in Poole Harbour, Chichester Harbour and the Solent. Therefore, the objective of the investigation was to compare the physiological performance of *O. edulis* between these locations. Key indicators of the physiological potential of *O. edulis* in this chapter include filtration rate, respiration rate and condition index.

4.2 Methods

The findings in Chapter 3 suggested that oysters had highest filtration and respiration rates in summer because higher temperatures facilitated increased physiological performance. Previous studies also suggested that *O. edulis* had maximum growth rates in the summer period (Brooks, 1994; Pogoda et al., 2011). Therefore, in order to investigate spatial variation in their filtration and respiration rates, wild oysters on the south coast of the UK (Poole Harbour, Chichester Harbour and the Solent) (Figure 4.1) were collected three times during summer (May 2014, June 2014 and August 2014). The Poole oysters (65-89 mm) were collected using oyster dredge by Othniel Oysters Ltd. Solent oysters (62-87 mm) were also collected using an oyster dredge on the RV *Bill Conway*, while Chichester oysters (61-83 mm) were collected using a grab sampler at the Itchenor pontoon. Environmental variables (total suspended solids and chlorophyll *a* concentration) as well as oyster respiration rate, filtration rate and condition index were measured and compared between the sampling locations.

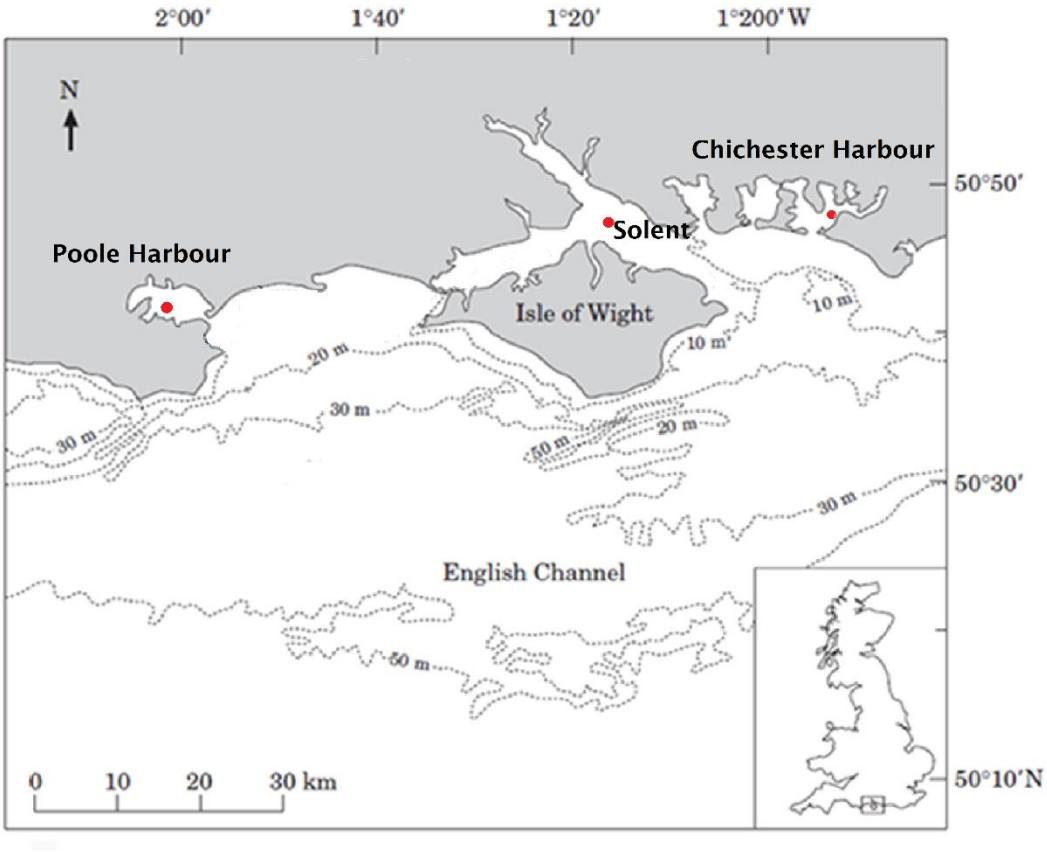

Figure 4.1 Sampling locations included Poole Harbour, the Solent and Chichester Harbour on the south coast of the UK.

4.2.1 Environmental conditions

The field temperature and salinity were recorded at the time of collection. One litre of water at each sampling location was also collected in order to measure chlorophyll *a* concentration and total suspended solids using the methods as described in Chapter 2.

4.2.2 Oyster samples

At each sampling interval, a total number of 45 individuals, 15 oysters from each location, were collected. They were carefully scrubbed to remove epifaunal species, measured for total length (mm), weight (g) and volume (determined by displacement of water (ml)). Oyster samples from each location were left in water baths with sea water collected from each sampling site, provided with oxygen via air pumps and regulated to the same temperature as

that in the field at the time of collection. *O. edulis* were acclimated for two days prior to commencement of experiments.

4.2.3 Oyster filtration and respiration rates

Filtration and respiration rate measurements were carried out using the protocol described in Chapter 3. For this study, filtration and respiration rate measurements were made twice. First, filtration and respiration measurements were carried out in natural field water collected from each location. Oyster samples were then left in water baths in NOCS aquarium seawater sourced from Southampton Water for two weeks. In the meantime, every 3 days water changes were made and all oysters were fed with *Isochrysis galbana* (20,000-30,000 cells ml^{-1}). After two weeks in aquarium conditions, the second filtration and respiration measurements were made. Oyster populations were maintained in the NOCS aquarium water to test short-term effects of environmental changes on *O. edulis* physiology and also to test the stability of respiration and filtration rates in oysters collected from each location. After the second measurement, the oysters were shucked, dried for 48 hours at 80°C (DW), then ashed for 6 hours at 500 °C (Rodhouse, 1977) and weighed again for ash weight (AW).

4.2.4 Condition index

Condition index was calculated using the same method as described in Chapter 3.

4.2.5 Statistical analyses

Data (filtration rate, respiration rate and condition index) were first tested for normality and homogeneity of variance. Two-way ANOVA was performed to test how those physiological indicators varied with locations and sampling months. Where appropriate, *post-hoc* pairwise multiple comparisons (Tukey's HSD tests) compared the effect of sampling months on the physiological indicators between sampling intervals. Tukey's HSD tests also compared the effect of locations on the physiological indicators between locations. Comparisons of environmental conditions (chlorophyll *a* concentration and total suspended solids) among three locations were made using Kruskal-Wallis

test and *post-hoc* pairwise multiple comparisons (Dunn's multiple comparison test) compared the concentration of chlorophyll *a* concentration and total suspended solids between locations. In all cases, statistical significance was accepted at $\alpha = 0.05$.

4.3 Results

4.3.1 Environmental conditions

There was no significant difference in field water temperatures between Poole Harbour, Chichester Harbour and the Solent for any sampling interval. The field temperatures were 13˚C (May 2014), 15˚C (June 2014) and 16˚C (August 2014). Salinities slightly varied between locations as showed in Table 4.1. There were significant differences in total suspended solids (TSS) and chlorophyll *a* concentrations between locations (Kruskal-Wallis test, $P<0.05$ and $P<0.05$, respectively). Additionally, TSS in the water column in Chichester Harbour were significantly higher than Poole Harbour and the Solent at each sampling interval (Dunn's test, $P<0.05$ and $P<0.05$, respectively) but there was no significant difference in TSS between Poole Harbour and the Solent (Figure 4.2). Chlorophyll *a* concentrations in the water column in the Solent were significantly lower compared with chlorophyll *a* concentrations in Poole and Chichester Harbours in May and June 2014 (Dunn's test, $P<0.05$ and $P<0.05$, respectively) but there were no significant differences in chlorophyll *a* concentrations between Poole and Chichester Harbours at every sampling interval (Figure 4.3).

Table 4.1 Water temperatures and salinities measured at sampling locations

Sampling months	Locations	Temperature (˚C)	Salinity
May	Poole Harbour	13	29
	Chichester Harbour	13	29
	The Solent	13	32
	NOCS aquarium	13	29
June	Poole Harbour	15	30
	Chichester Harbour	15	29
	The Solent	15	33
	NOCS aquarium	15	30
August	Poole Harbour	16	31
	Chichester Harbour	16	30
	The Solent	16	34
	NOCS aquarium	16	31

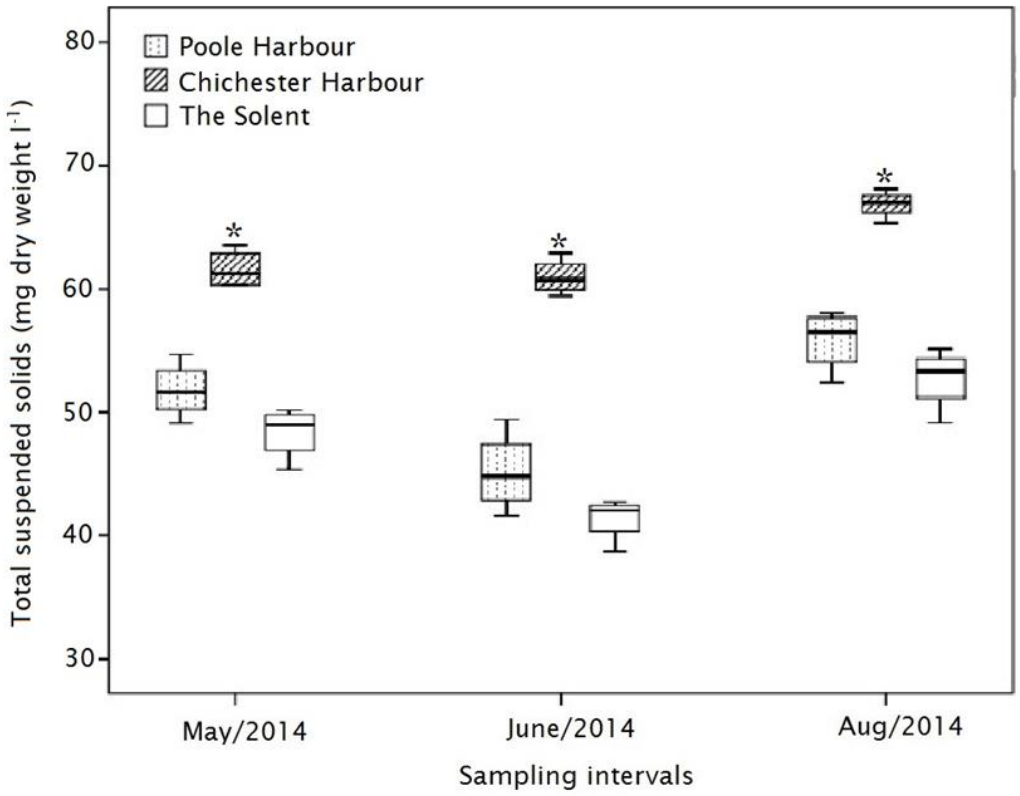

Figure 4.2 Total suspended solids (mg dry weight l^{-1}) at each sampling location (n=4). Significant differences (Dunn's test, P<0.05) between sampling locations are denoted by an asterisk for each sampling event.

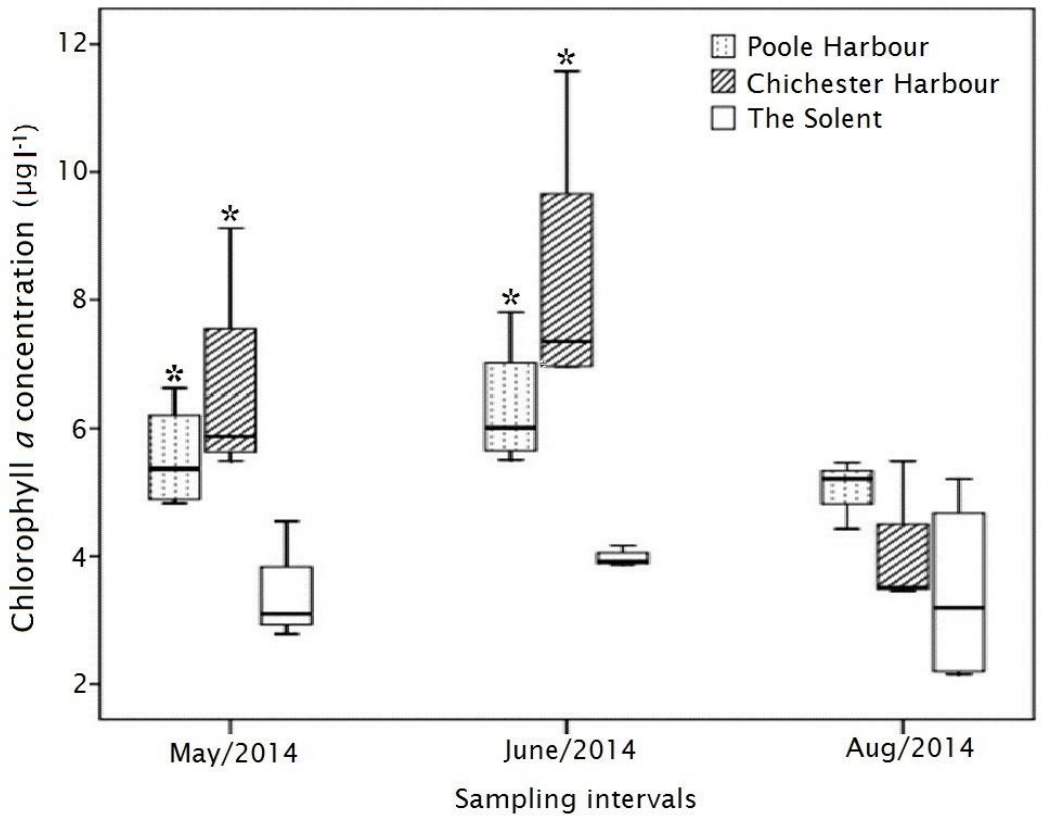

Figure 4.3 Chlorophyll *a* concentration (µg l^{-1}) at each sampling location (n=4). Significant differences between sampling locations are denoted by an asterisk for each sampling event.

4.3.2 Spatial variation in filtration rates of *Ostrea edulis* in field water conditions

At each sampling interval, oyster samples from Poole Harbour (n=15), Chichester Harbour (n=15) and the Solent (n=15) were collected to test the variation in filtration rates of oysters between different geographic locations. Filtration rates (mean ± SE) are shown in Figure 4.4. Two-way ANOVA identified a significant effect of location and sampling interval ($P< 0.001$ and $P< 0.001$, respectively) on *O. edulis* filtration rates but no interaction between the main two effects (Table 4.2). Tukey's HSD pairwise tests showed that filtration rates of oysters collected from Poole Harbour were significantly higher compared with oysters collected from Chichester Harbour and the Solent ($P<0.05$ and $P<0.001$, respectively) but there was no significant difference in filtration rates between Chichester and Solent oysters (Table 4.3). Tukey's HSD pairwise tests

also suggested that there was no significant difference in filtration rates of oysters collected in June and August 2014 but these rates were significantly higher compared with oysters sampled in May 2014 ($P<0.001$) (Table 4.4). The filtration rates of Poole Harbour (1.90±0.09 l h^{-1} g^{-1} AFDW), Chichester Harbour (1.73±0.10 l h^{-1} g^{-1} AFDW) and Solent oysters (1.70±0.11 l h^{-1} g^{-1} AFDW) in May 2014 were the lowest rates observed, while the highest filtration rates of Poole Harbour (3.09±0.08 l h^{-1} g^{-1} AFDW), Chichester Harbour (2.69± 0.09 l h^{-1} g^{-1} AFDW) and the Solent oysters (2.49±0.09 l h^{-1} g^{-1} AFDW) were observed in August 2014 when the water temperature was 16^0C.

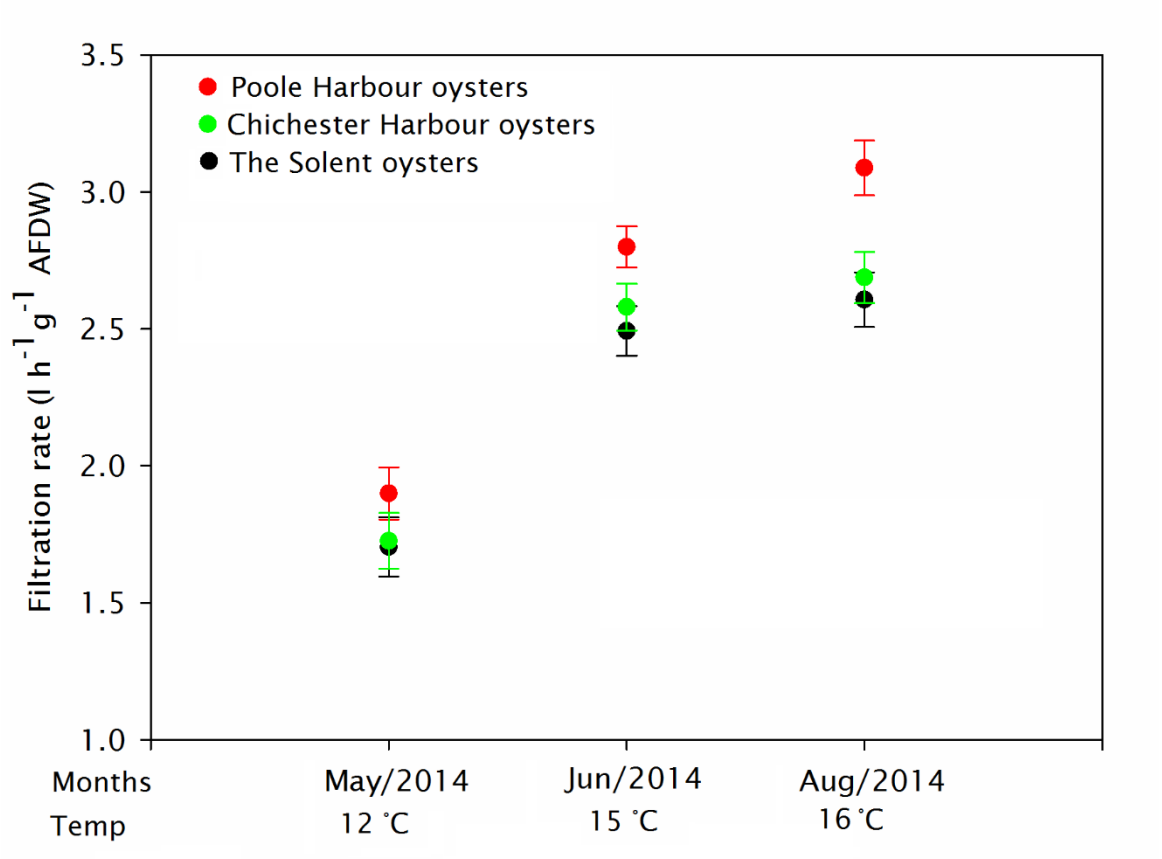

Figure 4.4 Filtration rates of *Ostrea edulis* (l h^{-1} g^{-1} AFDW, mean ± SE) collected from Poole Harbour (n=15), Chichester Harbour (n=15) and the Solent (n=15) on each sampling occasion. These filtration rates were measured after two day acclimation in field water collected from each location. The filtration rate was affected by sampling intervals and location (two-way ANOVA, $P<0.001$ and $P<0.001$, respectively).

Table 4.2 Comparing the effect of sampling interval, location, and the interaction month x location on filtration rate (l $h^{-1}g^{-1}$AFDW) after two day acclimation in field water. Significant differences are denoted by asterisks.

Sources of variation	df	F	P-value
Sampling interval	2	99.49	0.001*
location	2	10.13	0.001*
Sampling interval x location	4	2.11	0.611

Table 4.3 *Post-hoc* (Tukey's HSD test) results comparing the effect of location on filtration rate (l $h^{-1}g^{-1}$AFDW) after two day acclimation in field water. "ns" represents no significant difference.

Post-hoc test the effect of location	P value		
	Poole	Chichester	Solent
Poole Harbour		<0.05	<0.001
Chichester Harbour			ns

Table 4.4 *Post-hoc* (Tukey's HSD test) results comparing the effect of sampling interval or temperature on filtration rate (l $h^{-1}g^{-1}$AFDW) after two day acclimation in field water. "ns" represents no significant difference.

Post-hoc test the effect of sampling interval	P value		
	May 2014 (12˚C)	Jun 2014 (15˚C)	Aug 2014 (16˚C)
May 2014		<0.001	<0.001
Jun 2014			ns

4.3.3 Spatial variation in filtration rates of *Ostrea edulis* acclimated to NOCS aquarium water

Filtration rates (mean ± SE) are shown in Figure 4.5. Location and sampling interval significantly affected *O. edulis* filtration rates (two-way ANOVA, P< 0.001 and P< 0.005, respectively) but with no significant interaction between the main two effects (Table 4.5). Tukey's HSD pairwise tests showed that the filtration rates of oysters collected from Poole Harbour were significantly higher than in oysters collected from Chichester Harbour and the Solent (P<0.001and P<0.001, respectively) but there was no significant difference in filtration rates between Chichester and Solent oysters (Table 4.6). Tukey's HSD pairwise tests also suggested that there was no significant difference in filtration rates of oyster samples collected in June and August 2014 but these rates were significantly higher than oysters sampled in May 2014 (P<0.01and P<0.005, respectively) (Table 4.7). The filtration rates measured in the aquarium water of Poole Harbour (1.82±0.12 l h^{-1} g^{-1} AFDW), Chichester Harbour (1.71±0.07 l h^{-1} g^{-1} AFDW) and the Solent oysters (1.62±0.10 l h^{-1} g^{-1} AFDW) collected in May 2014 were at the lowest rate, while the highest filtration rates of Poole Harbour (2.88±0.11 l h^{-1} g^{-1} AFDW), Chichester Harbour (2.60± 0.11l h^{-1} g^{-1} AFDW) and the Solent oysters (2.44±0.11 l h^{-1} g^{-1} AFDW) were measured in oysters sampled from August 2014.

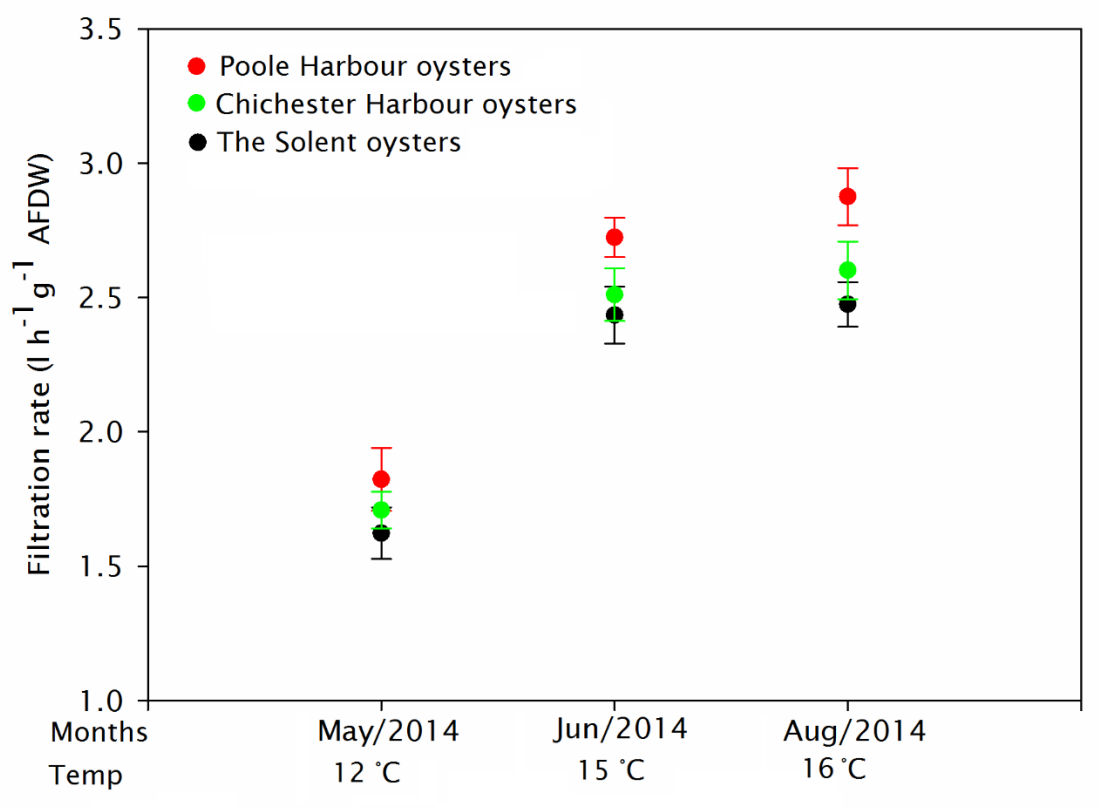

Figure 4.5 Filtration rates of *Ostrea edulis* (l h^{-1} g^{-1} AFDW, mean ± SE) collected from Poole Harbour (n=15), Chichester Harbour (n=15) and the Solent (n=15) on each sampling interval. These filtration rates were measured after two weeks acclimation to NOCS aquarium water. The filtration rate was affected by sampling interval and location (two-way ANOVA, P<0.001 and P<0.001, respectively).

Table 4.5 Two-way ANOVA results comparing the effect of sampling interval, location, and the interaction month x location on filtration rate (l $h^{-1}g^{-1}$AFDW) measured after two weeks acclimation to NOCS aquarium water. Significant differences are denoted by asterisks.

Sources of variation	df	F	P-value
Sampling interval	2	6.51	0.002*
location	2	38.86	0.001*
Sampling interval x location	4	2.18	0.075

Table 4.6 *Post-hoc* (Tukey's HSD test) results comparing the effect of location on filtration rate (l $h^{-1}g^{-1}$AFDW) measured after two weeks acclimation to NOCS aquarium water. "ns" represents no significant difference.

Post-hoc test the effect of location	P value		
	Poole	Chichester	Solent
Poole Harbour		<0.001	<0.001
Chichester Harbour			ns

Table 4.7 *Post-hoc* (Tukey's HSD test) results comparing the effect of sampling interval or temperature on filtration rate (l $h^{-1}g^{-1}$AFDW) measured after two weeks acclimation to NOCS aquarium water. "ns" represents no significant difference.

Post-hoc test the effect of sampling interval	P value		
	May 2014 (12˚C)	Jun 2014 (15˚C)	Aug 2014 (16˚C)
May 2014		<0.01	<0.005
Jun 2014			ns

4.3.4 Spatial variation in respiration rates of *Ostrea edulis* in field water conditions

Respiration rates were measured to identify differences in metabolic rate with geographic location. The respiration rate (mean ± SE) of oysters collected from each location at each sampling event is shown in Figure 4.6. The respiration rates of Poole Harbour (0.58±0.06 ml O_2 h^{-1} g^{-1} AFDW), Chichester Harbour (0.74±0.06 ml O_2 h^{-1} g^{-1} AFDW) and the Solent oysters (0.62±0.07 ml O_2 h^{-1} g^{-1} AFDW) in May 2014 were the lowest, while the highest respiration rates of Poole Harbour (0.68±0.08 ml O_2 h^{-1} g^{-1} AFDW), Chichester Harbour (0.82± 0.08 ml O_2 h^{-1} g^{-1} AFDW) and the Solent oysters (0.71±0.09 ml O_2 h^{-1} g^{-1}AFDW) were recorded in August 2014 when the water temperature was 16^0C. Two-way ANOVA indicated that there were no significant effects of sampling months (in summer) and location on respiration rates. Also, there was no interaction

between the two main effects (location and sampling interval) on respiration rate (Table 4.8).

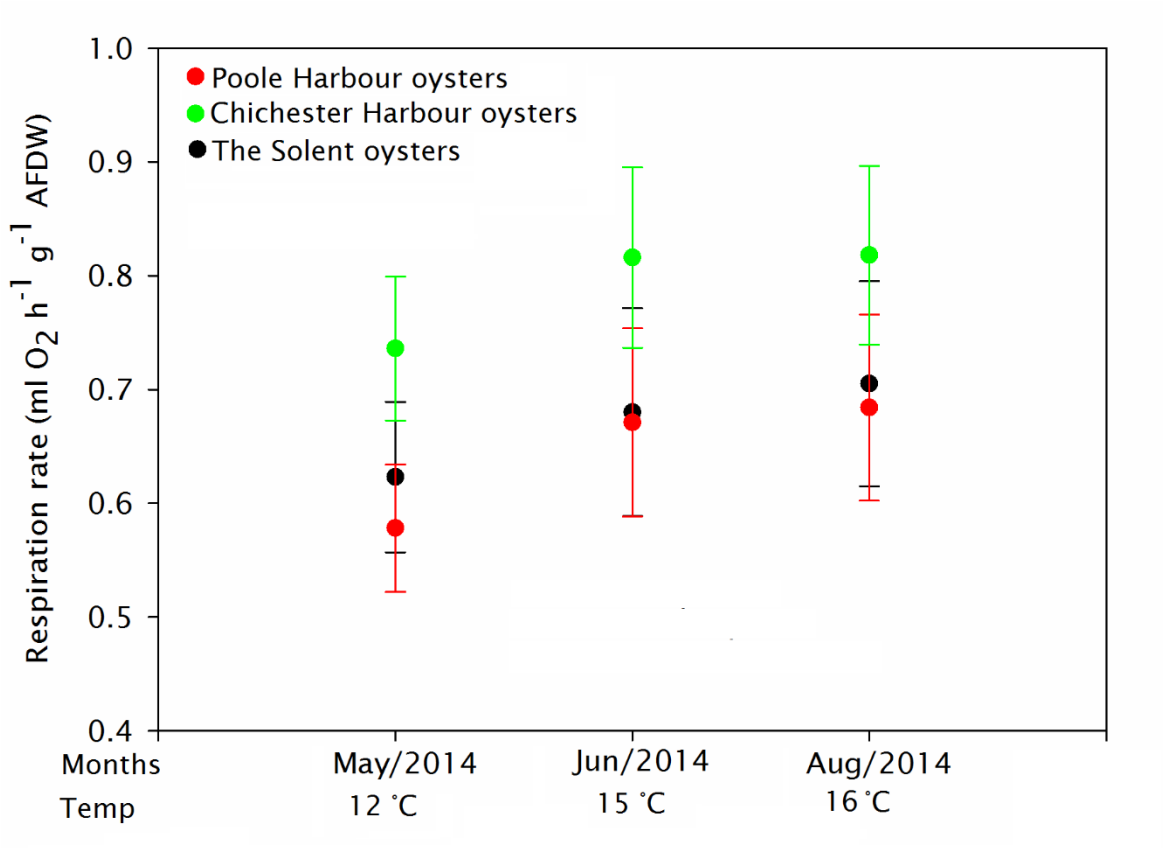

Figure 4.6 Respiration rates (l h^{-1} g^{-1} AFDW, mean ± SE) of *Ostrea edulis* collected from Poole Harbour (n=15), Chichester Harbour (n=15) and the Solent (n=15) on each sampling occasion. These respiration rates were measured after two day acclimation in field water collected from each location. There was no significant effect of spatial variation and sampling interval (in summer) on respiration rate (two-way ANOVA, $P>0.05$).

Table 4.8 Two-way ANOVA results comparing the effect of sampling interval, location, and the interaction month x location on respiration rate (ml $O_2 h^{-1} g^{-1}$ AFDW) of *O. edulis* measured after two day acclimation in field water.

Sources of variation	df	F	P-value
Sampling interval	2	2.06	0.132
location	2	2.17	0.118
Sampling interval x location	4	0.53	0.711

4.3.5 Spatial variation in respiration rates of *Ostrea edulis* acclimated to NOCS aquarium water

Oyster populations were reconditioned in NOCS aquarium water in order to test the respiration rate response after short-term environmental acclimation. After a 2 week acclimation period in the NOCS aquarium the respiration rates of Poole Harbour (0.53±0.09 ml $O_2 h^{-1} g^{-1}$ AFDW), Chichester Harbour (0.65±0.07 ml $O_2 h^{-1} g^{-1}$ AFDW) and the Solent oysters (0.52±0.06 ml $O_2 h^{-1} g^{-1}$ AFDW) were lowest in May 2014 (Figure 4.7). The highest respiration rates of Poole Harbour (0.60±0.06 ml $O_2 h^{-1} g^{-1}$ AFDW), Chichester Harbour (0.68± 0.09 ml $O_2 h^{-1} g^{-1}$ AFDW) and the Solent oysters (0.63±0.08 ml $O_2 h^{-1} g^{-1}$ AFDW) were observed in August 2014 when the water temperature was 16^0C. Two-way ANOVA suggested that there was no significant effects of sampling months (in summer) and location on respiration rates. Also, there was no interaction between the two main effects (location and sampling interval) on respiration rate (Table 4.9).

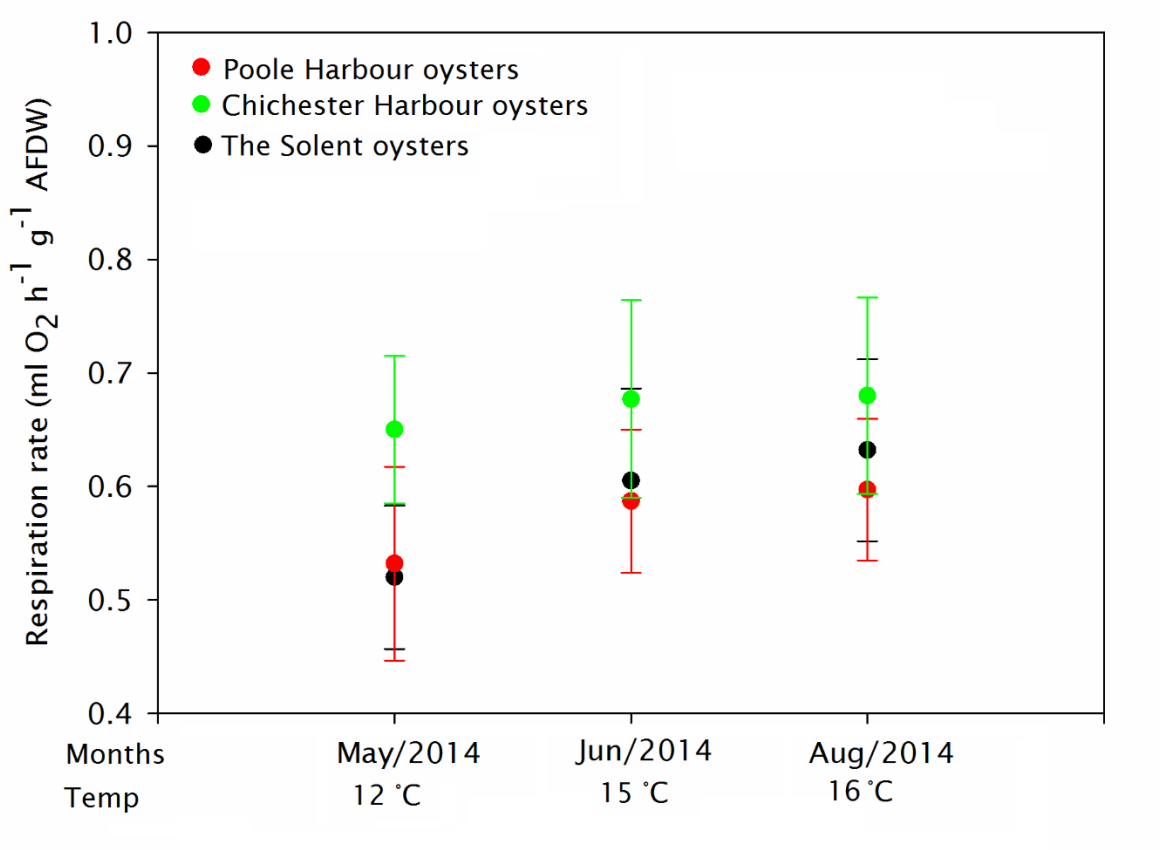

Figure 4.7 Respiration rates (ml O_2 h^{-1} g^{-1} AFDW, mean ± SE) of *Ostrea edulis* measured after two weeks acclimation to NOCS aquarium water during each sampling interval. Respiration rates of oysters collected from Poole Harbour (n=15), Chichester Harbour (n=15) and the Solent (n=15) were not significantly affected by spatial variation and sampling interval (in summer) (two-way ANOVA, P>0.05).

Table 4.9 Two-way ANOVA results comparing the effect of sampling interval, location, and the interaction month x location on respiration rate (ml O_2 h^{-1} g^{-1} AFDW) of oysters after two weeks acclimation to NOCS aquarium water.

Sources of variation	Df	F	P-value
Sampling interval	2	0.956	0.387
Location	2	1.107	0.334
Sampling interval x location	4	0.267	0.898

4.3.6 Spatial variation in condition index of *Ostrea edulis*

Condition index of each oyster collected from each location was measured as a general integrated measure of physiological performance. The mean condition index of oyster soft tissue varied between location and sampling interval (Figure 4.8). Two-way ANOVA indicated that location and sampling interval had a significant effect on the condition index ($P<0.05$ and $P<0.01$, respectively) but there was no interaction between the two main effects (location and sampling interval) on condition index (Table 4.10). Pairwise multiple comparisons (Tukey's HSD) showed that the condition index of oysters collected from Poole Harbour were significantly higher than oysters collected from Chichester Harbour and the Solent ($P<0.05$ and $P<0.01$, respectively) but there was no significant difference in the condition index between Chichester and Solent oysters (Table 4.11). Tukey's HSD pairwise tests also showed that the condition index of oysters collected in June 2014 was significantly higher compared with oysters collected in May and August 2014 ($P<0.001$ and $P<0.001$, respectively, Table 4.12). In May 2014, the lowest condition index (mean ± SE) of Poole Harbour, Chichester Harbour, and the Solent oysters were observed (2.91±0.16, 2.66±0.17 and 2.46±0.14, respectively), while the highest condition index of oysters were recorded in June 2014 (4.25±0.22, 3.80±0.20 and 3.61± 0.21, respectively).

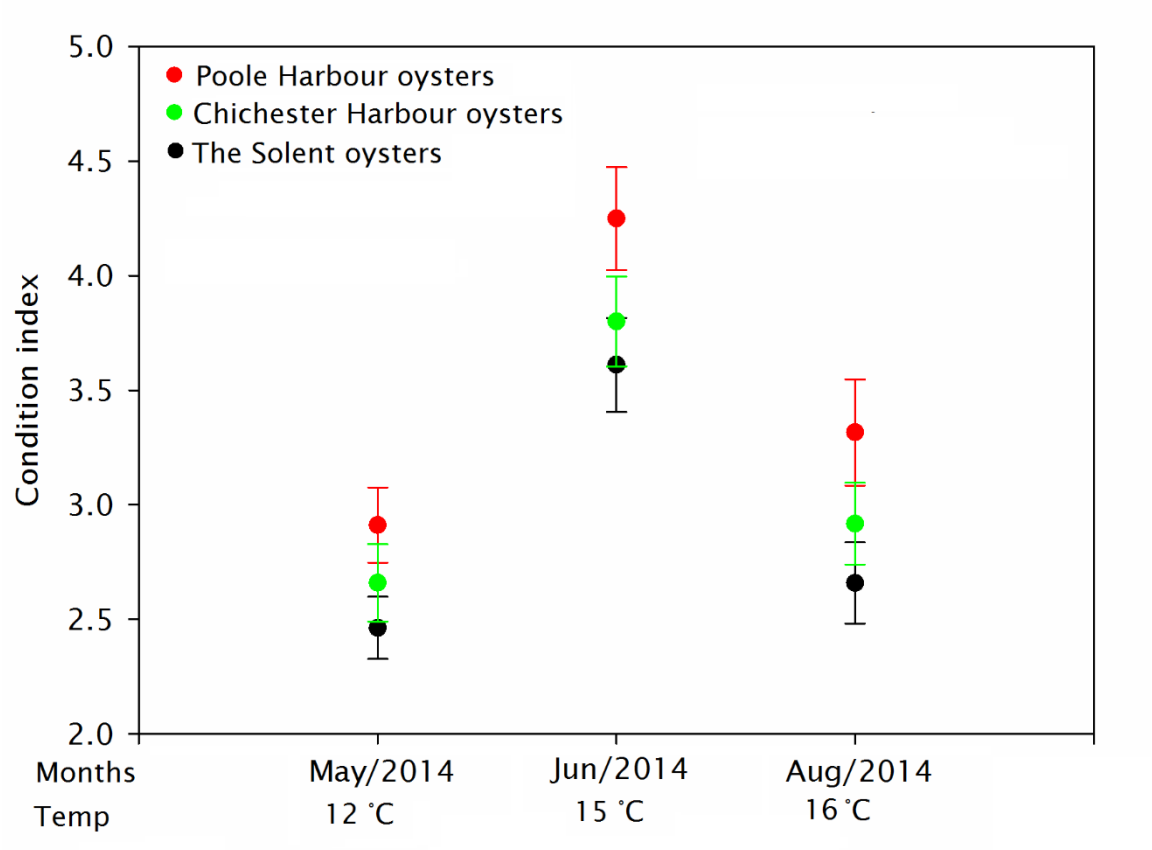

Figure 4.8 Condition index of *Ostrea edulis* collected from Poole Harbour (n=15), Chichester Harbour (n=15) and the Solent (n=15) on each sampling occasion. The condition index was affected by location and sampling interval (two-way ANOVA, $P<0.05$ and $P<0.01$, respectively).

Table 4.10 Two-way ANOVA results comparing the effect of sampling interval, location, and the interaction month x location on condition index. Significant differences are denoted by asterisks.

Sources of variation	df	F	P-value
Sampling interval	2	9.67	0.01*
Location	2	6.13	0.05*
Sampling interval x location	4	1.98	0.30

Table 4.11 *Post-hoc* (Tukey's HSD test) results comparing the effect of location on condition index. "ns" represents no significant difference.

Post-hoc test the effect of location	P value		
	Poole	Chichester	Solent
Poole Harbour		<0.05	<0.01
Chichester Harbour			ns

Table 4.12 *Post-hoc* (Tukey's HSD test) results comparing the effect of sampling interval or temperature on condition index. "ns" represents no significant difference.

Post-hoc test the effect of sampling interval	P value		
	May 2014 (12˚C)	Jun 2014 (15˚C)	Aug 2014 (16˚C)
May 2014		<0.001	ns
Jun 2014			<0.001

4.4 Discussion and conclusion

The relationship between environmental conditions and growth has been intensively studied in several bivalve species for aquaculture considerations (Dame, 1975; MacDonald and Thompson, 1985). In estuarine and marine systems, bivalve growth is related to a function of environmental factors e.g. temperature, salinity, water current and food quality (Cusson et al., 2005; Valero, 2006). Laing et al. (2005) reported that of all the abiotic factors that can affect the physiology of *O. edulis*, temperature and salinity are probably the two most important environmental variables affecting feeding, respiration, gonadal maturation, the time of spawning, recruitment, growth and mortality. Similarly, previous studies reported that temperature significantly influenced the physiological performance of bivalves. In particular, a temperature increase accelerated most physiological rates, including those involved in the energy balance, such as feeding, excretion and respiration of *O. edulis* (Haure et al., 1998; Johannesson et al., 1989; Korringa, 1952). Consistent with these publications, the growth rate of *O. edulis* was high in the summer when greater water temperatures and food availability allowed the oysters to consume the optimum amount of food dependent upon their water pumping capacity (Korringa, 1952). In this study, wild *O. edulis* were collected from three habitats (i.e. Poole Harbour, Chichester Harbour and the Solent) to assess and compare the physiological performance of these different oyster populations in response to local environmental conditions that routinely occur on the south coast of the UK. Environmental data suggested that there was no significant difference in water temperature between sampling sites during summer 2014. During this period, significant differences in the filtration rates of *O. edulis* collected between these sites (Poole Harbour, Chichester Harbour and the Solent) were observed. The filtration rates of oysters collected from Poole Harbour were significantly higher in comparison with Chichester Harbour and Solent oysters.

Hutchinson and Hawkins (1992) revealed that within a specific thermal range, filtration rates of *O. edulis* increased when salinity increased. Conversely, in this study Solent oysters living in higher salinity areas (32-34) had significantly lower filtration rates compared with Poole Harbour oysters (29-31). Korringa (1941) suggested that *O. edulis* is a stenohaline species having an optimum

salinity regime of 25-37. The salinity measured at field conditions at all locations, in this study, varied within the optimum range for *O. edulis* growth.

Recently, several studies (Marsden, 2004; Ward and Shumway, 2004; Yukihira et al., 2006) suggested that food availability was also a significant factor controlling filtration rates and growth in bivalve species. Therefore, as there was no difference in water temperature between sites, significantly higher chlorophyll *a* concentrations in the water column in Poole Harbour compared to the Solent appeared to be a possible reason to support higher filtration rates for Poole Harbour oysters. This agrees with reports that the feeding activity of filter-feeders is influenced by several environmental factors e.g. water temperature, food availability and particulate organic/inorganic matter in the water column (e.g. Bayne et al., 1993; Widdows, 1978; Wilbur and Owen, 1964).

Various clearance rates have been reported for *O. edulis* and these can be influenced not only by food availability but also by the size of the phytoplankton particles and/or by the quantity of the suspended particles (Mohlenberg and Riisgard, 1978). Hutchinson and Hawkins (1992) observed a drop in the clearance rate of *O. edulis* as the particulate inorganic concentration rose. Environmental data, in this study, suggested that although chlorophyll *a* concentration in the water column in Chichester Harbour was significantly higher than the Solent, there was no significant difference between filtration rates of Chichester Harbour and Solent oysters. It is likely that significantly higher total suspended solids in the water column in Chichester Harbour compared to Poole Harbour and the Solent was an important negative factor reducing feeding performance in Chichester Harbour oysters. Within nearshore environments, the particulate matter suspended as seston may be highly variable in both abundance and composition (Bayne and Widdows, 1978; Cranford and Hargrave, 1994; Navarro and Thompson, 1995; Rodhouse, 1977). Therefore, although temperature and salinity have been suggested as the main abiotic factors influencing oyster biology and physiology (Laing et al., 2005), when water temperature and salinity between habitats were not significantly different, it is likely that other field variables such as chlorophyll *a* concentration and total suspended solids influence filtration. Therefore, these factors need to be included in the assessment of physiology in order to identify favourable habitats for *O. edulis* populations.

Interestingly, when considering the questions addressed in the Discussion (section 3.4) of Chapter 3 which were "Is the improved physiology of reef oysters still notable when comparing between different populations?" or "Is the variation in physiological performance between population just as great as the difference between reef and sea bed habitats?", the results in this Chapter suggest the improved physiology of reef oysters in the summer is still noteworthy when comparing between different populations. Growing *O. edulis* (Poole oysters) on elevated reef structures increases filtration rate in the summer by 0.93 l h^{-1} g^{-1} AFDW above rates observed when they are re-laid on the sea bed, whilst the difference in filtration rates of *O. edulis* Poole oysters compared with Chichester oysters and Solent oysters was 0.4 l h^{-1} g^{-1} AFDW and 0.6 l h^{-1} g^{-1} AFDW and respectively. Therefore, as the transportation of oysters between locations is concerning due to the widespread nature of the parasite *B. ostreae*, using elevated reef structures to improve oyster physiology in their natural habitat is likely a better approach than relocating oysters to other habitats.

Temperature has widely been accepted as a significant factor affecting respiration rates in *O. edulis* and other bivalve species (Cusson et al., 2005; Korringa, 1941; Valero, 2006). The environmental data suggested that the water temperatures at Poole Harbour, Chichester Harbour and the Solent were similar. Therefore, unsurprisingly, no significant effects of location on the respiration rates of oysters were noted in this study. Respiration rates of oysters collected from all locations were highest in August 2014 (16ºC) and lowest in May 2014 when the water temperature was 12ºC. However, there was no significant effect of sampling interval in summer 2014 (12-16ºC) on respiration rate. Consistent with the findings in Chapter 3, there was no significant difference in the respiration rates of oysters collected in September 2012 and August 2013 when the temperature was 12ºC and 15ºC, respectively. Beiras et al. (1995) reported that *O. edulis* can maintain metabolic rates for short periods during minimal temperature fluctuations in order to maximize available energy budgets to enhance growth and survive the summer and winter mortalities. This strategy is often adopted by animals to survive in new environmental conditions (Hand and Hardewig, 1996).

Filtration and respiration rates of oysters, measured initially in water collected from the field, were not significantly different to the filtration and respiration

rates measured after acclimation to NOCS aquarium seawater for 2 weeks. Significantly higher filtration rates for Poole Harbour oysters in comparison with Chichester Harbour oysters and the Solent oysters were observed in both measurements conducted in field water and NOCS aquarium water. It could be hypothesised that the oysters used in this study would have adapted their physiological rates to local conditions at natural habitats where oysters were collected and the two week acclimations were not sufficiently long term. This is consistent with a previous study of bivalve transplantation. Montaudouin (1996) reported that growth rate of cockles (*Cerastoderma edule*) had not significantly changed during the first 4 months of manipulation from when they were located at the original site as they were in the adaptation period. Likewise, previous studies (Shpigel, 1989; Sunderlin et al., 1976) suggested that *O. edulis* maintain their physiological rates during short periods of minimal environmental changes. In contrast, in the case of large thermal changes, Beiras et al. (1995) reported that the scope for growth increased with temperature in juvenile *O. edulis* that were initially maintained at 20˚C and further exposed to 14˚C, 20˚C or 26˚C for 3 weeks. Unsurprisingly, in this study, spatial variation and sampling month (in summer) did not significantly affect respiration rates measured in those oysters maintained in NOCS aquarium water. The aquarium temperature conditions were consistent with the temperatures observed in Poole Harbour, Chichester Harbour and the Solent at the time of collection (Table 4.1) and were therefore not adjusted during this second acclimation period. As previously discussed, temperature was widely accepted as a main factor influencing the metabolic rate of bivalves. Therefore, it appeared likely that oysters conditioned in the same thermal regime would not have significantly different respiration rates.

The condition index has also been used to estimate growth differences between bivalves living in different environmental conditions (Austin et al., 1993). In this study, the condition index of Poole Harbour oysters was significantly higher compared to Chichester Harbour and the Solent oysters. Hawkins et al. (1998) suggested that increased clearance rates of suspension-feeding bivalve molluscs (e.g. *O. edulis, C. gigas* and *M. edulis*) was significantly linked to rapid growth in these bivalve organisms. As previously discussed, filtration rates of Poole Harbour oysters were significantly higher compared with Chichester Harbour oysters and the Solent oysters. However,

there was no significant difference in respiration rates (a main metabolic cost of growth) in oysters between sites. Therefore, it is likely that significantly higher filtration rates resulted in a significantly higher condition index for Poole Harbour oysters. Poole Harbour oysters could acquire more energy from significantly higher filtration rates compared to oysters collected from Chichester Harbour and the Solent.

The condition index showed seasonal variations for oysters from all habitats. Location and sampling interval significantly affected the condition index. Similar variations have also been reported for *C. gigas* and *O. edulis* in coastal areas of Western Europe (Abad et al., 1995; Linehan et al., 1999; Ruiz et al., 1992; Soletchnik et al., 2006) and South Australia (Li et al., 2009). These variations are generated by changing food supply and in adult individuals by gametogenesis and spawning (Li et al., 2009; Soletchnik et al., 2006). As an ecophysiological index, condition index can be affected by multiple biotic, abiotic factors and physiological activities, among which spawning is one of the most important. The observation that the condition index of oysters from all sites rapidly increased in June 2014 and slightly declined in August 2014 appears to be caused by the spawning cycle, a biological factor causing differences in oyster condition index over time (Nascimento and Pereira, 1980)

In conclusion, location in the context of environmental conditions, significantly influenced filtration rates of *O. edulis*. Poole Harbour oysters had significantly higher filtration rates compared to Chichester Harbour oysters and Solent oysters. These higher filtration rates in *O. edulis* from Poole Harbour resulted in a significantly higher condition index. Although, all oyster sampling sites were located on the southern coast of the UK, local variations in environmental conditions such as chlorophyll *a* concentration and total suspended solids were noted. It is likely that these environmental differences influenced the physiological performance of *O. edulis.* Moreover, previous studies reported that water current and tidal cycle affected the physiological rates of *O. edulis* (Valero, 2006) Therefore, these variables need to be considered in any future studies. In Chapter 3, findings indicated that artificial reef structures could improve the filtration rates of *O. edulis.* It is likely that although Poole Harbour had significantly higher filtration rates compared to Chichester Harbour and Solent oysters, cultured oysters (Poole Harbour oysters) on elevated reef modules had an overall greater physiological performance. Elevated reef

oysters had higher filtration rates in comparison with oysters directly planted on the sea bed. The improved physiology of reef oysters (Poole oysters) in summer was still important when comparing between different populations (Chichester Harbour and Solent oysters), suggesting that the improvement in physiology of *O. edulis* using elevated reefs is perhaps better than the relocation of oysters to other habitats. As the improvement in the physiological performance of *O. edulis* cultured on elevated reef structures is discussed in Chapter 3, in order to maintain a productive oyster population it is crucial to investigate and understand the impact of reef habitats on oyster reproduction. Therefore, the focus of Chapter 5 will be in understanding the effects of elevated reef habitats on the gonadal development of *O. edulis* in Poole Bay.

Chapter 5

The effects of elevated reef habitat on gonadal development of *Ostrea edulis*[2]

5.1 Introduction

Chapter 3 showed that filtration rates of *Ostrea edulis* reared on elevated reef structures were significantly higher than those of oysters on the sea bed. However, this represents just one important aspect of the life history of the adult oyster. In the long-term the success of the fishery enhancement programme is contingent upon the improved reproductive output and recruitment of oyster larvae and spat to the reef and surrounding sea bed/fishery. Therefore, it is important to establish if living on an elevated structure is of benefit to oyster reproduction, in turn, leading to increased recruitment and abundance. The assessment of the reproductive status, in terms of gonad development, of reef and sea bed oysters is therefore required to establish the potential long term benefits of rearing oyster broodstock on elevated protected reefs for fishery enhancement.

Transplanting of parental stocks into natural oyster beds has previously been applied elsewhere in order to supply brood stock, providing enough male and female stocks with which to enhance new recruits in natural populations (Key and Davidson, 1981; Korringa, 1952; Yonge, 1966). However, there has not been any assessment of the reproductive status, associated with gametogenic cycle, of the transplants after they have been moved. Management intervention must ensure that the reproductive capacity of transplanted stocks of *O. edulis* are maintained. In order to achieve this, the gametogenic cycle of relocated *O.*

[2] Some of these data have been published as Sawusdee et al. (2015), see Appendix 12.

edulis broodstock, used for restoring programmes including oyster artificial reefs, must be addressed.

Several studies have revealed that temperature is a key environmental determinant influencing gonad development in ostreids (Korringa, 1957; Loosanoff, 1962; Mann, 1979; Orton, 1920; Wilson and Simons, 1985) and other bivalves (e.g *Crassostrea gigas*: Fabioux et al., 2005; *Mytilus galloprovincialis*: Fearman and Moltschaniwskyj, 2010). The specific temperature requirement for the gametogenic cycle of *O. edulis* varies between populations, but 15˚C is a minimum requirement for ripening and releasing of eggs or sperm in UK populations (Korringa, 1957; Utting et al., 1991); however, this temperature minimum is not consistent for other geographic populations. For example, while Orton (1920) reported that *O. edulis* located in the northerly channel of the Firth of Forth were able to spawn above a temperature of 15˚C, Wilson and Simons (1985) reported that *O. edulis* in the Northern Adriatic spawned at 13-14˚C in 1960 but at 10-11˚C in 1964. Recently, several studies have shown that the quantity of food in the water column is also an essential parameter that influenced gonad maturation of *O. edulis* (Gonzalez-Araya et al., 2012, Millican and Helm, 1994; Utting and Millican, 1997) and other bivalves (*e.g. C. gigas*: Chávez-Villalba et al., 2002; Kang et al., 2000, *Argopecten purpuratus*: Martinez et al., 2000). Particle size, algal species and their surface properties have been shown to affect food selectivity and gamete maturity of bivalves (Rosa et al., 2013; Shumway et al., 1985; Ward et al., 1997). Gonzalez-Araya et al. (2012) demonstrated, from laboratory experiments, that gametogenesis was very active in *O. edulis* fed *Rhodomonas salina*, or *Thalassiosira weissflogii,* whilst Ronquillo et al. (2012) suggested that the best diet for rearing European oyster juveniles was a mixture of *Nannochloropsis oculata* and *Pavlova lutheri,* providing better growth rates and shorter gametogenic cycles. Previously, researchers have also established the effects of culture depth on reproduction of different oyster species. Ngo et al., (2003; 2006) compared the reproductive status of *C. gigas* when cultured at depths of 0-2m and 3-5m on long lines in Gosung Bay on the southern coast of South Korea. They found that oysters cultured at a depth of 0-2m had a faster cycle of gonad maturation than those cultured between 3-5m, particularly during the early stages. The gonadosomatic index (GSI) was shown to be significantly higher in the shallow oysters compared with the deep

oysters, which was attributed to increased food availability at shallow depths. The effect of water depth on the reproductive potential has also been documented for scallops (*Placopecten magallanicus*, Barber et al., 1988; MacDonald and Bourne, 1987), oysters (*C. virginica*: Loosanoff, 1965) and mussels (*M. edulis*: Pipe, 1985).

In general the physical and chemical properties of the environment must be seen as acting indirectly on the reproductive output of bivalves. As argued by Delgado et al. (2004), ultimately energy reserves offer key direct control on the maturation of gonadal tissue. Studies have reported that the reproductive cycle in bivalves was influenced by the energy storage and how the energy budget was distributed or utilized within an individual (Barber and Blake, 1981; Castro and de Mattio, 1987; Massapina et al., 1999; Perez-Camacho et al., 2003). The energy storage and utilization cycles determine the seasonal pattern and concentration of egg proteins. The concentration of egg proteins in turn influence the gametogenic cycle (Holland, 1978; Tlili et al., 2012), although this varies according to species and environmental conditions (Albentosa et al., 2007).

Generally, Vitellogenin (Vg) concentrations are detectable in sexually mature females (Matozzo and Marin, 2008). Vitellogenins (Vg) in female bivalves have been used as an indicator of reproductive status, especially during the spawning period. Vg are glycolipophosphoproteins, which consist of calcium (Ca^{2+}) and zinc (Zn^{2+}) ligands (Clayton, 1996). Vg provide energy reserves for embryo development in oviparous organisms (Suzuki et al., 1992; Wallace, 1985) and are considered to have similar characteristics in vertebrates, such as fish (Nagler et al., 1987), and invertebrates, particularly molluscs (Blaise et al., 1999). Vitellogenin proteins are produced in the liver or equivalent organs in response to endogenous estrogens and are released into the bloodstream, or stored in developing oocytes. The lipid content of Vg has been used as an indirect method to measure oocyte proteins (Gagne et al., 2002). Most studies concerning Vg induction in aquatic invertebrates have been conducted using the alkali-labile phosphate (ALP) method (Blaise et al., 1999) that detects inorganic phosphate liberated from phosphorylated proteins, including Vg-like proteins in molluscs, (e.g. *Mya arenaria* (Gagne et al., 2002) *M. galloprovincialis* (Pampanin et al., 2005), *Argopecten gibbus* (Quinn et al.,

2005), *M. edulis* (Zorita et al., 2006) and *Elliptio complanata* (Blaise et al., 2003)). To date, there has been no study of Vg levels in *O. edulis.*

As previously stated, an elevated reef habitat significantly improved the filtration rate of *O. edulis* in Poole Bay. Therefore, in light of the important role of energy intake in gonadal development in bivalves, this study investigated the indirect effect of rearing oysters on an elevated reef structure on the oyster *O. edulis* gonad maturation and the expression of vitellogenin-like protein. In this chapter data are presented comparing the gonad development and Vg expression between oysters reared on the sea bed and those cultured on an elevated reef structure (80 cm above the sea bed). In addition the sex ratio of oysters from Poole Bay is established. The initial working hypothesis was that culture on an elevated reef would accelerate and/or improve gonad maturation, in turn, leading to a potentially increased reproductive output. The explicit null hypothesis tested was that there would be no difference in the reproductive status between *O. edulis* cultured on the reef modules and those cultured at the sea bed.

5.2 Methods

The gonads of *O. edulis*, 57-99 mm shell height, were histologically examined during the 15-month interval after reef deployment. The field experimental design and sampling intervals have been described in Chapter 2. As has been described, the sampling frequency was designed to observe seasonal variation over 15 months (September 2012, April 2013, August 2013 and November 2013).

5.2.1 Gonadal examination and index

The soft tissue of each oyster, sampled as mentioned in Chapter 2, was carefully removed from the shell using a scalpel. A longitudinal section was taken through the mid-region of the body containing gonadal tissue and was fixed in Bouin's solution (Sigma-Aldrich™, Dorset, UK) for 24h. Tissues were dehydrated through an ethanol series (50%, 70%, 96% and dehydrated ethanol) overnight for each concentration. To clarify the dehydrated sections, they were

immersed in xylene and then xylene with wax overnight, respectively. Melted paraffin wax was poured onto the clarified tissues in glass vials. The tissues were then placed in the oven at 60˚C for 24 hours, allowing wax to infiltrate the whole tissue. The tissue was quickly transferred from melted wax and placed section-face down into a square mould cassette containing fresh melted wax. Wax blocks were sectioned at 7-8 µm using Cambridge microtome (Cambridge Instrument Co. Ltd, England), floated on a water bath at 45-47˚C and then transferred to glass slides for drying. Tissue sections were stained according to the following protocol (Howard and Smith, 1983):

1) de-waxed in xylene (5 seconds),

2) graded series of ethanol (96%, 70%, 50%),

3) stained with haematoxylin (15 minutes),

4) rinsed in tap water and counterstained with 1% eosin for 3 minutes.

Finally, the sections were dehydrated in 96% ethanol and clarified in xylene for 3 minutes. The slides were left to dry, mounted with DPX and a cover slip applied.

Sections were examined under bright field to determine the sex and reproductive stage. Phase identification of oogenesis and spermatogenesis in *O. edulis* was performed following da Silva et al. (2009) as summarized in Table 5.1and Figures 5.1-5.2. After the oyster samples were sexed, the developmental stages were classified based on a criteria described by da Silva et al. (2009) (Table 5.1). The gonad index, at any one sampling interval, was taken as the arithmetic mean of the individual rank values as shown in Table 5.1.

Table 5.1 Sex determination and gonad maturity (da Silva et al., 2009). The numerical characters (0-4) indicate the index of each developed gonadal stage.

Gonad index	Description	Gonad Properties
G 0	Inactive	No evidence of developing or ripe gametes. The gonad contained dilated and unfilled follicles. The follicles locate between the mantle and the digestive gland walled by abundant connective tissue.
G 1	Early	Gonad follicles are more spread into the connective tissue. Oogonia and spermatogonia were mostly attached to the follicle cells. In addition, for male oysters, primary and secondary spermatocytes are observed and developing oocytes of female oysters are obviously attached to developing lines.
G 2	Advanced	Gonad follicles are bigger than in the previous stage while connective tissue is reduced. Male oysters, development of few spermatogonia still occurs, but spermatocytes and spermatid balls are dominant; in females, oocytes in vitellogenesis are dominant but oocytes in post-vitellogenesis are thin.
G 3	Ripe	Large follicles can be observed in whole area between the mantle and digestive gland. Both functional male and female develop follicles contained gametes, plentiful spermatozoa balls and advanced oocytes.
G 4	Spent	Gonad follicles are smaller than ripe stage. More than 70% of gametes have been released. Residual spermatozoa/ oocytes appear in largely empty follicles and gonoducts.

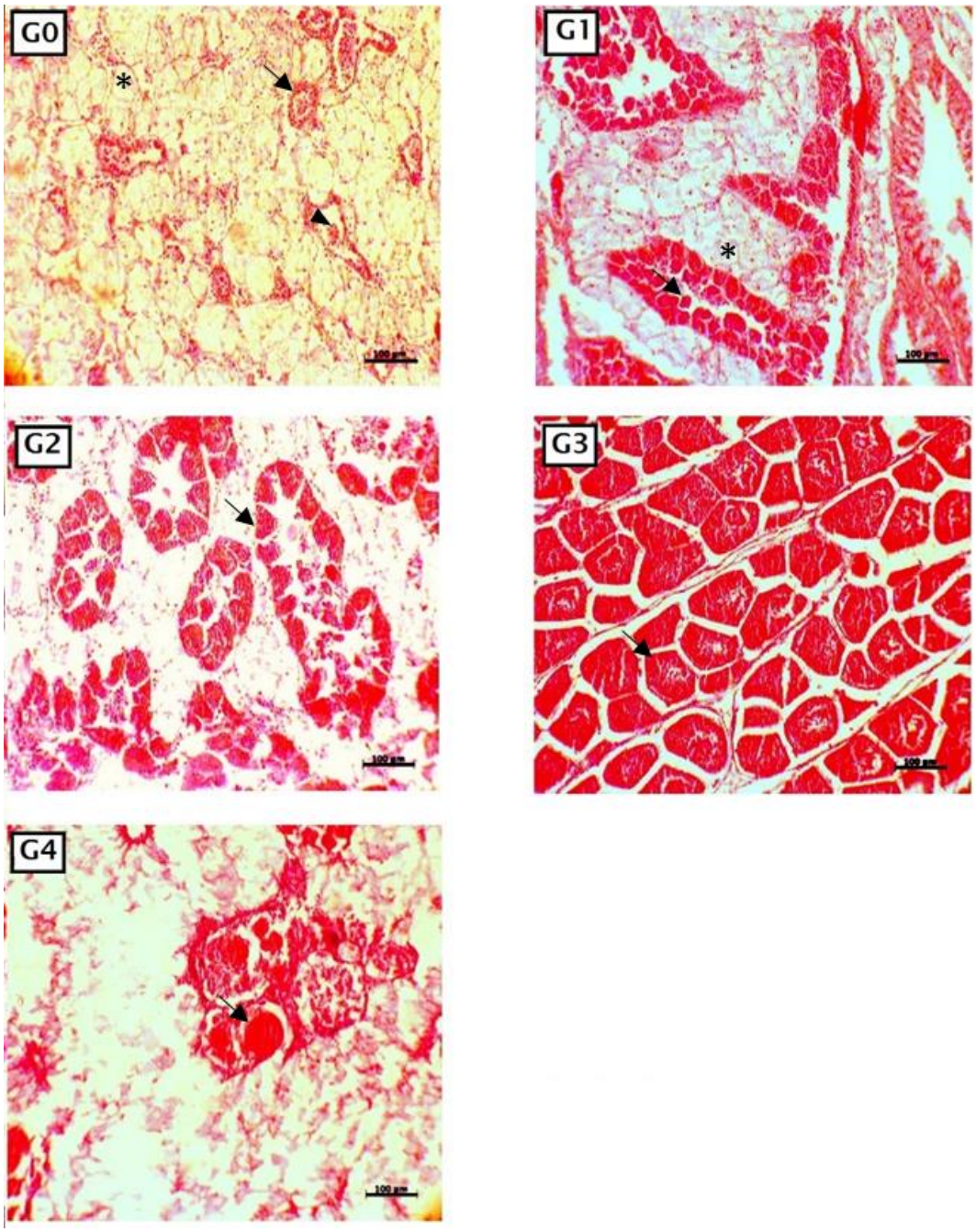

Figure 5.1 The reproductive stages of female *Ostrea edulis* sampled in oysters from the Poole Harbour reef in this study; GO = inactive stage; few small gonad follicles (arrow) surrounded by predominant connective tissue (*) some phagocytes (arrowhead) appear inside follicles, G1= early developed stage; predominant small oocytes (arrow, 15-30 µm diameter), G2 =advanced stage; oocytes (arrow, 30-80 µm diameter), G3= ripe stage; numerous advanced oocytes (arrow,90-110 µm diameter) and G4= spent stage; residual oocytes (arrow). Tissue sections were stained with haematoxylin and erosin. Scale bars are all 100 µm.

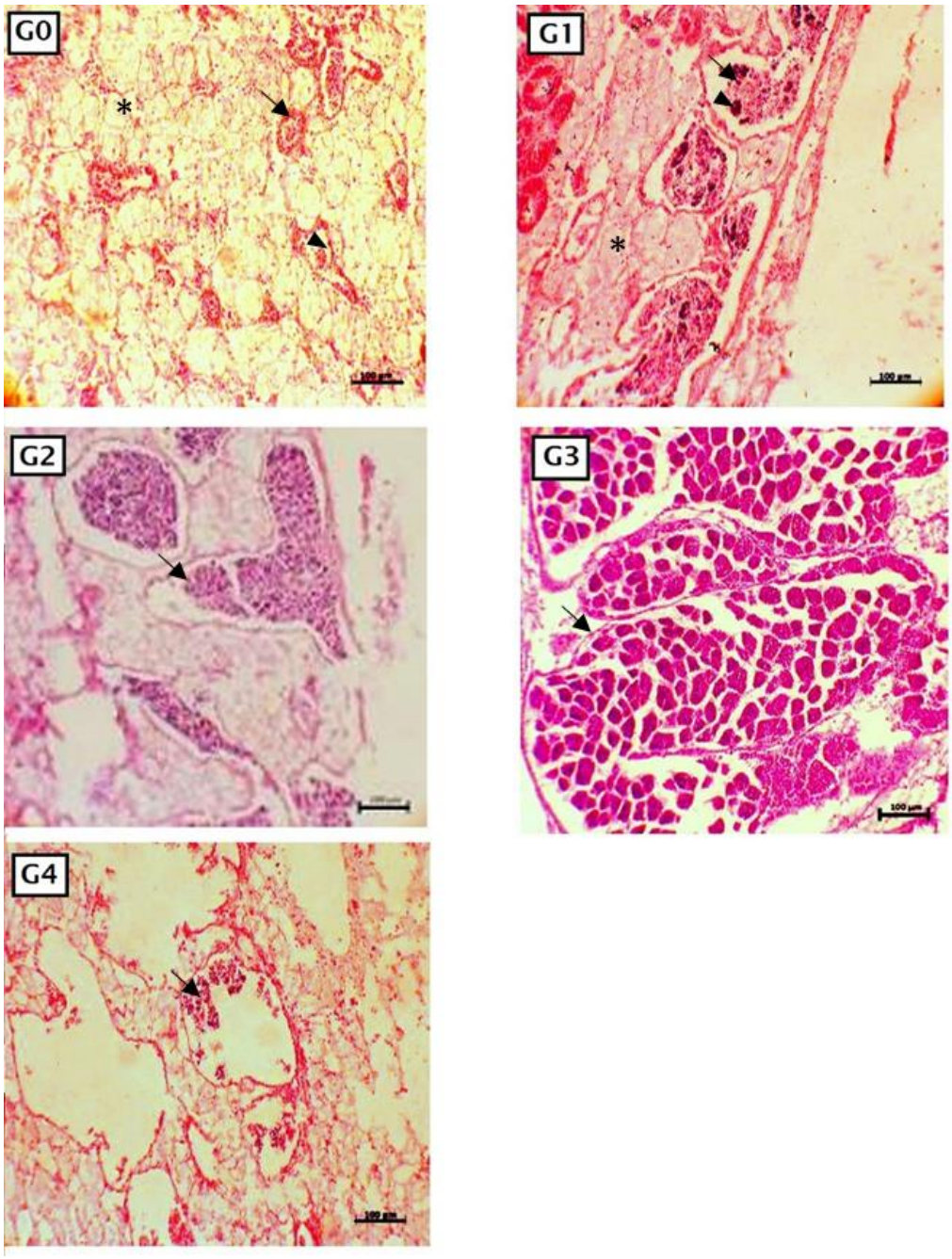

Figure 5.2 The reproductive stages of male *Ostrea edulis* sampled in oysters from the Poole Harbour reef in this study; G0 = inactive stage; few small gonad follicles (arrow) surrounded by predominant connective tissue (*) some phagocytes (arrowhead) appear inside follicles, G1= early developed stage; spermatogonia (arrowhead), spermatocyte balls (arrow), G2=advanced stage; predominant small spermatocytes (arrow), G3= ripe stage; numerous spermatozoa balls (arrow) and G4= spent stage; residual spermatozoa (arrow). Tissue sections were stained with haematoxylin and erosin. Scale bars are all 100 µm.

5.2.2 Vitellogenin-like (Vg-like) protein assays

Thirty oyster samples per sampling occasion (15 oysters from the reefs and 15 oysters from the sea bed) were used for determining Vg levels using an alkali-labile phosphate (ALP) assay (Blaise et al., 1999), which adapted the phosphomolybdate assay described by Stanton (1968). Oyster haemolymph (1.2 ml) was extracted from the adductor muscle sinus of each oyster sample, using a hypodermic syringe and 21G needle (Terumo, 0.5x25 mm). Haemolymph was centrifuged at 4˚C and 10,000 *g* for 15 minutes to pellet the haemocytes. After centrifugation, the supernatant was retained and the haemocyte pellet was discarded. The free-cell haemolymph (1000 µl) was extracted with 500 µl of 100% methyl-t-butyl ether, vortexed and centrifuged at 1000 *g* for 5 minutes and left at room temperature for 15 minutes. The ether phase of each sample (top phase) was removed from the phase extraction and mixed with 100 µl of 1M sodium hydroxide to release the labile phosphates, stirred and left in a dark at room temperature for 60 minutes.

After one hour the akali-digested phosphate emulsion was centrifuged for 2.5 minutes at 10000 *g* and total inorganic phosphate in the aqueous phase was determined. 1 ml of the aqueous phase was removed and mixed with 1 ml of the acid molybdate solution and 0.4 ml of aminonaphthol sulfonic acid (ANSA) reagent. The time at which the ANSA solution was added was recorded. The reaction was diluted to 10 ml with deionised water and reactions were incubated for 40 minutes in the dark at room temperature. The absorbance of each sample was measured using a spectrophotometer (Jenway 7315, Bibby Scienfific Ltd, Staffordshire, UK) at 815 nm and compared to a standard curve as shown in Figure 5.3 to determine the total phosphate concentration in the alkali extraction. ALP levels from each sample were corrected against the protein concentration of the haemolymph using an aliquot of the supernatant described above and the Bicinchoninic Acid (BCA) protein assay described in Chapter 3 (section 3.2.5).

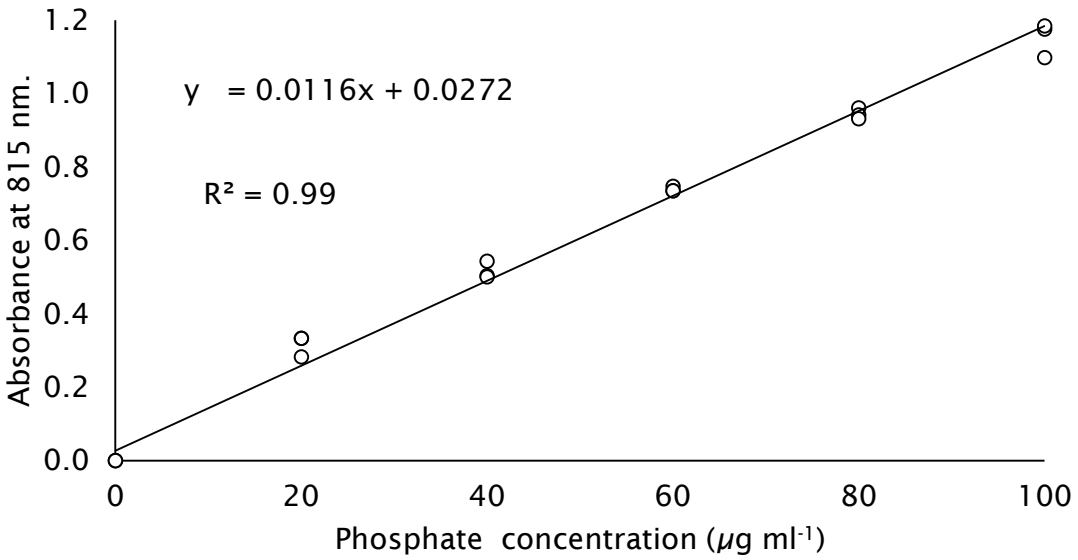

Figure 5.3 Typical standard curve response from the phosphomolybdenum assay (Stanton, 1968), showing a fitted regression (black line). Absorbance increased proportionally with increased phosphate concentration (R^2=0.9977).

5.2.3 Statistical analyses

Differences in gonad index and Vg levels between reef and sea bed oysters and between months were tested using two-way ANOVA. Assumptions of homogeneity of variance and normality were tested by Shapiro-Wilk method. Tukey's HSD tests were used to perform p*ost-hoc* pairwise multiple comparisons, as appropriate. Chi-squared tests were used to test the hypothesis of equal sex ratios (50:50) in each sampled population. In all cases, statistical significance was accepted at α = 0.05.

5.3 Results

5.3.1 Gonadal maturation

A total of 109 oysters were histologically processed. A total of 55 oysters were sampled from the elevated reef modules and 54 oysters from the sea bed cages. Of the elevated reef oysters, 25 were female, 19 were male and 11 were unsexed. From the sea bed cages 17 were identified as female and 25 were identified as male, with 12 undetermined. The sex ratio of male to female within the populations of reef or sea bed oysters showed no significant difference from a 1:1 ratio (Chi-square, $P>0.05$). The majority of individuals developed and spent their gametes once a year except three reef oysters and one sea bed oyster. These had evidence of a second-round gametogenic cycle as a male developing spermatocytes while residual oocytes from first round gametogenesis were not completely reabsorbed. This was evident in the histological samples in the August 2013 sampling (Figure 5.4).

At the first sampling event, September 2012, when the water temperature was 12˚C, 80% *O. edulis* from elevated reefs were spent while only 55% of sea bed oysters were spent and the remainder were ripe (Figure 5.5). In April 2013, with the water temperature at 7˚C, 50% of the elevated reef oysters were scored as being in an advanced stage of gonad maturation, compared to 40% of sea bed oysters. Interestingly, in August 2013, 46% of reef oysters were identified as spent and three oysters showed evidence of second round gametogenesis, whilst only 27% of sea bed oysters were spent and only one oyster showed the evidence of second round gametogenesis. In November 2013, evidence for gametogenesis of reef oysters was limited, the inactive stage accounting for 95% of individuals. Nevertheless, at this time point 22% of sea bed oysters (male oysters) were still either ripe or spent. Therefore, from these data it was concluded that that reef oysters matured earlier than those on the sea bed.

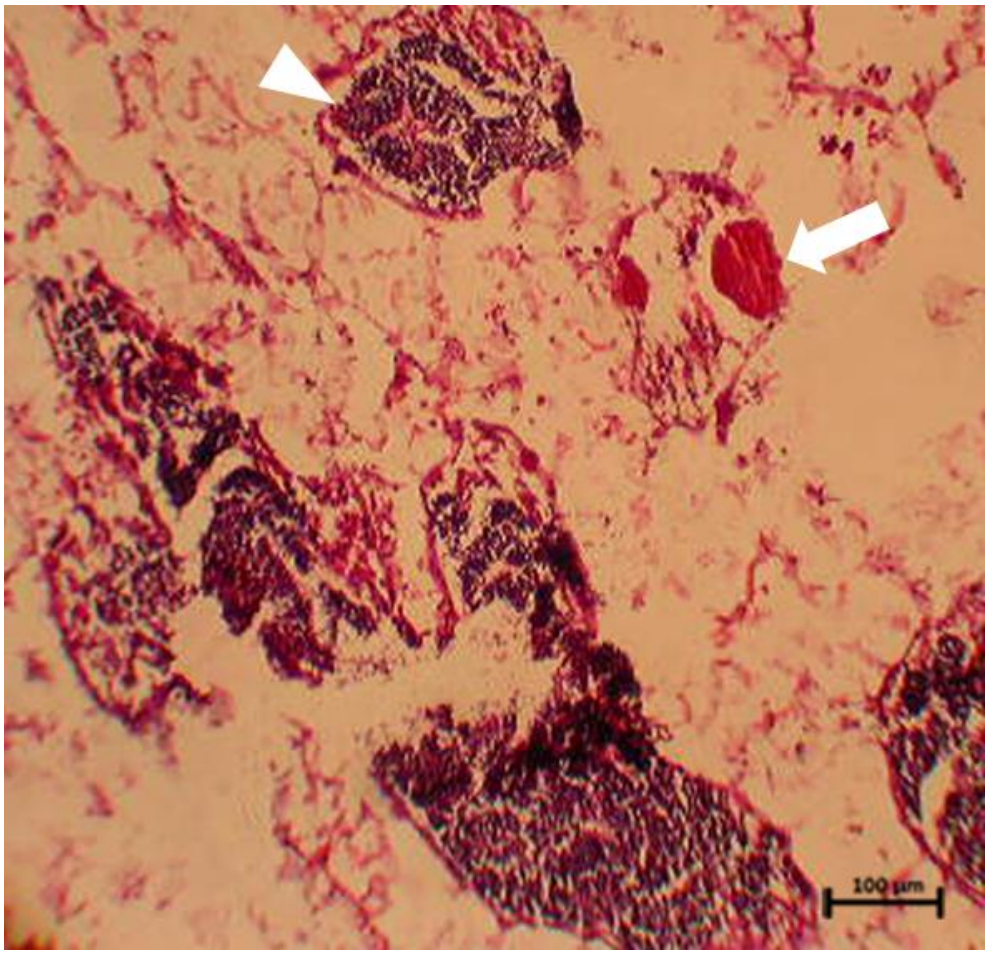

Figure 5.4 Histological section of *Ostrea edulis*, showing the evidence of second round gametogenic cycle, residual oocytes of first round gametogenesis as female (arrow), developing spermatocytes of second round gametogenesis as male (arrowhead). Tissue sections were stained with haematoxylin and erosin. Scale bars are all 100 µm.

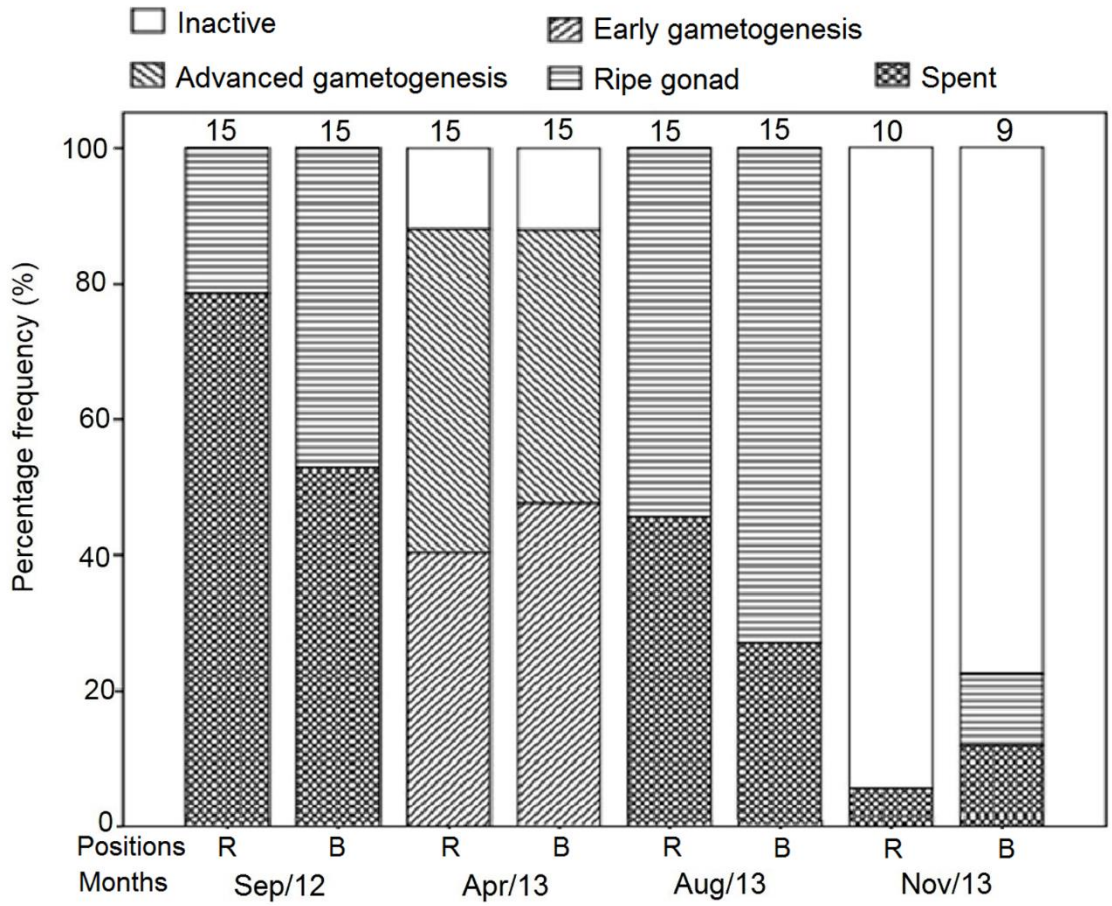

Figure 5.5 Percent frequency of gonad phases of *Ostrea edulis*, collected from elevated reefs (R) and sea bed (B) on each sampling occasion. The number of oysters analysed from each locations is indicated at the top of the bar chart.

The fluctuation in mean gonadal stage can be explained by the development of gonads (Figure 5.6). High gonadal indices were identified in September 2012 and August 2013 and remained low in April 2013 and November 2013, when oysters completed their spawning season and the water temperature was low. Two-way ANOVA revealed a significant effect of sampling month on gonadal development ($P<0.001$) but not for elevation and no interaction between the main effects (Table 5.2). Tukey's HSD pairwise tests showed that there was no difference in the gonadal stage of oyster samples collected in September 2012 and August 2013 and also between April 2013 and November 2013. However, there were significant differences in the gonadal stage of oysters between other sampling intervals i.e. the gonadal stage of oysters collected in

September 2012 and August 2013 were significantly higher than those oysters collected in April 2013 (P<0.001) and November 2013 (P<0.001) (Table 5.3).

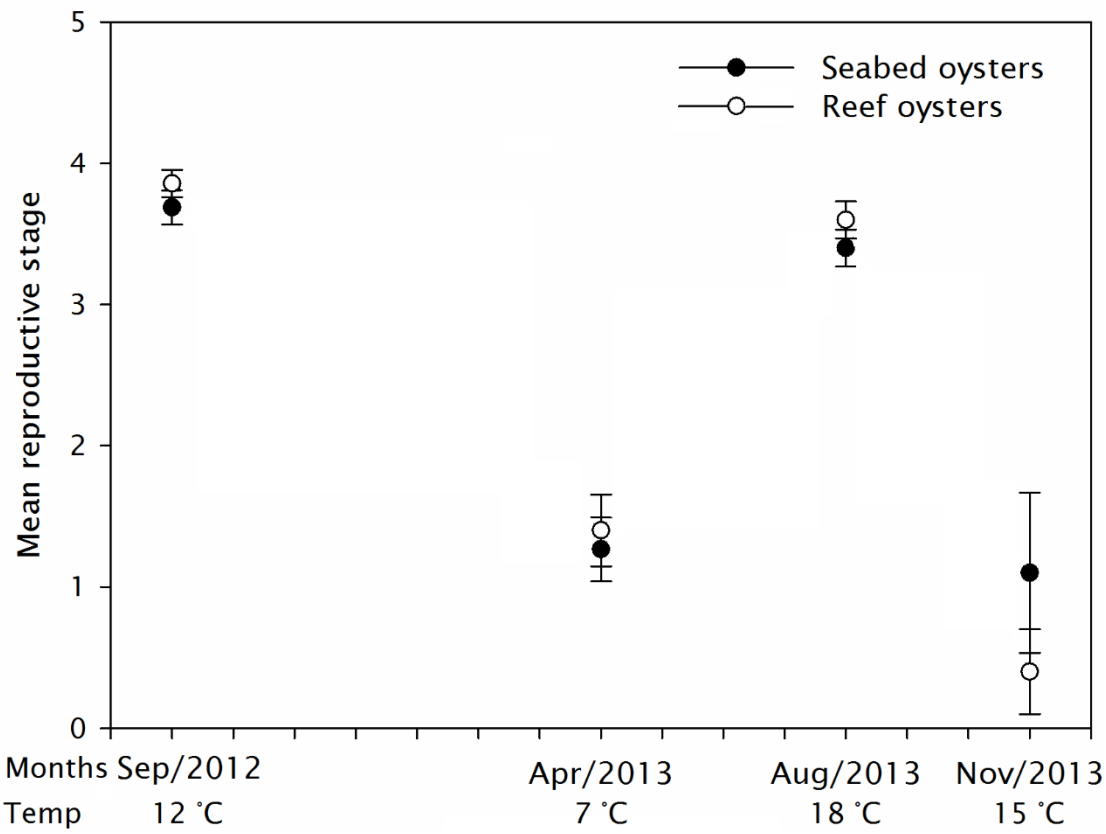

Figure 5.6 Temporal variation in gonad index of *Ostrea edulis*. Data points are the mean (±SE) of gonadal stages of each location at that time (n=15, except November sampling (reef oysters; n=10 and sea bed oysters; n=9) Gonad index was affected by sampling intervals (two-way ANOVA, P<0.001) but not elevation.

Table 5.2 Two-way ANOVA results comparing the effect of sampling interval, elevation (reef/sea bed), and the interaction sampling interval x elevation on gonad maturation. Significant differences are denoted by asterisks.

Sources of variation	df	F	P-value
Sampling interval	3	73.96	0.001*
Elevation (reef/sea bed)	1	0.18	0.676
Sampling interval x Elevation	3	1.68	0.176

Table 5.3 *Post-hoc* (Tukey's HSD test) results comparing the effect of sampling interval on gonad maturation. "ns" represents no significant difference.

Post-hoc test the effect of sampling interval	P-value		
	Sep 2012 (12 ˚C)	Apr 2013 (7 ˚C)	Aug 2013 (18 ˚C)
Apr 2013	<0.001		
Aug 2013	ns	<0.001	
Nov 2013 (15 ˚C)	<0.001	ns	<0.001

5.3.2 Vitellogenin-like protein concentration

The level of vitellogenin-like proteins in haemolymph of both female oysters collected from the elevated reefs and sea bed were examined by using an alkali labile phosphate (ALP) measurement assay (Blaise et al., 1999). Pearson's correlation indicated that the level of vitellogenin-like protein concentrations were strongly positively correclated with the gonadal index ($r=0.61$, $P<0.05$). The highest concentration (2.86 ± 0.38 and 2.68 ± 2.30 µg ALP mg^{-1} protein, mean±SE of reef and sea bed oysters, respectively) of female ALP was recorded in August 2013 (Figure 5.7), which corresponded to the reproductive period suggested by gonadal histology. The level of ALP was also high in September 2012, then it dropped rapidly to April 2013 (0.67 ± 0.23 and 0.35 ± 0.12 µg ALP mg^{-1} protein, mean±SE of reef and sea bed oysters, respectively). In November 2013, the female ALP concentration was below the detection limit of the spectrophotometric assay. This was because oysters were either in the

resting or inactive stages. During these stages gender could not be identified. Interestingly, this ALP response pattern was consistent with the results of the gonadal index of the oyster population (Figure 5.6) from both reef and sea bed habitats. ALP concentrations were high in August 2013 and September 2012 and remained low in April 2013 when water temperatures were low (7ºC). Two-way ANOVA revealed a significant effect of sampling month on the level of ALP but not for elevation and no interaction between the main effects (Table 5.4). Tukey's HSD pairwise tests showed that there was no significant difference in the ALP of oyster blood samples collected in September 2012 versus August 2013. In contrast, ALP levels of oysters collected in September 2012 and August 2013 were significantly higher than those oysters collected in April 2013 ($P<0.001$) (Table 5.5).

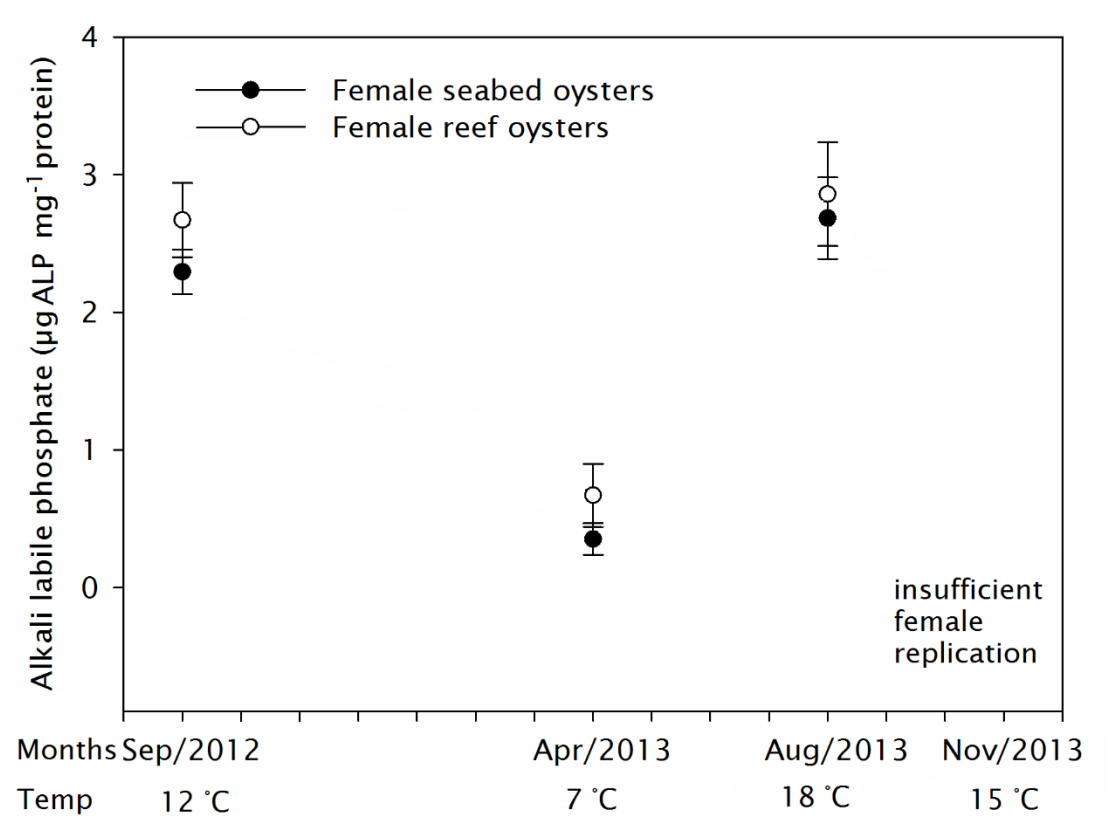

Figure 5.7 Temporal variation of haemolymph alkali labile phosphate level (ALP) (mean ± SE) in female oysters from both reef and sea bed oysters at each sampling interval (reef oysters; n=8, 8, 9, respectively, sea bed oysters; n= 6, 8, 6, respectively). There were no identifiable females in November 2013. Haemolymph ALP level was affected by sampling interval ($P<0.001$) but not elevation.

Table 5.4 Two-way ANOVA results comparing the effect of sampling interval, elevation (reef/sea bed), and the interaction sampling interval x elevation on haemolymph ALP. Significant differences are denoted by asterisks.

Sources of variation	df	F	P-value
sampling interval	2	26.70	<0.001*
Elevation (reef/sea bed)	1	0.03	0.862
Month x Elevation	2	0.14	0.868

Table 5.5 *Post-hoc* (Tukey test) results comparing the effect of sampling months or temperature on Haemolymph ALP. "ns" represents no significant difference.

Post-hoc test the effect of sampling months (Tukey test)	P-value		
	Sep 2012 (12 ˚C)	Apr 2013 (7 ˚C)	Aug 2013 (18 ˚C)
Apr 2013	<0.001		
Aug 2013	ns	<0.001	

5.4 Discussion and conclusion

The histological assessment of gonadal tissues of 109 oysters collected over 15 months after reef deployment indicated that *O. edulis* were spent during August and September. Other previous studies have also reported that in the UK *O. edulis* spawns just once a year in the summer period when temperatures reach up to about 15˚C (Korringa, 1957; Utting et al., 1991). Kamphausen et al. (2011) reported that the spawning season of *O. edulis* in the Solent was between March and October.

It is widely accepted that temperature not only controls the food intake of oysters and other bivalves (Comeau, 2014; Saucedo et al., 2004; Shumway, 1982), but also effects gonad maturity and spawning season (Mann, 1979; Nelson, 1928; Orton, 1920; Walne, 1974; Wilson and Simons, 1985). Moreover, the influence of diet composition to accelerate gonadal development of

molluscs has been recognised more recently (Gonzalez-Araya et al., 2012; Millican and Helm, 1994; Utting and Millican, 1997). However, no previous publications have reported the role of elevated structures in determining filtration rate and subsequent gonad maturation of *O. edulis.* In this study, the environmental data in Chapter 2 confirmed that both reef and sea bed oysters were subject to the same temperature regime. Condition index peaked during August 2013 (average water temperature was 18˚C), when more than half of the oyster gonads, from both reef and sea bed locations, were ripe. However, In August 2013, the percentage of reef oysters with spent gonads was higher than sea bed oysters. In November 2013, 95% of reef oysters had completed their spawning cycle, while 22% of sea bed oysters were either ripe or spent. It is concluded that, at the same temperature conditions, reef oysters can complete spawning earlier than sea bed oysters. In accordance with these data, Bataller et al. (1999) and Comeau et al. (2010) reported that warm summer months combined with elevated food in the upper water column enhanced growth and shortened the reproductive cycle in *C. virginica* (Comeau, 2013).

O. edulis can alternate its gender between male and female after initially reaching sexual maturity, about 50 mm in total length as a male (Orton, 1927). Previous studies reported that under exceptionally favourable conditions, e.g. high diet quality (Gonzalez-Araya et al., 2012) and extended periods of high water temperature in summer (minimum 15˚C) (Mann, 1979), *O. edulis* can reach maturity several times in one spawning season. This is because their gonads can begin to change into the opposite gender immediately after releasing eggs or sperm (Kamphausen, 2012). Moreover, Korringa (1957) and Kamphausen (2012) reported that *O. edulis,* under favourable conditions, can continue the second round of the gametogenic cycle without complete reabsorption of the first gonad. In this study, evidence for a second round gametogenesis was recorded for four oysters in total. Three oysters were observed on elevated reefs, whereas only one sea bed oyster continued the second round of gametogenesis. Pouvreau et al. (2006) explained that the reproductive cycle of a bivalve can be divided into two processes 'storage accumulation' and 'gametogenesis.' To complete the second process, gametogenesis, the previously accumulated energy from the first step, before the spawning period, is required. Therefore, the remaining energy budget during the spawning season or early summer is an important factor that

influences gametogenic activities. Energy reserves are intimately correlated with environmental conditions and the annual gametogenic cycles (Delgado et al., 2004; Holland, 1978; Tlili et al., 2012). Similarly, several previous studies have suggested that the differences in gonadal maturation were influenced by the storage energy budget and water depths in the context of surrounding water conditions. For example, Barber et al. (1988) studied the gonad maturation of deep-water scallops (*Placopecten magallanicus*) in the Gulf of Maine, USA and found that slower gonadal development and lower fecundity were recorded for the scallops inhabiting deeper water because of insufficient food sources. Ngo et al. (2003) also revealed that water depth significantly affected gonadal maturation of *C. gigas* i.e. the bottom oysters grew slower and produced relatively fewer eggs. Moreover, Ngo et al. (2006) reported that *C. gigas* cultured at the top (0-2 m) of longlines underwent gonad maturation at a faster rate than on-bottom oysters. In Chapter 3, data are presented that elevated reef oysters exhibited significantly higher filtration rates especially during warm summer months. It is hypothesised that this higher feeding rate supplements the energy reserves in the individuals from elevated reefs. Based on that, the energy used in the gametogenic cycle may be derived from the reserves previously stored during early summer, as described in *M. edulis* (Hilbish and Zimmerman, 1988). Accordingly, the presence of second round gametogenesis in three reef oysters may be the result of the significantly higher filtration rate in comparison with sea bed oysters. Therefore, it can be postulated that these oysters would have had a more favourable energy status which permitted a faster rate of gametogenesis, possibly enabling them to reach a ripe state earlier in comparison with sea bed oysters. However, these results must be interpreted cautiously as the numbers of oysters presenting second round gametogenesis are low.

In addition, it should be cautioned that rapid gametogenesis and spawning might result in poor larval quality. Although the investigation of larval quality was beyond the scope of current research, it could be argued that the larval quality of reef oysters was possibly low because of the shortened gametogenesis. Honkoop and Meer (1998) have reported that low quality reproductive output in bivalves (low fecundity, small eggs and low larval quality) was mainly caused by extremely high water temperatures as they were rapidly released without completing development (see also: Avendano and

Pennec, 1997; Enríquez-Díaz et al., 2009). Nevertheless, as previously discussed in Chapter 2, both reef and sea bed oysters were subject to the same temperature regime. It follows that there would be no differential impact on the quality of larvae between reef and sea bed oysters as a result of temperature. Korringa (1957) reported that temperature required for larval release in *O. edulis* was between 15-20ºC and environmental data confirmed that water temperature in August 2013 (18ºC), was consistent with Korringa's (1957) findings. Therefore, it was likely that there was an advantage in early spawning for reef oysters. Larvae spawned from reef oysters would possibly access food earlier because the peak of chlorophyll *a* was recorded in August 2013. They were then able to 'search' for hard substrates to settle earlier and avoid greater levels of interspecific competition with other filter-feeding epifaunas (Barnes et al., 1973; Fretter and Graham, 1981; Korringa, 1951; Walne, 1956). More detailed assessment of fecundity and larval quality are recommended for future research in order to ensure that elevated reefs promote not only the rate of gonad maturation, but also spawning success, larval quality and settlement success – all of which are essential for the successful recruitment of new cohorts from broodstock.

The Vg concentration in context of ALP levels in female bivalves, especially in the spawning period, has been used to indicate reproductive status (Blaise et al., 1999; Matozzo and Marin, 2008; Suzuki et al., 1992). Arcos et al. (2009) also confirmed that Vg protein concentration correlated with ovary maturation in *Crassostrea corteziensis* female oysters and recommended that the concentration of these proteins was a potential indicator of female reproductive investment. In this study, there was no significant difference of ALP concentrations between reef and sea bed oysters. However, this study is consistent with previous publications that propose seasonal effects on ALP concentration (Blaise et al., 1999; Matozzo and Marin, 2008; Morthorst et al., 2014). Suzuki et al. (1992) reported that the Vg proteins are synthesized within the ovary during the spawning season. The current study supports the contention that ALP concentration can be used as a proxy for Vg-like proteins and hence gametogenic stage in female *O. edulis*. Both the ALP concentrations and gonadal index were high during the spawning period and the ALP levels were nearly zero when the gonads of the oysters were inactive or during early maturity in April 2013.

In conclusion, the 15 months of reef deployment covered two spawning seasons for *O. edulis*. Culture of oysters on elevated reef modules had no effect on the gonad index and ALP concentration. However, when comparing the gonad maturation between reef and sea bed oysters, the data from this study suggested that oysters held on elevated reef structures completed their gametogenic cycle earlier than sea bed oysters. One possible explanation for these results is that reef oysters had significantly higher filtration rates, stored more energy and could therefore spawn and complete spawning earlier than the sea bed oysters. Improvements in the physiological performance of *O. edulis* cultured on elevated reef structures have been previously addressed in Chapter 3. Oyster reefs have been argued to enhance local ecosystem biodiversity, yet little is known about their associated epifaunal and mobile fauna community in UK waters. Chapter 6 establishes the benefits of reef structures created by *O. edulis* valves in terms of providing substrates for bivalve spat and improving local epifaunal biodiversity.

Chapter 6

Epifaunal utilization of elevated oyster reef habitat in Poole Bay, UK

6.1 Introduction

Improvements in the physiological performance of European flat oysters *Ostrea edulis* cultured on elevated reef structures have been discussed in Chapter 3 and Chapter 5, and published as Sawusdee et al. (2015) (Appendix 12). In this chapter, the role of elevated reef habitats in improving local diversity are reported. Artificial oyster reefs have been widely documented as key marine structures (Jackson et al., 2001; Meyer and Townsend, 2000; Steimle and Zetlin, 2000), which potentially provide ecological benefits (Thomsen and McGlathery, 2006) by enhancing local biodiversity (Luckenbach et al., 2005; Meyer and Townsend, 2000). The better environmental conditions (e.g. water flow, food availability, less sediment, light intensity) at the height of elevated reefs for epifaunal settlement have been reported (Dame et al., 2000; Howard et al., 1994). Moreover, the three-dimensional structure of the elevated reefs created by oyster valves, with its substantial roughness, also promotes greater turbulence, which can improve the availability of the organic particles found within the water column to suspension feeders living at the height of elevated reefs (Frechette et al., 1989; Reidenbach et al., 2013). Grabowski (2004) suggested that artificial oyster reefs (*Crassostrea virginica*) provided valuable substrates for epifaunal species that are important prey resources for fish and crustaceans. Moreover, several previous authors have reported that dead and live oyster valves release chemical cues that could attract epibiota to settle on their valves (Brumbaugh et al., 2006; Mesias-Gansbiller et al., 2013). Therefore, these environmental and biological advantages could potentially increase the biodiversity and abundance of organisms in reef habitats created by oyster valve cultch (Abelson and Denny, 1997), make elevated reef ecosystems more complex and consequently strengthen the stability and sustainability of the reef community (Burman et al., 2012; Thrush et al., 2008; Worm et al., 2006).

Moore (1977) described how suspended sediment and silted substrata restricted the settlement of planktonic larvae. The satisfactory settlement of many epifaunal organisms on hard substrata can be prevented by a layer of silt 1–2 mm in depth (Hutchinson and Hawkins, 1992; Gosling, 2003). In this study, environmental data in Chapter 2 showed that the total suspended solids in the water column at the height of the elevated reefs were significantly lower than in the water column adjacent to the sea bed. Therefore, it is very interesting to test the hypothesis that the epifaunal diversity on *O. edulis* valve cultch situated on elevated reefs was higher than that on oyster valve cultch on the sea bed. Most studies on oyster communities, such as those undertaken in oyster restoration projects for *C. virginica* in the USA, have focussed on the diversity of the oyster assemblages themselves and examined the shell surfaces which provide a large portion of the natural hard substrata for sessile filter-feeding organisms, including oysters, in coastal and estuarine ecosystems (Breitburg et al., 2000; Coen et al., 2007). Korringa (1951) listed over 250 species of epifauna and epiflora on the shells of *O. edulis* in the Oosterschelde, Holland. However, no previous study has compared species richness, diversity and abundance of epifauna on *O. edulis* valves at different heights above the sea bed.

In light of the paucity of available data on the influences of elevated artificial reef habitats created by *O. edulis* valves on local biodiversity in the UK, this chapter discusses whether or not an elevated artificial reef construction provides a large suitable substrate for epifauna including oyster spat. If this was shown to be the case, then elevated oyster reef structures should be considered as a solution for local biodiversity enhancement.

6.2 Methods

Three elevated reef boxes on the top of reef modules and three oyster cages on the sea bed were recovered at each sampling interval as described in Chapter 2. Every reef box and oyster cage, wrapped in fine mesh net *in situ* before recovery, were kept in plastic containers and the mesh soaked with sea water to prevent drying out of live oysters and epifauna on oyster valves. These were immediately transferred to NOCS on the day of collection. Sampling

frequency was designed to observe seasonal variation of epifaunal utilization of elevated oyster reef habitat over 15 months (September 2012; 1month after deployment, April 2013; 8 months after deployment, August 2013; 12 months after deployment and November 2013; 15 months after deployment). Each reef box and oyster cage was separately maintained for five days in the recirculating aquarium system: this facilitated live identification of the epifaunal species.

6.2.1 Live and dead oyster valve samples and processing

The complexity and the surface area of oyster valves influences species richness, diversity and abundance of epifaunal species (Bartol and Mann, 1997; Thomsen et al., 2007). Smyth and Roberts (2010) reported that *O. edulis* valves showed increased epifaunal diversity with increasing valve size. By considering the influence of valve size on epifaunal diversity and the different oyster positions (reef and sea bed), the valves were divided into 5 categories; i.e. 2 categories of live valves and 3 categories of oyster valve cultch viz.:

a) live oyster valves on elevated reefs (LVR)
b) live oyster valves on sea bed (LVS)
c) whole oyster valve cultch on elevated reefs (WVR)
d) fragmented oyster valve cultch on elevated reefs (FVR)
e) whole oyster valve cultch on sea bed (WVS)

At each sampling interval, the WVR and FVR in the reef boxes and the WVS in the sea bed cages were each haphazardly sampled for 30 valves. Therefore, 90 oyster valve cultch in total were used for epifaunal identification. The number of live oysters sampled was determined by surviving oysters at the time of sampling. Consequently, the number of live oyster samples varied across sampling interval and position. In September 2012, April 2013, August 2013 and November 2013, 25, 25, 16 and 11 live oysters were taken from the reef boxes (LVR) and 19, 16, 13 and 9 live oysters were taken from the sea bed oyster cages (LVS), respectively.

Shell roughness and the surface area of oyster valves were difficult to measure. Shell height (SH) and shell width (SW) were measured using a vernier caliper (±0.02 mm) in all valve samples at every sampling interval. SH and SW were compared between valve categories (LVR, LVS, WVR, FVR and WVS) and between

sampling intervals by using one way ANOVA. The oyster valves that were not significantly different in shell height (SH) and shell width (SW) were assumed to be not significantly different in terms of valve surface area (Figure 6.1, Results section 6.3.1). There was no significant difference in shell height and shell width between the oyster valve cultch (dead valve) categories (WVR, FVR and WVS) or between the live oyster valve categories (LVR and LVS). However, the size of live and dead oyster valve samples were significantly different (Tukey's HSD test, $P<0.05$). Due to this size difference, species richness, species diversity and abundance of epifauna on live oysters and on oyster valve cultch were presented separately.

Epifaunal species were identified under the light microscope (Nikon SMZ800, 10x) during the initial five days that they were maintained in the recirculating aquarium system at NOCS. Individual species of epifauna on each valve were taxonomically classified to either genus or species level following Hayward and Ryland (1996). The nonmenclature for identified species was updated as appropriate *via* the 'European Register of Marine Species' (accessible at http://www.marbef. org/data/erms.php). Solitary epifaunal species, including oyster spat, were enumerated. Non-encrusting colonial epibiota were counted as clumps (sponges, tunicates) or branches (hydroids) (Fariñas-Franco et al., 2013). Encrusting taxa such as bryozoans were recorded as present (Fariñas-Franco et al., 2013).

6.2.2 Statistical analyses

Species richness was calculated as the number of epifaunal species per valve, Species diversity index (Shannon's H) on each valve was also calculated as well as abundance which was expressed as the number of individuals per valve. These indices were used as indicators to investigate and compare the development of epifaunal colonisation between oyster valve cultch positioned on the elevated reef and sea bed.

Prior to statistical analyses, univariate data (i.e. species richness, diversity index and abundance) were first tested for normality and homogeneity of variance using the Shapiro-Wilk method**.** When assumptions of homogeneity of variances and normal distribution were not met, the data were log (x + 1)

transformed. Two-way ANOVA was carried out to test the influences of elevation (LVR vs LVS and WVR vs WVS) or sampling interval, or interaction between main factors, on species richness, diversity index and the abundance of epifauna on oyster valves. Where appropriate, *post-hoc* pairwise multiple comparisons (Tukey's HSD tests) compared the effect of sampling interval or elevation on species richness, diversity index and the abundance of epifauna on oyster valves. One-way ANOVA was applied to investigate temporal variation of the five most abundance species recorded on each valve type. In all cases, statistical analyses were carried out using SigmaPlot software (version 12.5) and statistical significance was accepted at $\alpha = 0.05$.

Multivariate statistical approaches (PRIMER software package, version 6.1.6) were utilized to compare the structure of epifaunal communities between the valves positioned on elevated reefs and the sea bed (Clarke, 1993). Abundance of epifauna were transformed to presence and absence. This transformation reduces weighting between the common species and rarer species (Clarke and Gorley, 2001). Cluster analysis was performed using the Bray–Curtis similarity index and group linkage was used to create dendrograms of epifaunal composition (Bray and Curtis 1957; Field et al., 1982). Epifaunal community assemblage on oyster valves was visualised using multidimensional scaling (MDS, Clarke, 1993). One-way Analyses of Similarity (ANOSIM, Clarke, 1993) on Bray–Curtis dissimilarity matrices (Bray and Curtis, 1957) was applied to test the differences in epifaunal species composition between valves positioned on the elevated reefs and the sea bed. SIMPER routines (Clarke, 1993) were used to identify the taxa that contributed most to multivariate dissimilarity and account for the observed assemblage differences. Taxa were considered to be a good discriminator between the elevated reef and sea bed communities if they possessed a dissimilarity to standard deviation ratio >1.3 (Clarke and Warwick, 1994). A dissimilarity >8% would indicate some key species were typical of only a single location (elevated reef or sea bed), indicating taxa were characteristic in discriminating between locations (Bulleri et al., 2005).

6.3 Results

6.3.1 Epifaunal colonisation on oysters valves

As described in section 6.2.1, the shell size can influence the numbers of species, abundance and diversity index of epifauna on oyster valves (Bartol et al., 1999; Thomsen et al., 2007). Therefore, every valve sample was measured in terms of height (mm) and width (mm) to test the range of shell sizes (Figure 6.1). One-way ANOVA confirmed that there were significant differences in shell height and width between valve categories ($P<0.05$). Pairwise multiple comparisons (Tukey's HSD test) suggested that there was no significant difference in valve size between the oyster valve cultch groups (WVR, FVR and WVS) or between the live oyster valve groups (LVS and LVR). In contrast, comparison between the live oysters and valve cultch groups were significantly different in shell height and width ($P<0.05$). Consequently, the comparison of species richness (numbers of species), diversity index (Shannon's H index) and abundance of epifauna on oyster valve cultch and live oyster valves were done separately.

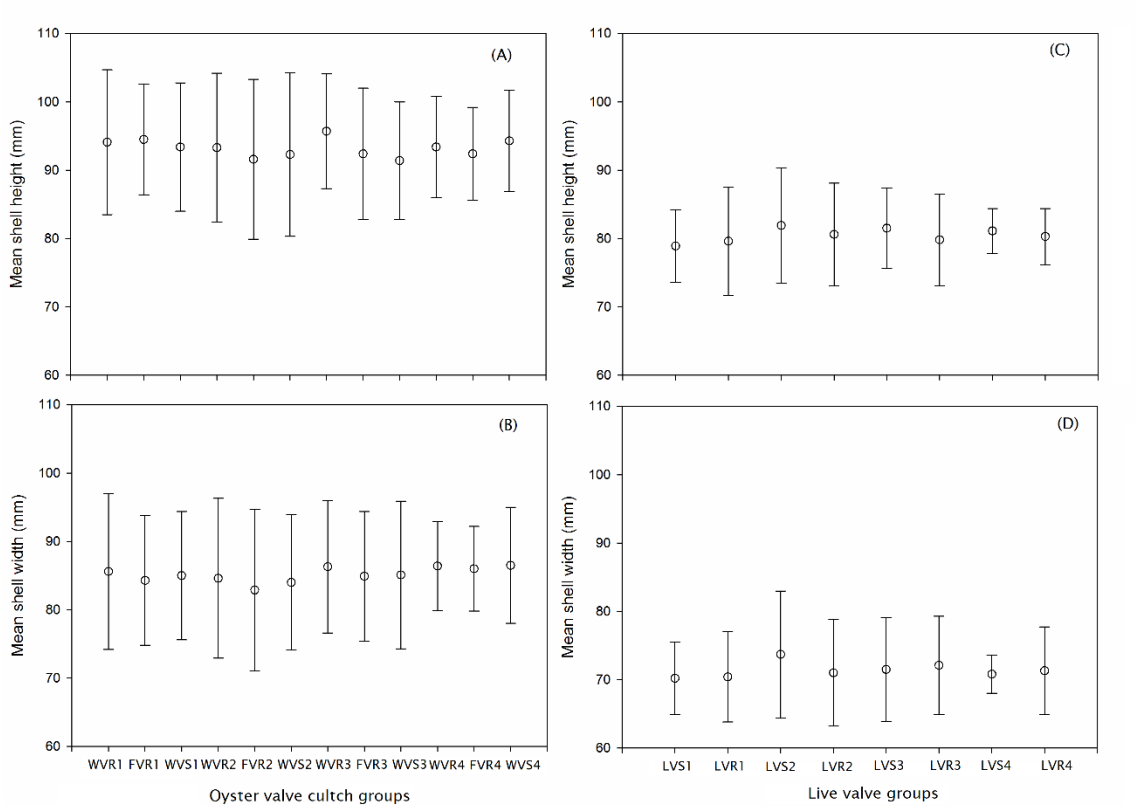

Figure 6.1 Shell height and width (mm) (mean ± SD) in each category and sampling interval; (A): shell height within the oyster valve cultch groups (WVR, FVR and WVS), (B): shell width within oyster valve cultch groups (C) shell height within live valve groups (LVS and LVR), (D) shell width within live valve groups. One-way ANOVA indicated that there was no significant difference in shell height and shell width within valve cultch or live valve categories but there were significant differences in shell size between valve cultch and live valve categories ($P<0.05$).

During the 15 months of reef deployment, a total of 54 epifaunal species, from 7 phyla, were recorded from the oyster valve artificial reefs in Poole Bay. The Tunicata were the most diverse taxa with 18 ascidian species identified, followed by Bryozoa (12 species) and Cnidaria (10 species). The Annelida were recorded as the most abundant, followed by Crustacea and Bryozoa, respectively. Moreover, artificial reef construction also served as the habitat for a variety of other epifauna such as molluscs and sponges. A total of 54 species, including *O. edulis* spat, were observed on valves collected from reef boxes on the top of the reef modules. Conversely, 23 species and no *O. edulis*

spat were found on oyster valves in oyster cages directly laid on the sea bed. A full list of species and abundance can be found in Appendix 2-6.

Of the total 54 taxa identified, the five most dominant species contributed 80.8% of the total individual abundance. These were keeled tubeworms (*Pomatoceros triqueter*), acorn barnacles (*Elminius modestus*), segmented worms (*Filograna implexa*), spiral tubeworms (*Spirorbis spirorbis*) and slipper limpets (*Crepidula fornicata*) (Appendix 7).

As can be seen from Figure 6.2, *P. triqueter* markedly increased in abundance on the dead oyster valves, WVR (40.5±4.6 individuals/valve) and FVR (25.0±3.1 individuals/valve) on elevated reefs and to a lesser degree on WVS (20.0 ±2.3 individuals/valve) on the sea bed in August 2013 and then were slightly reduced in abundance in November 2013. *E. modestus* also increased in abundance in August 2013, but only on those shells that were situated on the elevated reefs (WVR; 40.4±7.6 individuals/valve, FVR; 26.1±6.9 individuals/valve and LVR; 20.1±3.6 individuals) and remained low on both oyster valve cultch and live oysters situated on the sea bed. *F. implexa* was numerically dominant on valve cutch on the elevated reefs (WVR; 8.3±5.7 individuals/valve and FVR; 2.0 ±1.3 individuals/valve). *F. implexa* was first observed in August 2013 and increased in abundance through November 2013 (WVR and FVR only). *S. spirorbis* was initially observed in April 2013 in low abundance. Abundance of *S. spirorbis* increased in August 2013 on the valves of live oysters and valve cultch on the sea bed and only on valve cultch on the elevated reefs. Abundance of *S. spirorbis* continued to increase in November 2013, but only on both oyster valve cultch on the elevated reef (WVR; 3.0±0.7 individuals/valve, FVR; 2.7±0.2 individuals/valve) and sea bed (WVS; 3.5±1.3 individuals/valve). The abundance of *C. fornicata* on valves positioned on the sea bed (WVS and LVS) was consistent. Whilst the abundance of *C. fornicata* on live oysters on the elevated reefs (LVR) decreased with sampling intervals, the overall frequency of *C. fornicata* was low (note the scale differences on Figures 6.2).

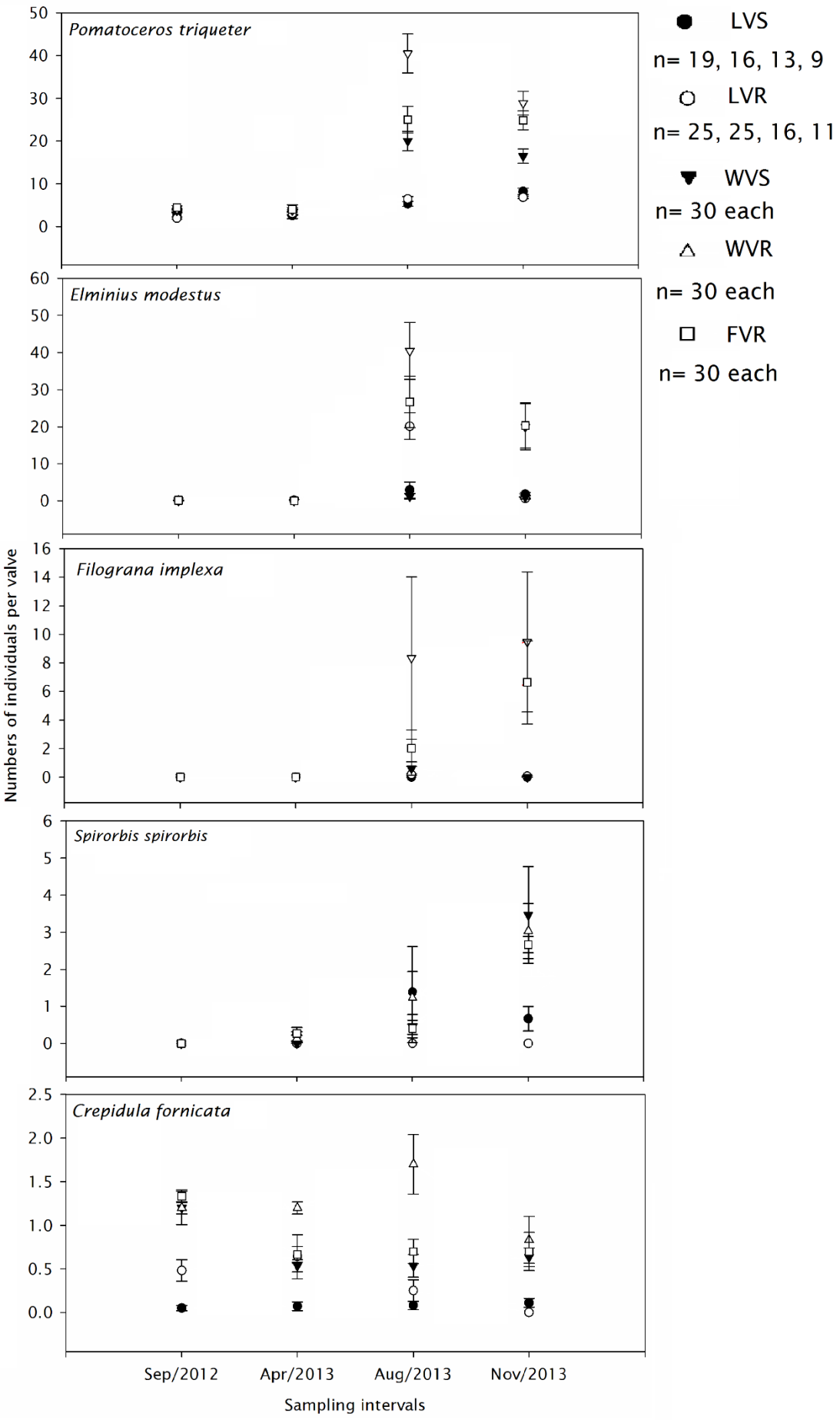
Pomatoceros triqueter
Elminius modestus
Filograna implexa
Spirorbis spirorbis
Crepidula fornicata
Numbers of individuals per valve
Sep/2012
Apr/2013
Aug/2013
Nov/2013
Sampling intervals
LVS
n= 19, 16, 13, 9
LVR
n= 25, 25, 16, 11
WVS
n= 30 each
WVR
n= 30 each
FVR
n= 30 each

Figure 6.2 (Previous page) Temporal variation in the abundance (mean ± SE) of the five most dominant species (numbers of individuals) colonised in each valve category. The graphs are ordered in terms of the most abundant species (top) to the least (bottom). The numbers of valve samples for each valve type at each sampling interval are indicated underneath the valve abbreviations in the figure legend. Note scale variation on each graph.

6.3.2 Epifaunal colonisation on live oyster valves

6.3.2.1 Epifauna settled on live oysters on the sea bed (LVS)

A total of 15 species of epifauna settled on LVS during the 15 month period (Appendix 2). The five most abundant species characterised 90.0% of total individuals. These were *Pomatoceros triqueter* (62.6%), *Elminius modestus* (14.3%), *Spirorbis spirorbis* (7.4%), *Electra pilosa* (2.9%) and *Plagioecia patina* (2.9%) (Appendix 7).

As can be seen from Figure 6.3, the most dominant epifaunal species in September 2012 was *P. triqueter* (2.7±0.5 individuals/oyster) contributing 85.2% of total individuals. There were concurrent significant increases in abundance over time, increasing from 5.3±0.6 individuals/oyster in August 2013 to 8.2±0.8 individuals/oyster in November 2013. *E. modestus* was first observed in August 2013 (2.9±2.2 individuals/oyster), while *S. spirorbis* was initially observed in April 2013 (0.2±0.2 individuals/oyster). The abundance of both of these species was significantly greater in August and November 2013 than in September 2012 and April 2013. *E. pilosa* was not found in April 2013 and there was no significant difference in the numbers of colonies between sampling intervals. The abundance of *P. patina* significantly increased with sampling intervals and their colonies were significantly greater in August 2013 (0.4±0.1 colonies/oyster) and November 2013 (0.7±0.2 colonies/oyster)

In conclusion, epibiota showed seasonal variations in abundance throughout the year. *P. triqueter* and *E. pilosa* were pioneer species found in September 2012, i.e. a month after reef deployment. *S. spirorbis* was identified in April 2013 whilst *E. modestus and P. patina* were observed from August 2013 onwards. Although *P. triqueter* was the dominant species during the 15 month experiment, the abundance of *E. modestus, S. spirorbis,* and *P. patina* notably increased in August 2013 and November 2013, which consequently reduced the contribution of *P. triqueter* in these months.

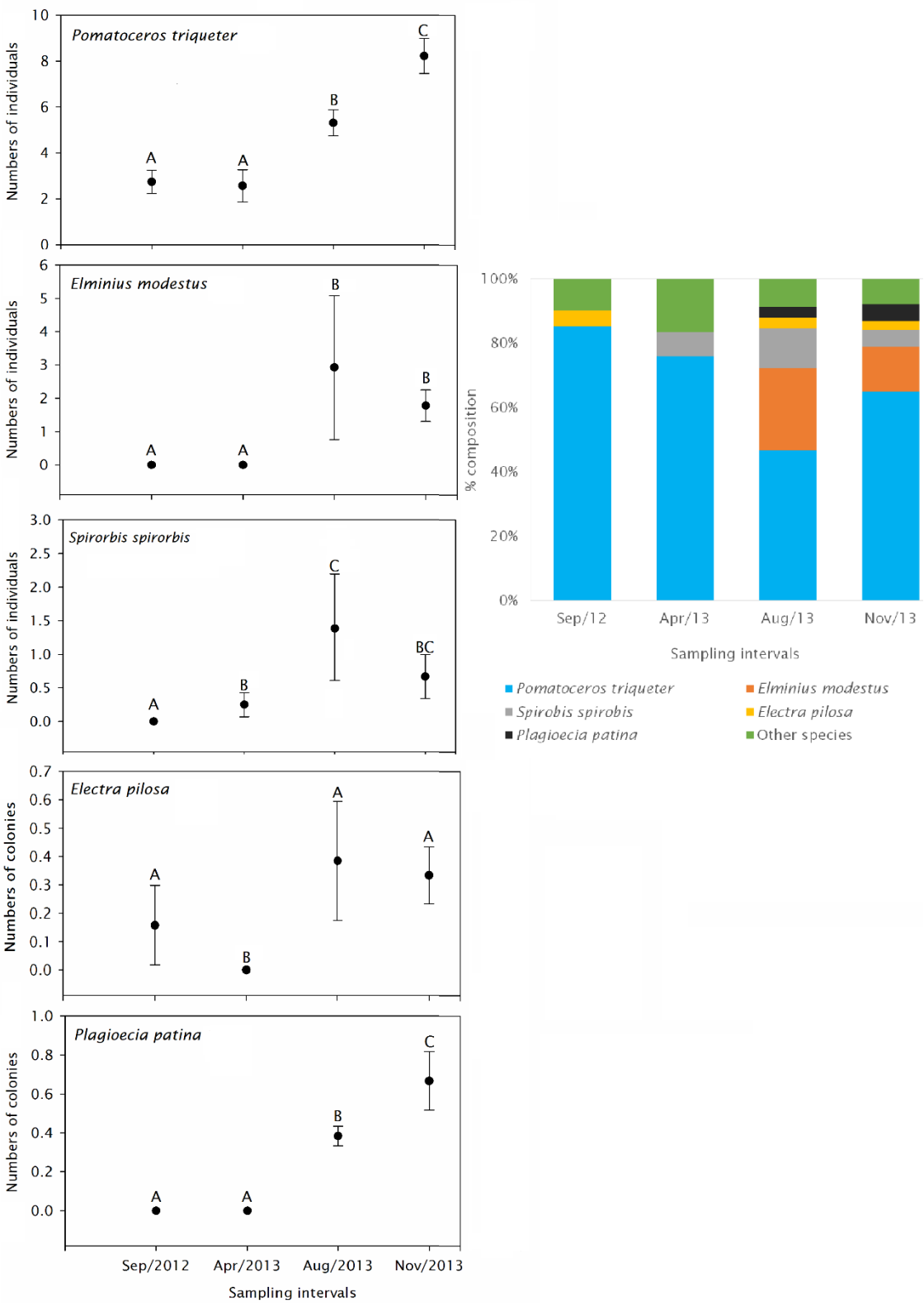
Pomatoceros triqueter
Numbers of individuals
Elminius modestus
Numbers of individuals
Spirorbis spirorbis
Numbers of individuals
Electra pilosa
Numbers of colonies
Plagioecia patina
Numbers of colonies
Sep/2012
Apr/2013
Aug/2013
Nov/2013
Sampling intervals
% composition
Sep/12
Apr/13
Aug/13
Nov/13
Sampling intervals
Pomatoceros triqueter
Elminius modestus
Spirobis spirobis
Electra pilosa
Plagioecia patina
Other species

Figure 6.3 (Previous page) Temporal variation in the abundances (mean ± SE) of the five dominant species colonised on LVS. The graphs are ordered in terms of the most abundant species (top) to the least (bottom), the stacked column showed relative abundance of the five species and other epifaunal species. Shared letters (A-C) between sampling intervals indicate no significant difference (one-way ANOVA, $P>0.05$). Note scale variation on each graph. Oyster samples at each sampling interval were 19, 16, 13 and 9 individuals, respectively.

6.3.2.2 Epifauna settled on live oysters on the elevated reefs (LVR)

There were a total of 32 species recorded on LVR during the 15 month experiment (Appendix 3). The five highest abundant species contributed 75.0% of total individuals recorded on LVR. *Elminius modestus* was the most dominant species (31.5%), followed by *Pomatoceros triqueter* (28.2%), *Disporella hispida* (8.1%), *Tubulipora plumosa* (3.7%) and *Dendrodoa grossularia* (3.5%) (Appendix 7).

As can be seen in Figure 6.4, the mean of *E. modestus* per oyster markedly increased from 0.2±0.1 individuals/oyster in April 2013 to 20.1±3.6 individuals/oyster in August 2013. *P. triqueter* was the first dominant species observed in September 2012 (1.9±0.2 individuals/oyster) and continued to significantly increase in abundance between April 2012 (3.0±0.2 individuals/oyster) and August 2013 (6.4±0.6 individuals/oyster), whilst there was no significant increase in abundance between August 2013 and November 2013. The abundance of bryozoans (*D. hispida* and *T. plumosa*) significantly increased in April 2013 (1.8±0.2 and 0.6±0.1 colonies /oyster respectively) and then significantly decreased in August 2013 (1.0±0.1 and 0.5±0.1 colonies /oyster respectively). Lastly, *D. grossularia* abundance slightly varied between sampling intervals.

To conclude, the two bryozoans (*D. hispida* and *T. plumosa*), and the 'baked bean' ascidian *D. grossularia* and the keel worm *P. triqueter* were pioneer species that had settled on LVR by September 2012, i.e. a month after reef deployment. A marked increase in abundance of *E. modestus* was observed in summer (August 2013) but this significantly reduced in November 2013.

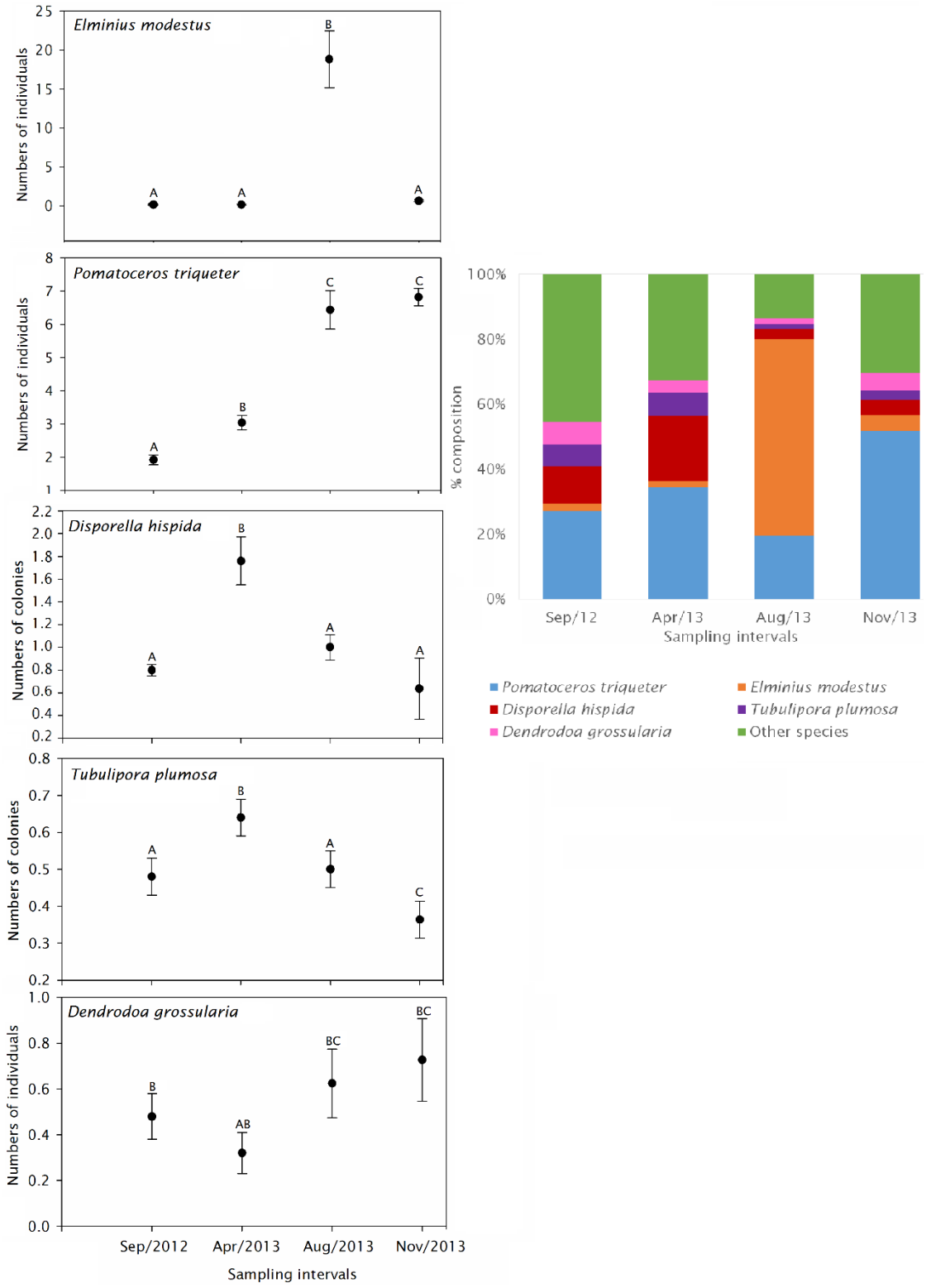
Elminius modestus
Numbers of individuals
Pomatoceros triqueter
Numbers of individuals
Disporella hispida
Numbers of colonies
Tubulipora plumosa
Numbers of colonies
Dendrodoa grossularia
Numbers of individuals
Sep/2012
Apr/2013
Aug/2013
Nov/2013
Sampling intervals
% composition
Sep/12
Apr/13
Aug/13
Nov/13
Sampling intervals
Pomatoceros triqueter
Elminius modestus
Disporella hispida
Tubulipora plumosa
Dendrodoa grossularia
Other species

Figure 6.4 (previous page) Temporal variation in the abundances (mean ± SE) of the five most dominant species colonised on LVR. The graphs are ordered in terms of the most abundant species (top) to the least (bottom), whist the stacked column showed relative abundance of the five species and other epifaunal species. Shared letters (A-C) between sampling intervals indicate no significant difference (one-way ANOVA, $P>0.05$). Note scale variation on each graph. Oyster samples at each sampling interval were 25, 25, 16 and 11 individuals, respectively.

6.3.2.3 Comparison of epifaunal species richness (number of epifaunal species per individual) between live oyster valves on the elevated reef (LVR) and sea bed (LVS)

The number of epifaunal species per individual (mean ± SE) was used to define species richness. Species richness on both LVR and LVS increased over time. The species richness for LVR and LVS were the lowest in September 2012, a month after reef deployment, (2.5±0.4 and 1.2±0.2, respectively) and increased in August 2013, 12 months after reef deployment, to 6.4±0.4 and 3.2±0.2, respectively (Figure 6.5). Two-way ANOVA indicated that elevation (reef/sea bed) and sampling interval had a significant effect on species richness, but no significant difference was found for the interaction between the two main effects on species richness (Table 6.1).

Pairwise multiple comparisons (Tukey's HSD test) comparing the effect of sampling interval on epifaunal species richness showed that the species richness of epifauna on live oyster valves collected in August 2013 was significantly higher compared with September 2012 and April 2013. Moreover, species richness observed in November 2013 was significantly higher than September 2012 and April 2013. However, there was no significant difference between the remaining sampling intervals i.e. between August and November 2013 samplings and between September 2012 and April 2013 samplings (Table 6.2).

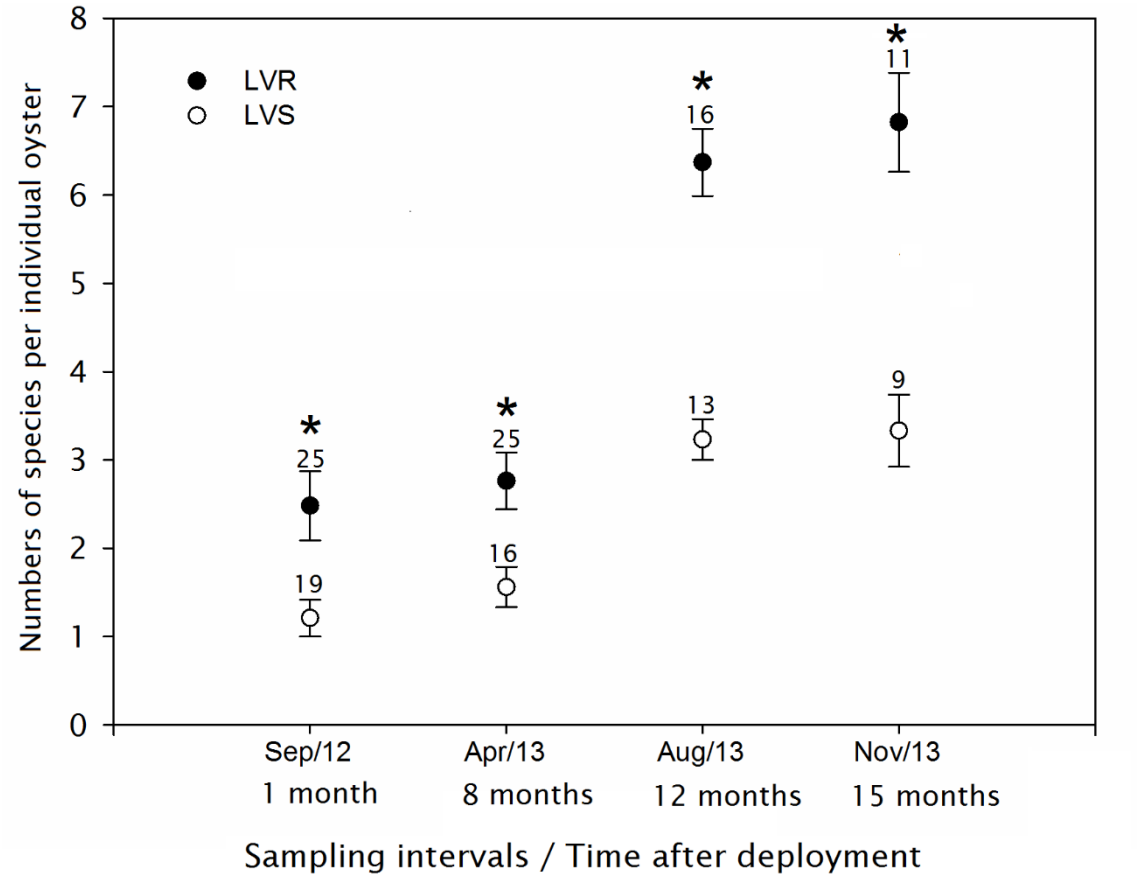

Figure 6.5 Species richness, number of epifaunal species per individual (mean ± SE), of live oysters positioned on elevated reefs (LVR) and the sea bed (LVS). Species richness was significantly affected by elevation (reef/sea bed) and sampling interval (two-way ANOVA, $P<0.001$ and $P<0.001$, respectively). Significant differences are denoted by asterisks. The numbers of live oysters analysed from each location is indicated above the error bars.

Table 6.1 Comparison of the effect of sampling interval, elevation (reef/sea bed), and the interaction between sampling interval and elevation on species richness. Significant differences are denoted by asterisks.

Sources of variation	df	F	P-value
Sampling interval	3	29.44	0.001*
Elevation (reef/sea bed)	1	30.86	0.001*
Sampling interval x Elevation	3	2.23	0.544

Table 6.2 *Post-hoc* (Tukey's HSD test) results comparing the effect of sampling interval on epifaunal species richness. "ns" represents no significant difference

Post-hoc test of the effect of sampling intervals	P-value		
	Sep 2012	Apr 2013	Aug 2013
Apr 2013	ns		
Aug 2013	<0.001	<0.001	
Nov 2013	<0.001	<0.001	ns

6.3.2.4 Comparison of species diversity (Shannon's H index) between live oysters collected from the elevated reef (LVR) and sea bed (LVS)

Shannon's H diversity index for LVR and LVS showed seasonal variation. The diversity index values for LVR and LVS were the lowest in April 2013 (0.5±0.1 and 0.3±0.1, respectively) and increased in August 2013 (1.2±0.1 and 0.9±0.1, respectively, Figure 6.6). Two-way ANOVA indicated that elevation (reef/sea bed) and sampling interval had a significant effect on the diversity index but no significant difference was found for the interaction between the two main effects (Table 6.3).

Pairwise multiple comparisons (Tukey's HSD test) showed that the species diversity on the valves of live oysters collected in August 2013, 12 months after reef deployment, was significantly higher in comparison to that seen in September 2012 and April 2013. The diversity index of epifauna on live oysters in November 2013 was significantly higher compared with September 2012 and April 2013. However, there was no significant difference in diversity index between other sampling intervals i.e. between August and November 2013 and between September 2012 and April 2013 samplings (Table 6.4).

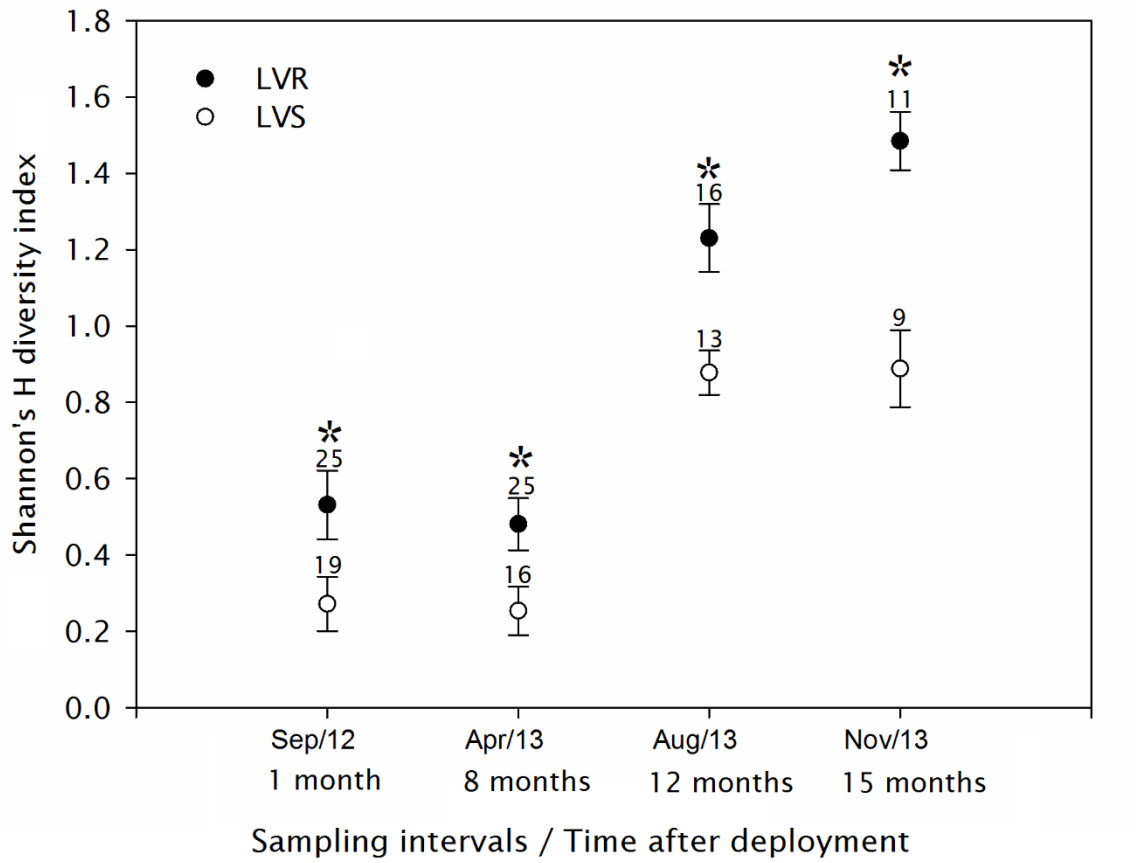

Figure 6.6 Shannon's H diversity index (mean ± SE), of live oysters positioned on the elevated reefs (LVR) and the sea bed (LVS). The diversity index was significantly affected by elevation (reef/sea bed) and sampling interval (two-way ANOVA, $P<0.001$ and $P<0.001$, respectively). Significant differences are denoted by asterisks. The numbers of oysters analysed from each location is indicated above the error bars.

Table 6.3 Comparison of the effect of sampling interval, elevation (reef/sea bed), and the interaction between sampling interval and elevation on diversity index. Significant differences are denoted by asterisks

Sources of variation	df	F	P-value
Sampling interval	3	44.87	0.001*
Elevation (reef/sea bed)	1	27.46	0.001*
Sampling interval x Elevation	3	0.387	0.763

Table 6.4 *Post-hoc* (Tukey's HSD test) results comparing the effect of sampling interval on epifaunal diversity index. "ns" represents no significant difference

Post-hoc test of the effect of sampling intervals	P-value		
	Sep 2012	Apr 2013	Aug 2013
Apr 2013	ns		
Aug 2013	<0.001	<0.001	
Nov 2013	<0.001	<0.001	ns

6.3.2.5 Comparison between abundance (number of individuals) of epifauna on live oysters collected from the elevated reefs (LVR) and sea bed (LVS)

Abundance (mean ± SE), was also used as a measure of the development of the epifaunal community on oyster valves. The abundance of epifauna on LVR and LVS varied between seasons. The highest abundance on LVR was recorded in August 2013 (33.1±4.1 individuals/oyster), whilst the highest abundance on LVS was recorded in November 2013 (12.7±1.3 individuals/oyster). The lowest abundance per valve on both LVR and LVS were recorded in April 2013 (8.8±0.7, 3.4±0.7 individuals/valves, respectively, Figure 6.7). Two-way ANOVA indicated that interaction between elevation (reef/sea bed) and sampling interval had a significant effect on the epifaunal abundance (Table 6.5).

Pairwise multiple comparisons (Tukey's HSD test) of the abundance of epifauna on live oyster valves at each sampling interval between different elevation (reef: LVR and sea bed: LVS) showed that epifaunal abundance on live oysters positioned on elevated reefs was significantly higher than those oysters on the sea bed at any sampling intervals (Table 6.6).

Pairwise multiple comparisons (Tukey's HSD test) of epifaunal abundance on LVS between different sampling intervals showed that abundance in August 2013 was significantly higher than in September 2012 and April 2013. Moreover, the number of individuals (abundance) recorded in November 2013 was also significantly higher compared with September 2012 and April 2013.

In addition, epifaunal abundance on LVS in September 2012 was significantly higher compared to April 2013. However, there was no significant difference in epifaunal abundance between August 2013 and November 2013 (Table 6.7).

Pairwise multiple comparisons (Tukey's HSD test) of epifaunal abundance on LVR between sampling intervals showed that the abundance of epifauna on LVR collected in August 2013 was significantly higher than in other sampling intervals. Moreover, the abundance recorded in November 2013 was also significantly higher compared with April 2013. There was no significant difference in epifaunal abundance observed in September 2012 in comparison with April 2013 and November 2013 (Table 6.7).

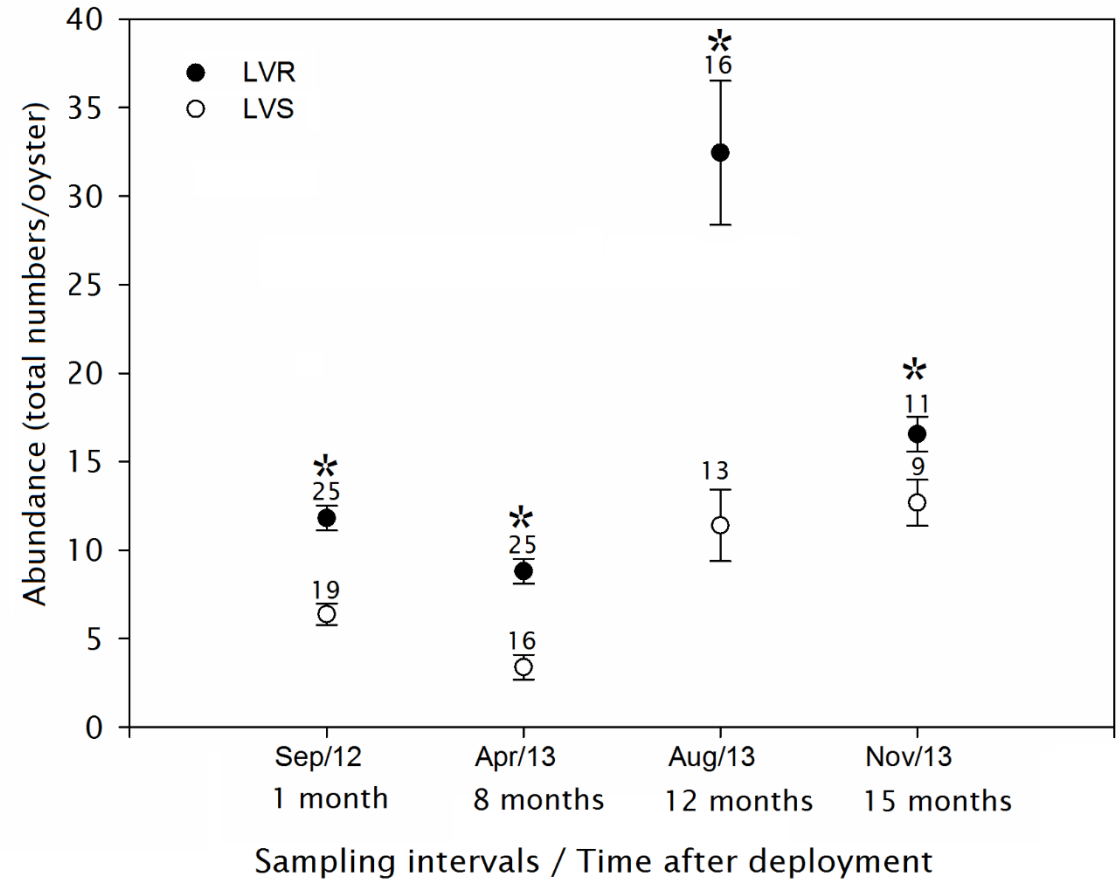

Figure 6.7 Abundance, total numbers of all species per individual (mean ± SE), on live oysters positioned on elevated reefs (LVR) and the sea bed (LVS). Abundance was significantly affected by interaction between elevation (reef/sea bed) and sampling interval (two-way ANOVA, $P<0.01$). Significant differences are denoted by asterisks. The number of oysters analysed from each locations is indicated above the error bars.

Table 6.5 Comparison of the effect of sampling interval, elevation (reef/sea bed), and the interaction between sampling interval and elevation on epifaunal abundance. Significant differences are denoted by asterisks.

Sources of variation	df	F	P-value
Sampling interval	3	45.67	0.001*
Elevation (reef/sea bed)	1	78.98	0.001*
Sampling interval x Elevation	3	4.18	0.007*

Table 6.6 *Post-hoc* (Tukey's HSD test) results comparing the effect of elevation (reef/sea bed) between sampling interval on the abundance of epifauna on live oysters.

Post-hoc test (Tukey's HSD)	P-value
Elevated reef vs Sea bed (Sep 2012)	<0.001
Elevated reef vs Sea bed (Apr 2013)	<0.001
Elevated reef vs Sea bed (Aug 2013)	<0.001
Elevated reef vs Sea bed (Nov 2013)	<0.05

Table 6.7 *Post-hoc* (Tukey's HSD test) results comparing the effect of sampling interval within elevation (reef: LVR/sea bed: LVS) on the abundance of epifauna colonised on live oysters. "ns" represents no significant difference

Post-hoc test (Tukey's HSD)	P-value		
	Sep 2012	Apr 2013	Aug 2013
Effect of sampling interval on the abundance of epifauna on LVS			
Apr 2013	<0.001		
Aug 2013	<0.05	<0.001	
Nov 2013	<0.01	<0.001	ns
Effect of sampling interval on the abundance of epifauna on LVR			
Apr 2013	ns		
Aug 2013	<0.001	<0.001	
Nov 2013	ns	<0.001	<0.01

6.3.3 Epifaunal colonisation on oyster valve cultch

6.3.3.1 Epifauna settled on whole oyster valve cultch re-laid on the sea bed (WVS)

There were a total of 23 species recorded on WVS (Appendix 4). The five most abundant species represented 88.9% of total individuals recorded on WVS. These were *Pomatoceros triqueter* (72.0%), *Spirorbis spirorbis* (6.3%), and *Crepidula fornicata* (4.7%), *Elminius modestus* (3.5%) and *Disporella hispada* (2.4%) (Appendix 7).

P. triqueter was the dominant species at every sampling interval (Figure 6.8). The most notable observation was the significant increase in abundance of *P. triqueter* in August 2013 (20.0±2.3 individuals/valve). *S. spirorbis* was not observed until August 2013 and significantly increased in abundance in November 2013 (3.5±1.3 individuals/valve). The abundance of *C. fornicata* in September 2012 (1.2±0.2 individuals/valve) was significantly greater compared to the abundance noted at successive sampling intervals. The abundance of *E. modestus* significantly increased in August 2013 (1.2±1.7 individuals/valve). Finally, *D. hispada* were observed in low numbers (0.3-0.4 individuals/valve) throughout the sampling period, but did not significantly change in abundance. Whilst some species' abundance significantly changed over the 15 months, individual numbers remained low for some species. As an example, the significant change in *E. modestus* varied from 0 individuals/valve (April 2013) to 1.2 individuals/valve (August 2013). In contrast, the significant increase in the abundance of *P. triqueter* between April and August 2013 varied from a mean of 4.5 to 20.0 individuals/valve.

In conclusion, *P. triqueter, C. fornicata* and *D. hispada* were the early pioneer species that settled on WVS in September 2012, i.e. a month after reef deployment. The numbers of *P. triqueter* peaked in August 2013 whilst *E. modestus and S. spirorbis* were not observed until August 2013.

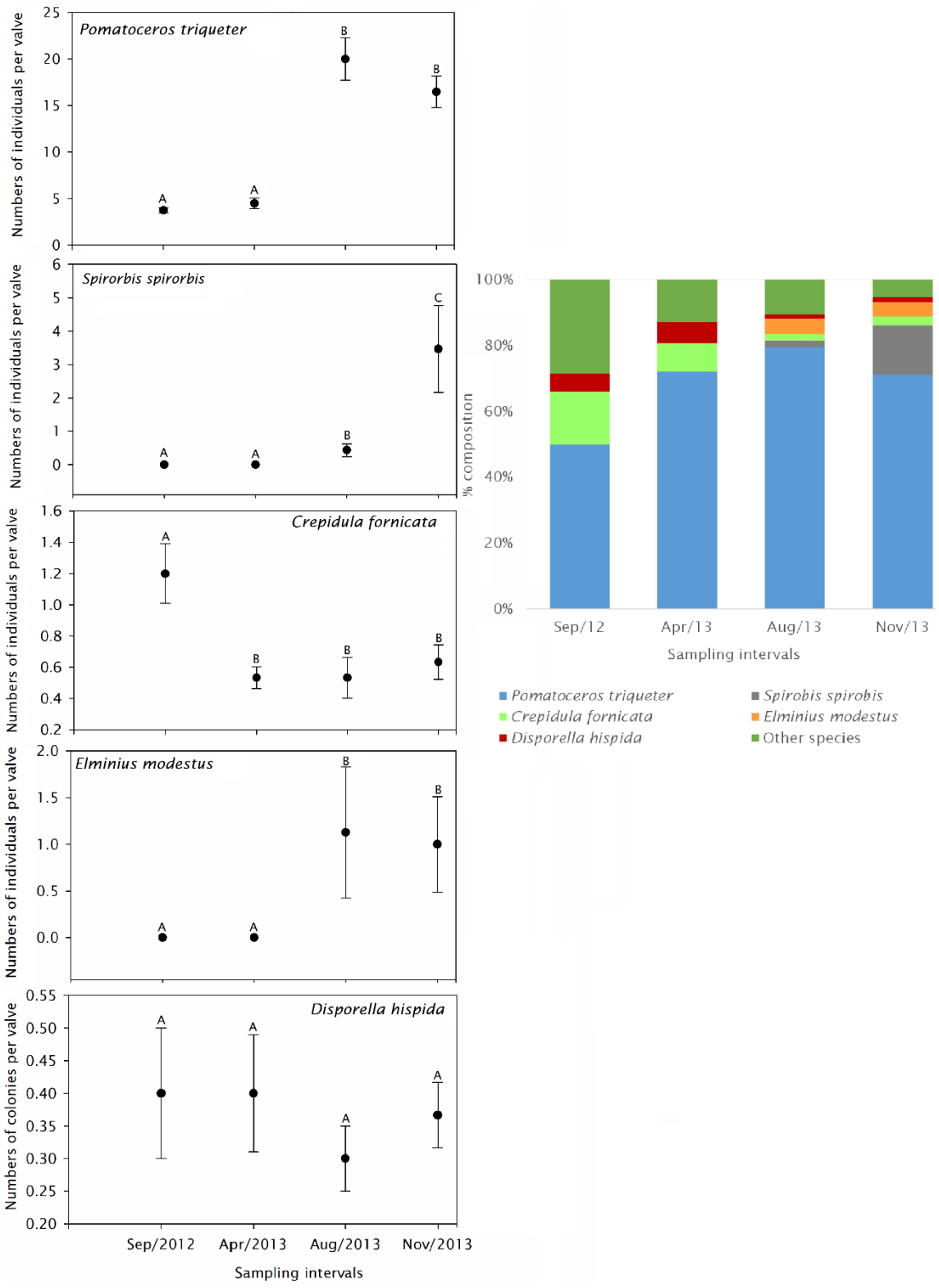
Pomatoceros triqueter
Spirorbis spirorbis
Crepidula fornicata
Elminius modestus
Disporella hispida
Numbers of individuals per valve
Numbers of colonies per valve
Sep/2012
Apr/2013
Aug/2013
Nov/2013
Sampling intervals
% composition
Sep/12
Apr/13
Aug/13
Nov/13
Pomatoceros triqueter
Spirobis spirobis
Crepidula fornicata
Elminius modestus
Disporella hispida
Other species

Figure 6.8 (Previous page) Temporal variation on the abundances per valve (mean ± SE) of the five most dominant species colonised on WVS. The graphs are ordered in terms of the most abundant species (top) to the least (bottom), whist the stacked column showed relative abundance of the five species and other epifaunal species. Shared letters (A-C) between sampling intervals indicate no significant difference (one-way ANOVA, $P>0.05$). Note scale differences in the number of colonies / individuals per valve. Valve samples at each sampling interval were 30 valves.

6.3.3.2 Epifauna settled on intact oyster valve cultch on the elevated reefs (WVR)

There were 48 species in total recorded on WVR during the experimental period (Appendix 5). The five most abundant species represented 85.3% of total individuals of epifauna recorded on WVR. These were *Pomatoceros triqueter* (39.9%), *Elminius modestus* (31.4%), *and Filograna implexa* (9.2%), *Crepidula fornicata* (2.5%) and *Spirorbis spirorbis* (2.3%) (Appendix 7).

P. triqueter was the most dominant species at each sampling interval (Figure 6.9). The numbers of *P. triqueter* significantly increased in August 2013, (40.5±4.5 individuals/valve) and significantly decreased in November 2013 (28.9±2.8 individuals/valve). Similarly to *P. triqueter, E. modestus* significantly increased in abundance in August 2013 (40.4±7.6 individuals/valve) then significantly decreased in abundance in November 2013 (20.0±6.2individuals/valve). *F. implexa* abundance significantly increased in August 2013 (8.3±5.7 individuals/valve). The abundance of *C. fornicata* also significantly increased in August 2013 (1.7±0.3 individuals/valve) and significantly reduced in November 2013 (0.8±0.3 individuals/valve). Lastly, *S. spirorbis* abundance significantly increased sequentially in August 2013 (1.2±0.7 individuals/valve) and November 2013 (3.0±0.7 individuals/valve).

Overall, the epibiota showed seasonal variations in abundance throughout the year and a progression in the numbers of species present. *P. triqueter, E. modestus* and *C. fornicata* were pioneer species observed a month after reef deployment in September 2012. *F. implexa* and *S. spirorbis* were observed on WVR in August 2013 and November 2013. The numbers of individuals for all five dominant species were low in September 2012 and then significantly increased in abundance in August 2013, particularly *E. modestus*.

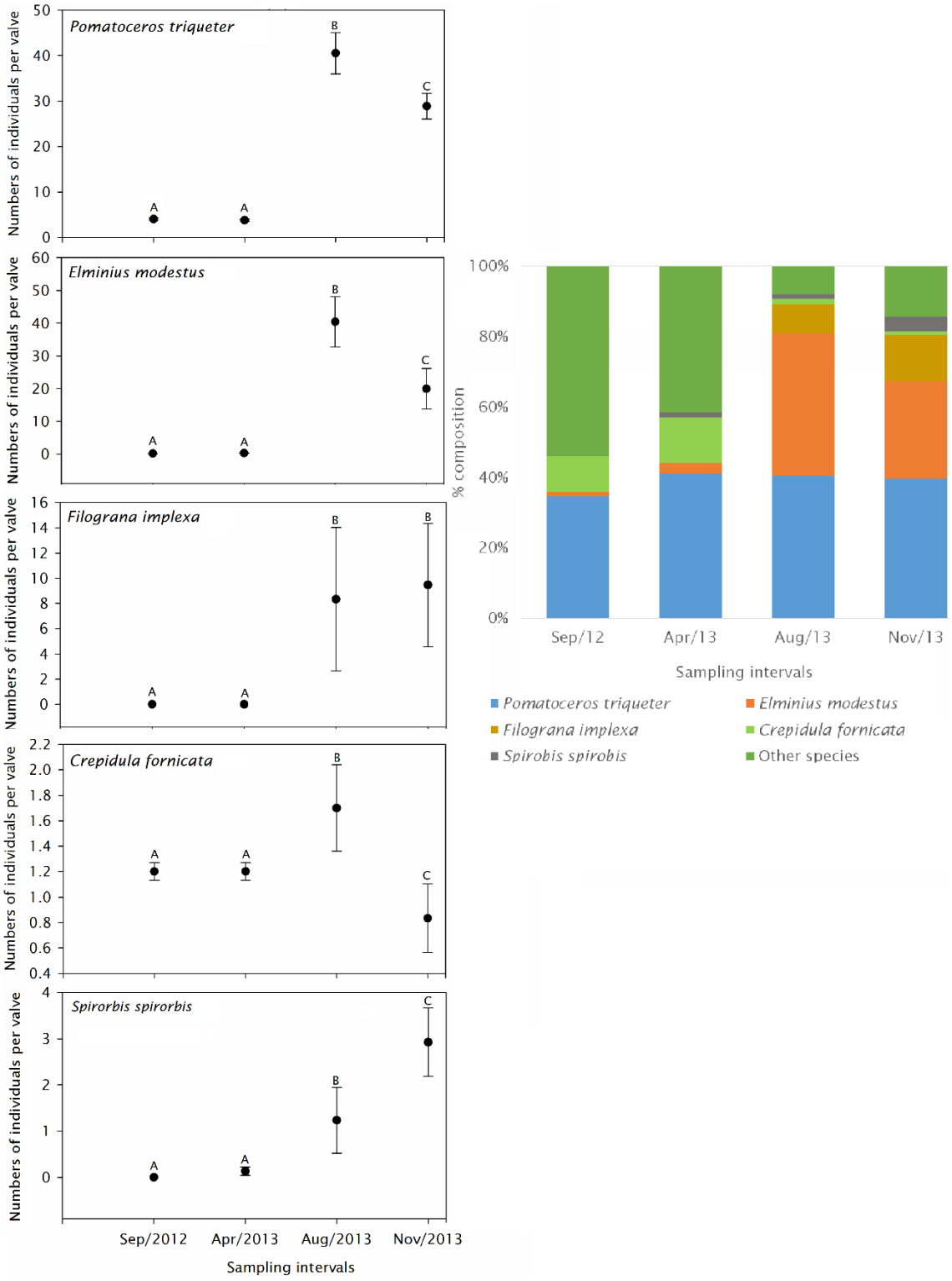
Pomatoceros triqueter
Elminius modestus
Filograna implexa
Crepidula fornicata
Spirorbis spirorbis
Numbers of individuals per valve
Sep/2012
Apr/2013
Aug/2013
Nov/2013
Sampling intervals
% composition
100%
80%
60%
40%
20%
0%
Sep/12
Apr/13
Aug/13
Nov/13
Sampling intervals
Pomatoceros triqueter
Elminius modestus
Filograna implexa
Crepidula fornicata
Spirobis spirobis
Other species

Figure 6.9 (Previous page) Temporal variation in the abundances per valve (mean ± SE) of the five most dominant species colonised on WVR. The graphs are ordered in terms of the most abundant species (top) to the least (bottom), whist the stacked column showed relative abundance of the five species and other epifaunal species. Shared letters (A-C) between sampling intervals indicate no significant difference (one-way ANOVA, $P>0.05$). Note scale differences in the number of individuals per valve. Valve samples at each sampling interval were 30 valves.

6.3.3.3 Epifauna settled on fragmented oyster valve cultch on the elevated reefs (FVR)

A total of 50 species were recorded on FVR (Appendix 6). The five most abundant species represented 85.3% of the total numbers of epifauna recorded on FVR. These were *Pomatoceros triqueter* (36.6%), *Elminius modestus* (29.6%), *Filograna implexa* (5.4%), *Crepidula fornicata* (2.1%) and *Spirorbis spirorbis* (2.1%) (Appendix 7).

As observed in the other categories of oyster valves, Figure 6.10 shows that *P. triqueter* was the early dominant species (September 2012, 4.4±0.4 individuals/valve). It was also the most abundant species during the successive sampling periods with the exception of *E. modestus* in August 2013 (26.7±6.9 individuals/valve). Abundance of *P. triqueter* significantly increased in August 2013, (25.0±3.1 individuals/valve). The profile of abundance over time of *E. modestus* was similar to that of *P. triqueter*, with a significant increase in August 2013. Abundance of *F. implexa* significantly increased successively in August 2013 (2.0±1.3 individuals/valve) and November 2013 (6.6±2.9 individuals/valve). The abundance of *C. fornicata* was greatest at the early state of reef deployment in September 2012 (1.3±0.1 individuals/valve) and significantly decreased in April 2013, thereafter remaining constant. *S. spirorbis* was first observed in April 2013 (0.2±0.1 individuals/valve) and significantly increased in November 2013 (2.7±0.2 individuals/valve)

In conclusion, seasonal variations in epifaunal abundance varied during the experimental period along with a progression in the number of species present. *P. triqueter, E. modestus* and *C. fornicata* were the pioneer species observed in September 2012. *F. implexa* and *S. spirorbis* were observed to settle on FVR from August 2013 onwards. The numbers of individuals were limited post early reef deployment, and then significantly increased in August 2013. The exception to this was *C. fornicata* which significantly decreased in April 2013. *P. triqueter* was the overall numerically dominant species with the exception of August 2013 in which *E. modestus* became most dominant.

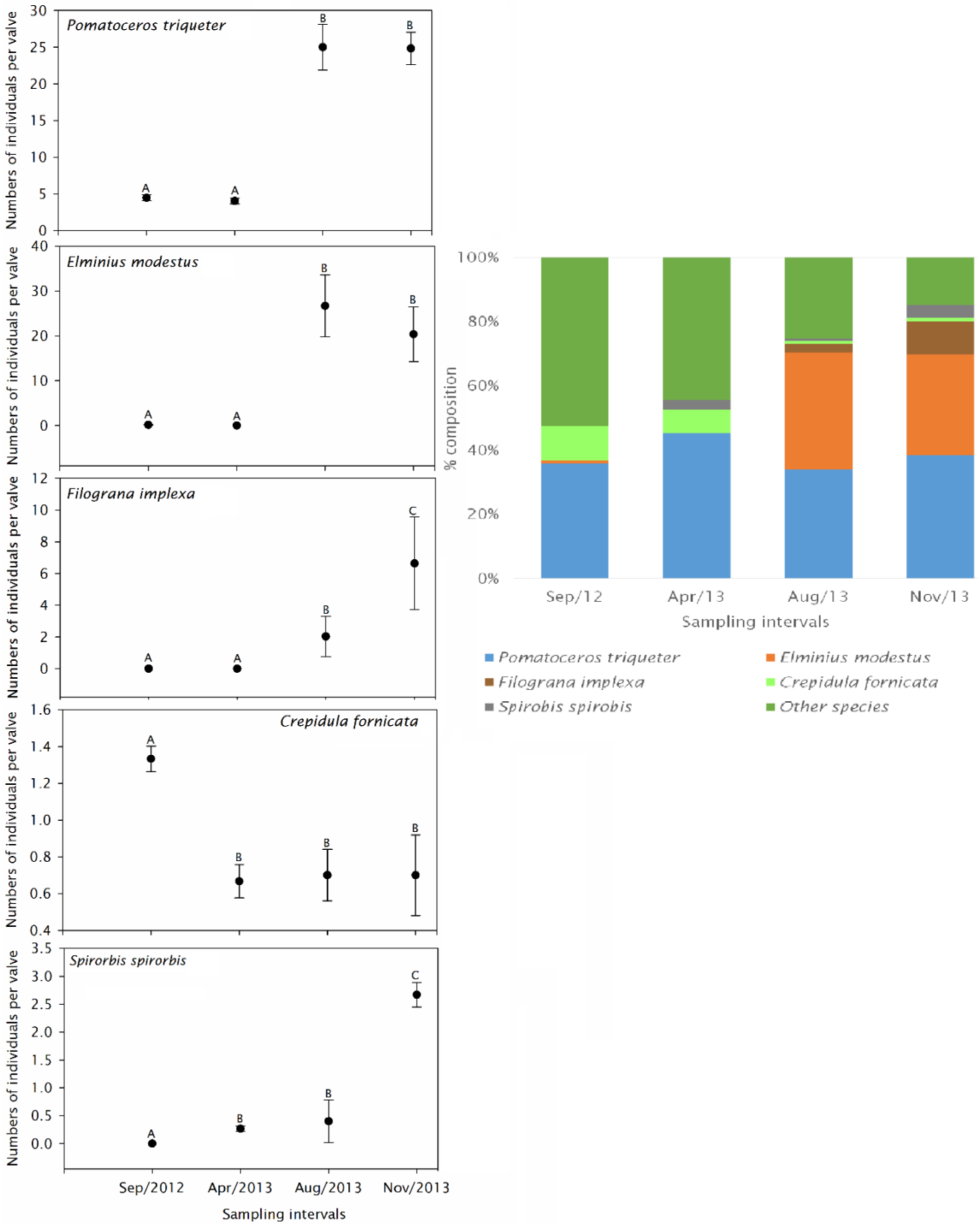
Pomatoceros triqueter
Elminius modestus
Filograna implexa
Crepidula fornicata
Spirorbis spirorbis
Numbers of individuals per valve
Sep/2012
Apr/2013
Aug/2013
Nov/2013
Sampling intervals
% composition
100%
80%
60%
40%
20%
0%
Pomatoceros triqueter
Elminius modestus
Filograna implexa
Crepidula fornicata
Spirobis spirobis
Other species

Figure 6.10 (Previous page) Temporal variation in the abundance per valve (mean ± SE) of the five most dominant species colonised on FVR. The figures are ordered in terms of the most abundant species (top) to the least (bottom), whist the stacked column showed relative abundance of the five species and other epifaunal species. Shared letters (A-C) between sampling intervals indicate no significant difference (one-way ANOVA, $P>0.05$). Note scale differences in the number of individuals per valve. Valve samples at each sampling interval were 30 valves.

6.3.3.4 Comparison of epifaunal species richness (numbers of species per valve) between oyster valve cultch on the elevated reefs (WVR) and sea bed (WVS)

Species richness on oyster valve cultch positioned on both elevated reefs and sea bed varied with sampling interval. Species richness on WVR and WVS were the lowest in April 2013 (3.0±0.2 and 1.5±0.1, respectively) and markedly increased in August 2013 (7.0±0.3 and 3.7±0.4, respectively, Figure 6.11). Two-way ANOVA indicated that the interaction between elevation and sampling interval had a significant effect on the species richness on oyster valve cultch (Table 6.8).

Pairwise multiple comparisons (Tukey's HSD test) of species richness of epifauna on dead oyster valves at each sampling interval between different elevations showed that the numbers of species on oyster valve cultch positioned on elevated reefs (WVR) was significantly higher than those valve cultch re-laid on the sea bed (WVS) at any sampling intervals (Table 6.9).

Pairwise multiple comparisons (Tukey's HSD test) of species richness on WVS between different sampling intervals showed that the numbers of species on WVS in August 2013 was significantly higher than in September 2012 and April 2013 but not for November 2013. Moreover, the number of species recorded in November 2013 was also significantly higher compared with September 2012 and April 2013. However, there was no significant difference in species richness between August 2013 and November 2013 (Table 6.10).

Pairwise multiple comparisons (Tukey's HSD test) of species richness on WVR between sampling intervals showed that the numbers of species on WVR collected in August 2013 was significantly higher than in September 2012 and April 2013 but not for November 2013. Moreover, the number of species recorded in November 2013 was also significantly higher compared with September 2012 and April 2013. There was no significant difference in the numbers of species observed in September 2012 in comparison with April 2013. Also, there was no significant difference in the numbers of species between August 2013 and November 2013 sampling (Table 6.10).

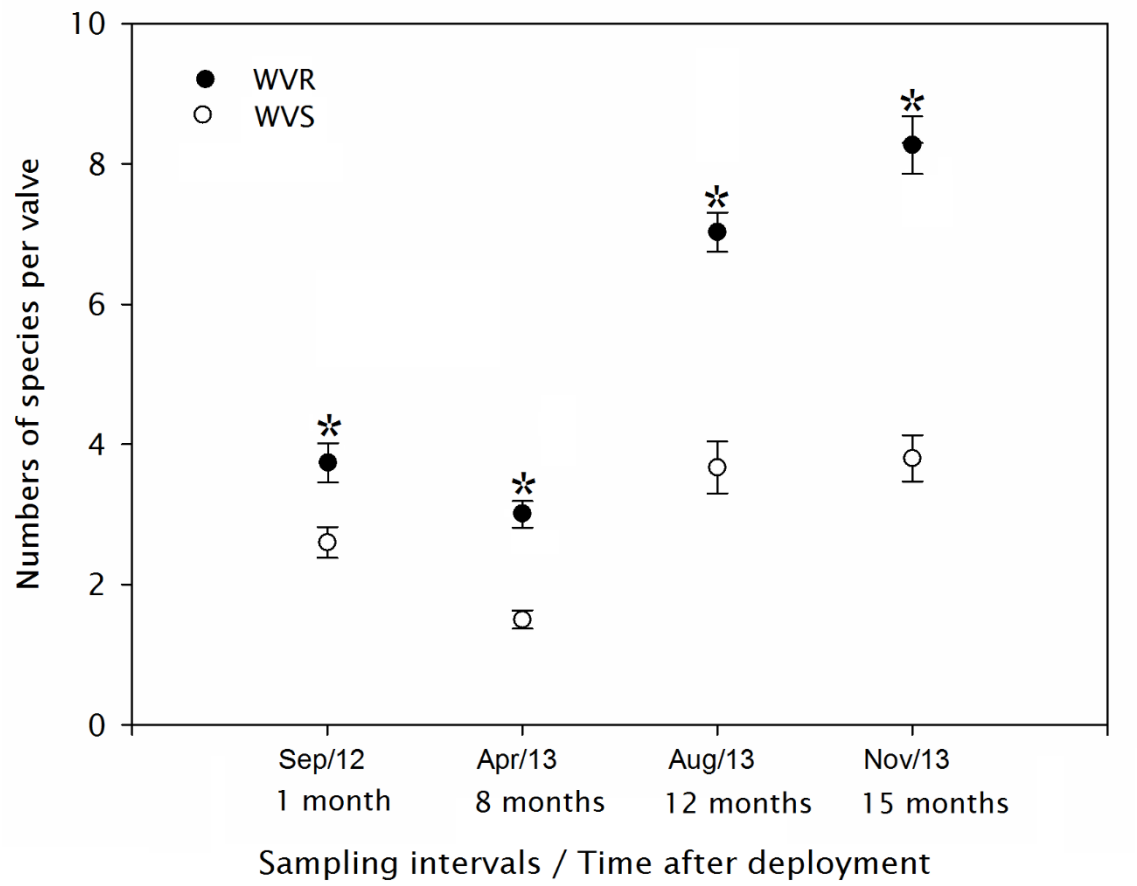

Figure 6.11 Species richness, number of epifaunal species per valve (mean ± SE), on WVR (oyster valve cultch on elevated reefs) and WVS (oyster valve cultch on the sea bed). Species richness was significantly affected by interaction between elevation (reef/sea bed) and sampling interval (two-way ANOVA, $P<0.01$). The number of valve samples analysed from each location at any sampling intervals were 30 valves.

Table 6.8 Comparison of the effect of sampling interval, elevation (reef/sea bed), and the interaction between sampling interval and elevation on species richness of epifauna colonised on oyster valve cultch. Significant differences are denoted by asterisks.

Sources of variation	df	F	P-value
Sampling interval	3	158.68	0.001*
Elevation (reef/sea bed)	1	66.66	0.001*
Sampling interval x Elevation	3	4.97	0.002*

Table 6.9 *Post-hoc* (Tukey's HSD test) results comparing the effect of elevation (reef/sea bed) between sampling interval on the number of species colonised on oyster valve cultch.

Post-hoc test (Tukey's HSD)	P-value
Elevated reef vs Sea bed (Sep 2012)	<0.001
Elevated reef vs Sea bed (Apr 2013)	<0.001
Elevated reef vs Sea bed (Aug 2013)	<0.001
Elevated reef vs Sea bed (Nov 2013)	<0.001

Table 6.10 *Post-hoc* (Tukey's HSD test) results comparing the effect of sampling interval within elevation (reef/sea bed) on the numbers of species colonised on oyster valve cultch. "ns" represents no significant difference

Post-hoc test (Tukey's HSD)	P-value		
	Sep 2012	Apr 2013	Aug 2013
Effect of sampling interval on species richness on WVS			
Apr 2013	<0.001		
Aug 2013	<0.05	<0.001	
Nov 2013	<0.05	<0.001	ns
Effect of sampling interval on species richness on WVR			
Apr 2013	ns		
Aug 2013	<0.001	<0.001	
Nov 2013	<0.001	<0.001	ns

6.3.3.5 Comparison of species diversity (Shannon's H) between oyster valve cultch collected from the elevated reefs (WVR) and sea bed (WVS)

Shannon's H diversity index of epifaunal species on oyster valve cultch on both elevated reefs and the sea bed varied with sampling intervals. The diversity index per valve on WVR and WVS were the lowest in April 2013 (0.5±0.1and 0.1±0.1 per valve, respectively) and increased in August 2013 (1.1±0.1and 0.6±0.1 per valve, respectively, Figure 6.12). Two-way ANOVA indicated that the interaction between elevation and sampling interval had a significant effect on diversity (Table 6.11). Pairwise multiple comparisons (Tukey's HSD test) of the diversity index of epifauna on oyster valve cultch at each sampling interval between different elevations (reef/sea bed) showed that the diversity index on WVR was significantly higher than those on WVS valves at any sampling interval (Table 6.12).

Pairwise multiple comparisons (Tukey's HSD test) of the diversity index values for WVS between different sampling intervals showed that the diversity index on WVS collected in April 2013 was significantly lower compared with values related to WVS valves in September 2012, August 2013 and November 2013.

However, there was no significant difference in the diversity index achieved between other samplings (Table 6.13).

Pairwise multiple comparisons (Tukey's HSD test) of the diversity index on WVR between different sampling intervals showed that the diversity index on WVR collected in August 2013 was significantly higher compared with those WVR valves in September 2012 and April 2013 but not for November 2013. The diversity index on WVR in November 2013 was also significantly higher than September 2012 and April 2013. Diversity in September 2012 was significantly higher than in April 2013. However, there was no significant difference in the diversity index between August 2013 and November 2013 (Table 6.13).

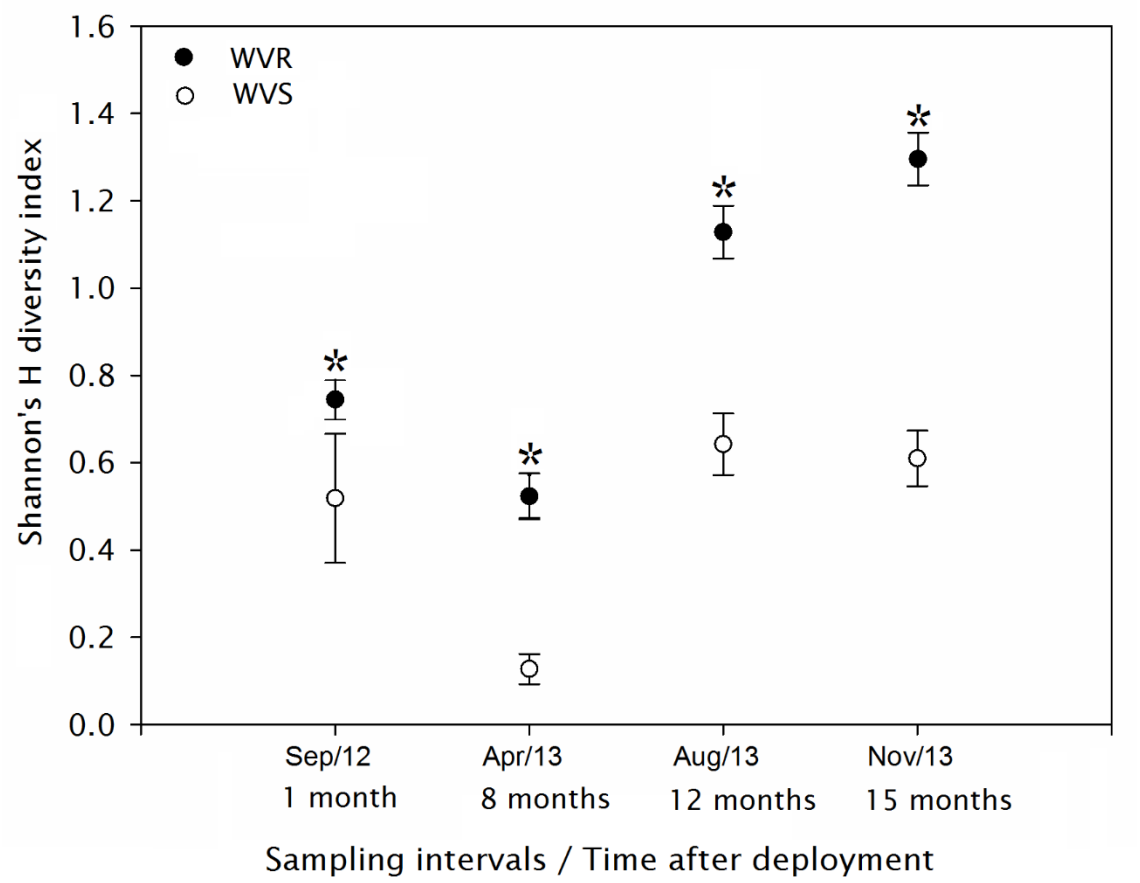

Figure 6.12 Shannon's H diversity index (mean ± SE), on WVR (oyster valve cultch on elevated reefs) and WVS (oyster valve cultch on the sea bed). The diversity index was significantly affected by interaction between elevation (reef/sea bed) and sampling interval (two-way ANOVA, $P<0.01$). N = 30 valve samples for each location and sampling interval.

Table 6.11 Comparison of the effect of sampling interval, elevation (reef/sea bed), and the interaction between sampling interval and elevation on diversity index on oyster valve cultch. Significant differences are denoted by asterisks.

Sources of variation	df	F	P-value
Sampling interval	3	51.06	0.001*
Elevation (reef/sea bed)	1	127.19	0.001*
Sampling interval x Elevation	3	4.20	0.007*

Table 6.12 *Post-hoc* (Tukey's HSD test) results comparing the effect of elevation (reef/sea bed) between sampling interval on diversity index on oyster valve cultch.

Post-hoc test (Tukey's HSD)	P-value
Elevated reef vs Sea bed (Sep 2012)	<0.01
Elevated reef vs Sea bed (Apr 2013)	<0.001
Elevated reef vs Sea bed (Aug 2013)	<0.001
Elevated reef vs Sea bed (Nov 2013)	<0.001

Table 6.13 *Post-hoc* (Tukey's HSD test) results comparing the effect of sampling interval within elevation (reef/sea bed) on diversity index on oyster valve cultch. "ns" represents no significant difference.

Post-hoc test (Tukey's HSD)	P-value		
	Sep 2012	Apr 2013	Aug 2013
Effect of sampling interval on the diversity index on WVS			
Apr 2013	<0.001		
Aug 2013	ns	<0.001	
Nov 2013	ns	<0.001	ns
Effect of sampling interval on the diversity index on WVR			
Apr 2013	<0.05		
Aug 2013	<0.001	<0.001	
Nov 2013	<0.001	<0.001	ns

6.3.3.6 Comparison of epifaunal abundance (total numbers per valve) between oyster valve cultch collected from the elevated reefs (WVR) and sea bed (WVS)

The abundance of epifauna on oyster valve cultch positioned on both the elevated reefs and the sea bed varied between seasons. The abundances of epifauna on WVR and WVS were low in September 2012 and April 2013. Peak abundance of both epifauna on WVR and WVS was recorded in August 2013 (100.3±10.1, and 25.2±2.5 individuals/valve, respectively). However, the abundance per valve slightly decreased in November 2013 (Figure 6.13). Two-way ANOVA indicated that interaction between elevation (reef/sea bed) and sampling interval had a significant effect on the abundance of epifauna (Table 6.14).

Pairwise multiple comparisons (Tukey's HSD test) of the abundance of epifauna on oyster valve cultch between different elevations (reef and sea bed) at each sampling interval showed that the abundance of epifauna on WVR was significantly higher than those WVS valves on the sea bed at any sampling interval (Table 6.15).

Pairwise multiple comparisons (Tukey's HSD test) of the abundance of epifauna on WVS between different sampling intervals showed that the abundance of epifauna on WVS collected in August 2013 was significantly higher than those WVS valves in September 2012 and April 2013. The abundance in November 2013 was also significantly higher compared with September 2012 and April 2013. The abundance of epifauna observed in September 2012 was significantly higher than April 2013. However, there was no significant difference in the abundance of epifauna between August 2013 and November 2013 (Table 6.16).

Pairwise multiple comparisons (Tukey's HSD test) of the abundance of epifauna on WVR between different sampling intervals showed that the abundance of epifauna on WVR collected in August 2013 was significantly higher than those WVR valves in September 2012 and April 2013 but not for November 2013. Moreover, the abundance observed in November 2013 was also significantly higher compared with September 2012 and April 2013. However, there was no significant difference in the abundance of epifauna recorded in September 2012 in comparison to April 2013 (Table 6.16).

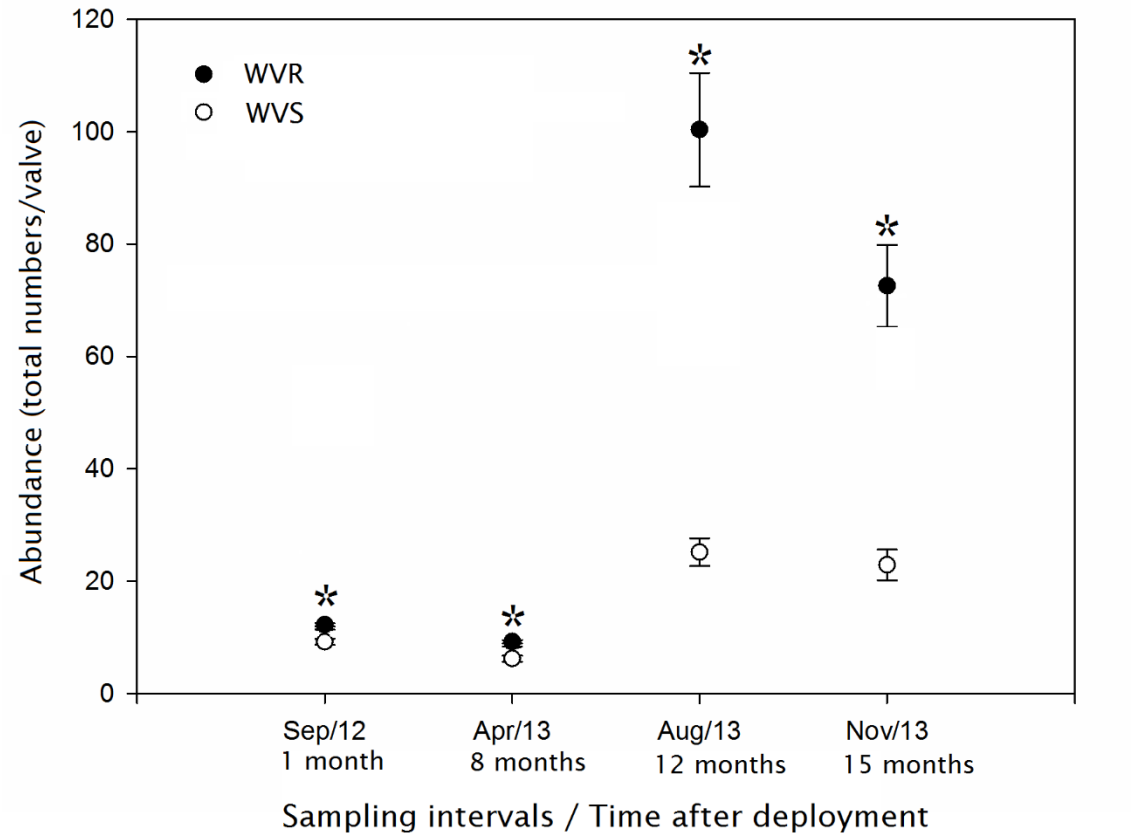

Figure 6.13 Abundance, number of individuals per valve (mean ± SE), on WVR (oyster valve cultch on elevated reefs) and WVS (oyster valve cultch on the sea bed). Abundance was significantly affected by interaction between elevation (reef/sea bed) and sampling interval (two-way ANOVA, $P<0.01$). The number of valve samples analysed from each location at any sampling intervals was 30.

Table 6.14 Comparison of the effect of sampling interval, elevation (reef/sea bed), and the interaction between sampling interval and elevation on the abundance of epifauna on oyster valve cultch. Significant differences are denoted by asterisks.

Sources of variation	df	F	P-value
Sampling interval	3	177.82	0.001*
Elevation (reef/sea bed)	1	160.28	0.001*
Sampling interval x Elevation	3	16.04	0.001*

Table 6.15 *Post-hoc* (Tukey's HSD test) results comparing the effect of elevation (reef/sea bed) between sampling interval on the abundance of epifauna colonised on oyster valve cultch.

Post-hoc test (Tukey's HSD)	P-value
Elevated reef vs Sea bed (Sep 2012)	<0.05
Elevated reef vs Sea bed (Apr 2013)	<0.001
Elevated reef vs Sea bed (Aug 2013)	<0.001
Elevated reef vs Sea bed (Nov 2013)	<0.001

Table 6.16 *Post-hoc* (Tukey's HSD test) results comparing the effect of sampling interval within elevation (reef/sea bed) on the abundance of epifauna colonised on oyster valve cultch. "ns" represents no significant difference

Post-hoc test (Tukey's HSD)	P-value		
	Sep 2012	Apr 2013	Aug 2013
Effect of sampling interval on the abundance of epifauna on WVS			
Apr 2013	<0.01		
Aug 2013	<0.001	<0.001	
Nov 2013	<0.001	<0.001	ns
Effect of sampling interval on the abundance of epifauna on WVR			
Apr 2013	ns		
Aug 2013	<0.001	<0.001	
Nov 2013	<0.001	<0.001	ns

6.3.4 Epifaunal community structure responses to the presence of elevated oyster reefs

During 15 months of reef deployment, a total of 54 epifaunal species were observed on oyster valve cultch and live oysters from experimental structures in Poole Bay. All 54 species were found on oyster valves in reef boxes on the top of elevated reefs, 80 cm above the sea bed, whilst only 23 species were found on oyster valves in oyster cages laid on the sea bed (Appendix 2-6).

Thirty one suspension feeding species were only observed on oyster valves in reef boxes (Table 6.17). Interestingly, a total of 19 *O. edulis* spat were recorded on the oyster valve cultch collected from reef boxes with a maximum of four spat per reef box but no oyster spat were observed on valve cultch and live oysters laid on the sea bed. No epifaunal species were specifically identified occurring only on oyster valves on the sea bed.

P. triqueter, C. fornicata and *D. hispida* were pioneer dominant species observed at the first sampling interval. There were some new sedentary species which were additionally recorded on oyster valves in April 2013, e.g. *Actinothoe sphyrodeta*, *S. spirorbis*, *Bispira volutacornis, Leptochiton cinerea, Styela clava* and the spat of *O. edulis*. Conversely, some of the species, recorded in September 2012, were not observed, especially on oyster valves on the sea bed, or were only recorded at reduced abundance in the winter sampling (April 2013), especially in the case of the tunicates (*Corella eumyota*, *Ciona intestinalis* and *Molgula* sp.). There were several epifauna species that additionally settled on oyster valves in summer (August 2013) such as the hydroids (*Halecium beanie*, *Kirchenpaueria pinnata, Sertularella gaudichaudi*), bryozoans (*Bicellariella ciliata*, *Bugulina flabellata*, *Chartella papyracea*, *Epistomia bursaria, Flustra foliacea, Plagioecia patina, Cellepora* sp.), tunicates (*Ascidia mentula*, *Ascidia virginea*, *Pyura microcosmus*, *Perophora* sp., *Botrylloides diegense*, *Botrylloides violacea*, *Morchellium argus*, *Didemnum* sp.) and a chiton (*Acanthochitona fascicularis*). Whilst some epifaunal species e.g. *Dysidea fragilis* (sponge), *Eudendrium ramosum* (cnidarian) and *Harmothoe* sp. (annelid) were first observed in November 2013 (Table 6.17).

To visualise the structure of the oyster valve epifaunal community, a MDS (multidimensional scaling) plot, based on a Bray-Curtis similarity matrix of present and absent species (Appendix 8) on oyster valves corresponding to sampling intervals, was produced. The MDS plot showed a useful two-dimensional representation with stress value of 0.10, suggesting good ordination with potentially no misleading features (Clarke, 2001) (Figure 6.14). The MDS plot showed the difference of epifaunal composition on oyster valves between the elevated reefs and sea bed. ANOSIM was used to test the difference of epifaunal community on oyster valve between the elevated reefs and sea bed using elevation (reef/ sea bed) as a factor. This test provided a Global R value of 0.497 (P=0.01), indicating that epifaunal community on oyster valves positioned on elevated reefs was significantly different from those oyster valves on the sea bed but also had some species in common (Clarke and Gorley, 2001)

Table 6.17 Temporal variation of epifaunal settlement on oyster valves during a 15 month period. Green represents epifauna only observed on oyster valves in reef boxes. Grey represents epifauna observed on both oyster valves in reef boxes and oyster cages. There was no epifaunal species that was only observed on oyster valves in oyster cages laid on the sea bed.

	Sampling intervals			
Phylum and species	Sep/2012	Apr/2013	Aug/2013	Nov/2013
Porifera				
Dysidea fragilis				
Halichondria panicea				
Cnidaria				
Eudendrium ramosum				
Halecium beanii				
Halecium halecinum				
Kirchenpaueria pinnata				
Laomedea flexuosa				
Sertularia argentea				
Sertularella gaudichaudi				
Ectopleura larynx				
Actinothoe sphyrodeta				
Sagartia troglodytes				
Annelida				
Filograna implexa				
Pomatoceros triqueter				
Sabella pavonina				
Spirorbis spirorbis				
Harmothoe sp.				
Crustacea				
Elminius modestus				
Mollusca				
Acanthochitona fascicularis				
Crepidula fornicata				
Anomia ephippium				
Chlamys varia				
Aequipecten opercularis				
Ostrea edulis				

Table 6.17 (Cont.)

Phylum and species	Sampling intervals			
	Sep/2012	Apr/2013	Aug/2013	Nov/2013
Bryozoa				
Alcyonidium diaphanum				
Amathia lendigera				
Bicellariella ciliata				
Bugulina flabellata				
Chartella papyracea				
Epistomia bursaria				
Flustra foliacea				
Disporella hispida				
Electra pilosa				
Plagioecia patina				
Tubulipora plumosa				
Cellepora sp.				
Tunicata				
Ascidia conchilega				
Ascidia mentula				
Ascidia virginea				
Ascidiella aspersa				
Ciona intestinalis				
Corella eumyota				
Dendrodoa grossularia				
Pyura microcosmus				
Styela clava				
Molgula sp.				
Perophora sp.				
Botrylloides diegense				
Botrylloides leachii				
Botrylloides violacea				
Botryllus schlosseri				
Morchellium argus				
Diplosoma listerianum				
Didemnum sp.				

SIMPER comparisons showed that the similarity of epifaunal composition within elevated reefs to that within the seabed valves was 61.81% and 58.95%. This SIMPER result suggested that there was an overlap of epifaunal species, between the elevated reefs and sea bed. The most frequently recorded species, based on percentage of contribution on oyster valves situated on elevated reefs with ascending order were *P. triqueter*, *D. hispida, Electra pilosa, T. plumosa, Molgula* sp.*, Laomedea flexuosa, C. fornicata, E. modestus, Ciona intestinalis, Ascidiella aspersa, Dendrodoa grossularia,* and *Botrylloides leachii*. These species together accounted for 72.91% of similarity within elevated reefs. Whilst the key species on oyster valves positioned on the sea bed in ascending order were *P. triqueter*, *C. fornicata*, *D. hispida*, *E. pilosa* and *T. plumosa*. These species together accounted for 76.51% of similarity within the sea bed oyster valves. The SIMPER outputs clearly show that there was some crossover of the most frequently recorded species between the elevated reef and sea bed valve communities i.e. *P. triqueter*, *C. fornicata*, *D. hispida*, *E. pilosa* and *T. plumosa*.

SIMPER analysis also showed that the average dissimilarity value of epifauna on oyster valves between the elevated reefs and sea bed was 51.80%. There were two species that possessed a dissimilarity to standard deviation ratio more than 1.3. These were *C. intestinalis* (Diss/SD=1.63) and *D. grossularia* (Diss/SD=1.44). However, no epifaunal species accounted for more than 8% of dissimilarity. Therefore, there was no single species that specifically characterised the dissimilarity between the elevated reefs and sea bed valve communities. A total of 22 species together accounted for more than 70% of the dissimilarity of epifaunal communities between elevated reef and sea bed valves. The lists of species and contribution to similarity within group and dissimilarity between groups (reef/sea bed), in ascending order, can be found in Appendix 9.

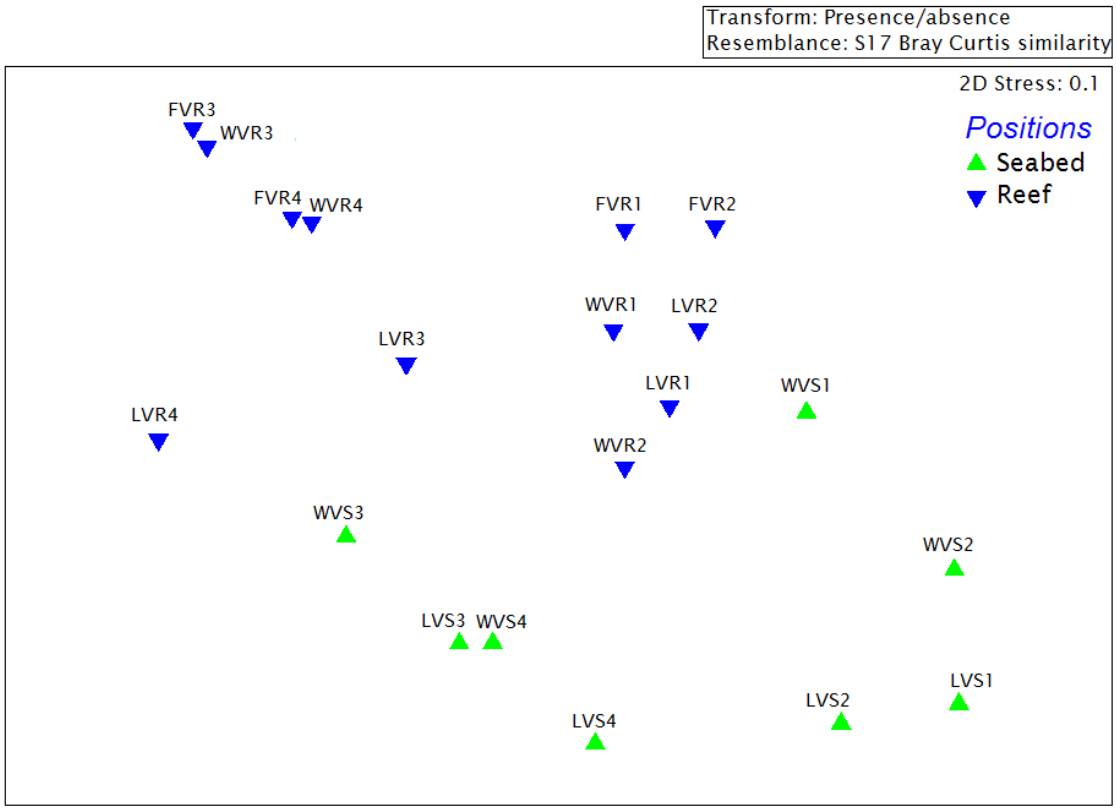

Figure 6.14 MDS plot based on Bray-Curtis similarity matrix of presence/absence of epifaunal species recorded on each valve category at each sampling interval. The first 3 characters indicate valve categories (LVS: live oyster on the sea bed, LVR: live oyster on elevated reefs, WVS: whole oyster valve cultch on the sea bed, WVR: whole oyster valve cultch on elevated reefs and FVR: fragmented oyster valve cultch on elevated reefs) and the last number represents sampling interval (1: September 2012, 2: April 2013, 3: August 2013 and 4: November 2013). Stress level (0.10) indicates a useful two-dimensional data representation. The MDS plot identified the separation of epifaunal composition on oyster valves between the elevated reefs and sea bed.

6.4 Discussion and conclusion

It has been argued that artificial oyster reefs enhance oyster stocks and ecosystem complexity by providing additional habitats for marine biota. Although the benefits of bivalve shells in terms of creating ecological habitats for epifaunal species has been widely published (Harding and Mann, 2001; Korringa, 1951; Lenihan and Peterson, 1998; Lenihan et al., 2001; Raj, 2008; Smyth and Roberts, 2010), little is known about the potential effect that artificial reefs created with *O. edulis* valves may have on associated epifaunal communities in the UK. Moreover, comparisons of the epifaunal community on *O. edulis* valves situated on elevated reefs compared with those valves directly laid on the sea bed have not been explored yet. To the best of our knowledge, this is the first study comparing species richness, diversity index and abundance of epifauna on *O. edulis* valves at different heights above the sea bed. Based on a 15 month experiment, a total 54 epifaunal species, including *O. edulis* spat, were identified on oyster valves in reef boxes on top of reef modules. On the contrary, only 23 species and no *O. edulis* spat were observed on oyster valves in oyster cages laid on the sea bed. Species richness, diversity and the abundance of epifauna on oyster valve cultch situated on the elevated reefs were significantly higher than those valve cultch on the sea bed. These findings suggest that holding live oysters or oyster shell cultch in elevated reefs could enhance epifaunal biodiversity compared to re-laying the oysters (or cultch) directly onto the sea bed. Unfortunately, as oyster valves are not a big component of the surface sea bed in Poole Bay, there is no previous study reporting the numbers of epifaunal species on *O. edulis* valves in the Poole Bay to make comparison of the numbers of epifaunal species on oyster valves between elevated reefs and natural habitat. However, Smyth and Roberts (2010) investigated the spatial variation of epibiotic (fauna and flora) species on *O. edulis* valves in two different locations in Strangford Lough, Ireland and showed that there were 21 epifaunal species recorded on *O. edulis* valves collected from Ballyreagh while 49 epifaunal species were recorded on Greyabbey where lower turbidity was observed. This previous study combined with data in this study suggests that elevated reef habitat can improve and accelerate local biodiversity and this method could be applied as a tool for restoring biodiversity especially in high turbidity areas.

Several previous studies reported that turbid conditions influenced the epifaunal colonisation on hard substrata (Moore, 1977; Gosling, 2003). Gosling (2003) explained that suspended sediment can be an influencing factor that reduces epifaunal species richness because siltation on substrata restricts the settlement of planktonic larvae. It seems that sediment in the water column or similar turbid conditions can be an influencing factor on faunal diversity. Indeed, the differences in environmental conditions between the elevated reefs and sea bed have been already discussed in Chapter 2. Total suspended solids in the water column at the height of elevated reefs were significantly lower than the water column adjacent to the sea bed. Therefore, it is possible that the significantly higher total suspended solids at the sea bed could have affected epifaunal settlement on the sea bed adversely. Smyth and Roberts (2010) reported that some epifaunal species that are sensitive to turbidity are unlikely to colonise surfaces in such limiting conditions found at the sea bed; including species such as sponges (*Halichondria panacea*, *Dysidea fragilis*). Similarly, Bartol and Mann (1997) reported that Porifera are unlikely to survive siltation for any significant length of time as the colonies rely on water movement for feeding and respiration and they have a mechanism for shedding their complete outer tissue layer together with any debris. Smyth and Roberts (2010) supported that sponges are intolerant to long periods of siltation. In this study, two species of sponges (*Halichondria panacea*, *Dysidea fragil*) were not observed on oyster valve cultch directly laid on the sea bed. This could be a reason that might explain why fewer epifaunal species were found on oyster valve cultch laid on the sea bed compared to valve cultch on the elevated reefs.

Harding (1996) and Laing et al. (2005) reported that the larvae of *O. edulis* require a firm substrate with low levels of suspended sediment. A high silt fraction has the potential to increase *O. edulis* mortality in both juvenile and adult oysters, especially when water temperatures are low (Yonge, 1966). Coen et al. (1999) reported that oyster shells and the interstitial spaces provide space for settlement and refuge from predation, thereby increasing the recruitment, growth and survival of oysters on reefs. These previous publications support the findings in this study that a total of 19 *O. edulis* spat were identified on the oyster valve cultch in reef boxes with a maximum of four spat per reef box, but no oyster spat were found on cultch or live oyster

valves on the sea bed. From these observations, the hypothesis that an elevated reef construction provides a large suitable substrate with less sediment for oyster spat is tentatively supported.

Species richness of epifauna on oyster valve cultch at each position (reef/sea bed) was higher than on live oyster valves at each location. On elevated reefs, there were 48 and 50 species observed on WVR and FVR respectively, whist 32 species were recorded on LVR. In the same way, on the sea bed, the numbers of species observed on oyster valve cultch were higher than live oysters (23 and 15 species, respectively). Although unequal valve samples and the differing sizes of live oysters and oyster valve cultch could not be excluded from explaining these differences, there are several other possible reasons that could provide an explaination for these observations. Previous studies by Summerhayes et al. (2009) suggested that single dead valves, which increase the availability of interstitial spaces and provide greater surface areas for attachment, supported the greater species richness of epibiota compared with the matrices comprised solely of live oysters. Tamburri et al. (1996) identified that live oysters (*C. virginica*) decrease the abundance and diversity of epibiota on their valves compared to oyster valve cultch as live oysters would have the ability to filter planktonic larvae that come to settle on their valves. Although data in this study suggested that, at the same elevation, oyster valve cultch could provide better substrates for epifaunal settlement compared with live oysters, this was not the case for differences in elevation. In this study, when considering the numbers of species observed on LVR compared to WVS, data suggested that species richness on LVR (32 species) was higher than WVS (23 species). It is possible that total suspended solids in the water column adjacent to the sea bed had a marked effect on larval settlement on both live oysters and oyster valve cultch. The fact that most epifaunal species are suspension feeders, which require water flow and reduced suspended sediment, means that elevated reef structures are more likely to provide preferable conditions for epifauna to settle.

Biodiversity is frequently used as a measure of the health of ecosystems (Doak et al., 1998; Tilman et al., 1998). A basic assumption is that the more diverse an ecosystem, the greater its ability to resist the imposition of environmental stress because of its enhanced resilience through increased complexity (Elmqvist et al., 2003; Wilson, 1998). The more species present in a

community, the higher the functional or niche redundancy, and thus the greater stability afforded to a given community (Burman et al., 2012; Thrush et al., 2008; Worm et al., 2006). Data collected in this study demonstrate that the interaction between elevation and sampling interval significantly influenced the epifaunal diversity on oyster valve cultch, such that the epifaunal diversity on oyster valve cultch situated on elevated reefs was significantly higher than valves obtained from the sea bed. Previous studies have suggested that the key variables determining diversity in benthic communities are not only abiotic factors e.g. the sediment types, organic content and current velocities (Warwick et al., 1991) but also depend on biotic factors e.g. reproductive cycle, larval recruitment phase, survival, mortality and so forth (Chia,1989; Greene and Schoener, 1982). As previously discussed, the data from this study identifies that elevation had a significant effect on abiotic factors between the elevated reefs and the sea bed, with reference to e.g. total suspended solids. Other studies have also suggested that water current and light availability, at the height of elevated reefs, usually increase (Howard et al., 1994). Therefore, it could be confirmed that abiotic environmental conditions, at the height of elevated reefs, are suitable for epifaunal colonisation. However, the sampling intervals were correlated with biotic factors such as spawning season and settlement period. Taken together these could explain why there was an interaction between elevation and sampling interval in determining epifaunal diversity on oyster valve cultch.

The interaction between elevation and sampling interval also had a significant effect on the abundance of epifauna, such that the abundance of epifauna on the oyster valves situated on elevated reefs was significantly higher than that on seabed oyster valves at every sampling interval. Abelson and Denny (1997) explained that water currents at the height of elevated reefs would clean valve surfaces and render them more suitable for epifaunal settlement, in turn leading to increased epifaunal abundance. Frechette et al. (1989) have also identified that elevated reef structures enhance vertical diffusive turbulent water flow which in turn further enrich water column nutrient concentration and improve the survival and growth rates of filtering feeding species on elevated reefs. Keough and Downes (1982) described how invertebrate larvae may preferentially settle into oyster reefs. Physical factors such as crevices, the curvature and complexity of valves may act as a preferential settlement area

for larvae, creating sheltered zones in an otherwise turbulent environment. Ultimately, these biotic and abiotic advantages provided by elevated reefs may increase the chance of settlement and consequently cause an increase in the abundance of epifauna on live oysters and oyster valve cultch on the elevated reefs.

Although species composition on elevated reefs and sea bed were different, there was some conservation of the most dominant species between the elevated reefs and sea bed. The first dominant and conserved species was *P. triqueter*. As reported by Jensen et al. (2000) *P. triqueter* is a locally dominant species in Poole Bay and is also widely distributed along the south coast of the UK. Ecologically, *P. triqueter* has been noted to occur in very exposed to extremely sheltered locations, with very sheltered to exposed water flow rate, and in areas where there is heavy or no silt present (Price et al., 1980). Therefore, unsurprisingly, the abundance of *P. triqueter* on both elevated reefs and sea bed were higher than other species. Moreover, *P. triqueter* has been considered to be an initial fouling organism (Crisp, 1965), colonizing new substrates including buoys, ships hulls, docks and offshore oil rigs (Organisation for Economic Co-operation and Development, 1967). Cotter et al. (2003) studied variations in settlement rate of this species in Bantry Bay, Ireland, and concluded that, although the species settled all year round, a single peak in recruitment during summer (especially July and August) with very little recruitment at other times of the year. These reports are in agreement with the observed increase in abundance of *P. triqueter* on every valve category in August 2013.

C. intestinalis and *D. grossularia* were identified as good discriminators between the elevated reefs and sea bed community as they possessed a dissimilarity to standard deviation ratio >1.3 (Clarke and Warwick, 1994). The Marine Biological Association (1957) reported that *C. intestinalis* spawns throughout the year and they preferred sheltered habitats. *C. intestinalis* has been observed on elevated artificial surfaces such as metal and concrete and other hard substrates e.g. oyster shells collected from suspended oyster cultures (Mazouni et al., 2001). The spawning and settlement of this species can be controlled by manipulation of light levels (Berrill, 1947; Woollacott, 2005). Whittingham (1967) noted that *C. intestinalis* spawned within 4 minutes (±2.6) of exposure to light. It appears likely that light exposure may

synchronize spawning of this species in some instances. Therefore, at the height of elevated reefs (80 cm above sea bed), the light intensity was possibly better than at the sea bed and this difference is possibly an reason explaning the presence of *C. intestinalis* at the height of elevated reefs.

Elevated reefs provided more suitable conditions e.g. some water flow and low suspended sediment for epifaunal settlement. These advantages possibly attracted *D. grossularia* to settle on elevated reefs rather than on the sea bed. Previous studies reported that *D. grossularia* was particularly abundant on substrates that are within enhanced water flow as it is a suspension feeder. Morover, the fact that the habitat complexity created by the added ~1,200 oyster valves in reef boxes potentially provides shelter habitats for epifauna (Breitburg et al., 2000; Tolley and Volety, 2005), thereby increasing the chance of settlement of epifaunal species that prefer particular habitats, in this study e.g. *C. intestinalis* and *D. grossularia*. It can be concluded that an elevated reef construction provides a large suitable substrate for diverse epifaunal settlement including *O. edulis* spat. This consequently improves local diversity by increasing the chance of settlement of fouling species, suspension feeders, on oyster valves that which might influence the food web by attracting mobile species to use reef habitats for food resources.

In conclusion, data presented in this chapter indicates that improvement of ecosystem diversity through the construction of artificial elevated structures represents a better solution than the continued relaying of live oysters on the sea bed or than the distribution of oyster valve cultch directly onto the sea bed. Species richness, diversity index and the abundance of epifaunal species on live oysters or oyster valve cultch situated on elevated reefs were significantly higher than those valves on the sea bed at every sampling interval. The complex structure and elevation of reef boxes increased the suitable space, which resulted in an increase in epifaunal diversity on elevated reef structures. Whilst Poole Bay is not currently recognised locally as a site for the recruitment of *O. edulis* the observation of 19 oyster spat on elevated reefs, with no spat being found on the sea bed oyster valves, suggests that *O. edulis* valves on elevated reef structures provide more suitable settlement habitat than valves placed on the sea bed. Therefore, based on data from this 15-month experiment, it can be concluded that artificial *O. edulis* valve reef construction provides a suitable space for epifaunal colonisation. As a

consequence, complex reef habitat will attract competing species or predators (e.g. mobile species) to coexist within a structurally complex environment in the elevated reef ecosystem. Therefore, the following chapter presents the utilization of the elevated oyster reef habitat by the mobile faunal community.

Chapter 7

Mobile faunal community structure responses to elevated oyster valve reef habitat.

7.1 Introduction

The goal of much artificial reef construction is mainly to enhance the production of reef-associated species (Jensen, 2002). General agreement exists in the scientific community that artificial reef structures can effectively accumulate mobile species and fish (Bohnsack and Sutherland 1985). Several studies reported positive impacts of the presence of artificial reef habitats in terms of providing spatial complexity, and refuge-rich habitats which are superior habitats for mobile fauna and associated fish (Charbonnel et al., 2002; Wenner et al., 1996). Additionally, three-dimensional structures of artificial reefs increase habitat heterogeneity and supply spaces to support diverse assemblages of mobile and nektonic organisms (Breitburg et al., 1995; Dame, 1979). This led to the observation that the increase in the abundance, species richness and species diversity of mobile species were recorded after artificial reefs were installed (Fujita et al., 1996). Studies also supported that the transformation of low-relief habitats to one having increased areas of high relief can increase the abundance and biodiversity of commercial species such as crustaceans and fish (Osenberg et al., 2002; Shipp and Bortone, 2009).

As previously discussed in Chapter 6, elevated reef habitats may enhance local biodiversity by providing suitable spaces for settlement of epifaunal organisms. Consequently, an increase in epifauna, including suspension feeders, has been shown to attract mobile species of higher trophic levels to reef habitats (Harding and Mann, 1999). For example, Bavestrello et al. (1996) reported that hydroid colonies can constitute the major regulators of zooplankton, playing an important role in the mass development of copepod species. Hydroid colonies also act as a food item for many mobile species, e.g.

amphipods. Among mobile marine species, crustaceans comprise more than 70% of individuals (Bavestrello et al., 1996). This relative abundance of crustaceans was a common feature in most benthic substrates and habitats (Navarro-Barranco et al., 2012). The abundance of crustaceans also attracts predators moving to use elevated reef as forage habitats (Markus et al., 2014). It is clear that the development of the reef community can be explained by the way of predator and prey relationships. Moreover, environmental conditions e.g. turbidity and sedimentation were also reported as the most important variables affecting mobile community structure in the South Western North Sea (Schratzberger et al., 2006). Therefore, as Chapter 2 suggested that sediment levels at the height of the elevated reefs were significantly lower than at the adjacent sea bed and this environmental advantage supported higher epifaunal species on the elevated reef valves in comparison with those on the sea bed, it is very interesting to investigate how mobile species respond to the presence of elevated reef structure composed of oyster valves.

The contributions of artificial reefs to enhancing ecological services by means of increasing biodiversity have been discussed in Chapter 1. However, to date, there is no essential data in the response of mobile fauna community to the presence of elevated reefs created by *Ostrea edulis* valves. Therefore, in this chapter colonisation of elevated reef structures by the mobile fauna community was investigated.

7.2 Methods

Three reef boxes and three oyster cages were deployed and recovered as explained in Chapter 2 (section 2.2.2) and Chapter 6 (section 6.2).

7.2.1 Mobile faunal samples and processing

At each time interval, mobile fauna were separated from the oyster valves collected from each reef box and cage by washing onto a 0.5 mm mesh sieve (Endecotts Ltd., England). Samples were relaxed with 5% ethanol in sea water for 15 minutes in order to prevent limb loss in some species, that could have impeded species identification (Smyth and Roberts, 2010), before preservation in 10% buffered formalin in sea water (Summerhayes et al., 2009). Species were transferred to 70% ethanol (Fariñas-Franco et al., 2013) after seven days. Following fixation, the sieved samples were washed onto a sorting tray and the fauna picked out using forceps under a dissecting microscope (Nikon SMZ800, 10x). Individual species were taxonomically identified at least to genus level using Hayward and Ryland (1996). The nomenclature for identified species was updated as appropriate via the 'European Register of Marine Species'.

As the sizes of reef boxes and oyster cages were different, direct comparison of the respective mobile faunal communities between reef and sea bed positions were not considered. However, the abundance and species richness of mobile species in reef boxes and oyster cages were separately reported in order to show the structure of the mobile fauna community present on the elevated reefs and the sea bed.

7.2.2 Statistical analyses

Prior to statistical analyses, univariate data (i.e. species richness, diversity index and abundance (total numbers of individuals per box)), were first tested for normality and homogeneity of variance using the Shapiro-Wilk method. When assumptions of homogeneity of variances and normal distribution were not met, the data were log (x + 1) transformed. One-way ANOVA was carried out to test the influence of sampling month on species richness, diversity and abundance of mobile fauna in reef boxes or oyster cages. Where appropriate,

post-hoc pairwise multiple comparisons (Tukey's HSD tests) compared the effect of sampling interval on richness, diversity and the abundance of mobile fauna. One-way ANOVA was also applied to investigate temporal variation in the five most abundant species recorded in the reef boxes or oyster cages. In all cases, statistical significance was accepted at $\alpha = 0.05$.

Mobile fauna found in reef boxes and oyster cages were aggregated into five functional groups (feeding guilds, see details in Appendix 10-11) i.e. suspension feeders, deposit feeders, herbivores, omnivores and carnivores according to: Craft and Sacco, 2003; Fanelli et al., 2014; Fauchald and Jumars, 1979; Guerra-Garcia et al., 2014. The temporal variation of these functional groups in each location (reef/sea bed) were investigated using one-way ANOVA. Relative abundance between functional groups was also determined, in terms of percentage of composition, in order to explain the general structure of mobile animals at each sampling interval.

The numbers of mobile fauna, based on functional groups, were log (x+1) transformed (using PRIMER software package version 6.1.6). The Draftsman's plot showed that the data was not heavily skewed following this transformation. Cluster analysis of each location (elevated reef/sea bed) was performed using the Bray–Curtis similarity index and group linkage was used to create dendrograms to illustrate the similarity of functional groups between sampling intervals (Bray and Curtis 1957; Field et al., 1982). Patterns of mobile communities were visualised using two-dimensional non-metric multidimensional scaling (MDS, Clarke, 1993) plots. One-way ANOSIM (Clarke, 1993) on Bray–Curtis dissimilarity matrices (Bray and Curtis, 1957) were applied to test the separation of faunal assemblages between sampling intervals.

7.3 Results

During the 15 month experiment, a total of 68 mobile species belonging to 5 phyla were observed at the experimental site in Poole Bay. The Crustacea was the most diverse phylum with 37 species identified, followed by Mollusca (15 species), Pisces (12 species), Pycnogonida (3 species) and Echinodermata (1 species). The total number of individuals recorded within the phylum Crustacea represented 95.0% (37,908 individuals) of total abundance, followed by the Mollusca (1,850 individuals) and Pisces (82 individuals). Eleven of the 12 fish species identified were observed in reef boxes on the top of elevated reefs while only 5 fish species with limited numbers of individuals were occasionally observed in oyster cages on the sea bed.

7.3.1 The structure of mobile faunal community responses to the formation of elevated reefs

A total of 65 mobile species were observed. The species lists and abundance of animals in reef boxes at each sampling interval are presented in Appendix 10. When considering the numerically dominant species, based on total numbers of individuals in reef boxes, the first dominant species was the small filter-feeding porcelain crab (*Pisidia longicornis*, 2,576.5 ± 348.5 individuals/box), followed by hooded shrimp (*Athanas nitescens,* 197.6 ± 35.7 individuals/box), an amphipod (*Jassa* spp., 78.5 ± 15.2 individuals/box), squat lobster (*Galathea squamifera,* 65.8 ± 4.3 individuals/box), and common spire shell (*Rissoa parva*, 47.2 ± 26.8 individuals/box). These mobile species represented 92.2% of the total mobile faunal abundance recorded across four sampling intervals over 15 months.

As can be seen from Figure 7.1, *P. longicornis* was the most abundant species in reef boxes at every sampling interval. The abundance of *P. longicornis* varied with sampling interval. The highest abundance of *P. longicornis* was recorded in September 2012 (3,240.7±135.7 individuals/box). The abundance of *P. longicornis* significantly decreased in April 2013 (2,527.6±164.6 individuals/ box) before increasing to 2,905.1±69.4 individuals/box in August 2013 and then decreasing to 1,628.7±123.9 individuals/box in November 2013. The highest abundance of *A. nitescens* was recorded in November 2013 (300.7±21.9 individuals/box) and was significantly higher than at other

sampling intervals. The abundance of amphipods (*Jassa* spp.) significantly increased in August 2013 (109.1±6.5 individuals/box) and dropped in November 2013 (37.1±6.7 individuals/box), whilst the abundance of *G. squamifera* was significantly greater in April 2013 (69.7±6.4 individuals/box) and remained consistent from April 2013 onwards. The abundance of *R. parva* in August 2013 was significantly higher compared with the other sampling intervals.

To conclude, the abundance of *P. longicornis* decreased with sampling interval with the exception of August 2013, whilst the abundance of *A. nitescens*, *Jassa* spp. and *G. squamifera* and other faunal species increased with sampling interval. The increase in abundance of these species reduced the contribution of *P. longicornis* with time during the experiment.

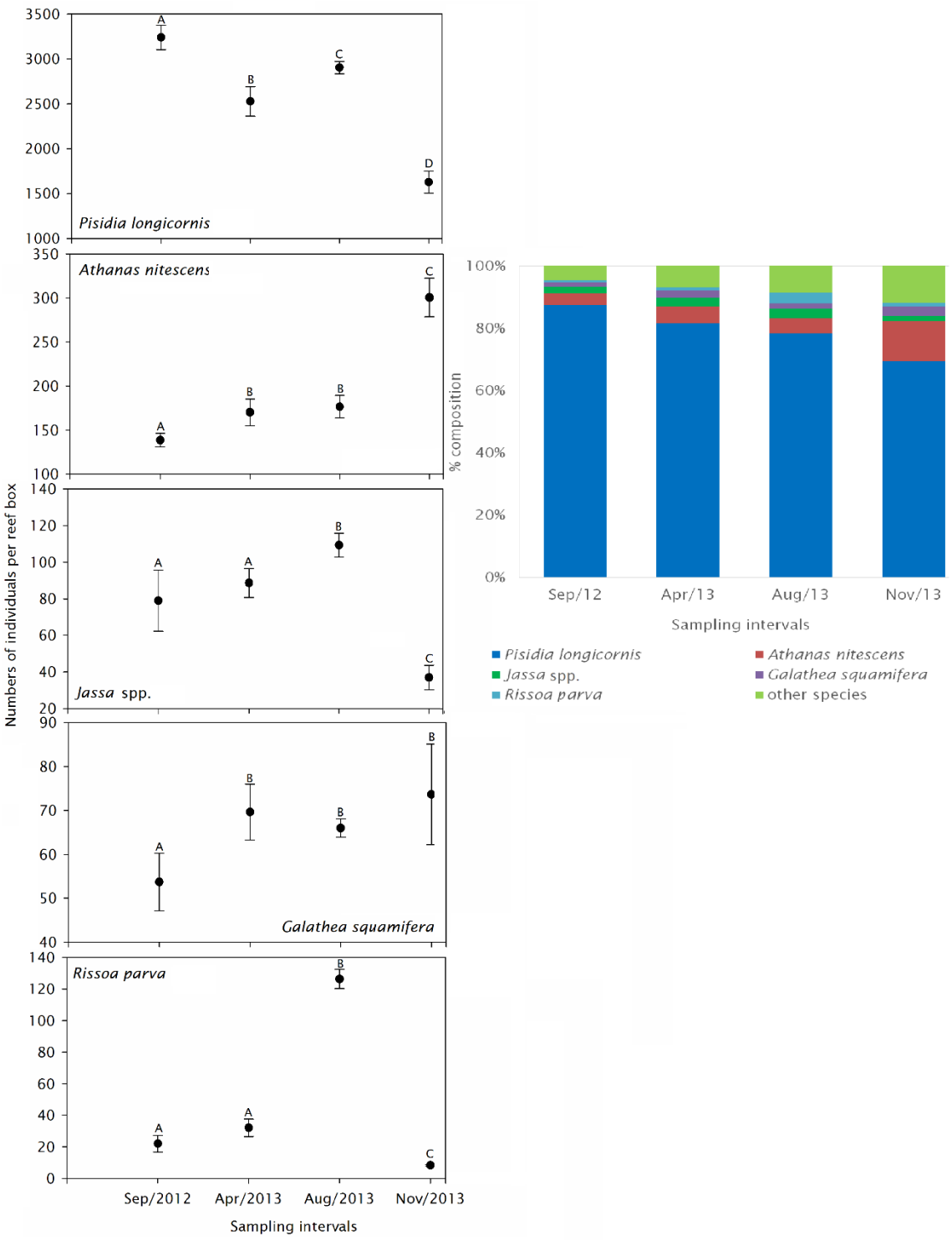
Pisidia longicornis
Athanas nitescens
Jassa spp.
Galathea squamifera
Rissoa parva
Numbers of individuals per reef box
Sep/2012
Apr/2013
Aug/2013
Nov/2013
Sampling intervals
% composition
100%
80%
60%
40%
20%
0%
Sep/12
Apr/13
Aug/13
Nov/13
Sampling intervals
Pisidia longicornis
Athanas nitescens
Jassa spp.
Galathea squamifera
Rissoa parva
other species

Figure 7.1 (Previous page) Temporal variation in the abundance per reef box (mean ± SE) of the five numerically dominant mobile species on the elevated reefs. The graphs are ordered in terms of the most abundant species (top) to the least (bottom) and the stacked column presents the relative abundance of the five dominant species and other species. Shared letters (A-C) above the error bars indicate no significant difference (one-way ANOVA, $P>0.05$) between sampling intervals. The numbers of reef boxes were three boxes at every sampling interval. Note scale variation on each graph.

The abundance of feeding guilds of mobile fauna varied with time within the reef boxes (Figure 7.2, see also Appendix 10). Suspension feeders were the most dominant mobile functional group in reef boxes. Suspension feeders were observed in large numbers (3,370.3±136.6 individuals/ box) at an early phase of reef development in September 2012. A decrease in suspension feeders was subsequently recorded in April 2013 (2,645.3±158.1 individuals/box) and November 2013 (1,739.7±115.2 individuals/box). The abundance of deposit feeders significantly increased in August 2013 (178±15.0 individuals/box), whilst the abundance of herbivores and carnivores significantly increased in April 2013 (233.3±18.9 and 26.3±5.2 individuals/ box). The abundance of omnivorous animals was significantly greater in April 2013 (64.3±7.5 individuals/box) compared to other sampling intervals.

The species richness of mobile species in reef boxes also varied with time. One-way ANOVA identified a significant effect of sampling interval ($P < 0.01$) on the species richness in reef boxes. Tukey's HSD pairwise tests showed that the species richness in reef boxes were significantly different ($P < 0.01$) between September 2012 (a month after reef deployment), April 2013 (8 months after reef deployment) and August 2013 (12 months after reef deployment). However, there was no significant difference in faunal species richness between September 2012 and November 2013 (15 months after reef deployment). Additionally, the lowest species richness (mean ± SE) of mobile species was observed in April 2013 (33.7±0.3 species/box), whilst the highest species richness was observed in August 2013 (37.3±0.9 species/box, Figure 7.3).

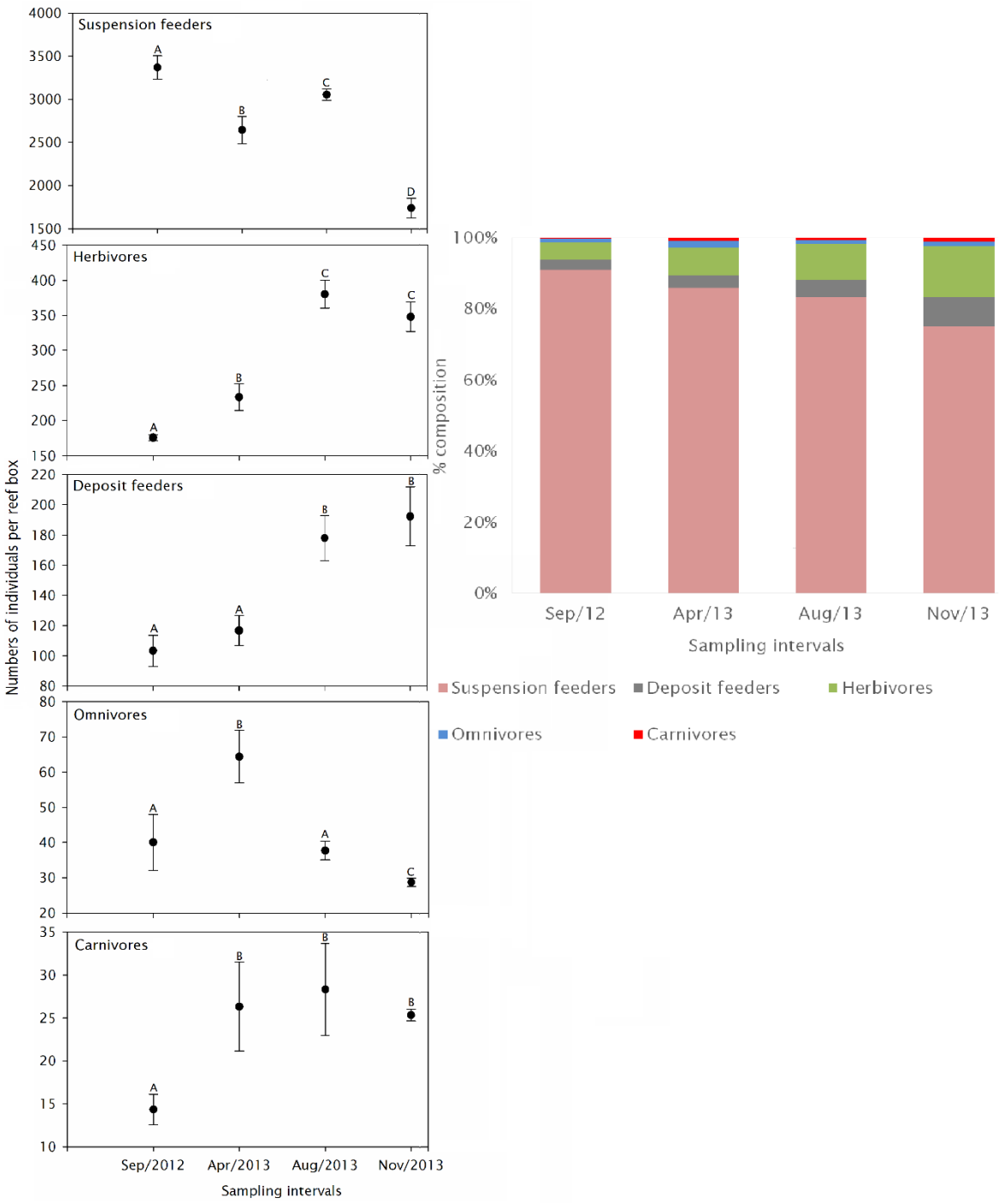
Suspension feeders
Herbivores
Deposit feeders
Omnivores
Carnivores
Numbers of individuals per reef box
Sep/2012
Apr/2013
Aug/2013
Nov/2013
Sampling intervals
% composition
100%
80%
60%
40%
20%
0%
Sep/12
Apr/13
Aug/13
Nov/13
Sampling intervals
Suspension feeders
Deposit feeders
Herbivores
Omnivores
Carnivores

Figure 7.2 (Previous page) Temporal variation in the abundance (mean ± SE, per reef box) of mobile species, in terms of functional groups, on elevated reef modules. The graphs are ordered in terms of the most abundant groups (top) to the least (bottom) and the stacked column presents the relative abundance of the five functional groups. Shared letters (A-C) above the error bars indicate no significant difference (one-way ANOVA, $P>0.05$) between sampling intervals. Three boxes were sampled at every interval. Note scale variation on each graph.

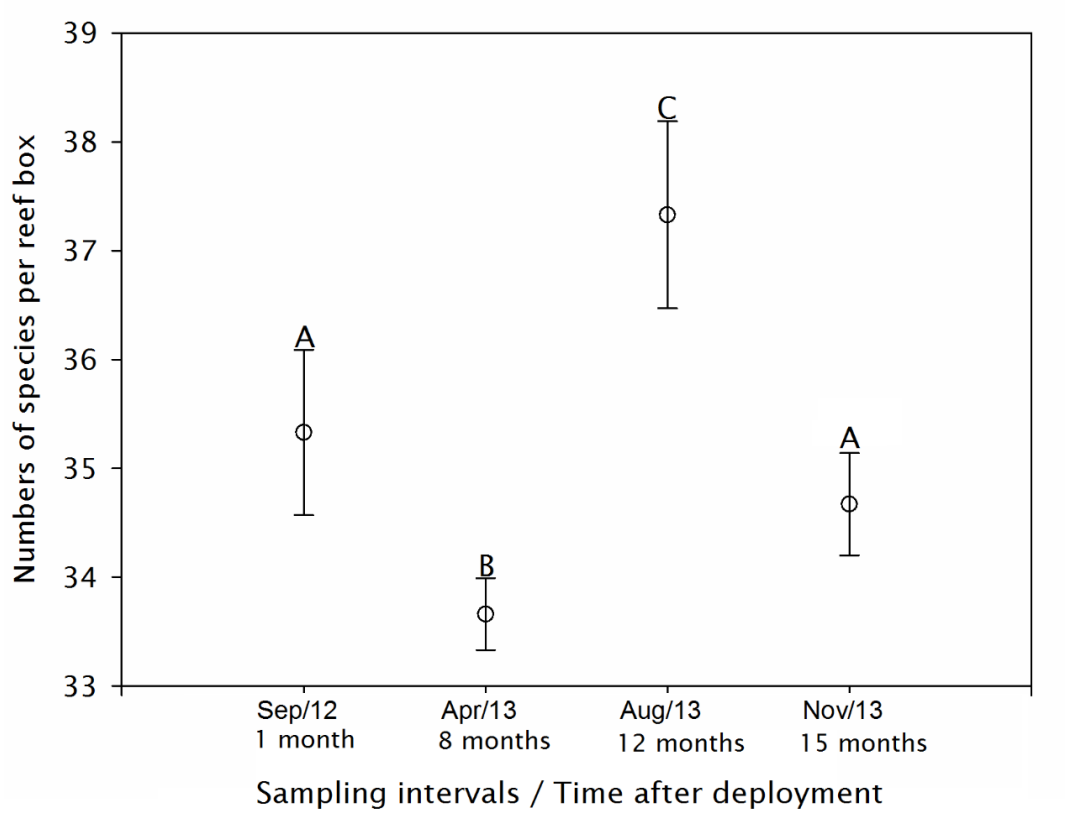

Figure 7.3 Species richness (number of mobile species per reef box, mean ± SE), observed on the elevated reefs. Species richness was significantly affected by sampling interval (one-way ANOVA, $P<0.01$). Shared letters above the error bars indicate no significant difference (one-way ANOVA, $P>0.05$) between sampling intervals. Three reef boxes were sampled at every interval.

One-way ANOVA also identified a significant effect of sampling interval ($P< 0.01$) on the abundance of mobile fauna in reef boxes. Tukey's HSD pairwise tests showed that the abundance of mobile fauna significantly differed between September 2012 (1 month after reef deployment), April 2013 (8 months after reef deployment) and November 2013 (15 months after reef deployment) ($P< 0.01$). However, there was no significant difference in mobile faunal abundance between September 2012 and August 2013. The lowest abundance (mean ± SE) of mobile species was observed in November 2013 (2,334±97.2 individuals/box), whilst the highest abundance was observed in September 2012 (3,704±134.9 individuals/box, Figure 7.4).

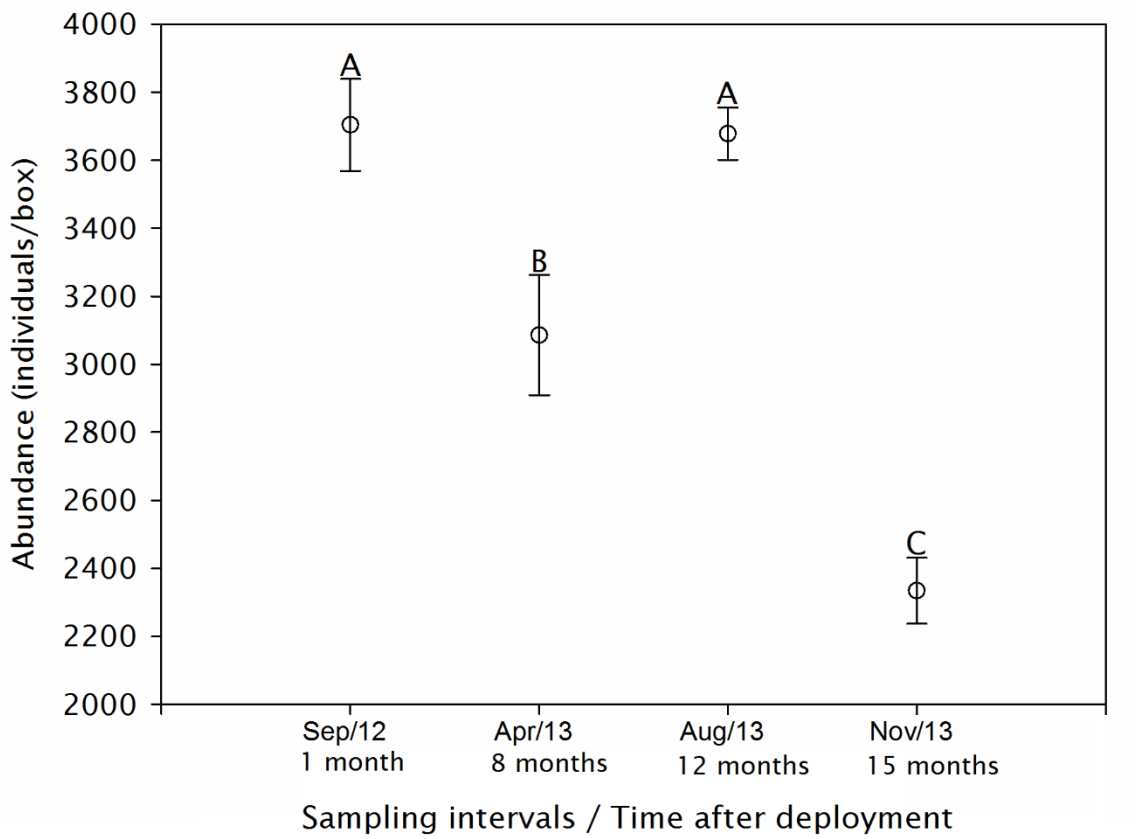

Figure 7.4 Abundance (total numbers of individuals per reef box, mean ± SE), observed on the elevated reefs. The abundance was significantly affected by sampling interval (one-way ANOVA, $P<0.01$). Shared letters above the error bars indicate no significant difference (one-way ANOVA, $P>0.05$) between sampling intervals. Three reef boxes were sampled at every interval.

The dendrogram of cluster analysis and an MDS plot, based on the log (x+1) transformation of the abundance of faunal species associated with functional groups, were produced in order to illustrate the temporal variation of mobile fauna in the reef community. From cluster analysis on the species similarity matrix (Figure 7.5), two main groups have been identified (SIMPROF, $P<0.05$). The first assemblage were grouped between sampling intervals that represent early state of reef deployment, before 12 months of experiment, (September 2012 and April 2013). The latter assemblage was grouped between sampling intervals that had been done after 12 months of reef deployment (August 2013 and November 2013). The MDS plot illustrated a two-dimensional representation with stress value of 0.06 (Figure 7.6). The MDS also confirmed

the separation of faunal community between sampling months. ANOSIM Global R value was 0.901 (P=0.01) indicating that the mobile faunal assemblage, in terms of functional groups, observed in September 2012 and April 2013 differed significantly from August 2013 and November 2013.

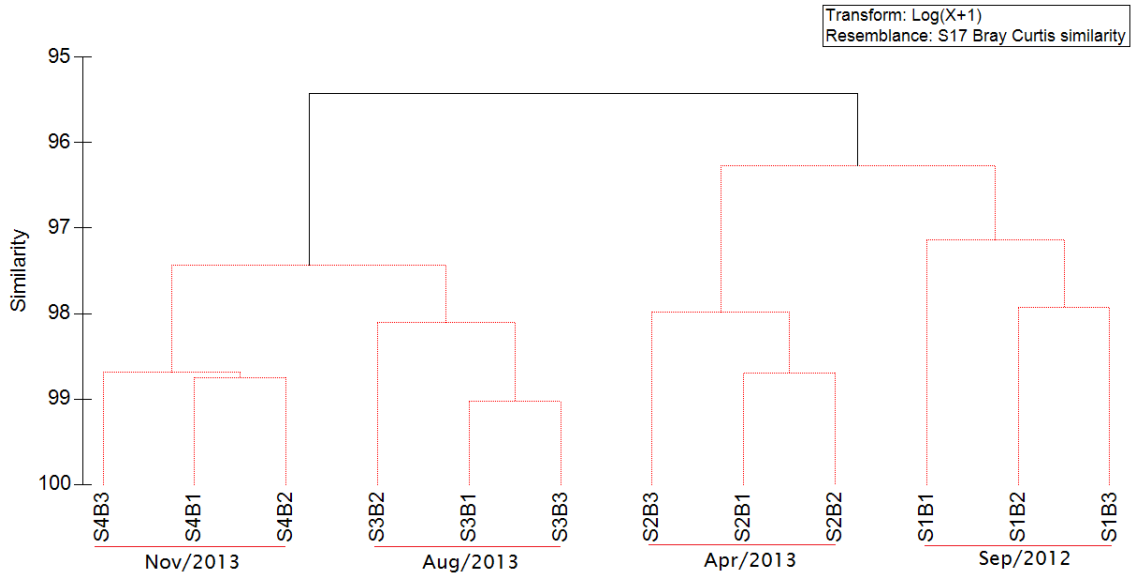

Figure 7.5 Hierarchical clustering (Bray and Curtis index method) of the abundance (log (x+1) transformed) of mobile fauna, associated with feeding groups. The dendrogram shows two groups of faunal community (SIMPROF, P<0.05). The first two characters represent sampling intervals (S1=September 2012, S2=April 2013, S3=August 2013, S4=November 2013) and the latter two characters (B1, B2, B3) represent the replicated numbers of reef boxes. There were three replicates at every sampling interval.

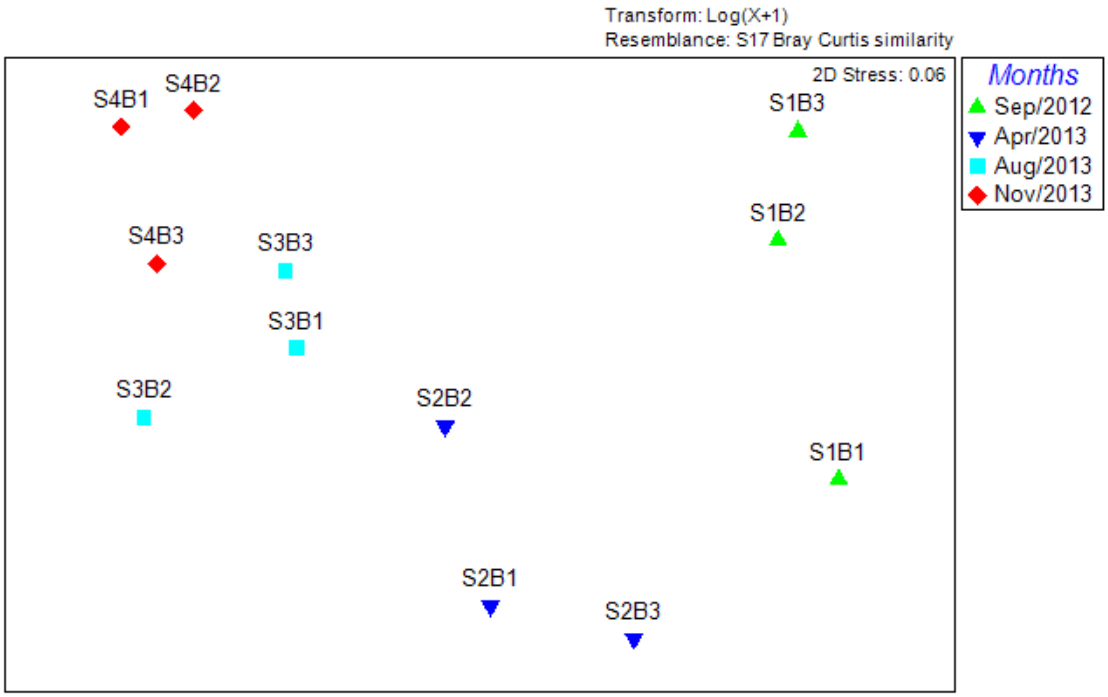

Figure 7.6 MDS diagram based on Bray-Curtis similarity matrix log (x+1) transformed mobile abundance in terms of functional groups in reef boxes at each sampling interval. The first two characters represent sampling intervals (S1=September 2012, S2=April 2013, S3=August 2013, S4=November 2013) and the latter two characters represent the replicated numbers of reef boxes. Stress level (0.06) indicates a well established two-dimensional data representation. The MDS plot illustrates the separation of sampling months associated with functional groups (ANOSIM Global R value =0.901, P=0.01).

7.3.2 The structure of the mobile faunal community in oyster cages on sea bed

A total of 47 mobile species were recorded in oyster cages on the sea bed. When considering the numerically dominant mobile species, based on the total numbers of individuals, the small filter-feeding porcelain crab (*P. longicornis*) was the first dominant fauna species, followed by the common spire shell (*R. parva*), amphipods (*Jassa* spp.), European pheasant shell (*Tricolia pullus*) and thick-lipped dogwhelk (*Nassarius incrassatus*). These five faunal species represented 71.6% of the total abundance recorded during 15 months of reef deployment. The species lists and abundance of each species in oyster cages are presented in Appendix 11.

P. longicornis was the primary dominant species with the exception of September 2012. The abundance of *P. longicornis* varied with sampling intervals (Figure 7.7). The highest abundance of *P. longicornis* was recorded in August 2013 (76.3±7.5 individuals/cage) and significantly dropped in November 2013 (50.7±1.9 individuals/cage). The abundance of *P. longicornis* in August 2013, was significantly higher compared with other sampling intervals. The number of *R. parva* significantly increased in August 2013 (43.6±4.3 individuals/cage) but the species was absent in November 2013. The highest abundance of *Jassa* spp. was observed in August 2013 (9.7±0.6 individuals/cage) and significantly decreased in November 2013 (6.3±0.2 individuals/cage). The lowest abundance of *T. pullus* was recorded in August 2013 (1.7±0.3 individuals/cage) and significantly increased in November 2013 (20.0±2.5 individuals/cage). The abundance of *N. incrassatus* in August 2013 was significantly higher compared with other sampling intervals.

In short, from April 2013 onwards, the porcelain crab (*P. longicornis*) was the most dominant species. However, the relative abundance (stacked column) of *P. longicornis* decreased in August 2013, whist the abundance of *R. parva*, *Jassa* spp. and *N. incrassatus* significantly increased. These three species significantly decreased in abundance in November 2013, especially *R. parva* that was absent at this time.

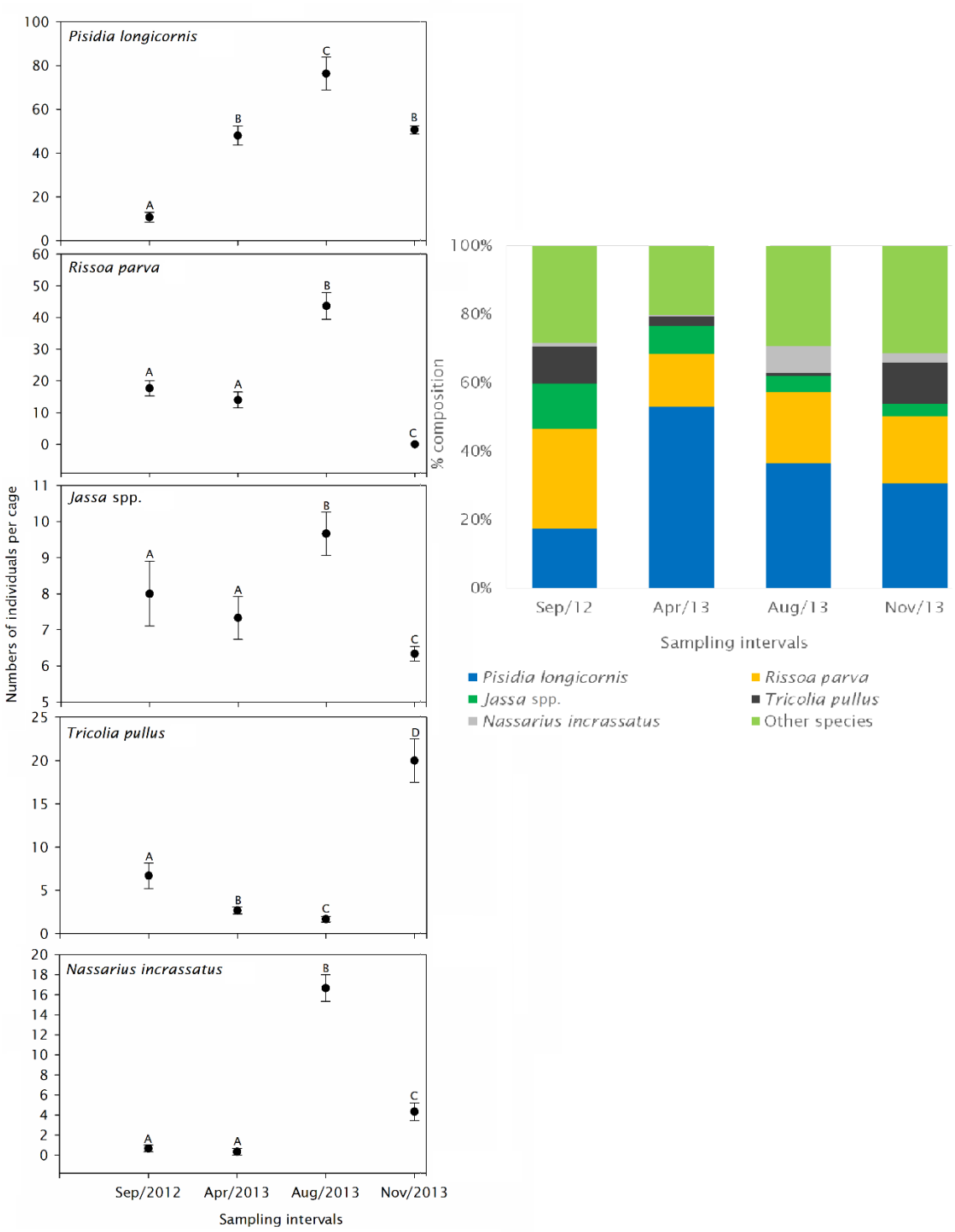
Pisidia longicornis
Rissoa parva
Jassa spp.
Tricolia pullus
Nassarius incrassatus
Numbers of individuals per cage
Sep/2012
Apr/2013
Aug/2013
Nov/2013
Sampling intervals
% composition
100%
80%
60%
40%
20%
0%
Sep/12
Apr/13
Aug/13
Nov/13
Sampling intervals
Pisidia longicornis
Rissoa parva
Jassa spp.
Tricolia pullus
Nassarius incrassatus
Other species

Figure 7.7 Temporal variation on the abundance per cage (mean ± SE) of the five dominant mobile species on the sea bed. The graphs are ordered in terms of the most abundant species (top) to the least (bottom) and the stacked column presents the relative abundance of the five dominant species and other species. Shared letters (A-C) above the error bars indicate no significant difference (one-way ANOVA, $P>0.05$) between sampling intervals. The number of oyster cages was three at every sampling interval.

The abundance of mobile fauna associated with feeding guilds in oyster cages (Appendix 11) varied with sampling interval. Suspension feeders were the most dominant functional group with the exception of September 2012 when herbivores were dominant (Figure 7.8). The highest abundance of all functional groups was observed in August 2013, with the exception of omnivores that demonstrated no significant variation with sampling interval. Suspension feeders and carnivores presented similar patterns of variation in abundance. The abundance of these groups significantly increased with sampling interval from September 2012 (23.7±3.2 and 0.7±0.3 individuals/cage, respectively) to August 2013 (90.7±6.2 and 8.0±0.6 individuals/cage, respectively) and significantly decreased in November 2013 (64.0±1.0 and 4.0±0.6 individuals/cage, respectively). Herbivores and deposit feeders presented a similar trend. Additionally, there was no significant difference in abundance of these groups between September 2012 and April 2013. A significant increase was observed in August 2013(67.7±3.3 and 37.7±5.5 individuals/cage, respectively) and the abundance reduced in November 2013 (42.7±0.3 and 16.3±2.0 individuals/cage, respectively).

The species richness of mobile species in sea bed oyster cages varied with time. One-way ANOVA identified a significant effect of sampling interval ($P< 0.01$) on the species richness of mobile fauna in the oyster cages. Tukey's HSD pairwise tests indicated that the species richness was significantly different between every sampling interval ($P< 0.01$).The lowest species richness (mean ± SE) was observed in September 2012 (14.0±0.6 species/cage), whilst the highest species richness was recorded in August 2013 (24.3±0.7 species cage, Figure 7.9). There was a significant effect of sampling interval (ANOVA; $P< 0.01$) on the abundance of mobile fauna in oyster cages. *Post hoc* tests showed that the abundance of mobile fauna significantly differed between every sampling interval ($P< 0.01$). The lowest abundance (mean ± SE) was recorded in November 2013 (60.3±1.7 individuals/cage), whilst the highest abundance was observed in September 2012 (209.3±7.5 individuals/cage, Figure 7.10).

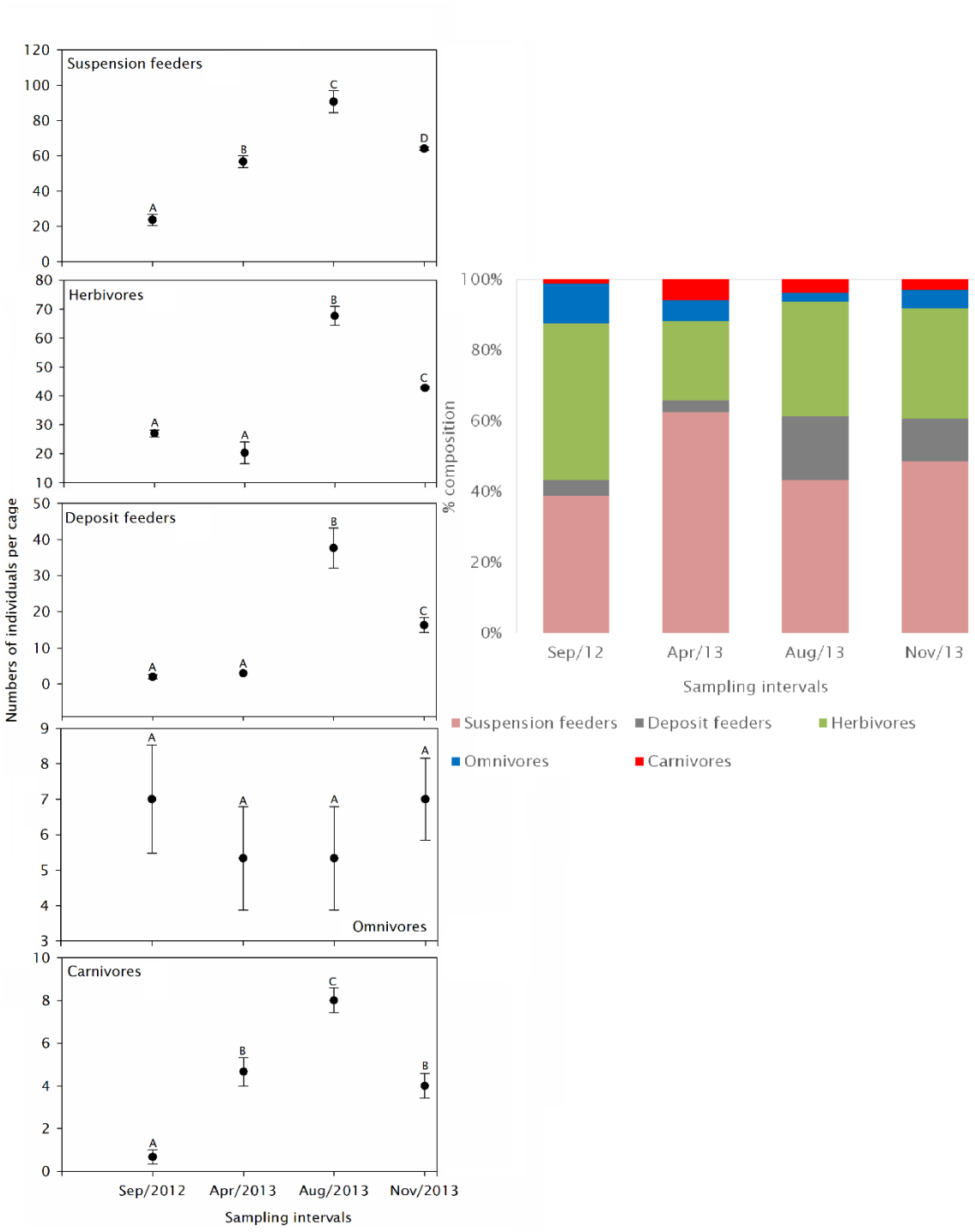
Suspension feeders
Herbivores
Deposit feeders
Omnivores
Carnivores
Numbers of individuals per cage
Sep/2012
Apr/2013
Aug/2013
Nov/2013
Sampling intervals
% composition
Sep/12
Apr/13
Aug/13
Nov/13
Sampling intervals
Suspension feeders
Deposit feeders
Herbivores
Omnivores
Carnivores

Figure 7.8 Temporal variation on the abundance (mean ± SE, per reef box) of mobile species, in terms of functional groups, on the sea bed. The graphs are ordered in terms of the most abundant groups (top) to the least (bottom). The stacked column shows the relative abundance of the five functional groups. Shared letters (A-C) above the error bars indicate no significant difference (one-way ANOVA, $P>0.05$) between sampling intervals. The numbers of oyster cages was three at every sampling interval. Note scale variation on each graph.

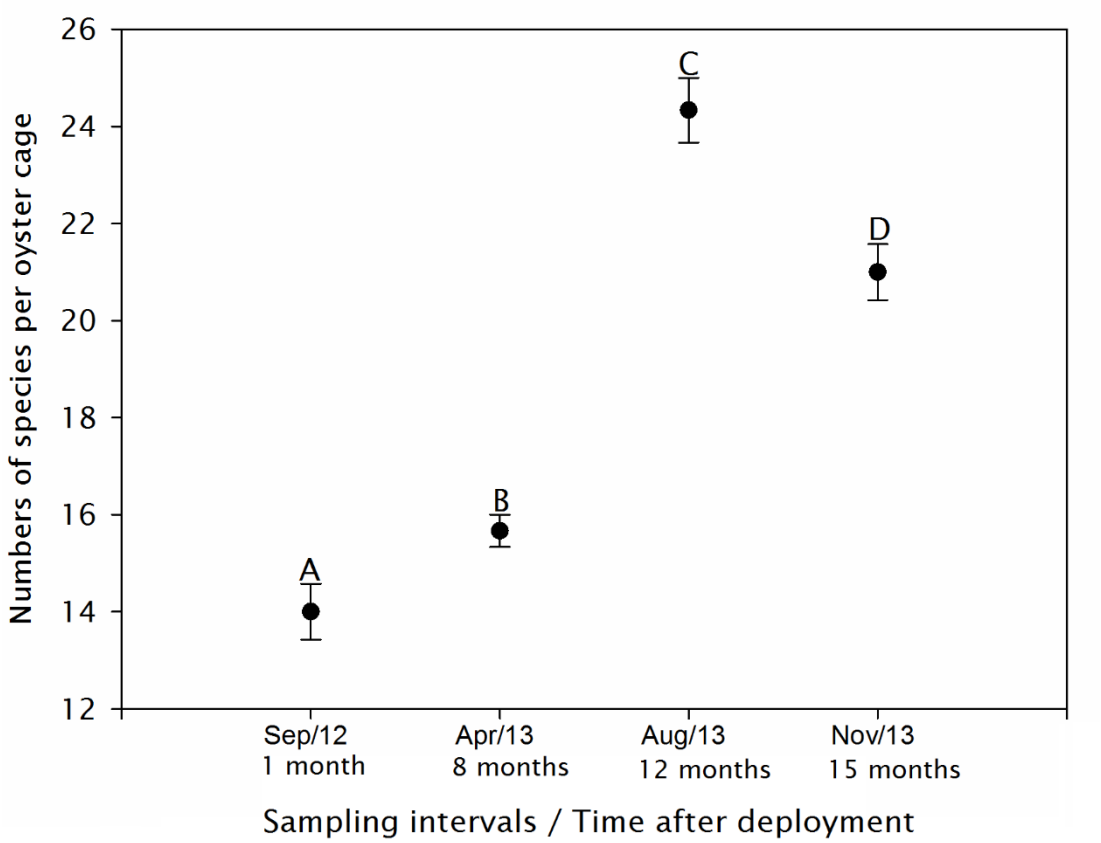

Figure 7.9 Species richness, expressed as the number of mobile species per cage, (mean ± SE), observed in oyster cages on the sea bed. Richness was significantly affected by sampling interval (one-way ANOVA, $P<0.01$). Shared letters above the error bars indicate no significant difference (one-way ANOVA, $P>0.05$) between sampling intervals. Three oyster cages sampled at each interval.

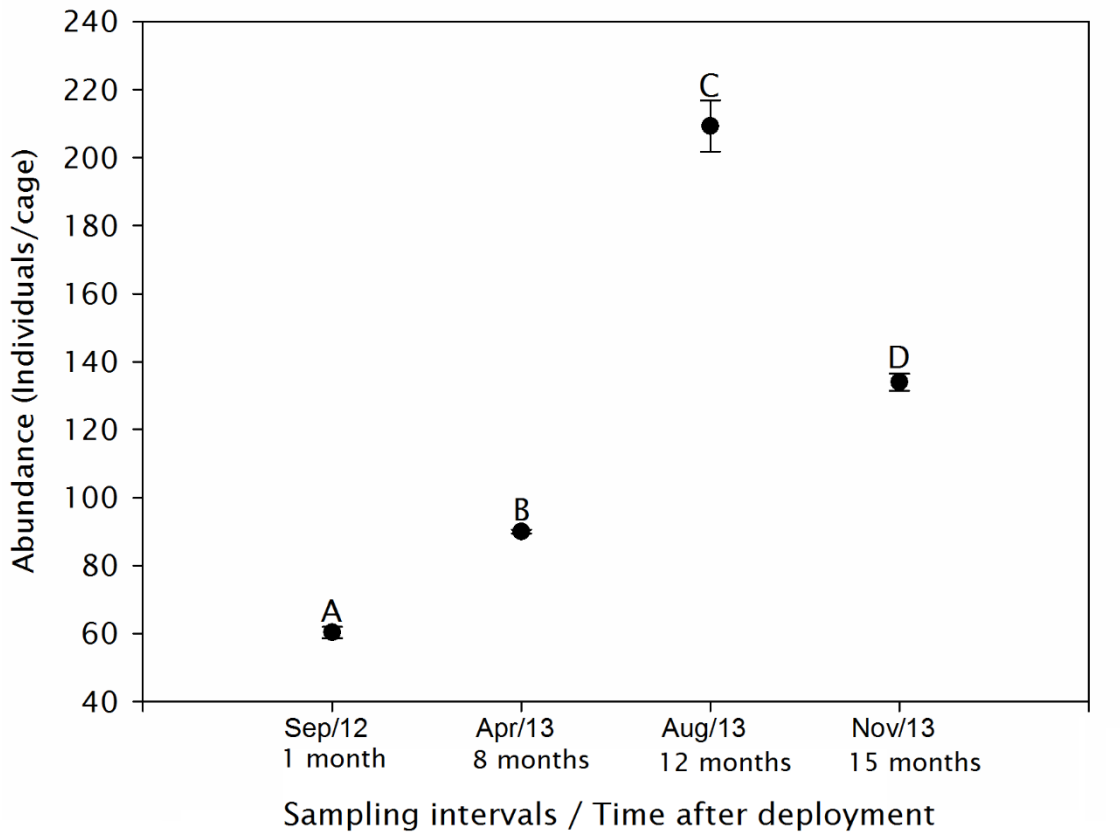

Figure 7.10 Abundance, expressed as the total number of individuals per cage (mean ± SE), in the oyster cages Abundance was significantly affected by sampling interval (one-way ANOVA, $P<0.01$). Shared letters above the error bars indicate no significant difference between sampling intervals (one-way ANOVA, $P>0.05$). Three cages sampled at each interval.

The dendrogram of cluster analysis and an MDS plot, based on the log (x+1) transformation of the abundance of functional groups, illustrated the temporal variation of mobile fauna in the oyster cages laid on the sea bed. From cluster analysis on the species similarity matrix (Figure 7.11) four main groups have been identified. Additionally, the hierarchical clustering indicates that the faunal community differed between sampling intervals (SIMPROF, $P<0.05$). The MDS plot illustrated a well established two-dimensional representation with a stress value of 0.04 (Figure 12). The MDS also presented the separation of mobile faunal composition between sampling intervals. ANOSIM Global R value was 0.920 ($P=0.01$) indicating that the faunal composition, in terms of functional groups, significantly differed between sampling intervals.

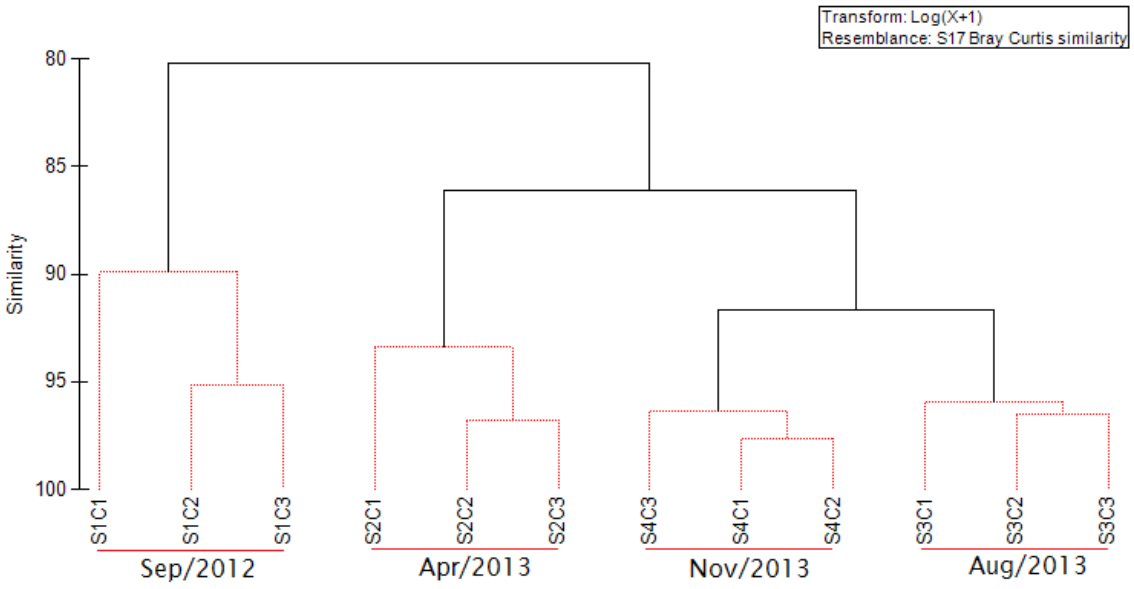

Figure 7.11 Hierarchical clustering (Bray and Curtis index method) of the abundance (log (x+1) transformed) data for mobile fauna, associated with functional groups. The dendrogram shows that the faunal composition differed between sampling intervals (SIMPROF, $P<0.05$). The first two characters represent sampling intervals (S1=September 2012, S2=April 2013, S3=August 2013, S4=November 2013) and the latter two characters (C1, C2, C3) represent the replicated numbers of oyster cages. n = 3 at every sampling interval.

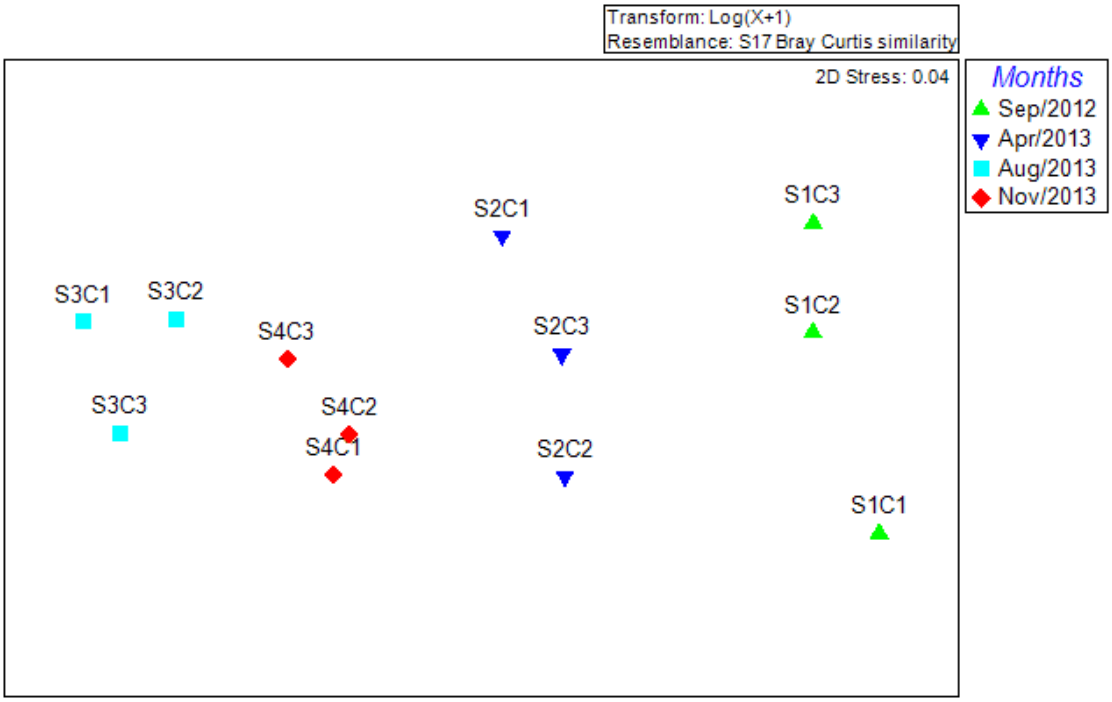

Figure 7.12 MDS diagram based on Bray-Curtis similarity matrix log (x+1) transformed mobile abundance, in terms of feeding groups, in oyster cages at each sampling interval. The first two characters represent sampling intervals (S1=September 2012, S2=April 2013, S3=August 2013, S4=November 2013) and the latter two characters represent the replicated numbers of oyster cages. Stress level (0.04) indicates a well established two-dimensional data representation. The MDS plot illustrates the separation of sampling months associated with animal functional groups (ANOSIM Global R value =0.901, P=0.01).

7.4 Discussion and conclusion

Artificial oyster reefs are not only beneficial for epifaunal species as discussed in Chapter 6 but also provide shelter and forage habitats for interstitial and mobile species (Coen and Luckenbach, 2000; Meyer and Townsend, 2000; Rodney and Paynter, 2006). Previous studies have reported that artificial oyster reef habitats provided abundant epifaunal species as prey for higher trophic animals and also create shelters for a large variety of macroinvertebrates and fish (Grabowski et al., 2005; Lehnert and Allen, 2002). Similarly, in Chapter 6, an increase of epifauna on oyster valves on the elevated reef may well have provided a greater opportunity for grazing by mobile species. There were 65 mobile species observed in reef boxes, whilst 47 species were recorded in oyster cages. Asmus and Asmus (2011) reported that epifauna or sessile suspension feeders often show a tendency for aggregation on oyster reefs and can, in this way, form communities offering space and nourishment for both mobile vertebrate and invertebrate fauna. Consistent with this study, the small filter-feeding porcelain crab (*P. longicornis*) was the first dominant species. Habitat complexity may influence predator-prey interactions and have significant impacts on the local faunal community (Humphries et al., 2012). In this study, the presence of some mobile species could be explained by predator and prey interactions. The molluscs *Trivia arctica* and *Trivia monacha*, which feed on ascidians (Fretter and Manly, 1977), were observed on elevated reefs in August 2013, when the highest abundance of ascidians (Chapter 6) was simultaneously recorded. Previous observations in the Poole Bay reported that the swimming crab (*Necora puber*) was observed browsing on barnacles attached to artificial reef block surfaces (Jensen et al., 2000). In this study *N. puber* was also often found in reef boxes where an abundance of barnacle (*Elminius modestus*) was recorded. *Cancer pagurus,* a commercial crab species that feed on gastropods, amongst other species, were also observed in reef boxes within a month after reef deployment whilst only occasionally observed on the sea bed after a year of deployment. Therefore, it is clear that the large aggregation of epifaunal species in reef boxes on elevated reef modules could potentially attract mobile species and consequently enhance reef community complexity by food web mechanisms (Grabowski et al., 2005).

The enhanced abundance of epifauna (Chapter 6) and mobile fauna on elevated oyster reefs has been shown to lead to the immigration of fish species to forage (Harding and Mann, 2001; Meyer and Townsend, 2000). Many resident and transient fishes often move into oyster reefs to search for food items, thus facilitating the transfer of energy from the benthos to higher trophic levels (Harding and Mann, 1999). It has been argued that the increased density of mobile fauna in oyster reef habitats could potentially cause an increase in fish productivity (Grabowskiet al., 2005). Studies in the USA using a variety of sampling methods have found 11–42 fish species on *Crassostrea virginica* reefs (Coen and Mark, 2000; Harding and Mann, 1999; Lehnert and Allen, 2002; Lenihan et al., 2001). In this study, within 15 months of reef deployment, 11 of a total record of 12 different fish species identified were observed in reef boxes on the top of elevated reefs while only 5 fish species with a few individuals were observed in oyster cages on the sea bed. This is possibly because prey items for the fish such as crustaceans, prawns, molluscs, hydroids and polychaetes were recorded in increased numbers in reef boxes on the elevated reefs. Conversely, Beukers and Jones (1997) reported that habitats that are more complex often contain greater spaces of refuges from predators, thus reducing the intensity of predation by restricting predator manoeuvrability and/or the ability to visually detect prey (Finke and Denno, 2002). This could explain why the large numbers of porcelain crab (*P. longicornis*) and hooded shrimp (*A. nitescens*), both species preyed upon by fish, were recorded on elevated reefs. Posey et al. (1999) reported the value of oyster reefs as refuges from fish predators for grass shrimps (*Palaemonetes pugio*). Consistent with this study, the grass shrimps (*Palaemon* spp.) were also observed in reef boxes but absent in sea bed cages.

Herbert (2013) investigated the contribution of an artificial surf reef, a basal area of 50 x 70 m, to inshore fisheries in Poole Bay between August 2011 and May 2012. The author reported that the most dominant group was crustaceans whilst a total of 14 fish taxa were observed at the artificial surf reef. In this study, a total of 12 fish species were also observed on the elevated oyster reefs. It is likely that at this initial phase of the construction the presence of the elevated reefs has so far not had an observable impact on the numbers of fish species. Moreover, the fish species observed in this study do not differ

from the typical fish which have previously been recorded in the localised area around the Poole Bay artificial reef by Mallinson et al. (1999).

Increased oyster predation is an important issue that requires consideration when constructing artificial reefs for oyster restoration. Whilst Collins et al. (1994) reported the presence of the tingle *Oceanebra erinacea*, a predatory gastropod mollusc, in Poole Bay. The licence granted to place the oyster reef for this project was restricted to the Poole Bay site. Predictably, *O. erinacea* was found on both the elevated reef and the sea bed. The highest numbers of individuals in both areas were observed in August 2013, coincident with the highest abundance of molluscs. Data presented in this chapter indicate that elevated reefs can provide suitable substrates for *O. edulis* settlement; however, the height of elevated reefs in this study (80 cm) did not afford sufficient protection from predators (*O. erinacea*) to this target species. However, the potential enhancement of settlement would serve to promote survival of oysters as 19 spat that had survived to reach a size large enough to identify, were observed. This evidence suggests that predation by *O. erinacea* is not 100% on a reef.

Kang et al. (2006) have identified that seasonal variations in food supplies influence growth, reproduction, and survival rate of organisms, and consequently affects the temporal variation in species richness and abundance of mobile fauna in reef communities. Similarly, in this study, the species richness and abundance of mobile animals in reef boxes varied seasonally. The highest abundance of mobile fauna was observed in August 2013 when the highest abundance of epifaunal species (Chapter 6), key food items of mobile species, was also recorded. In contrast, the lowest abundance was observed in April 2013 when the lowest abundance of epifauna (Chapter 6) was observed. Cluster analysis indicated that the mobile faunal feeding guilds in September 2012 and April 2013 were significantly different from August 2013 and November 2013. This is perhaps because the abundance of suspension feeders (e.g. *P. longicornis*), the dominant species on elevated reefs, varied seasonally and significantly decreased in November 2013. At the same time, the relative abundance of minor abundant groups (i.e. herbivores, deposit feeders and carnivores) increased with sampling time. Consistent with findings in this study, previous studies have shown that suspension feeders were the most abundant benthic invertebrates on artificial structures, especially shortly after

deployment (Baird et al., 2004; Markus et al, 2014). However, the abundance of suspension feeders varied with season and age of artificial structures as a result of the later addition of higher trophic species. This addition of higher trophic animals increased the complexity of the oyster reef community itself by enhancing the number and diversity of feeding guilds (Markus et al., 2014), thereby affecting overall ecosystem functioning (Van der Zee et al., 2015)

In oyster cages, the relative abundance of suspension feeders was not high (20-50% of total mobile individuals, Figure 7.8), compared to that found in reef boxes (70-85%, figure 7.7). This variation in relative abundance of suspension feeders could be a function of the improved water conditions on the elevated reefs (e.g. increased water current, food supply and reduced suspended sediment; Chapter 2). In August 2013, there was a notable increase in the proportion of deposit feeders, i.e. an increase abundance of *Nassarius incrassatus* that prefers living on detritus-rich substrates (Tallmark, 1980). Indeed, Castel et al. (1989) suggested that an increase in the number of deposit feeders on the sea bed nearby oyster reefs was due to the deposition of organic matter from elevated reef modules.

The structure of mobile faunal communities in oyster cages was significantly different between every sampling interval, whilst the mobile faunal community in elevated reefs differed before and after 12 months post-deployment. Elmqvist et al. (2003) argued that a more diverse ecosystem is more resilient to the impacts of environmental stress or seasonal variation because of its enhanced stability through redundancy. As such, the data from this study indicates that the faunal community on the sea bed was more fragile than that of the elevated reef community. It can be concluded that the presence of elevated reef habitats created by *O. edulis* valves can enhance local diversity and ecosystem stability in the Poole Bay. However, Jensen et al. (2000) reported that European artificial reefs have been shown to develop as successful ecosystems over prolonged periods of time. Five years seems to be sufficient time for a relatively stable community to develop in water other than the most oligotrophic areas of the Mediterranean. Therefore, long term monitoring, of at least 5 years, of the reef colonisation by epifaunal and mobile species is needed to be included in any future study.

Chapter 8

Conclusions and future work

After elevated reefs were deployed in August 2012, four sampling intervals were carried out during 15 months of deployment. The reef boxes and oyster cages were recovered in September 2012 (Autumn, 12˚C), April 2013 (Spring, 7˚C), August 2013 (Summer, 18˚C) and November 2013 (Autumn, 15˚C). The environmental data indicated that temperature and salinity were vertically fully mixed and there was no significant difference in temperature or salinity between the elevated reefs and sea bed. Water temperatures at the date of sampling varied between 7-18˚C while salinities were between 33-35. The purpose of this elevated reef construction was to place oysters at depths where water qualities were improved e.g. reducing the amount of suspended sediment and increasing food availability in order to subsequently enhance physiological performance of *Ostrea edulis*. The environmental data showed that the total suspended solids (TSS) in the water column at the height of elevated reefs were significantly lower than at the adjacent sea bed in every sampling interval. This project sought to establish the effect of reef habitat on physiological performance (Chapter 3) and gonad maturation (Chapter 5) of *O. edulis* in comparison with sea bed oysters. The geographic variation in *O. edulis* physiology was also investigated (Chapter 4). Secondarily, the benefits of elevated reefs in terms of enhancing epifaunal settlement and mobile fauna abundance were also discussed in Chapter 6 and 7, respectively.

8.1 Achievement of research objectives

To the best of knowledge obtained from this study, this project presented the novel observation that elevation had a significant effect on the filtration rate of oysters, such that the European flat oyster (*O. edulis*) on top of elevated reefs (80 cm above sea bed) had significantly higher filtration rates compared with oysters on the sea bed. The highest filtration rate was observed in August 2013 when the temperature rose to 18^0C. There was no significant effect of

elevation (reef/sea bed) on the respiration rate, condition index and haemolymph protein levels of *O. edulis*. Those parameters were mainly influenced by sampling interval.

Geographical variation in filtration rate were also reported in this study. Significant differences in the filtration rates of *O. edulis* collected between Poole Harbour, Chichester Harbour and the Solent were observed. The filtration rate of oysters collected from Poole Harbour were significantly higher in comparison with Chichester Harbour and Solent oysters. However, unsurprisingly, no significant effects of location on the respiration rates of oysters were noted. Respiration rates of oysters collected from all locations were the highest in August 2014 (16^0C) and the lowest in May 2014 when the water temperature was 12^0C. These results in Chapter 4 compared with findings in Chapter 3 suggest the improved physiology of reef oysters in summer is still notable when comparing between different populations. Growing *O. edulis* (Poole oysters) on elevated reef structures increases filtration rate in the summer by 0.93 l h^{-1} g^{-1} AFDW above the performance when they are re-laid on the sea bed while the magnitude of difference in filtration rates of *O. edulis* from Poole compared with Chichester oysters and Solent oysters are 0.4 l h^{-1} g^{-1} AFDW and 0.6 l h^{-1} g^{-1} AFDW, respectively. Therefore, as the transportation of oysters between locations is prohibited in order to limit the spread of the parasite *Bonamia ostreae*, using elevated reef structures to improve oyster physiology in their natural habitats is considered to be a more tractable solution than that of relocating oysters between habitats.

Culture of oysters on elevated reef modules had no effect on the gonad index and ALP (alkali-labile phosphate) concentration. However, when comparing the gonad maturation between reef and sea bed oysters, the data from this study suggested that oysters held on elevated reef structures completed their gametogenic cycle earlier than sea bed oysters. One possible explanation for these results is that reef oysters had significantly higher filtration rates, stored more energy and could therefore spawn and complete spawning earlier than the sea bed oysters.

The prevalence of *Bonamia* sp. infection for natural Poole Harbour oysters was superficially higher compared with relocated oysters in the experimental site,

Poole Bay (reef and sea bed oysters). It could be argued that increasing the filtration rate for reef oysters may increase the likelihood of uptake of the protozoan parasite, *Bonamia* sp. However, the intensity of *Bonamia* sp. infection was very low. Based on the fact that haemocytes are important in terms of immune defence in the host, and reef oysters had significantly higher numbers of total haemocytes and granulocytes than sea bed oysters, this may increase the potential of reef oysters to survive infection. Moreover, no significant differences in the concentration of haemolymph proteins (Section 3.3.4) between reef and sea bed oysters were found. Therefore, there appears to be no differential impact on physiology between reef and sea bed oysters as a result of *Bonamia* sp. infection.

The assessment of new-recruits is essential to consider when assessing the success of management strategy on stock enhancement. Whilst Poole Bay is not locally recognised as a site for the recruitment of *O. edulis,* the observation of 19 oyster spat on elevated reefs, with no spat being found on the sea bed oyster valves, suggests that *O. edulis* valves on elevated reef structures provide more suitable settlement habitat than valves laid on the sea bed.

During 15 months of reef deployment, a total of 54 epifaunal species were observed on oyster valve cultch and live oysters in the Poole Bay. All 54 species were found on oyster valves in reef boxes on the top of elevated reefs, 80 cm above the sea bed, whilst only 23 species were found on oyster valves in oyster cages laid on the sea bed. This finding indicates that restoring the native oyster population and ecosystem diversity through the construction of artificial elevated structures represents a better solution than the continued relaying of live oysters on the sea bed or than the distribution of oyster valve cultch directly onto the sea bed. Species richness, diversity and the abundance of epifaunal species on live oysters or oyster valve cultch situated on elevated reefs were significantly higher than those valves on the sea bed at every sampling interval.

Multidimensional scaling plot (MDS) showed that epifaunal community on oyster valves positioned on elevated reefs was significantly different from the community on oyster valves on the sea bed but also had some species in common. The five most dominant species contributed 80.8% of the total individual abundance. These were keeled tubeworms (*Pomatoceros triqueter*),

acorn barnacles (*Elminius modestus*), segmented worms (*Filograna implexa*), spiral tubeworms (*Spirorbis spirorbis*) and slipper limpets (*Crepidula fornicata*). These dominant species were commonly found on both valves collected from the elevated reefs and from the sea bed.

There were 65 mobile species observed in reef boxes, whilst 47 species were recorded in oyster cages. The first dominant species was the small filter-feeding porcelain crab (*Pisidia longicornis*) followed by hooded shrimp (*Athanas nitescens*), an amphipod (*Jassa* spp.), squat lobster (*Galathea squamifera*), and common spire shell (*Rissoa parva*). Cluster analysis on the species similarity matrix suggested that two assemblages of mobile fauna in reef boxes have been identified. The first assemblage were grouped between sampling intervals that represent the early stage of reef deployment, in the first 12 months of the experiment, (September 2012 and April 2013). The latter assemblage was grouped between sampling intervals after 12 months of reef deployment (August 2013 and November 2013). The MDS plot also confirmed that the mobile faunal assemblage, in terms of functional groups, observed in September 2012 and April 2013 differed significantly from August 2013 and November 2013. Cluster analysis suggested that four main groups of mobile fauna in oyster cages on the sea bed have been identified. Additionally, the hierarchical clustering showed that the faunal community significantly differed between sampling intervals. The MDS also indicated that the faunal composition, in terms of functional groups, in oyster cages laid on the sea bed significantly differed between sampling intervals. As such, the data from this study indicate that the faunal community on the sea bed was more sensitive to seasonal variation than the elevated reef community. Based on epifaunal and mobile faunal data, it can be concluded that the presence of elevated reef habitats created by *O. edulis* valves can enhance and accelerate local diversity and ecosystem stability in the Poole Bay.

8.2 Problems and limitations

This thesis represents the results of a pilot project to investigate potential benefits of elevated reefs. Benefits were identified in terms of improved physiological performance and condition of the target species *O. edulis*. However, the work was also limited by finite resources and the licence obtained to construct the reefs. Based on these limitations, the elevated reefs were deployed at only one site, Poole Bay. Sampling was dictated by the prevailing weather conditions, particularly during the winter months, when it was not possible to dive to sample the reefs. The original plan of this study was to investigate the influences of artificial reef habitat on oyster physiology and local biodiversity over 24 months of experimental reef deployment. Unfortunately, after 15 months of reef deployment, the elevated artificial reefs and sea bed cages were damaged or removed. The SCUBA divers surveyed the experimental site in April 2014 and found that there were no reef boxes or sea bed cages remaining. This issue was possibly caused by fishing activities or winter storms between January and February 2014. Nevertheless, data from this study, albeit limited in replication, spans at least one annual cycle and our results confirm by multiple measures that elevated reef habit significantly improves the physiological performance of *O. edulis*. Thus, this thesis has merit as a reseach report to support the continued research investment in proving this approach as a fishery management strategy for restoration of the European flat oysters.

8.3 Recommendations for future study

Whilst we report that a number of key physiological variables were increased by an elevated reef habitat in *O. edulis*, these pilot data do not represent definitive proof of the benefits of rearing oysters on reef structures. Further, and longer-term, replicated studies are necessary to confirm that the results we have described are confirmed in other indices of performance and health, incorporating assays such as Scope for Growth and growth rates to confirm the health status of the reef oysters. Future work should also address the assessment of physiological performance *in situ*, including respiration and filtration measurements, in order to field-validate the findings of our laboratory

assays. Formal assessments of spawning output including more detailed assessment of fecundity and larval quality are recommended for the future research in order to ensure that elevated reefs promote not only the rate of gonad maturation, but also spawning success, larval quality and settlement success – all of which are essential for the successful recruitment of new cohorts from broodstock. The measurement of additional water conditions e.g. water current, tidal cycle, light intensity and also lab-based assessment of physiological performance of the oysters related to these parameters need to be considered.

It is also important that these experiments are conducted at multiple locations across the European margin to establish the broader scale validity of our findings. Identifying suitable locations that do not interfere with, and are not damaged by, fishing activities such as trawling and dredging prior to reef construction and deployment is prerequisite. Reef design suitable for the UK hydrodynamic conditions is also a major area where further work is desired. Using two steel pins holding a reef module is recommended in order increase durabity of reef structures which were possibly frustrated by strong water current and fishing activities.

Future work should establish the importance of *Bonamia* sp. in long-term oyster reef health as well as investigating long-term studies of the reservoirs of infection. Understanding the real role of haemocytes in an immune defence in response to *Bonamia* infection in *O. edulis* is still unclear. To fulfil this knowledge gap, direct assessments of *Bonamia* sp. impacts on physiological performance of *O. edulis* (e.g. phagocytosis and respiratory burst) needs to be addressed in future investigations. As the life cycle of this protozoan parasite is still unknown, the significance of intermediate hosts on the reefs e.g. *Ophiotrix fragilis* remains uncertain. Therefore, this knowledge gap also needs to be resolved by further research. Due to limited resources, in this study the abundances of mobile infauna and epifauna were assessed by counting individuals only. However, in future study, the author recommends that the assessment of biomass should be reported as ash-free dry weight as a more robust means to determine changes in community composition. Moreover, to investigate the overall success of reef habitats as an ecosystem enhancement tool, long term monitoring of the epifaunal, mobile faunal and fish assemblage in the elevated reefs needs to be included in any future study

Appendices

Pearson's product-moment correlation coefficients between physiological parameters

Parameters	Statistical values	Respiration	Total haemocytes	Granulocytes	Hyalinocytes	Haemolymph protein	Condition index
Filtration	Correlation Coefficient	0.75	0.64	0.65	0.54	0.48	0.45
	P Value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Respiration	Correlation Coefficient		0.32	0.32	0.28	0.30	0.21
	P Value		<0.05	<0.05	<0.05	<0.05	<0.05
Total haemocytes	Correlation Coefficient			0.97	0.93	-0.05	0.47
	P Value			<0.05	<0.05	>0.05	<0.05
Granulocytes	Correlation Coefficient				0.81	-0.11	0.48
	P Value				<0.05	>0.05	<0.05
Hyyanulocytes	Correlation Coefficient					-0.04	0.40
	P Value					>0.05	<0.05
Haemolymph protein	Correlation Coefficient						-0.12
	P Value						<0.05

Appendix 2

List of epifaunal species and the number of individuals recorded on live oysters collected from sea bed (LVS) at each sampling interval.

Species name	Sampling intervals				Total
	Sep/12 n=19	Apr/13 n=16	Aug/13 n=13	Nov/13 n=9	n=57
Porifera					**0**
Dysidea fragilis	0	0	0	0	0
Halichondria panicea	0	0	0	0	0
Cnidaria					**2**
Eudendrium ramosum	0	0	0	0	0
Halecium beanii	0	0	0	0	0
Halecium halecinum	0	0	0	0	0
Kirchenpaueria pinnata	0	0	0	0	0
Laomedea flexuosa	1	1	0	0	2
Sertularia argentea	0	0	0	0	0
Sertularella gaudichaudi	0	0	0	0	0
Tubularia larynx	0	0	0	0	0
Actinothoe sphyrodeta	0	0	0	0	0
Sagartia troglodytes	0	0	0	0	0
Annelida					**265**
Filograna implexa	0	0	0	0	0
Pomatoceros triqueter	52	41	69	74	236
Sabella pavonina	0	0	1	0	1
Spirobis spirobis	0	4	18	6	28
Harmothoe sp.	0	0	0	0	0
Crustacea					**54**
Elminius modestus	0	0	38	16	54
Mollusca					**4**
Acanthochitona fascicularis	0	0	0	0	0
Crepidula fornicata	1	1	1	1	4
Anomia ephippum	0	0	0	0	0
Chlamys varia	0	0	0	0	0
Aequipecten opercularis	0	0	0	0	0
Ostrea edulis	0	0	0	0	0
Bryozoa					**48**
Alcyonidium diaphanum	0	0	0	0	0
Amathia lendigera	0	0	3	2	5
Bicellariella ciliata	0	0	0	0	0
Bugula flabellata	0	0	0	0	0
Chartella papyracea	0	0	0	0	0
Epistomia bursaria	0	0	0	0	0
Flustra foliacea	0	0	0	0	0
Disporella hispida	4	3	1	2	10
Electra pilosa	3	0	5	3	11

Appendix 2 (Cont.)

Species name	Sampling intervals				Total
	Sep/12 n=19	Apr/13 n=16	Aug/13 n=13	Nov/13 n=9	n=57
Plagioecia patina	0	0	5	6	11
Tubulipora plumosa	0	2	3	4	9
Cellepora sp.	0	0	2	0	2
Tunicata					**4**
Ascidia conchilega	0	0	0	0	0
Ascidia mentula	0	0	0	0	0
Ascidia verginea	0	0	0	0	0
Ascidiella aspersa	0	0	0	0	0
Ciona intestinalis	0	0	0	0	0
Corella eumyota	0	0	0	0	0
Dendrodoa grossularia	0	0	0	0	0
Pyura microcosmus	0	0	0	0	0
Styela clava	0	0	0	0	0
Molgula sp.	0	0	1	0	1
Perophora sp.	0	0	0	0	0
Botrylloides diegense	0	0	0	0	0
Botrylloides leachi	0	0	0	0	0
Botrylloides violacea	0	0	0	0	0
Botryllus schlosseri	0	0	1	0	1
Morchelium argus	0	0	0	0	0
Diplosoma listerianum	0	2	0	0	2
Didemnum sp.	0	0	0	0	0

Appendix 3

List of epifaunal species and the number of individuals recorded on live oysters collected from elevated reefs (LVR) at each sampling interval.

Species name	Sampling intervals				Total
	Sep/12 n=25	Apr/13 n=25	Aug/13 n=16	Nov/13 n=11	n=77
Porifera					**0**
Dysidea fragilis	0	0	0	0	0
Halichondria panicea	0	0	0	0	0
Cnidaria					**79**
Eudendrium ramosum	0	0	0	3	3
Halecium beanii	20	4	2	0	26
Halecium halecinum	0	0	0	0	0
Kirchenpaueria pinnata	0	0	0	0	0
Laomedea flexuosa	20	16	1	0	37
Sertularia argentea	0	0	0	0	0
Sertularella gaudichaudi	0	0	0	0	0
Tubularia larynx	0	0	0	7	7
Actinothoe sphyrodeta	0	0	0	0	0
Sagartia troglodytes	4	0	0	2	6
Annelida					**316**
Filograna implexa	0	0	3	1	4
Pomatoceros triqueter	48	76	103	75	302
Sabella pavonina	0	0	0	0	0
Spirobis spirobis	0	0	9	1	10
Harmothoe sp.	0	0	0	0	0
Crustacea					**337**
Elminius modestus	4	4	322	7	337
Mollusca					**36**
Acanthochitona fascicularis	0	0	0	0	0
Crepidula fornicata	12	16	4	0	32
Anomia ephippum	0	0	0	1	1
Chlamys varia	0	0	0	0	0
Aequipecten opercularis	0	0	0	2	2
Ostrea edulis	0	0	0	1	1
Bryozoa					**205**
Alcyonidium diaphanum	0	0	0	0	0
Amathia lendigera	0	0	0	0	0
Bicellariella ciliata	0	0	0	0	0
Bugula flabellata	0	0	0	0	0
Chartella papyracea	0	0	2	0	2
Epistomia bursaria	0	0	0	0	0
Flustra foliacea	0	0	0	0	0
Disporella hispida	20	44	16	7	87
Electra pilosa	4	16	9	5	34

Appendix 3 (Cont.)

Species name	Sampling intervals				Total
	Sep/12 n=25	Apr/13 n=25	Aug/13 n=16	Nov/13 n=11	n=77
Plagioecia patina	0	0	21	8	29
Tubulipora plumosa	12	16	8	4	40
Cellepora sp.	0	0	8	5	13
Tunicata					**98**
Ascidia conchilega	0	4	0	0	4
Ascidia mentula	0	0	0	0	0
Ascidia verginea	0	0	0	0	0
Ascidiella aspersa	0	4	1	0	5
Ciona intestinalis	0	4	1	1	6
Corella eumyota	0	0	0	0	0
Dendrodoa grossularia	12	8	10	8	38
Pyura microcosmus	0	0	3	2	5
Styela clava	0	4	0	0	4
Molgula sp.	4	4	1	1	10
Perophora sp.	0	0	0	0	0
Botrylloides diegense	0	0	1	0	1
Botrylloides leachi	4	0	1	0	5
Botrylloides violacea	0	0	2	2	4
Botryllus schlosseri	8	0	0	0	8
Morchelium argus	0	0	0	0	0
Diplosoma listerianum	4	0	0	0	4
Didemnum sp.	0	0	2	2	4

Appendix 4

List of epifaunal species and the number of individuals recorded on dead valves in oyster cages laid on sea bed (WVS) at each sampling interval.

Species name	Sampling intervals				Total
	Sep/12 n=30	Apr/13 n=30	Aug/13 n=30	Nov/13 n=30	n=120
Porifera					**0**
Dysidea fragilis	0	0	0	0	0
Halichondria panicea	0	0	0	0	0
Cnidaria					**22**
Eudendrium ramosum	0	0	0	0	0
Halecium beanii	20	0	0	0	20
Halecium halecinum	0	0	0	0	0
Kirchenpaueria pinnata	0	0	0	0	0
Laomedea flexuosa	0	0	2	0	2
Sertularia argentea	0	0	0	0	0
Sertularella gaudichaudi	0	0	0	0	0
Tubularia larynx	0	0	0	0	0
Actinothoe sphyrodeta	0	0	0	0	0
Sagartia troglodytes	0	0	0	0	0
Annelida					**1,497**
Filograna implexa	0	0	18	0	18
Pomatoceros triqueter	112	134	600	494	1,340
Sabella pavonina	0	0	22	0	22
Spirobis spirobis	0	0	13	104	117
Harmothoe sp.	0	0	0	0	0
Crustacea					**66**
Elminius modestus	0	0	36	30	66
Mollusca					**89**
Acanthochitona fascicularis	0	0	0	0	0
Crepidula fornicata	36	16	16	19	87
Anomia ephippum	0	0	2	0	2
Chlamys varia	0	0	0	0	0
Aequipecten opercularis	0	0	0	0	0
Ostrea edulis	0	0	0	0	0
Bryozoa					**144**
Alcyonidium diaphanum	0	0	0	0	0
Amathia lendigera	0	0	4	10	14
Bicellariella ciliata	0	0	0	0	0
Bugula flabellata	0	0	0	0	0
Chartella papyracea	0	0	0	0	0
Epistomia bursaria	0	0	0	0	0
Flustra foliacea	0	0	0	0	0
Disporella hispida	12	12	9	11	44
Electra pilosa	12	8	11	8	39

Appendix 4 (Cont.)

Species name	Sampling intervals				Total
	Sep/12 n=30	Apr/13 n=30	Aug/13 n=30	Nov/13 n=30	n=120
Plagioecia patina	0	0	5	8	13
Tubulipora plumosa	12	8	0	3	23
Cellepora sp.	0	0	6	5	11
Tunicata					**42**
Ascidia conchilega	0	0	0	0	0
Ascidia mentula	0	0	1	0	1
Ascidia verginea	0	0	0	0	0
Ascidiella aspersa	0	4	1	0	5
Ciona intestinalis	4	0	0	0	4
Corella eumyota	0	0	0	0	0
Dendrodoa grossularia	0	0	1	0	1
Pyura microcosmus	0	0	0	0	0
Styela clava	0	4	0	0	4
Molgula sp.	8	0	8	2	18
Perophora sp.	0	0	0	0	0
Botrylloides diegense	0	0	0	0	0
Botrylloides leachi	0	0	0	0	0
Botrylloides violacea	0	0	0	0	0
Botryllus schlosseri	4	0	0	1	5
Morchelium argus	0	0	0	0	0
Diplosoma listerianum	4	0	0	0	4
Didemnum sp.	0	0	0	0	0

Appendix 5

List of epifaunal species and the number of individuals recorded on intact dead valves in reef boxes on elevated reefs (WVR) at each sampling interval.

Species name	Sampling intervals				Total
	Sep/12 n=30	Apr/13 n=30	Aug/13 n=30	Nov/13 n=30	n=120
Porifera					**8**
Dysidea fragilis	0	0	0	4	4
Halichondria panicea	0	0	1	3	4
Cnidaria					**183**
Eudendrium ramosum	0	0	0	0	0
Halecium beanii	40	0	1	0	41
Halecium halecinum	0	0	3	0	3
Kirchenpaueria pinnata	0	0	0	0	0
Laomedea flexuosa	40	24	27	2	93
Sertularia argentea	0	0	2	3	5
Sertularella gaudichaudi	0	0	11	0	11
Tubularia larynx	0	0	10	0	10
Actinothoe sphyrodeta	0	0	4	0	4
Sagartia troglodytes	0	4	11	1	16
Annelida					**3,084**
Filograna implexa	0	0	250	284	534
Pomatoceros triqueter	121	114	1,216	866	2,317
Sabella pavonina	0	0	15	74	89
Spirobis spirobis	0	4	37	91	132
Harmothoe sp.	0	0	0	12	12
Crustacea					**1,824**
Elminius modestus	4	8	1,213	599	1,824
Mollusca					**175**
Acanthochitona fascicularis	0	0	0	3	3
Crepidula fornicata	36	36	51	25	148
Anomia ephippum	0	0	3	9	12
Chlamys varia	0	0	2	1	3
Aequipecten opercularis	0	0	0	0	0
Ostrea edulis	0	4	1	4	9
Bryozoa					**349**
Alcyonidium diaphanum	0	0	1	0	1
Amathia lendigera	0	0	10	24	34
Bicellariella ciliata	0	0	3	0	3
Bugula flabellata	0	0	0	0	0
Chartella papyracea	0	0	0	0	0
Epistomia bursaria	0	0	10	0	10
Flustra foliacea	0	0	2	0	2
Disporella hispida	12	24	14	27	77
Electra pilosa	12	11	18	23	64

Appendix 5 (Cont.)

Species name	Sampling intervals				Total
	Sep/12 n=30	Apr/13 n=30	Aug/13 n=30	Nov/13 n=30	n=120
Plagioecia patina	0	0	27	42	69
Tubulipora plumosa	20	8	13	22	63
Cellepora sp.	0	0	13	13	26
Tunicata					**182**
Ascidia conchilega	0	0	5	4	9
Ascidia mentula	0	0	2	2	4
Ascidia verginea	0	0	0	0	0
Ascidiella aspersa	12	4	4	5	25
Ciona intestinalis	4	8	4	5	21
Corella eumyota	4	0	2	1	7
Dendrodoa grossularia	20	0	2	9	31
Pyura microcosmus	0	0	2	4	6
Styela clava	0	0	4	1	5
Molgula sp.	8	20	5	4	37
Perophora sp.	0	0	1	0	1
Botrylloides diegense	0	0	1	0	1
Botrylloides leachi	4	0	0	1	5
Botrylloides violacea	0	0	3	2	5
Botryllus schlosseri	8	0	3	1	12
Morchelium argus	0	0	1	0	1
Diplosoma listerianum	4	0	1	2	7
Didemnum sp.	0	0	1	4	5

Appendix 6

List of epifaunal species and the number of individuals recorded on fragmented dead valves in reef boxes on elevated reefs (FVR) at each sampling interval.

Species name	Sampling intervals				Total
	Sep/12 n=30	Apr/13 n=30	Aug/13 n=30	Nov/13 n=30	n=120
Porifera					**4**
Dysidea fragilis	0	0	0	2	2
Halichondria panicea	0	0	1	1	2
Cnidaria					**186**
Eudendrium ramosum	0	0	0	0	0
Halecium beanii	44	0	27	0	71
Halecium halecinum	0	0	2	0	2
Kirchenpaueria pinnata	0	0	11	0	11
Laomedea flexuosa	44	20	10	2	76
Sertularia argentea	0	0	4	3	7
Sertularella gaudichaudi	0	0	11	0	11
Tubularia larynx	0	0	1	0	1
Actinothoe sphyrodeta	0	4	0	0	4
Sagartia troglodytes	0	0	2	1	3
Annelida					**2,211**
Filograna implexa	0	0	61	199	260
Pomatoceros triqueter	134	121	750	745	1,750
Sabella pavonina	0	0	29	60	89
Spirobis spirobis	0	8	12	80	100
Harmothoe sp.	0	0	0	12	12
Crustacea					**1,414**
Elminius modestus	4	0	800	610	1,414
Mollusca					**187**
Acanthochitona fascicularis	0	0	54	2	56
Crepidula fornicata	40	20	21	21	102
Anomia ephippum	0	0	1	8	9
Chlamys varia	4	4	2	1	11
Aequipecten opercularis	0	0	0	0	0
Ostrea edulis	0	4	1	4	9
Bryozoa					**541**
Alcyonidium diaphanum	0	0	3	0	3
Amathia lendigera	0	0	13	30	43
Bicellariella ciliata	0	0	27	0	27
Bugula flabellata	0	0	1	0	1
Chartella papyracea	0	0	0	0	0
Epistomia bursaria	0	0	90	0	90
Flustra foliacea	0	0	7	0	7
Disporella hispida	24	28	10	22	84
Electra pilosa	12	11	52	21	96

Appendix 6 (Cont.)

Species name	Sampling intervals				Total
	Sep/12 n=30	Apr/13 n=30	Aug/13 n=30	Nov/13 n=30	n=120
Plagioecia patina	0	0	82	40	122
Tubulipora plumosa	12	8	3	21	44
Cellepora sp.	0	0	13	11	24
Tunicata					**242**
Ascidia conchilega	4	4	11	4	23
Ascidia mentula	0	0	6	2	8
Ascidia verginea	0	0	1	0	1
Ascidiella aspersa	8	4	6	5	23
Ciona intestinalis	4	8	2	5	19
Corella eumyota	8	0	1	1	10
Dendrodoa grossularia	16	0	27	9	52
Pyura microcosmus	0	0	5	4	9
Styela clava	0	0	2	1	3
Molgula sp.	8	20	14	4	46
Perophora sp.	0	0	5	0	5
Botrylloides diegense	0	0	5	0	5
Botrylloides leachi	4	4	2	1	11
Botrylloides violacea	0	0	10	2	12
Botryllus schlosseri	0	0	1	1	2
Morchelium argus	0	0	1	0	1
Diplosoma listerianum	4	0	1	2	7
Didemnum sp.	0	0	1	4	5

Appendix 7

Relative abundance of the 30 most abundant epifaunal species that colonised on each valve category. The valve abbreviations were defined as following LVS: live oyster on the sea bed, LVR: live oyster on elevated reefs, WVS: whole oyster valve cultch on the sea bed, WVR: whole oyster valve cultch on elevated reefs and FVR: fragmented oyster valve cultch on elevated reefs.

Epifaunal species	Shell categories					Positions		Total
	LVS	LVR	WVS	WVR	FVR	Reef	Seabed	
Pomatoceros triqueter	62.6	28.2	72.0	39.9	36.6	38.0	70.5	42.8
Elminius modestus	14.3	31.5	3.5	31.4	29.6	31.4	5.4	26.6
Filograna implexa	0.0	0.4	1.0	9.2	5.4	7.8	0.8	5.9
Spirorbis spirorbis	7.4	0.9	6.3	2.3	2.1	2.1	6.5	2.8
Crepidula fornicata	1.1	3.0	4.7	2.5	2.1	2.6	4.1	2.7
Disporella hispida	2.7	8.1	2.4	1.3	1.8	2.4	2.4	2.2
Electra pilosa	2.9	3.2	2.1	1.1	2.0	1.4	2.2	1.8
Plagioecia patina	2.9	2.7	0.7	1.2	2.0	1.4	1.1	1.8
Laomedea flexuosa	0.5	3.5	0.1	1.6	1.6	1.9	0.2	1.5
Sabella pavonina	0.3	0.0	1.2	1.5	1.9	1.3	1.0	1.4
Tubulipora plumosa	2.4	3.7	1.2	1.1	0.9	1.5	1.4	1.3
Halecium beanii	0.0	2.4	1.1	0.7	1.5	1.0	0.9	1.1
Dendrodoa grossularia	0.0	3.5	0.1	0.5	1.1	1.0	0.0	0.9
Molgula sp.	0.3	0.9	1.0	0.6	1.0	0.7	0.8	0.8
Epistomia bursaria	0.0	0.0	0.0	0.2	1.9	0.1	0.0	0.7
Amathia lendigera	1.3	0.0	0.8	0.6	0.9	0.5	0.8	0.7
Cellepora sp.	0.5	1.2	0.6	0.4	0.5	0.6	0.6	0.5
Acanthochitona fascicularis	0.0	0.0	0.0	0.1	1.2	0.0	0.0	0.4
Ascidiella aspersa	0.0	0.5	0.3	0.4	0.5	0.4	0.2	0.4
Ciona intestinalis	0.0	0.6	0.2	0.4	0.4	0.4	0.2	0.4
Ascidia conchilega	0.0	0.4	0.0	0.2	0.5	0.2	0.0	0.3
Bicellariella ciliata	0.0	0.0	0.0	0.1	0.6	0.0	0.0	0.2
Botryllus schlosseri	0.3	0.7	0.3	0.2	0.0	0.3	0.3	0.2
Ostrea edulis	0.0	0.1	0.0	0.3	0.2	0.3	0.0	0.2
Sagartia troglodytes	0.0	0.6	0.0	0.3	0.1	0.3	0.0	0.2
Harmothoe sp.	0.0	0.0	0.0	0.2	0.3	0.2	0.0	0.2
Anomia ephippium	0.0	0.1	0.1	0.2	0.2	0.2	0.1	0.2
Diplosoma listerianum	0.5	0.4	0.2	0.1	0.1	0.2	0.3	0.2
Sertularella gaudichaudi	0.0	0.0	0.0	0.2	0.2	0.2	0.0	0.2
Botrylloides leachii	0.0	0.5	0.0	0.1	0.2	0.1	0.0	0.2
Total (%)	100.0	97.0	99.7	98.8	97.7	98.5	99.8	98.4

Remark: Reef shells (LVR+WVR) and sea bed shells (LVS+WVS) and see total lists of epifaunal species and composition in appendix 1-5

Appendix 8

Presence and absence of epifaunal species observed on each valve type at each sampling interval. 1-4 indicate sampling intervals (1: September 2012, 2: April 2013, 3: August 2013 and 4: November 2013)

Species	Valves on sea bed								Valves on elevated reefs											
	LVS				WVS				LVR				WVR				FVR			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Porifera																				
Dysidea fragilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	P
Halichondria panicea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	P	-	-	P	P
Cnidaria																				
Eudendrium ramosum	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-
Halecium beanii	-	-	-	-	P	-	-	-	P	P	P	-	P	-	P	-	P	-	P	-
Halecium halecinum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	P	-
Kirchenpaueria pinnata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-
Laomedea flexuosa	P	P	-	-	-	-	P	-	P	P	P	-	P	P	P	P	P	P	P	P
Sertularia argentea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	P	-	-	P	P
Sertularella gaudichaudi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	P	-
Ectopleura larynx	-	-	-	-	-	-	-	-	-	-	-	P	-	-	P	-	-	-	P	-
Actinothoe sphyrodeta	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	P	-	-
Sagartia troglodytes	-	-	-	-	-	-	-	-	P	-	-	P	-	P	P	P	-	-	P	P
Annelida																				
Filograna implexa	-	-	-	-	-	-	P	-	-	-	P	P	-	-	P	P	-	-	P	P
Pomatoceros triqueter	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Sabella pavonina	-	-	P	-	-	-	P	-	-	-	-	-	-	-	P	P	-	-	P	P
Spirorbis spirorbis	-	P	P	P	-	-	P	P	-	-	P	P	-	P	P	P	-	P	P	P
Harmothoe sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	P
Crustacea																				
Elminius modestus	-	-	P	P	-	-	P	P	P	P	P	P	P	P	P	P	P	-	P	P
Mollusca																				
Acanthochitona fascicularis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	P	P
Crepidula fornicata	P	P	P	P	P	P	P	P	P	P	P	-	P	P	P	P	P	P	P	P
Anomia ephippium	-	-	-	-	-	-	P	-	-	-	-	P	-	-	P	P	-	-	P	P
Chlamys varia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	P	P	P	P	P
Aequipecten opercularis	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-
Ostrea edulis	-	-	-	-	-	-	-	-	-	-	-	P	-	P	P	P	-	P	P	P
Bryozoa																				
Alcyonidium diaphanum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	P	-
Amathia lendigera	-	-	P	P	-	-	P	P	-	-	-	-	-	-	P	P	-	-	P	P
Bicellariella ciliata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	P	-
Bugulina flabellata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-

Remark: presence (P), absence (-)

Appendix 8 (Cont.)

Species name	Seabed valves								Reef valves											
	LVS				WVS				LVR				WVR				FVR			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Chartella papyracea	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-
Epistomia bursaria	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	P	-
Flustra foliacea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	P	-
Disporella hispida	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Electra pilosa	P	-	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Plagioecia patina	-	-	P	P	-	-	P	P	-	-	P	P	-	-	P	P	-	-	P	P
Tubulipora plumosa	-	P	P	P	P	P	-	P	P	P	P	P	P	P	P	P	P	P	P	P
Cellepora sp.	-	-	P	-	-	-	P	P	-	-	P	P	-	-	P	P	-	-	P	P
Tunicata																				
Ascidia conchilega	-	-	-	-	-	-	-	-	-	P	-	-	-	-	P	P	P	P	P	P
Ascidia mentula	-	-	-	-	-	-	P	-	-	-	-	-	-	-	P	P	-	-	P	P
Ascidia virginea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-
Ascidiella aspersa	-	-	-	-	-	P	P	-	-	P	P	-	P	P	P	P	P	P	P	P
Ciona intestinalis	-	-	-	-	P	-	-	-	-	P	P	P	P	P	P	P	P	P	P	P
Corella eumyota	-	-	-	-	-	-	-	-	-	P	-	-	P	-	P	P	P	-	P	P
Dendrodoa grossularia	-	-	-	-	-	-	P	-	P	P	P	P	P	-	P	P	P	-	P	P
Pyura microcosmus	-	-	-	-	-	-	-	-	-	-	P	P	-	-	P	P	-	-	P	P
Styela clava	-	-	-	-	P	P	-	-	-	P	-	-	-	-	P	P	-	-	P	P
Molgula sp.	-	-	P	-	P	-	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Perophora sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	P	-
Botrylloides diegense	-	-	-	-	-	-	-	-	-	-	P	-	-	-	P	-	-	-	P	-
Botrylloides leachii	-	-	-	-	-	-	-	-	P	-	P	-	P	-	-	P	P	P	P	P
Botrylloides violacea	-	-	-	-	-	-	-	-	-	-	P	P	-	-	P	P	-	-	P	P
Botryllus schlosseri	-	-	P	-	P	-	-	P	P	-	-	-	P	-	P	P	-	-	P	P
Morchellium argus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	P	-
Diplosoma listerianum	-	P	-	-	P	-	-	-	P	-	-	-	P	-	P	P	P	-	P	P
Didemnum sp.	-	-	-	-	-	-	-	-	-	-	P	P	-	-	P	P	-	-	P	P

Remark: presence (P), absence (-)

Appendix 9

The lists of species and contribution to similarity within group and dissimilarity between groups (reef/sea bed), in ascending order.

SIMPER
Similarity Percentages - species contributions

One-Way Analysis

Data worksheet
Name: Data1
Data type: Other
Sample selection: All
Variable selection: All

Parameters
Resemblance: S17 Bray Curtis similarity
Cut off for low contributions: 90.00%

Factor Groups

Sample	Positions
LVS1	Seabed
LVS2	Seabed
LVS3	Seabed
LVS4	Seabed
WVS1	Seabed
WVS2	Seabed
WVS3	Seabed
WVS4	Seabed
LVR1	Reef
LVR2	Reef
LVR3	Reef
LVR4	Reef
WVR1	Reef
WVR2	Reef
WVR3	Reef
WVR4	Reef
FVR1	Reef
FVR2	Reef
FVR3	Reef
FVR4	Reef

Group Seabed
Average similarity: 58.95

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Pomatoceros triqueter	1.00	10.61	3.93	18.00	18.00
Crepidula fornicata	1.00	10.61	3.93	18.00	35.99
Disporella hispida	1.00	10.61	3.93	18.00	53.99
Electra pilosa	0.88	7.61	1.52	12.90	66.89
Tubulipora plumosa	0.75	5.67	1.03	9.62	76.51
Spirorbis spirorbis	0.63	3.19	0.71	5.41	81.91
Elminius modestus	0.50	1.71	0.51	2.90	84.81
Amathia lendigera	0.50	1.71	0.51	2.90	87.71
Plagioecia patina	0.50	1.71	0.51	2.90	90.61

Group Reef
Average similarity: 61.81

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Pomatoceros triqueter	1.00	4.54	2.96	7.34	7.34
Disporella hispida	1.00	4.54	2.96	7.34	14.69
Electra pilosa	1.00	4.54	2.96	7.34	22.03
Tubulipora plumosa	1.00	4.54	2.96	7.34	29.37
Molgula sp.	1.00	4.54	2.96	7.34	36.71
Laomedea flexuosa	0.92	3.76	1.68	6.09	42.80
Crepidula fornicata	0.92	3.76	1.68	6.09	48.89
Elminius modestus	0.92	3.64	1.71	5.90	54.78
Ciona intestinalis	0.92	3.62	1.72	5.86	60.64
Ascidiella aspersa	0.83	2.93	1.23	4.74	65.39
Dendrodoa grossularia	0.83	2.81	1.26	4.55	69.94
Botrylloides leachii	0.67	1.83	0.79	2.97	72.91
Spirorbis spirorbis	0.67	1.58	0.78	2.55	75.46
Halecium beanii	0.58	1.44	0.62	2.33	77.79
Ascidia conchilega	0.58	1.17	0.62	1.89	79.69
Diplosoma listerianum	0.58	1.16	0.63	1.88	81.57
Sagartia troglodytes	0.58	1.16	0.62	1.87	83.44
Ostrea edulis	0.58	1.15	0.63	1.85	85.29
Botryllus schlosseri	0.50	0.76	0.51	1.23	86.52
Chlamys varia	0.50	0.75	0.51	1.21	87.73
Corella eumyota	0.50	0.74	0.51	1.20	88.93
Filograna implexa	0.50	0.68	0.52	1.11	90.04

Groups Seabed & Reef

Average dissimilarity = 51.80

Specie	Seabed Av.Abund	Reef Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Ciona intestinalis	0.13	0.92	2.59	1.63	5.00	5.00
Dendrodoa grossularia	0.13	0.83	2.36	1.44	4.56	9.56
Ascidiella aspersa	0.25	0.83	2.16	1.21	4.17	13.73
Botrylloides leachii	0.00	0.67	2.11	1.23	4.07	17.80
Laomedea flexuosa	0.38	0.92	1.94	1.10	3.75	21.55
Halecium beanii	0.13	0.58	1.84	1.01	3.56	25.11
Molgula sp.	0.50	1.00	1.80	0.90	3.47	28.58
Elminius modestus	0.50	0.92	1.75	0.90	3.37	31.95
Ascidia conchilega	0.00	0.58	1.67	1.01	3.23	35.18
Sagartia troglodytes	0.00	0.58	1.66	1.00	3.21	38.39
Ostrea edulis	0.00	0.58	1.65	1.01	3.18	41.57
Diplosoma listerianum	0.25	0.58	1.65	0.95	3.18	44.75
Spirorbis spirorbis	0.63	0.67	1.57	0.85	3.02	47.78
Plagioecia patina	0.50	0.50	1.56	0.94	3.01	50.79
Botryllus schlosseri	0.38	0.50	1.53	0.90	2.96	53.75
Amathia lendigera	0.50	0.33	1.51	0.93	2.92	56.67
Cellepora sp.	0.38	0.50	1.45	0.94	2.80	59.47
Chlamys varia	0.00	0.50	1.32	0.88	2.54	62.01
Corella eumyota	0.00	0.50	1.30	0.89	2.52	64.53
Filograna implexa	0.13	0.50	1.27	0.93	2.45	66.98
Pyura microcosmus	0.00	0.50	1.20	0.93	2.32	69.30
Botrylloides violacea	0.00	0.50	1.20	0.93	2.32	71.61
Didemnum sp.	0.00	0.50	1.20	0.93	2.32	73.93
Styela clava	0.13	0.42	1.19	0.77	2.30	76.23
Anomia ephippium	0.13	0.42	1.06	0.83	2.05	78.28
Sabella pavonina	0.25	0.33	1.05	0.80	2.03	80.30
Ascidia mentula	0.13	0.33	0.84	0.74	1.63	81.93
Tubulipora plumosa	0.75	1.00	0.81	0.53	1.56	83.49
Halichondria panacea	0.00	0.33	0.66	0.70	1.28	84.77
Sertularia argentea	0.00	0.33	0.66	0.70	1.28	86.06
Ectopleura larynx	0.00	0.25	0.57	0.54	1.10	87.16
Botrylloides diegense	0.00	0.25	0.57	0.54	1.09	88.25
Acanthochitona fascicularis	0.00	0.25	0.51	0.57	0.98	89.23
Actinothoe sphyrodeta	0.00	0.17	0.50	0.41	0.96	90.18

Appendix 10

List of mobile species (65 species) and individual numbers of each species observed in reef boxes at each sampling interval (1: September 2012, 2: April 2013, 3: August 2013, 4: November 2013). Functional groups of each species were classified using the following publications (Craft and Sacco, 2003; Fanelli et al., 2014; Fauchald and Jumars, 1979; Guerra-Garcia et al., 2014; and Mikel et al., 2014)

Phylum	Species	Functional groups	Individuals/sampling interval				
			1	2	3	4	Total
Pycnogonida	*Endeis spinosa*	C	0	1	1	1	3
	Nymphon gracile	C	0	3	2	1	6
	Pycnogonum littorale	C	0	5	0	11	16
Crustacea	*Gammarus* spp.	O	75	49	68	62	254
	Jassa spp.	S	237	266	328	111	942
	Bathyporeia spp.	D	34	9	0	0	43
	Pseudoprotella phasma	H	0	0	0	1	1
	Caprella acanthifera	H	0	3	1	4	8
	Caprella linearis	H	0	2	12	0	14
	Maera spp.	H	14	5	15	52	86
	Gummarella spp.	O	0	0	12	16	28
	Dexamine spp.	S	22	1	51	6	80
	Ahenusa spp.	H	0	4	5	1	10
	Chelura spp.	H	0	0	2	0	2
	Leucothoe spp.	D	10	12	1	0	23
	Amphithoe spp.	H	7	10	5	0	22
	Urothoe spp.	H	3	0	0	0	3
	Melita spp.	H	5	2	0	0	7
	Idotea spp.	O	38	141	33	7	219
	Athanas nitescens	H	416	511	530	902	2359
	Alpheus macrocheles	H	1	4	1	2	8
	Thoralus cranchii	S	52	86	61	207	406
	Hippolyte longirostris	S	4	0	1	3	8
	Hippolyte varians	S	75	0	7	6	88
	Palaemon serratus	C	1	0	0	0	1
	Palaemon elegans	C	1	0	0	0	1
	Palaemonetes varians	C	0	0	1	0	1
	Pasiphaea sivado	C	0	0	1	1	2
	Galathea squamifera	D	161	209	198	221	789
	Pagurus cuanensis	D	8	4	27	5	44
	Necora puber	O	7	3	0	1	11

Remarks C: carnivore, O: omnivore, S: suspension feeders, D: deposit feeders and H: herbivore

Appendix 10 (Cont.)

Phylum	Species	Functional groups	Individuals/sampling interval				
			1	2	3	4	Total
	Macropodia rostrata	D	8	0	0	8	16
	Macropodia tenuirostris	D	7	0	0	0	7
	Pilumnus hirtellus	D	61	38	196	189	484
	Pisa tetraodon	H	3	0	0	0	3
	Pisidia longicornis	S	9722	7583	8715	4886	30906
	Cancer pagurus	C	3	3	3	2	11
	Pisa armata	H	0	0	1	0	1
Mollusca	*Gibbula umbilicalis*	H	7	4	0	0	11
	Gibbula cineraria	H	0	31	3	32	66
	Gibbula magus	H	1	1	0	4	6
	Nassarius incrassatus	D	16	76	95	150	337
	Nassarius reticulatus	D	1	1	6	0	8
	Nassarius pygmaeus	D	1	0	7	0	8
	Ocenebra erinacea	C	3	10	33	23	69
	Rissoa parva	H	66	96	379	25	566
	Pusillina sarsii	H	1	0	35	0	36
	Pusillina inconspicua	H	0	0	141	0	141
	Trivia arctica	C	1	6	16	6	29
	Trivia monacha	C	22	27	10	6	65
	Tricolia pullus	H	2	27	11	20	60
	Bittium reticulatum	H	1	0	0	1	2
	Calliostoma zizyphinum	C	0	2	0	0	2
Echinodermata	*Ophiothrix fragilis*	D	3	1	4	4	12
Pisces	*Apletodon dentatus*	C	1	0	0	0	1
	Lepadogaster lepadogaster	C	2	2	2	3	9
	Lepadogaster candolii	C	3	5	5	12	25
	Gaidropsarus vulgaris	C	2	1	3	0	6
	Ciliata mustela	C	2	0	2	3	7
	Pholis gunnellus	C	1	0	0	0	1
	Gobius paganellus	C	1	7	1	1	10
	Trigloporus lastoviza	C	0	0	2	1	3
	Gobius niger	C	0	1	3	1	5
	Ctenolabrus rupestris	C	0	6	0	1	7
	Parablennius gattorugine	C	0	0	0	3	3

Remarks C: carnivore, O: omnivore, S: suspension feeders, D: deposit feeders and H: herbivore

Appendix 11

List of mobile species (47 species) and individual numbers of each species observed in oyster cages on seabed at each sampling interval (1: September 2012, 2: April 2013, 3: August 2013, 4: November 2013). Functional groups of each species were classified using the following publications (Craft and Sacco, 2003; Fanelli et al., 2014; Fauchald and Jumars, 1979; Guerra-Garcia et al., 2014; and Mikel et al., 2014)

Phylum	Species	Functional groups	Individuals/sampling interval				
			1	2	3	4	Total
Pycnogonida	*Endeis spinosa*	C	0	0	1	1	2
	Nymphon gracile	C	0	5	0	0	5
	Pycnogonum littorale	C	0	7	0	1	8
Crustacea	*Gammarus* spp.	O	18	4	7	11	40
	Jassa spp.	S	24	22	29	19	94
	Bathyporeia spp.	D	0	1	0	0	1
	Caprella acanthifera	H	0	0	1	3	4
	Maera spp.	H	1	0	4	6	11
	Gummarella spp.	O	0	0	1	10	11
	Dexamine spp.	S	2	2	9	7	20
	Ahenusa spp.	H	0	0	0	1	1
	Leucothoe spp.	D	0	0	9	0	9
	Amphithoe spp.	H	0	6	28	0	34
	Urothoe spp.	H	0	0	1	0	1
	Melita spp.	H	0	3	6	0	9
	Idotea spp.	O	2	12	8	0	22
	Athanas nitescens	H	5	0	16	42	63
	Thoralus cranchii	S	6	0	4	14	24
	Hippolyte longirostris	S	3	1	0	0	4
	Hippolyte varians	S	4	1	1	0	6
	Galathea squamifera	D	0	3	39	18	60
	Pagurus cuanensis	D	1	1	10	1	13
	Necora puber	O	1	0	0	0	1
	Macropodia rostrata	D	4	2	0	3	9
	Pilumnus hirtellus	D	1	1	5	10	17
	Pisa tetraodon	H	0	0	2	0	2
	Pisidia longicornis	S	32	144	229	152	557
	Cancer pagurus	C	0	0	2	1	3
	Pisa armata	H	0	0	0	1	1
	Liocarcinus navigator	D	0	0	0	2	2
	Macropodia deflexa	D	0	0	0	1	1

Remarks C: carnivore, O: omnivore, S: suspension feeders, D: deposit feeders and H: herbivore

Appendix 11 (Cont.)

Phylum	Species	Functional groups	Individuals/sampling interval 1	2	3	4	Total
Mollusca	*Gibbula umbilicalis*	H	1	1	0	0	2
	Gibbula cineraria	H	1	1	9	14	25
	Nassarius incrassatus	D	2	1	50	13	66
	Ocenebra erinacea	C	2	2	14	4	22
	Rissoa parva	H	53	42	131	0	226
	Trivia arctica	C	0	0	1	2	3
	Trivia monacha	C	0	0	1	2	3
	Tricolia pullus	H	20	8	5	60	93
	Bittium reticulatum	H	0	0	0	1	1
	Calliostoma zizyphinum	C	0	2	1	0	3
Echinodermata	*Ophiothrix fragilis*	D	0	0	0	1	1
Pisces	*Gobius paganellus*	C	0	0	1	0	1
	Trigloporus lastoviza	C	0	0	0	1	1
	Gobius niger	C	0	0	1	0	1
	Ctenolabrus rupestris	C	0	0	1	0	1
	Symphodus melops	C	0	0	1	0	1

Remarks C: carnivore, O: omnivore, S: suspension feeders, D: deposit feeders and H: herbivore

Appendix 12

Sawusdee, A., Jensen, A.C., Collins, K.J., Hauton, C. 2015. Improvement in the physiological performance of European flat oysters *Ostrea edulis* (Linnaeus, 1758) cultured on elevated reef strctures: Implications for oyster restoration. Aquaculture, 444, 41-48.

Aquaculture 444 (2015) 41–48

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Improvements in the physiological performance of European flat oysters *Ostrea edulis* (Linnaeus, 1758) cultured on elevated reef structures: Implications for oyster restoration ☆

Amonsak Sawusdee [a,b,*], Antony C. Jensen [a], Ken J. Collins [a], Chris Hauton [a]

[a] Ocean and Earth Science, University of Southampton, National Oceanography Centre, Southampton SO14 3ZH, UK
[b] School of Engineering and Resources Management, Walailak University, Nakhon Si Thammarat 80160, Thailand

ARTICLE INFO

Article history:
Received 27 January 2015
Received in revised form 19 March 2015
Accepted 21 March 2015
Available online 26 March 2015

Keywords:
Ostrea edulis
Oyster physiology
Filtration rate
Haemocyte
Respiration rate

ABSTRACT

The precarious status of flat oyster *Ostrea edulis* stocks in Europe is widely acknowledged. To build a scientific basis for oyster restoration, an elevated experimental reef stocked with *O. edulis* was established within Poole Bay (Dorset, UK). Oysters were out planted on twenty four oyster reef modules (80 cm above sea bed) and compared with oysters held on the sea bed close to each reef module to test the hypothesis that a reef habitat enhanced physiological performance. Filtration and respiration rates, condition index, haemolymph protein concentration, haemocyte counts and gonad maturation were measured as indicators of physiological performance. During the first 15 months of oyster reef deployment, water samples were collected at regular intervals at the sea bed and at a height of 80 cm from the sea bed to determine chlorophyll *a* concentration, total suspended solids and bacterial abundance. Total suspended solids were significantly higher at the sea bed than at 80 cm above the sea bed at every sampling interval, while bacterial abundance adjacent to the sea bed was significantly higher than 80 cm above the sea bed in August and November 2013 when temperature was 18 °C and 15 °C, respectively. The filtration rates of oysters varied with elevation (reef/sea bed) and months. Filtration rates of 'reef oysters' (oysters on elevated reefs) were significantly higher than 'sea bed oysters' (oysters held on the sea bed). Respiration rates varied among months but were not significantly affected by elevation (reef/sea bed). Elevation and month also affected the total number of haemocytes and the granulocyte population; reef oysters had significantly higher numbers of haemocytes than sea bed oysters. As current stocks of European flat oysters (*O. edulis*) in Europe have declined in both abundance and distribution, the results of this pilot study suggest that the culture of oysters on elevated reef structure represents at least a partial solution to improve *O. edulis* physiology for restoration in Europe.

1. Introduction

The European flat oyster, *Ostrea edulis* (Linnaeus, 1758) occurs throughout the Atlantic and Mediterranean coasts of Europe (Airoldi and Beck, 2007; Lallias et al., 2007). At one time the species was among the most commercially important marine resources in the European waters (Orton, 1937). Today, stocks in France, Spain, Ireland, Croatia, Holland and United Kingdom are exploited commercially (Smith et al., 2006; Kamphausen et al., 2011). However, stocks of *O. edulis* have been in decline since before 1970s, principally as a result of over exploitation (through technological improvements in fishing and a lack of effective management) (Edwards, 1997), low rates of recruitment (Laing et al., 2006), the effects of the extremely cold winters in the 1930s and 1940s (Crisp, 1964), the prevalence of parasitic organism *Bonamia ostreae* (Lallias, 2008) and the destruction of natural oyster beds (Mackenzie et al., 1997). Jackson et al. (2001) stated that destruction of oyster reef habitats resulted to reduce a number of dominant filter-feeding bivalves and consequently influenced degradation of marine environmental ecosystems. Officer et al. (1984) and Jackson et al. (2001) reported that the evidences of hypoxia, anoxia and eutrophication were observed after the over exploited to oyster stock (*Crassostrea virginica*) in Chesapeake Bay. Therefore, the decline of *O. edulis* represents a financial loss to European coastal economies and also reduces the quality status of the marine environment. As filter feeders, oysters are a keystone species that play a major role in dissolved nutrient cycling by removing phytoplankton, suspended solids, and organic particles from the water column and therefore can contribute to the control of eutrophication in marine ecosystems (Newell, 1965; Ward and Shumway, 2004; Fulford et al., 2010). As such, the critical status of *O. edulis* has been recognised by its inclusion in the list of UK

☆ *Statement of relevance*: Improvements in the physiological performance of *O. edulis*.

* Corresponding author at: Ocean and Earth Science, University of Southampton, National Oceanography Centre, Southampton SO14 3ZH, UK. Tel.: +44 2380 592011.
E-mail addresses: as12e11@soton.ac.uk (A. Sawusdee), acj@noc.soton.ac.uk (A.C. Jensen), kjc@noc.soton.ac.uk (K.J. Collins), ch10@noc.soton.ac.uk (C. Hauton).

http://dx.doi.org/10.1016/j.aquaculture.2015.03.022
0044-8486/

Biodiversity Action Plan species (BRIG, 2007) as a response to the UN Rio Convention on Biological Diversity.

The restoration of shellfish stocks, especially reef-forming bivalves, using artificial reefs has become a popular management strategy, especially as applied to the restoration of *C. virginica* in USA. Often the common goals have been for habitat restoration, fisheries enhancement and recovery of species diversity (Bartol et al., 1999; Coen and Mark, 2000; Beck, 2011). Lenihan et al. (1999) suggested that *C. virginica* culture above sea bed can increase growth rates, survival rates and resistance to diseases, especially during periods of summer hypoxia. Moreover, Frechette et al. (1989) revealed that complexity and elevation of mussel beds enhanced vertical diffusive turbulent water flow past the mussels (*Mylitus edulis*), which in turn further enriched nutrients and improved growth rates of filtering feeding species. However, there has been no study evaluating the merits of building artificial reefs with which to restore communities of *O. edulis* and explicit evidence that artificial oyster reefs in the European region improve physiological potential is generally lacking. In light of the paucity of available data, it is first necessary to establish the potential benefit of reef culture of *O. edulis* at a pilot-scale before the fishery-scale introduction of this approach as a management strategy. The objective of the research herein was, therefore, to compare the physiological performance between sub-tidal *O. edulis* held off the sea bed on elevated structures and oysters (individuals) held on the sea bed. Indicators of physiological health included filtration and respiration rates, haemolymph protein content, and haemocyte counts.

2. Materials and methods

2.1. Study area and artificial reef construction

The experimental reef was located in Poole Bay, Dorset (50°40′ N 01°55′ W, Fig. 1A) at a depth of ~10 m below chart datum (tidal range = 2 m) (Jensen et al., 2000). Each individual reef module consisted of a breeze block base (40 cm high) with a 'reef box', a 0.064 m^3 cube of cleaned oyster valves encased within a Netlon™ mesh, attached to the top of the base (Fig. 1 B). The total height of the base plus reef box was 80 cm from the sea bed (Fig. 1 C) and reef boxes were held in place on top of the breeze block mount using 150 cm steel pins. Oyster shells, collected from the Solent, 50°46′ N and 1°14′ W, were washed, scrubbed of epibiota, and dried and were used as the material or 'fill' for each reef box. Reef boxes were3/4 filled with oyster shells – comprising approximately 1200 shells each box. Oyster cages (0.4 × 0.4 × 0.2 m), used to hold oysters on the sea bed, were located close to each of the reef box units. Twenty-four reef boxes and twenty-four oyster cages were deployed on the sea bed at Poole Bay in August 2012. Twenty live *O. edulis* (Othniel Oysters Ltd., Poole), 57–99 mm in shell height, were then added as a top layer to each reef box and oyster cage by divers.

2.2. Sampling intervals and oyster samples

After deployment in August 2012, replicate reef boxes were recovered on four occasions during the 15-month field experiment:

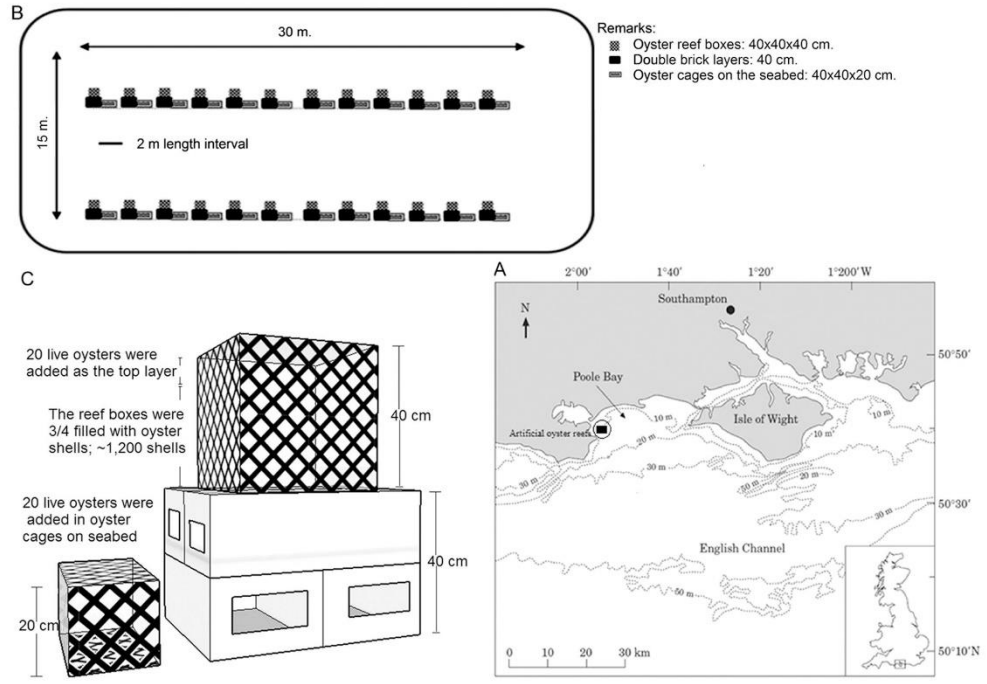

Fig. 1. Study area and artificial oyster reef design: (A); location of Poole Bay on the south coast of the UK, (B); Schematic of the general arrangement of the 24 replicate reef modules, (C); An artificial reef module; breeze block height plus reef box height is 80 cm from the sea bed.

September 2012 (autumn), April 2013 (spring), August 2013 (summer) and November 2013 (autumn). At each sampling interval, triplicate elevated reef boxes and sea bed cages were recovered by divers. Upon recovery, five oysters from each box and cage were removed for physiological assessment. These individuals were immediately returned to the National Oceanography Centre, Southampton (NOCS) aquarium in sea water taken from Poole Bay at the time of collection. Live oysters were acclimated for two days (Jo et al., 2008) at the collection temperature in sea water collected from Poole Bay. In-situ salinity and water temperature at the height of elevated oyster reefs and at the sea bed were measured using a YSI probe. Four replicates of water samples (1 litre) were collected at both heights above the sea bed by divers for total suspended sediment (TSS), bacterial abundance and chlorophyll *a* (Chl_*a*) analysis.

2.3. Environmental conditions

Total suspended sediment (TSS) was determined by filtration, dried to constant weight on GF/C filters (Fisherbrand™). Chl_*a* was extracted from four replicates of water samples collected from reef and sea bed positions in 6 ml of 90% acetone overnight at 4 °C. Chlorophyll fluorescence was measured by using a 10-AU Fluorometer (Parsons et al., 1984). Eight 20 μl replicates of sea water from reef and sea bed positions were inoculated on individual marine agar plates (Zobell 2216E, Difco™, France). They were incubated at room temperature for two days before bacterial abundance was determined as colony forming units (CFU ml^{-1}).

2.4. Oyster filtration rate measurement

Filtration rate was determined by measuring the consumption of a fixed ration of the prymnesiophyte *Isochrysis galbana*. Concentrated algae were diluted to 2×10^6 cells ml^{-1} using GF/C-filtered sea water collected from Poole Bay. Clearance rates were measured at the same temperature as in the field on the day of collection. Oysters were left undisturbed in 300 ml chambers until they had opened their valves, whereupon sufficient *I. galbana* was added to produce a final concentration of 25,000 cells ml^{-1}. Algal cells were sampled at time points after their addition to the clearance rate chambers and were fixed with Lugol's solution for 24 h in the dark before enumeration using a 0.1 mm deep Neubauer haemocytometer (Marienfeld; Germany) viewed under bright field optics. The clearance rates were calculated using the following equation (Coughlan, 1969)

$$CR\left(l\ g^{-1}h^{-1}\right) = V \times \frac{(\ln C1 - \ln C2)}{T}$$

where *CR* represents the clearance rate (volume of water cleared of particles per unit time, in litres per hour per animal ash free dry weight (*g*)); *V* = volume of water (*l*); *T* = the time (*h*); *C*1 = initial algal concentration and *C*2 = the final algal concentration.

2.5. Oyster respiration rate measurement

The respiration rate was measured in a 1000 ml flow-through respirometer filled with GF/C–filtered surface water collected in Poole Bay. Oxygen measurements were made using a Fibox 3™ fibre-optic oxygen meter (PreSens; Germany). Respirometers were placed in waterbath regulated to the same temperature as that in the field and were held in the dark. Oxygen saturation in each chamber was measured hourly for six hours. The oxygen values were corrected against a control respirometer to correct for bacterial respiration. The respiration rates were calculated using the following equation (Bayne, 1998)

$$RR = \left(Ct_o - Ct_1\right) \times (V_c - V_a) \times \frac{60}{(t_1 - t_0)}$$

where *RR* represents respiration rate; Ct_0 = oxygen saturation (mg l^{-1}) at t_0; Ct_1 = oxygen saturation (mg l^{-1}) at t_1; V_c = volume of chamber (cm^3); V_a = volume of animal (litre: measured by displacement of water). Filtration and respiration rates were corrected to ash free dry weight.

2.6. Condition index

Soft tissues and cleaned shells of oyster samples were oven-dried at 80 °C on pre-dried foil dishes for 48 h. Condition index was then calculated using the following equation (Lucas and Beninger, 1985)

$$\frac{Dry\ tissue\ weight\ \times\ 100}{Dry\ shell\ weight}.$$

2.7. Haemolymph protein analysis

Haemolymph protein was measured using the Bicinchoninic Acid assay kit (Sigma-Aldrich™, Dorset UK) according to the manufacturer's instructions.

2.8. Haemocyte counts

10 μl of haemolymph was withdrawn from adductor muscle sinus using a syringe and 23G needle and mixed with 10 μl of marine saline (Schlieper, 1972). Total and differential haemocyte cell counts were made with a 0.1 mm deep Neubauer haemocytometer using bright field. Cells were classified as hyalinocytes and granulocytes (according to Hauton et al., 2000; Malham et al., 2009).

2.9. Gonad maturation

The soft tissue of each oyster was longitudinally dissected through the mid region of the visceral mass to create a transverse section containing gonadal tissue. The tissue was placed into Bouin's solution (Sigma-Aldrich™, USA, HT10132) and left for 24 h. Tissues were subsequently dehydrated through an ethanol and clarified by overnight immersion in xylene and then xylene with wax. Wax blocks were thin-sectioned and stained with haematoxylin and eosin (Kamphausen et al., 2011). Slides were dehydrated in 96% ethanol and clarified in xylene before mounting with DPX. Phase identification of oogenesis and spermatogenesis in *O. edulis* were identified according to Silva et al. (2009).

2.10. Statistical analyses

Data were first tested for normality and homogeneity of variance. Two-way ANOVA was performed to test how physiological performance (i.e., respiration and filtration rates, condition index, haemolymph protein and haemocyte counts) varied with elevation (reef/sea bed) and sampling month. Where appropriate, post-hoc pairwise multiple comparisons (Tukey tests) compared the effect of sampling months on physiological indicators between sampling intervals. Environmental conditions were compared using Mann–Whitney U tests.

3. Results

3.1. Environmental conditions

The field temperatures and salinities at the experimental site were 12 °C and 35 (September 2012), 7 °C and 33 (April 2013), 18 °C and 34 (August 2013) and 15 °C and 34 (November 2013), respectively. Vertical profiles of temperature and salinity indicated a fully mixed water column at each sampling event; there were no significant differences in temperature or salinity between the elevated reefs and sea bed.

Total suspended solids at the height of elevated reef modules were significantly lower than that at the sea bed at every sampling interval (Fig. 2 A; Mann–Whitney U, $p < 0.05$). There was no significant difference in chlorophyll *a* concentration (Fig. 2 B) with height above the sea bed, whilst bacterial abundance (Fig. 2 C) in the water column at the height of the elevated reefs was significantly lower than that at the sea bed (Mann–Whitney U, $p < 0.05$) in August (18 °C) and November (15 °C) 2013.

3.2. O. edulis filtration rate

The mean filtration rate of oysters at each sampling event is shown in Fig. 3. Two-way ANOVA identified a significant effect of month and elevation (reef/sea bed) on *O. edulis* filtration rate but no interaction between the main effects (Table 1). Overall, filtration rates of reef oysters were significantly higher than sea bed oysters. Filtration rates also differed significantly among all sampling months (Supplementary Table 1).

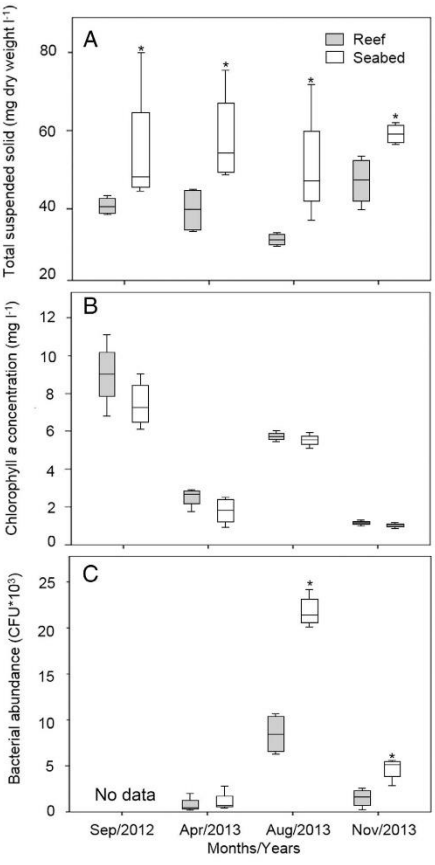

Fig. 2. Total suspended solid (A), chlorophyll *a* concentration (B) and bacterial abundance (C) at the height of the elevated reef modules (n = 4) and at sea bed (n = 4). Significant differences (Mann–Whitney U, $p < 0.05$) between sea bed values and values at an elevation of 80 cm are denoted by an asterisk for each sampling event.

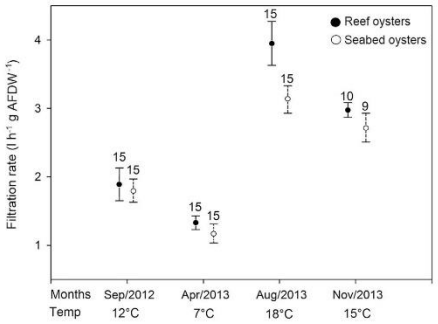

Fig. 3. Filtration rates (l h^{-1} g^{-1} AFDW, mean ± SE) of *O. edulis* collected from elevated reefs and sea bed on each sampling occasion. The filtration rate was affected by sampling month and elevation (Two-way ANOVA, $p < 0.001$ and 0.005, respectively). The number of oysters analysed from each location is indicated beside the error bars.

3.3. O. edulis respiration rate

The mean respiration rate of oysters at each sampling event is shown in Fig. 4. Two-way ANOVA identified a significant effect of sampling month but no effect of elevation and no interaction between the main effects on respiration rate (Table 1). Tukey pairwise tests showed that there was no difference in the respiration rates of oyster samples collected in August 2013 (18 °C), November 2013 (15 °C) and September 2012 (12 °C) but these rates were significantly higher than oysters sampled in April 2013 when water temperature was lowest at 7 °C (Supplementary Table 1).

Table 1
Two-way ANOVA comparing the effect of sampling month, elevation (reef/seabed), and the interaction month x elevation on physiological performance of *O. edulis*. Significant differences are denoted by asterisks.

Physiological parameters	df	F	*p*-Value
Filtration (l h^{-1} g $AFDW^{-1}$)			
Month	3	61.06	0.001*
Elevation (reef/seabed)	1	8.35	0.005*
Month × elevation	3	2.11	0.104
Respiration (ml O_2 h^{-1} g $AFDW^{-1}$)			
Month	3	9.16	0.001*
Elevation (reef/seabed)	1	3.14	0.080
Month × elevation	3	0.71	0.547
Condition index			
Month	3	62.28	0.001*
Elevation (reef/seabed)	1	1.93	0.167
Month × elevation	3	2.23	0.090
Haemolymph protein (mg ml^{-1})			
Month	3	4.029	0.009*
Elevation (reef/seabed)	1	2.129	0.148
Month × elevation	3	0.052	0.984
Total haemocytes (cells ml^{-1})			
Month	3	57.016	0.001*
Elevation (reef/seabed)	1	5.385	0.022*
Month × elevation	3	1.406	0.245
Hyalinocytes (cells ml^{-1})			
Month	3	29.187	0.001*
Elevation (reef/seabed)	1	2.033	0.157
Month × elevation	3	0.134	0.939
Granulocytes (cells ml^{-1})			
Month	3	64.598	0.001*
Elevation (reef/seabed)	1	7.012	0.009*
Month × elevation	3	2.774	0.045

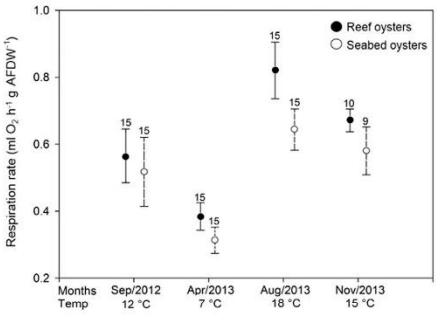

Fig. 4. Respiration rates (ml O_2 h^{-1} g^{-1} AFDW, mean ± SE) of *O. edulis* collected from elevated reefs and sea bed on each sampling occasion. The respiration rate was affected by sampling month (Two-way ANOVA, $p < 0.001$). The number of oysters analysed from each locations is indicated on top of the error bars.

3.4. Condition index

The mean condition index of oyster soft tissue varied among sampling months (Fig. 5). Two-way ANOVA indicated that the sample month had a significant effect on condition index (Table 1). Pairwise multiple comparisons showed that the condition index of oysters collected in August 2013 was significantly higher than in the other sampling months. Moreover, condition index in April 2013 was significantly higher than November 2013 but not statistically significant different between remaining months (Supplementary Table 1).

3.5. Haemolymph protein concentrations

Protein concentrations of reef and sea bed oysters during the experiment varied between 2.16 ± 0.46 mg ml^{-1} (Mean ± SE) and 1.94 ± 0.34 mg ml^{-1}, respectively. Protein concentration differed significantly between sampling months but there was no significant effect of elevation on haemolymph protein (Table 1). Haemolymph protein concentration was highest in August 2013 and was significantly higher than April 2013, whilst there was no difference between other sampling intervals (Supplementary Table 1).

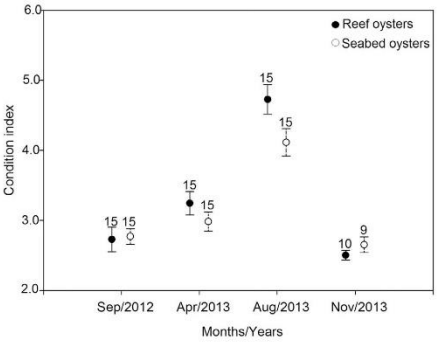

Fig. 5. Condition index (mean ± SE) of *O. edulis* collected from elevated reefs and the sea bed on each sampling occasion. Condition index was affected by sampling month (two-way ANOVA, $p < 0.001$).

3.6. Total haemocyte, hyalinocyte and granulocyte concentrations

Total haemocyte and granulocyte counts differed significantly between sampling months and with elevation (Table 1). Total haemocyte counts (Mean ± SE) of reef and sea bed oysters remained low in September 2012 (4.01 ± 0.25 and 4.10 ± 0.37 × 10^6 cells ml^{-1}, respectively) and April 2013 (5.06 ± 0.69 and 4.06 ± 0.43 × 10^6 cells ml^{-1}, respectively) but peaked in August 2013 (16.20 ± 1.45 and 12.71 ± 1.62 × 10^6 cells ml^{-1}, respectively) (Fig. 6). Tukey pairwise tests showed that there was no difference in total haemocytes, hyalinocytes and granulocytes between oysters collected in September 2012 and April 2013. In general, haemocyte numbers were low when in situ water temperatures were 12 °C and 7 °C (Supplementary Table 1).

3.7. Gonad maturation

The reproductive development of *O. edulis* is shown in Fig. 7. At the first sampling event, when the water temperature was 12 °C, 80% of animals from elevated reefs were spent while 55% of sea bed oysters were spent and the remainder were identified as ripe. In April 2013, with the water temperature at 7 °C, 48% of the elevated reef oysters were scored as being in an advanced state of gonad maturation, compared to 40% of sea bed oysters. Interestingly, in August 2013, 46% of reef oysters were identified as spent, whilst only 27% of sea bed oysters were spent. In November 2013, we found that 22% of sea bed oysters were still either ripe or spent whilst 95% of reef oysters were identified as spent. From these data it was concluded that the gametogenic cycle was more rapid for oysters held on elevated reef structures.

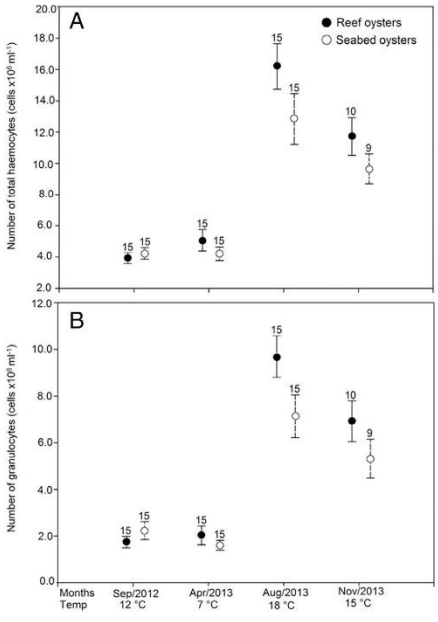

Fig. 6. Temporal variation of total haemocyte concentration (A) and granulocytes (B) (in 10^6 cells ml^{-1}, mean ± SE) of *O. edulis* collected from experimental reefs and sea bed on each sampling occasion. The granulocytes and total haemocyte counts were affected by temperature and elevation (Two-way ANOVA, $p < 0.001$ and 0.05 respectively).

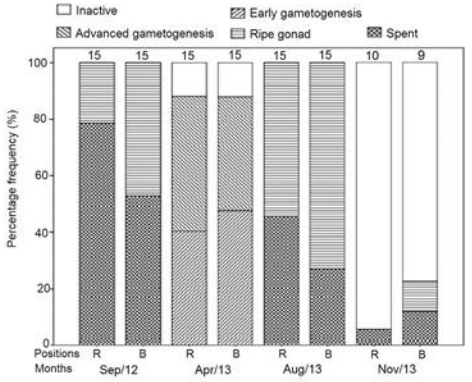

Fig. 7. Percentage frequency of gonad phases of *O. edulis*, collected from elevated reefs (R) and sea bed (B) on each sampling occasion. The number of oysters analysed from each locations is indicated at the top of the bar chart.

4. Discussion

Artificial oyster reefs have been created globally in order to increase the abundance of target oyster species and also restore ecosystem complexity. However, the benefits to physiological performance, especially of the native oyster *O. edulis*, have not been established. Previous studies have reported that environmental factors and food quality control the filter feeding activity of bivalves (Ward and Shumway, 2004). Whilst temperature and salinity are the most significant abiotic factors affecting *O. edulis* filtration and respiration (Hutchinson and Hawkins, 1992; Beiras et al., 1995; Haure et al., 1998), particle size, algae species and their surface properties have been shown to affect food selectivity of oysters and other molluscs as well (Shumway et al., 1985; Ward et al., 1997; Rosa et al., 2013) However, to our knowledge this is the first study to compare the filtration and respiration rates of oysters at the same in situ temperature and salinity but at different heights above the sea bed. Our data indicate that elevation had a significant effect on the filtration rate of oysters, such that the reef oysters in this study had higher filtration rates than sea bed oysters. Filtration rate is a key determinant of the energy available for oysters and will ultimately influence their ability to meet the energetic demands (Moore, 1977; Winter et al., 1984). Indeed, filtration rate has been considered to be the most significant driver of scope for growth (Barber et al., 1991; Hutchinson and Hawkins, 1992). The observations that reef oysters showed the highest filtration rates at times of elevated temperature and chlorophyll *a* indicate that these individuals would maximise their energy intake during the important growing season; as a result these elevated oysters would have an advantage over oysters on the sea bed. Conversely, data presented herein demonstrate that TSS and bacterial abundance adjacent to the sea bed were significantly higher than 80 cm above the sea bed and we cannot exclude the possibility that these environmental conditions might have impacted the physiological performance of sea bed oysters by reducing their filtration rate. Although oysters and other bivalves can reject unwanted particles (Yonge, 1926; Newell and Jordan, 1983; Shumway et al., 1985) with occasional rapid closure of their valves to expel accumulated silt and through psuedofaecal production (Yonge, 1926, 1960), an increase in suspended sediment has been shown to cause a decrease in the filtration rate of *O. edulis* (Korringa, 1952; Grant et al., 1990). In fact, oysters can sustain periods of valve closure in response to environmental perturbation (Orton, 1937; Cavanaugh, 1983; Vismann, 1991; Pennec et al., 1995). In accordance with our results, Wheat and Ruesink (2013) found that the field filtration rate of on-bottom Pacific oysters *Crassotrea gigas* was lower than those suspended above the bottom on long-line aquaculture.

There was no significant effect of elevation (reef/sea bed) on the respiration rate of oysters in this study. Respiration rate has been used as a physiological indicator of bivalve catabolism (Goulletquer et al., 1999; Huang and Newell, 2002) and several studies have reported that the metabolic performance of marine bivalves can respond to seasonal change of sea water temperature (Buxton et al., 1981; Dame et al., 1992; Bayne et al., 1999; Sytnik and Zolotnitskiy, 2014). The data from this study support previous work in showing that sampling month, in context of temperature, is also a key factor controlling oxygen consumption in subtidal *O. edulis*. Respiration rate of both sea bed and reef oysters showed significant seasonal variation that reached maximum values in August 2013 (18 °C) and the lowest values in April 2013 (7 °C). It can be therefore concluded that there was no significant difference in the metabolic rate, inferred from respiration rate, between reef and sea bed oysters but that the metabolic rate of all oysters was positively influenced by increased temperature.

It is likely that variation in condition index, among sampling months, was driven by temperature and reproductive cycles. It is widely accepted that temperature controls food intake of oysters and other bivalves (Shumway, 1982; Saucedo et al., 2004; Comeau, 2014). Korringa (1957) reported that *O. edulis* in the UK requires a minimum temperature of 15–16 °C to spawn fertilised eggs. The influence of diet composition in accelerating gonadal development of molluscs has been recognised more recently (Millican and Helm, 1994; Utting and Millican, 1997; Gonzalez Araya et al., 2012). However, no previous publications have reported the role of elevated structures in determining filtration rate and subsequent gonad maturation of *O. edulis*. In this study, the environmental data confirmed that both reef and sea bed oysters were subject to the same temperature regime. Although condition index of *O. edulis*, in this study, was not significantly affected by elevated reef structures, the variation in condition index of the oysters during the period of study can be linked to the gametogenic cycle. Condition index was highest in August 2013. At that time, there was a tendency for reef oysters to be higher in condition index than sea bed oysters and the percentage of reef oysters with spent gonads (46%) was higher than sea bed oysters (27%). Condition index of reef oysters was slightly lower than sea bed oysters in November 2013 when 95% of reef oysters had completed their gametogenic cycle while 22% of sea bed oysters were either ripe or spent. Therefore, it is likely that, at the same temperature condition, reef oysters can complete spawning earlier in the season than sea bed oysters. In accordance with our results, Bataller et al. (1999) and Comeau et al. (2010) reported that warm summer months combined with elevated food in the upper water column enhanced growth and shortened the reproductive cycle in *C. virginica* (Comeau, 2013).

Pouvreau et al. (2006) explained that the reproductive cycle of a bivalve can be divided into two processes viz., 'storage accumulation' and 'gametogenesis'. To complete the second process, gametogenesis, the previously accumulated energy from the first step, before the spawning period, is required. Therefore, the remaining energy budget during the spawning season or early summer is an important factor that influences gametogenic activities. Our data suggested that elevated reef oysters exhibited significantly higher filtration rates in every time interval sampled, but especially during warm summer months. It is hypothesised that this higher feeding rate supplements the reserve energy stores in the individuals from elevated reefs. Based on that, the energy used in the gametogenic cycle may be derived from the reserves previously stored and obtaining during early summer, as described in *Mytilus edulis* (Hilbish et al., 1994). In contrast, the energy used for gonad restoration may be derived directly from feeding later during the summer, as described in *Mytilus galloprovincialis* (Villalba, 1995). Therefore, it can be postulated that reef oysters would have had a more favourable energy status which permitted a faster rate of

gametogenesis, possibly enabling them to reach a ripe state earlier in comparison with sea bed oysters.

Bivalve haemocytes form an important line of defence against pathogens but are also susceptible to environment change (Fisher, 1987; Hauton et al., 2000; Comesana et al., 2012). Bivalve haemocyte counts have been used previously as an indicator of physiological performance. Silva et al. (2008) identified that oysters from different geographical areas with different environmental conditions had significant differences in their circulating haemocyte population. However, to date, no study has reported the effects of elevation (reef versus sea bed) on the circulating haemocyte population of *O. edulis*. In this study, the total haemocyte and granulocyte counts were significantly affected by elevation and sampling months. The concentration of total haemocytes was highest in August 2013 and lowest in September 2012. We have shown that elevated reef oysters exhibited higher filtration rates especially during warm summer months. Therefore we contend that these oysters would have had a favourable energy budget which permitted higher haemocyte, and specifically granulocyte, densities. Granulocytes have a higher phagocytic ability than hyalinocytes (Fisher, 1988; Chu, 2000) and contain abundant lysosomes that contribute to intracytoplasmic pathogen degradation (Cheng, 1981; Carballal et al., 1997; Lopez et al., 1997) through the release of lysosomal enzymes (Cheng, 1981; Montes et al., 1995). Therefore, higher total haemocyte and granulocyte counts would suggest that the elevated reef habitat benefitted the immune system – contributing to a reduced susceptibility to disease.

Whilst we report that a number of key physiological variables were increased by an elevated reef habit in *O. edulis*, these pilot data do not represent definitive proof of the benefits of rearing oysters on reef structures. Further, and longer-term, replicated studies are necessary to confirm that the results we have described are confirmed in other indices of performance and health, incorporating assays such as Scope for Growth, formal assessments of spawning output and measures of disease pathology to confirm the health status of the reef oysters. Future work should also address the assessment of physiological performance in situ, including respiration and filtration measurements, in order to field-validate the findings of our laboratory assays. It is also important that these experiments are conducted at multiple locations across the European margin to establish the broader scale validity of our findings.

5. Conclusions

We have shown that elevation (reef/sea bed) affected the physiological performance of *O. edulis*. Oysters on top of elevated reefs (80 cm above sea bed) had significantly higher filtration rates as a function of improved water conditions, including reduced sediment and bacteria loads in water column at the reef height. Whilst the data presented herein are essentially preliminary findings, when combined with earlier research on other species of oysters, they represent an important first step in supporting the use of elevated and fishery-protected reefs for brood stock as at least a partial solution to the problem of native oyster *O. edulis* restoration across Europe.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.aquaculture.2015.03.022.

Acknowledgements

This research was partly funded by Natural England (http://www.naturalengland.org.uk/) (GA No. RP0479). The authors are grateful to the Royal Thai Government which contributed to the full funding of a PhD grant for the first author. The authors thank the Ocean and Earth Science scientific diving team for their assistance in oyster reef construction and in reef recovery at each sampling. The authors gratefully acknowledge the assistance of Poole Harbour Commissioners for their assistance in deploying the breeze blocks in Poole Bay. The authors also gratefully acknowledge the assistance of the Southern Inshore Fisheries and Conservation Authority (Southern IFCA) for providing *Tenacity* from which the reef modules were deployed and thanks to Mike Markey for providing *Peveril Myth* for recovering reef modules. The authors acknowledge the assistance of Dr. Ben Ciotti (University of Southampton) for the statistical advice.

References

Airoldi, L.B., Beck, M.W., 2007. Loss status and trends for coastal marine habitats of Europe. Oceanogr. Mar. Biol. Annu. Rev. 45, 345–405.
Barber, B.J., Ford, S.E., Littlewood, D.T.J., 1991. Aphysiological comparison of resistant and susceptible oysters (*Crassostrea virginica*) exposed to the endoparasite *Haplosporidium nelsoni*. J. Exp. Mar. Biol. Ecol. 146, 101–112.
Bartol, I.K., Mann, R., Luckenbach, M., 1999. Growth and mortality of oysters (*Crassostrea virginica*) on constructed intertidal reefs: effects of tidal height and substrate level. J. Exp. Mar. Biol. Ecol. 237, 157–184.
Bataller, E., Boghen, A.D., Burt, M.D., 1999. Comparative growth of the eastern oyster *Crassostrea virginica* (Gmelin) reared at low and high salinities in New Brunswick, Canada. J. Shellfish Res. 18, 107–114.
Bayne, B.L., 1998. The physiology of suspension feeding bivalve molluscs: an introduction to the Plymouth 'TROPHEE' workshop. J. Exp. Mar. Biol. Ecol. 219, 1–19.
Bayne, B.L., Hedgecock, D., Mcgolrick, D., Ress, R., 1999. Feeding behaviour and metabolic efficiency contribute to growth heterosis in Pacific oysters *Crassostrea gigas*. J. Exp. Mar. Biol. Ecol. 233, 115–130.
Beck, M.W., 2011. Oyster reefs at risk and recommendations for conservation, restoration, and management. Bioscience 61, 107–116.
Beiras, R., Camach, P.A., Albentosa, M., 1995. Short-term and long-term budget of young oyster *Ostrea edulis* L. in response to temperature. J. Exp. Mar. Biol. Ecol. 186, 221–236.
BRIG, 2007. Report on the species and habitat review. Biodiversity Reporting and Information Group (BRIG) to the UK Standing Committee (172 pp.).
Buxton, C.D., Newell, R.C., Field, J.G., 1981. Response–surface analysis of the combined effects of exposure and acclimation temperatures on filtration, oxygen consumption and scope for growth in the oyster *Ostrea edulis*. Mar. Ecol. Prog. Ser. 6, 73–82.
Carballal, M.J., López, C., Azevedo, C., Villalba, A., 1997. In vitro study of phagocytic ability of *Mytilus galloprovincialis* Lmk. haemocytes. Fish Shellfish Immunol. 7, 403–416.
Cavanaugh, C.M., 1983. Symbiotic chemoautotrophic bacteria in marine invertebrates from sulphide-rich habitats. Nature 302, 58–61.
Cheng, T.C., 1981. Bivalves. In: Ratcliffe, N.A., Rowley, A.F. (Eds.), Invertebrate Blood Cells. Academic Press, London, pp. 233–300.
Chu, F.E., 2000. Defense mechanisms of marine bivalves. In: Fingerman, M., Nagabhushanam, R. (Eds.), Recent Advances in Marine Biotechnology Immunology and Pathology. Science Publishers Inc., USA.
Coen, L.D., Mark, W.L., 2000. Developing success criteria and goals for evaluating oyster reef restoration: ecological function or resource exploitation? Ecol. Eng. 15, 323–343.
Comeau, L.A., 2013. Suspended versus bottom oysters culture in eastern Canada: comparing stocking densities and clearance rates. Aquaculture 410–411, 57–65.
Comeau, L.A., 2014. Spring awaking temperature and survival of sediment-covered eastern oysters *Crassostrea virginica*. Aquaculture 430, 188–194.
Comeau, L.A., Sonier, R., Lanteigne, L., Landry, T., 2010. A novel approach to measuring chlorophyll uptake by cultivated oysters. Aquacult. Eng. 43, 71–77.
Comesana, P., Casas, M.S., Cao, A., Abollo, E., Arzul, I., Morga, B., Villalba, A., 2012. Comparison of haemocytic parameters among flat oyster *Ostrea edulis* stocks with different susceptibility to bonamiosis and the Pacific oyster *Crassostrea gigas*. J. Invertebr. Pathol. 109, 274–286.
Coughlan, J., 1969. The estimation of filtering rate from the clearance of suspensions. Mar. Biol. 2, 356–358.
Crisp, D.J., 1964. The effect of the severe winter of 1962–1963 on marine life in Britian. J. Anim. Ecol. 33, 165–210.
Dame, R.F., Spurrier, J.D., Zingmark, R.G., 1992. In situ metabolism of an oyster reef. J. Exp. Mar. Biol. Ecol. 164, 147–159.
Edwards, E., 1997. Molluscan Fisheries in Britain. National Oceanic and Atmospheric Admistration & U.S. Department of Commerce, Washington.
Fisher, W.S., 1987. Response of European flat oyster (*Ostrea edulis*) hemocytes to acute salinity and temperature change. Aquaculture 67, 179–190.
Fisher, W.S., 1988. Environmental influence on bivalve hemocyte function. Am. Fish. Soc. Spec. Publ. 18, 225–237.
Frechette, M., Butman, C.A., Geyer, W.R., 1989. The importance of boundary-layer flows in supplying phytoplankton to the benthic suspension feeder, *Mytilus edulis* L. Limnol. Oceanogr. 34, 19–36.
Fulford, R.S., Breitburg, D.L., Luckenbach, M., Newell, R.I.E., 2010. Evaluating ecosystem response to oyster restoration and nutrient load reduction with a multispecies bioenergetics model. Ecol. Appl. 20, 915–934.
Gonzalez Araya, R., Mingant, C., Petton, B., Robert, R., 2012. Influence of diet assemblage on *Ostrea edulis* broodstock conditioning and subsequent larval development. Aquaculture 364–365, 272–280.
Goulletquer, P., Heral, M., Deslouspaoli, J.M., Prou, J., Garnier, J., Razet, D., Boromthanarat, W., 1999. Ecophysiology and energy balance of the Manila clam, *Ruditapes philippinaram*. J. Exp. Mar. Biol. Ecol. 132, 85–108.
Grant, J., Enright, C.T., Griswold, A., 1990. Resuspension and growth of *Ostrea edulis*: a field experiment. Mar. Biol. 104, 51–59.
Haure, J., Penisson, C., Bougrier, S., Baud, J.P., 1998. Influence of temperature on clearance and oxygen consumption rates of the flat oyster *Ostrea edulis*: determination of allometric coefficients. Aquaculture 169, 211–224.

Hauton, C., Hawkins, L.E., Hutchinson, S., 2000. The effects of salinity on the interaction between a pathogen (*Listonella anguillarum*) and components of a host (*Ostrea edulis*) immune system. Comp. Biochem. Physiol. B 127, 203–212.
Hilbish, T.J., Bayne, B.L., Day, A., 1994. Genetics of physiological differentiation within the mussel genus Mytilus. Evolution 48, 267–286.
Huang, S.C., Newell, R.I.E., 2002. Seasonal variations in the rates of aquatic and aerial respiration and ammonium excretion of the ribbed mussel, *Geukensia demissa* (Dillwyn). J. Exp. Mar. Biol. Ecol. 270, 241–255.
Hutchinson, S., Hawkins, L.E., 1992. Quantification of the physiological responses of the European flat oyster *Ostrea edulis* to temperature and salinity. J. Molluscan Stud. 58, 215–216.
Jackson, J.B., Kirby, M.X., Berger, W.H., Bjorndal, K.A., Botsford, L.W., Bourque, B.J., Bradbury, R.H., Cooke, R., Erlandson, J., Estes, J.A., Hughes, T.P., Kidwell, S., Lange, C.B., Lenihan, H.S., Pandolfi, J.M., Peterson, C.H., Steneck, R.S., Tegner, M.J., Warner, R.R., 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science 293, 629–638.
Jensen, A.C., Collins, K.J., Lockwood, A.P.M., 2000. Artificial Reefs in European Seas. Kluwer Academic Publishers, Dortrecht.
Jo, P.G., Choi, Y.K., Choi, C.Y., 2008. Cloning and mRNA expression of antioxidant enzymes in the Pacific oyster, *Crassostrea gigas*, in response to cadmium exposure. Comp. Biochem. Physiol. C 147, 460–469.
Kamphausen, L., Jensen, A., Hawkins, L., 2011. Unusually high proportion of males in a collapsing population of commercially fished oysters (*Ostrea edulis*) in the Solent, United Kingdom. J. Shellfish Res. 30, 217–222.
Korringa, P., 1952. Recent advances in oyster biology. Q. Rev. Biol. 27, 339–365.
Korringa, P., 1957. Water temperature and breeding throughout the geographical range of *Ostrea edulis*. Ann. Biol. 33, 109–116.
Laing, I., Walker, P., Areal, F., 2006. Return of the native-is European oyster (*Ostrea edulis*) stock restoration in the UK feasible? Aquat. Living Resour. 19, 283–287.
Lallias, D.A., 2008. *Bonamia ostreae*-induced mortalities in one-year old European flat oysters *Ostrea edulis*: experimental infection by cohabitation challenge. Aquat. Living Resour. 21, 423–439.
Lallias, D., Lapegue, S., Haley, C., Heurtebise, S., Boudry, P., Beaunmont, A., 2007. A preliminary genetic linkage map for the European flat oyster *Ostrea edulis*. Aquaculture 272, 281–282.
Lenihan, H.S., Micheli, F., Shelton, S.W., Peterson, C.H., 1999. The influence of multiple environmental stressors on susceptibility to parasites: an experimental determination with oysters. Limnol. Oceanogr. 44, 910–924.
Lopez, C., Carballal, M.J., Azevedo, C., Villalba, A., 1997. Differential phagocytic ability of the circulating haemocyte types of the carpet shell clam *Ruditapes decussatus* (Mollusca: Bivalvia). Dis. Aquat. Org. 30, 209–215.
Lucas, A., Beninger, P.G., 1985. The use of physiological condition indices in marine bivalve aquaculture. Aquaculture 44, 187–220.
Mackenzie, C.L.J., Burrell, V.G., Rosenfield, A., Hobart, W.L., 1997. The History, Present Condition, and Future of the Molluscan Fisheries of North America and Europe. National Oceanic and Atmospheric Administration & U.S. Department of Commerce, Washington.
Malham, S., 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, 128–138.
Millican, P.F., Helm, M.M., 1994. Effects of nutrition on larvae production in the European flat oyster, *Ostrea edulis*. Aquaculture 23, 83–94.
Montes, J.F., Durfort, M., García-Valero, J., 1995. Cellular defence mechanism of the clam *Tapes semidecussatus* against infection by the protozoan *Perkinsus* sp. Cell Tissue Res. 279, 529–538.
Moore, P.G., 1977. Inorganic particulate suspensions in the sea and their effects on marine animals. Oceanogr. Mar. Biol. 15, 225–363.
Newell, R.C., 1965. The role of detritus in the nutrition of two marine deposit feeders, the prosobranch *Hydrobia ulvae* and the bivalve *Macoma balthica*. Proc. Zool. Soc. London 144, 25–45.
Newell, R.I.E., Jordan, S.J., 1983. Preferential ingestion of organic material by the American oyster *Crassostrea virginica*. Mar. Ecol. Prog. Ser. 13, 47–53.
Officer, C.B., Biggs, R.B., Taft, J.L., Cronin, L.B., Tyler, M.A., Boynton, W.R., 1984. Chesapeake Bay anoxia: oringin, development, and significance. Science 223, 22–27.
Orton, J.H., 1937. Oyster Biology and Oyster-Culture. Butler & Tanner Ltd, London.
Parsons, T.R., Maita, Y., Lalli, C.M., 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford.
Pennec, L.M., Beninger, P.G., Herry, A., 1995. Feeding and digestive adaptions of bivalve mollusc to sulphide-rich habitats. Comp. Biochem. Physiol. 111, 183–189.
Pouvreau, S., Bourles, Y., Lefebvre, S., Gangnery, A., Bruscia, M.A., 2006. Application of a dynamic energy budget model to the Pacific oyster, *Crassostrea gigas*, reared under various environmental conditions. J. Sea Res. 56, 156–167.
Rosa, M., Ward, J.E., Shumway, S.E., Wikfors, G.H., Espinosa, E.P., Allam, B., 2013. Effects of particle surface properties on feeding selectivity in the eastern oyster *Crassostrea virginica* and the blue mussel *Mytilus edulis*. J. Exp. Mar. Biol. Ecol. 466, 320–327.
Saucedo, P.E., Ocampo, L., Monteforte, M., Bervera, H., 2004. Effect of temperature on oxygen consumption and ammonia excretion in the Calafia mother-of-pearl oyster, *Pinctada mazatlanica* (Hanley, 1856). Aquaculture 229, 377–387.
Schlieper, C., 1972. Research Methods in Marine Biology. Sidgwick & Jackson, London.
Shumway, S.E., 1982. Oxygen consumption in oysters: an overview. Mar. Biol. Lett. 3, 1–23.
Shumway, S.E., Cucci, T.L., Newell, R.C., Selvin, R., Guillard, R.R.L., Yentsch, C.M., 1985. Flow cytometry: a new method for characterization of differential ingestion, digestion and egestion by suspension feeders. Mar. Ecol. Prog. Ser. 24, 201–204.
Silva, P.M.D., Comesana, P., Fuentes, J., Villalba, A., 2008. Variability of haemocyte and haemolymph parameters in European flat oyster *Ostrea edulis* families obtained from brood stocks of different geographical origins and relation with infection by the protozoan *Bonamia ostreae*. Fish Shellfish Immunol. 24, 551–563.
Silva, P.M.D., Fuentes, J., Villalba, A., 2009. Difference in gametogenic cycle among strains of the European flat oyster *Ostrea edulis* and relationship between gametogenesis and bonamiosis. Aquaculture 287, 253–265.
Smith, I.P., Low, P.J., Moore, P.G., 2006. Legal aspects of conserving native oysters in Scotland. Mar. Pollut. Bull. 52, 479–483.
Sytnik, N.A., Zolotnitskiy, A.P., 2014. On relation of filtration and respiration in the flat oyster (*Ostrea edulis*) at different water temperature. Hydrobiol. J. 50, 93–99.
Utting, S.O., Millican, P.F., 1997. Techniques for the hatchery conditioning of bivalve broodstock and the subsequent effect on egg quality and larval viability. Aquaculture 155, 45–54.
Villalba, A., 1995. Gametogenic cycle of cultured mussel, *Mytilus galloprovincialis*, in the bays of Galicia (N. W. Spain). Aquaculture 130, 269–277.
Vismann, B., 1991. Sulfide tolerance; physiological mechanisms and ecological implications. Ophelia 34, 1–27.
Ward, J.E., Shumway, S.E., 2004. Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. J. Exp. Mar. Biol. Ecol. 300, 83–130.
Ward, J.E., Levinton, J.S., Shumway, S.E., Cucci, T., 1997. Site of particle selection in a bivalve mollusc. Nature 390, 131–132.
Wheat, W., Ruesink, L.J., 2013. Commercially-cultured oysters (*Crassostrea gigas*) exert top-down control on intertidal pelagic resources in Wilapa Bay, Washington, USA. J. Sea Res. 81, 33–39.
Winter, J.E., Acevedo, M.A., Navarro, J.M., 1984. Quempil'n estuary, an experimental oyster cultivation station in Southern Chile. Energy balance in *Ostrea chilensis*. Mar. Ecol. Prog. Ser. 20, 151–164.
Yonge, C.M., 1926. Structure and physiology of the organs of feeding and digestion in *Ostrea edulis*. J. Mar. Biol. Assoc. UK 14, 295–386.
Yonge, C.M., 1960. Oysters. Collins, London.

List of References

Abad, M., Ruiz, C., Martínez, D., Mosquera, G., Sánchez, J.L., 1995. Seasonal variations of lipid classes and fatty acids in flat oyster, *Ostrea edulis*, from San Cibran (Galicia Spain). Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 110,109-118.

Abelson, A., Denny, M., 1997. Settlement of marine organisms in flow. Annual Review of Ecology and Systematics 28, 317-339.

Airoldi, L. B., Beck, M.W., 2007. Loss status and trends for coastal marine habitats of Europe. Oceanography and Marine Biology: An Annual Review 45, 345-405.

Albentosa, M., Fernández-Reiriz, M.J., Labarta, U., Pérez-Camacho, A., 2007. Response of two species of clams, *Ruditapes decussatus* and *Venerupis pullastra*, to starvation: Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology 146, 241-249.

Allam, B., Paillard, C., Auffret, M., 2000. Alterations in Hemolymph and Extrapallial Fluid Parameters in the Manila Clam, *Ruditapes philippinarum*, challenged with the Pathogen *Vibrio tapetis*. Journal of Invertebrate Pathology 76, 63-69.

Allam, B., Raftos, D., 2015. Immune responses to infectious diseases in bivalves. Journal of Invertebrate Pathology: (cited 17/07/15):*http://dx.doi.org/10.1016/j.jip.2015.05.005*.

Allee, W., Emerson, A.E., Park, O., Park, T., Schmidt K.P., 1949. Principles of Animal Ecology. W.B. Saunders Company, London, UK. 864 pp.

Andrews, J., 1979. Pelecypoda: Ostreidae. In: Giese, A. (Ed.). Reproduction of Marine Invertebrates, Volume 5: Molluscs: Pelecypods and lesser classes. Academic Press, New York, USA, pp 293-341.

Anon, 2007. Conservation of the Native Oyster *Ostrea edulis* in Scotland. Technical report. University Marine Biological Station Millport. Scottish Natural Heritage Commissioned Report No. 251 (ROAME No. F02AA408), UK. 173 pp.

Arcos-Ortega, F.G., Ibarra, A.M., Rodríguez-Jaramillo, M.C., García-Latorre E.A., Vazquez-Boucard, C., 2009. Quantification of vitellin/vitellogenin-like proteins in the oyster *Crassostrea corteziensis* (Hertlein, 1951) as a tool to predict the degree of gonad maturity. Aquaculture Research 40, 644–655.

Arzul, I., Gagnaire, B., Bond, C., Chollet, B., 2009. Effects of temperature and salinity on the survival of *Bonamia ostreae*, a parasite infecting flat oysters *Ostrea edulis*. Diseases of Aquatic Organisms 85, 67-75.

Arzul, I., Miossec, L., Blanchet, E., Garcia, C., Francois, C., Joly, J.P., 2006. *Bonamia ostreae* and *Ostrea edulis*: a stable host-parasite system in France? Symposia Proceedings, ISVEE conference XI, Cairns, Australia. 5 pp.

Ashton, E.C., 2010. The native oyster rediscovered in the Firth of Forth. BRISC Recorder News 76: 1-3.

Asmus, H., Asmus, R., 2011. Food web of intertidal mussel and oyster beds. Reference Module in Earth Systems and Environmental Sciences 6, 287-304.

Austin, H., Haven, D.S., Moustafa, M.S., 1993. The relationship between trends in a condition index of the American oyster, *Crassostera virginica* and environmental parameters in three Virginia estuaries. Estuaries 16, 362-374.

Avendaño, M., Pennec M.L., 1997. Intraspecific variation in gametogenesis in two populations of the Chilean mollusc bivalve, *Argopecten purpuratus* (Lamarck). Aquaculture Research 28, 175-183.

Baek, M.J., Lee, Y.J., Choi, K.S., Lee, W.C., Park, H.J., Kwak, J.H., Kang, C.K., 2014. Physiological disturbance of the Manila clam, *Ruditapes philippinarum*, by altered environmental conditions in a tidal flat on the west coast of Korea. Marine Pollution Bulletin 78, 137-145.

Baggett, L.P., Powers, S.P., Brumbaugh, R., Coen, L.D., DeAngelis, B., Greene, J., Hancock, B., Morlock, S., 2014. Oyster Habitat Restoration Monitoring and Assessment Handbook. The Nature Conservancy, Arlington, USA. 96 pp. (cited 12/05/15): *http://www.oyster-restoration.org/wp-content/uploads/2014/01/Oyster-Habitat-Restoration-Monitoring-and-Assessment-Handbook.pdf*.

Bahr, L.M., Lanier, W.P., 1981. The ecology of intertidal oyster reefs of the south Atlantic coast: a community profile. US Fish and Wildlife Service. Report no. FWS/OBS-81/15, USA. 118 pp. (cited 10/05/14): *http://www.nwrc.usgs.gov/ techrpt/81-15.pdf*.

Baird, D., Christian, R.R., Peterson, C.H., Johnson, G.A., 2004. Consequences of hypoxia on estuarine ecosystem function: Energy diversion from consumers to microbes. Ecological Applications 14, 805–822.

Balvanera, P., Siddique, I., Dee, L., Paquette, A., Isbell, F., Gonzalez, A., Byrnes, J., O'Connor, M.I., Hungate, B. A., Griffin, J.N., 2013. Linking biodiversity and ecosystem services: Current uncertainties and the necessary next steps. BioScience 64, 49–57.

Barber, B.J., Blake, N.J., 1981. Energy storage and utilization in relation to gametogenesis in *Argopecten irradians* concentricus (Say). Journal of Experimental Marine Biology and Ecology 52, 121–134.

Barber, B.J., Ford, S.E., Littlewood, D.T.J., 1991. Aphysiological comparison of resistant and susceptible oysters (*Crassostrea virginica*) exposed to the endoparasite *Haplosporidium nelsoni*. Journal of Experimental Marine Biology and Ecology 146, 101-112.

Barber, B.J., Getcheii, R., Shumway, S., Schick, D., 1988. Reduced fecundity in a deep-water population of the giant scallop *Placopecten magelldnicus* in the Gulf of Maine, USA. Marine Ecology Progress Series 42, 207-212.

Barnes, R. S.K., Coughlan, J., Holmes, N.J., 1973. A preliminary survey of the macroscopic bottom fauna of the Solent, with particular reference to *Crepidula fornicata* and *Ostrea edulis*. Proceedings of the Malacological Society of London 40, 253-257.

Bartol, I. K., Mann, R., 1997. Small-scale settlement patterns of the oyster *Crassostrea virginica* on a constructed intertidal reef. Bulletin of Marine Science 61, 881-897.

Bartol, I. K., Mann, R., Luckenbach, M., 1999. Growth and mortality of oysters (*Crassostrea virginica*) on constructed intertidal reefs: effects of tidal height and substrate level. Journal of Experimental Marine Biology and Ecology 237, 157-184.

Bataller, E., Boghen, A.D., Burt, M.D., 1999. Comparative growth of the eastern oyster *Crassostrea virginica* (Gmelin) reared at low and high salinities in New Brunswick, Canada. Journal of Shellfish Research 18, 107–114.

Baud, J. P., Gerard, A., Graven, N.Y., 1997. Comparative growth and mortality of *Bonamia ostrea* resistant and wild flat oyster, *Ostrea edulis*, in an intensive system. Marine Biology 130, 71-79.

Bavestrello, G., Cerrano, C., Cattaneo-Vietti, R., Sara, M., 1996. Relations between *Eudendrium glomeratum* (Cnidaria, Hydromedusae) and its associated vagile fauna. Scientia Marina 60, 137-143.

Bayne, B.L., 1969. The gregarious behaviour of the larvae of *Ostrea edulis* L. at settlement. Journal of the Marine Biological Association of the United Kingdom 49, 327-356.

Bayne, B.L., 1998. The physiology of suspension feeding bivalve molluscs: an introduction to the Plymouth 'TROPHEE' workshop. Journal of Experimental Marine Biology and Ecology 219, 1–19.

Bayne, B.L., Hedgecock, D., Mcgolrick, D., Ress, R., 1999. Feeding behaviour and metabolic efficiency contribute to growth heterosis in Pacific oysters *Crassostrea gigas*. Journal of Experimental Marine Biology and Ecology 233, 115-130.

Bayne, B.L, Iglesias, J.I.P., Hawkins, A.J.S., Navarro, E., Héral, M., Deslous-Paoli, J.M., 1993. Feeding behaviour of the mussel *Mytilus edulis*: Responses to variations in quantity and organic content of the seston. Journal of the Marine Biological Association of the United Kingdom 73, 813-829.

Bayne, B. L., Widdows, J., 1978. The physiological ecology of two populations of *Mytilus edulis* L. Oecologia 37,137-162.

Beaumont, A., Gowland, D., 2002. Do genes or the environment control growth of flat oyster seed? Results from a European Union Craft study. Shellfish News 14, 15-16.

Beaumont, N.J., Austen, M.C., Atkins, J.P., Burdon, D., Degraer, S., Dentinho, T.P., Derous, S., Holm, P., Horton, T., van Ierland, E., Marboe, A.H., Starkey, D.J., Townsend, M., Zarzycki, T., 2007. Identification, definition and quantification of goods and services provided by marine biodiversity: implications for the ecosystem approach. Marine Pollution Bulletin. 54, 253–265.

Beck, M. W., 2011. Oyster reefs at risk and recommendations for conservation, restoration, and management. Biological Science 61, 107–116.

Beiras, R., Camach, P.A., Albentosa, M., 1995. Short-term and long-term budget of young oyster *Ostrea edulis* L. in response to temperature. Journal of Experimental Marine Biology and Ecology 186, 221-236.

Bergquist, D.C., Hale, J.A., Baker, P., Baker, S.M., 2006. Development of ecosystem indicators for the Suwannee River estuary: Oyster reef habitat quality along a salinity gradient. Estuaries and Coasts 29, 353-360.

Berghahn, R., Ruth, M., 2005. The disappearance of oysters from the Wadden Sea: a cautionary tale for no-take zones. Aquatic Conservation Marine and Freshwater Ecosystems15, 91-104.

Berrill, N.J., 1947. The development and growth of *Ciona*. Journal of the Marine Biological Association of the United Kingdom 26, 606-615.

Berthe, F., Le Roux, F., Adlard, R., Figueras, A., 2004. Marteiliosis in molluscs: a review. Aquatic Living Resources 17, 433–448.

Beukers, J.S., Jones, G.P., 1997. Habitat complexity modifies the impact of piscivores on a coral reef fish population. Oecologia 114, 50-59.

Biodiversity Reporting and Information Group, 2007. Report on the species and habitat review. Biodiversity Reporting and Information Group (BRIG) to the UK Standing Committee. UK. 172 pp. (cited 23/12/12): *http://jncc.defra.gov.uk /pdf/UKBAP_Species-HabitatsReview-2007.pdf*.

Blaise, C., Gagné, F., Pellerin, J., Hansen, P.D., 1999. Determination of vitellogenin-like properties in *Mya arenaria* haemolymph (Saguenay Fjord, Canada): a potential biomarker of endocrine disruption Environ. Journal of Toxicology 14, 455-465.

Blaise, C., Gagné, F., Salazar, M., Salazar, S., Trottier, S., Hansen, P.D., 2003. Experimentally induced feminisation of freshwater mussels after long-term exposure to a municipal effluent. Fresenius Environmental Bulletin 12, 865– 870.

Bohnsack, J.A., Johnson, D.L., Ambrose, R.F., 1991. Ecology of Artificial Reef Habitats and Fishes. In: Seaman, W., Sprague, L.M. (Eds.), Artificial Habitats for Marine and Freshwater Fisheries. Academic Press, New York, USA, pp 61-107.

Bohnsack, J.A., Sutherland, D.L., 1985. Artificial reef research: a review with recommendations for future priorities. Bulletin of Marine Science 37, 11-39.

Bonar, D.B., Weiner, R.M., Colwell, R.R., 1986. Microbial-invertebrate interactions and potential for biotechnology. Microbial Ecology 12, 101-110.

Bonsdorff, E., Wenne, R., 1989. A comparison of condition indices of *Macoma balthica* (L.) from the northern and southern Baltic Sea. Netherlands Journal of Sea Research 23, 45-55.

Boscolo, R., Cornello, M., Giovanardi, O., 2003. Condition index and air survival time to compare three kinds of Manila clam *Tapes philippinarum* (Adams & Reeve) farming systems. Aquaculture International 11, 243-254.

Bray, J.R., Curtis, J.T., 1957. An ordination of upland forest communities of southern Wisconsin. Ecological Monographs 27, 325-349.

Breitburg, D.L., Coen, L.D., Luckenbach, M.W., Mann, R., Posey, M., Wesson, J.A., 2000. Oyster reef restoration: Convergence of harvest and conservation strategies. Journal of Shellfish Research 19, 371–377.

Breitburg, D.L., Palmer, M.A., Loher, T., 1995. Larval distributions and the spatial patterns of settlement of an oyster reef fish: Responses to flow and structure. Marine Ecology Progress Series 125, 45-60.

Brooks, J.D., 1994. The Aetiology of Environmental Stress Responses and Disease in Bivalve Molluscs. PhD Thesis, University of Southampton, UK. 280 pp.

Brown, R.C., Lockwood, A.H., Sonawane, B.R., 2005. Neurodegenerative diseases: An overview of environmental risk factors. Environmental Health Perspectives 113, 1250-1256.

Brumbaugh, R.D., Beck, M.W., Coen, L.D., Craig, L., Hicks, P., 2006. A practitioner's guide to the design and monitoring of shellfish restoration projects: an ecosystem services approach. The Nature Conservancy, Arlington, USA. 28 pp.

Brumbaugh, R.D., Coen, L.D., 2009. Contemporary approaches for small-scale oyster reef restoration to address substrate versus recruitment limitation: a review and comments relevant for the Olympia oyster, *Ostrea lurida* Carpenter 1864. Journal of Shellfish Research 28, 147-161.

Brumbaugh, R.D., Sorabella, L.A., Johnson, C., Goldsborough, W.J., 2000. Small-scale aquaculture as a tool for oyster restoration in Chesapeake Bay. Journal of Marine Science and Technology 34, 79-86.

Brumbaugh, R.D., Toropova, C., 2008. Economic valuation of ecosystem services: A new impetus for shellfish restoration? Basins and Coasts News 2, 8-15.

Bucke, D., 1988. Pathology of bonamiasis. Parasitology Today 4, 174-l76.

Bulleri, F., Chapman, M.G., Underwood A.J., 2005. Intertidal assemblages on seawalls and vertical rocky shores in Sydney Harbour (Australia). Austral Ecology 30, 655–667.

Burman, S.G., Aronson, R.B., van Woesik, R., 2012. Biotic homogenization of coral assemblages along the Florida reef tract. Marine Ecology Progress Series 467, 89-97.

Butt, D.T., Raftos, D.A., 2008. Phenoloxidase associated cellular defence in the Sydney rock oyster, *Saccostrea glomerata*, provides resistance against QX disease infections. Developmental and Comparative Immunology 32, 299–306.

Buxton, C.D., Newell, R.C., Field, J.G., 1981. Response-surface analysis of the combined effects of exposure and acclimation temperatures on filtration, oxygen consumption and scope for growth in the oyster *Ostrea edulis*. Marine Ecology Progress Series 6, 73-82.

Cano, J., Rosique, M.J., Rocamora, J., 1997. Influence of environmental parameters on reproduction of the European flat oyster (*Ostrea edulis*) in a coastal lagoon (Mar Menor, southeastern Spain). Journal of Molluscan Studies 63, 187–196.

Carballal, M.J., Lo´pez, M.C., Azevedo, C., Villalba, A., 1997. *In vitro* study of phagocytic ability of *Mytilus galloprovincialis* Lmk. haemocytes. Fish and Shellfish Immunology 7, 403-416.

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., Mace, G.M., Tilman, D., Wardle, D. A., Kinzig, A.P., Daily, G.C., Loreau, M., Grace, J.B., 2012. Biodiversity loss and its impact on humanity. Nature 486, 1–9

Carnegie, R.B., Laureau, C.N., 2004. Microcell parasites of oysters: Recent insights and future trends. Aquatic Living Resources 17, 519–528.

Carroll, J.C., Gobler, C.J., Peterson, B.P., 2008. Resource limitation of eelgrass in New York estuaries; light limitation and nutrient stress alleviation by hard clams. Marine Ecology Progress Series 369, 39–50.

Castel, J., Abourg, P. J. L., Scaravage, V.E., Auby, I., Garcia, M.E., 1989. Influence of seagrass beds and oyster parks on the abundance and biomass patterns of meio- and macro-benthos in tidal flats. Estuarine Coastal and Shelf Science 28, 71–85.

Castro, N.F., de Mattio, N.D.V., 1987. Biochemical composition, condition index and energy value of *Ostrea peulchana* (D'Orbigny): Relationships with the reproductive cycle. Journal of Experimental Marine Biology and Ecology 108, 113-126.

Cavanaugh, C.M., 1983. Symbiotic chemoautotrophic bacteria in marine invertebrates from sulphide-rich habitats. Nature 302, 58-61.

Chanley, P.E., 1958. Survival of some juvenile bivalves in water of low salinity. Proceedings of the National Shellfisheries Association 48, 52–65.

Charbonnel, E., Serre, C., Ruitton, S., Harmelin, J.G., Jensen, A., 2002. Effects of increase habitat complexity on fish assemblages associated with large artificial reef units (French Mediterranean coast). ICES Journal of Marine Science 59, 208-213.

Chávez-Villalba, J., Barret, J., Mingant, C., Cochard, J.C., Le Pennec, M., 2002. Autumn conditioning of the oyster *Crassostrea gigas*: A new approach. Aquaculture 210, 171-186.

Cheng, T.C., 1981. Bivalves, in: Ratcliffe, N.A., Rowley, A.F. (Eds.), Invertebrate Blood Cells. Academic Press, London, UK, pp 233-299.

Cheng, T.C., Howland, K.H., 1979. Chemotactic attraction between hemocytes of the oyster, *Crassostrea virginica*, and bacteria. Journal of Invertebrate Pathology 33, 204-210.

Chia, F.S., 1989. Differential Larval Settlement of Benthic Marine Invertebrates. In: Ryland, J.S., Tyler, P.A. (Eds.) Reproduction, Genetics, and Distribution of Marine Invertebrate Larvae. Olsen and Olsen, Fredenborg, Denmark. pp 283-285.

Chu, F.E., 2000. Defence Mechanisms of Marine Bivalves, in: Fingerman, M., Nagabhushanam, R. (Eds.), Recent Advances in Marine Biotechnology Immunology and Pathology. Science Publishers Inc., USA. pp 1-12.

Clarke, B., 2001. UK Biodiversity Action Plan for the Native Oyster. Shellfish News, 11, 7-8.

Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18, 117-143.

Clarke, K.R., Gorley, R.N., 2001. PRIMER Version 6 User Manual/Tutorial. Primer-E Ltd, Plymouth, UK. 192 pp.

Clarke, K.R., Warwick, R.M., 1994. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation. PRIMER-E, Plymouth Marine Laboratory, Plymouth, UK. 144 pp.

Clayton, M. E., 1996. Lipoprotein and heat shock proteins as measures of reproductive physiology in the soft shell clam *Mya arenaria*. PhD thesis, Massachusetts Institute of Technology, USA. 195 pp.

Cochennec, N., Le-Roux, F., Berthe, F.C.J., Gerard, A., 2000. Detection of *Bonamia ostreae* based on small subunit ribosomal probe. Journal of Invertebrate Pathology 76, 26-32.

Coen, L.D, Brumbaugh, R.D., Bushek, D., Grizzle, R., Luckenbach, M.W., Posey, M.H., Powers, S.P., Tolley, S.G., 2007. Ecosystem services related to oyster restoration. Marine Ecology Progress Series 341, 303-307.

Coen, L.D., Luckenbach, M.W., 2000. Developing success criteria and goals for evaluating oyster reef restoration: ecological function or resource exploitation? Ecological Engineering 15, 323-343.

Coen, L.D., Luckenbach, M.W., Breitburg, D.L., 1999. The role of oyster reefs as essential fish habitat: a review of current knowledge and some new perspectives. in Benaka L.R., (ed.). Fish habitat: essential fish habitat and rehabilitation. American Fisheries Society, Symposium 22, Bethesda, MD, pp 438-454.

Coen, L. D., Mark, W.L., 2000. Developing success criteria and goals for evaluating oyster reef restoration: ecological function or resource exploitation? Ecological Engineering 15, 323-343.

Cole, H.A., Knight-Jones, E.W., 1949. The setting behaviour of larvae of the European flat oyster, *Ostrea edulis*, and its influence on methods of cultivation and spat collection. Fisheries Investigations 17, 2-9.

Collins, K.J., Jensen, A.C., Lockwood, A.P.M., and Lockwood, S.J., 1994. Coastal structures, waste materials and fishery enhancement. Bulletin of Marine Science 55, 1253–1264.

Collins, K.J., Jensen, A.C., Lockwood, A.P.M., 1991. Artificial reef project - Poole Bay programme. Progress in Underwater Science 16, 75-84.

Collins, K.J., Mallinson, J.J., 1984. Colonisation of the “Mary Rose” excavation. Progress in Underwater Science 9, 67-74.

Comeau, L.A. 2013. Suspended versus bottom oysters culture in eastern Canada: comparing stocking densities and clearance rates. Aquaculture 410, 57-65.

Comeau, L.A., 2014. Spring awaking temperature and survival of sediment-covered eastern oysters *Crassostrea virginica*. Aquaculture 430, 188-194.

Comeau, L.A., Sonier, R., Lanteigne, L., Landry, T., 2010. A novel approach to measuring chlorophyll uptake by cultivated oysters. Aquacultural Engineering 43, 71-77.

Comesana, P., Casas, M.S., Cao, A., Abollo, E., Arzul, I., Morga, B., Villalba, A., 2012. Comparison of haemocytic parameters among flat oyster *Ostrea edulis* stocks with different susceptibility to bonamiosis and the Pacific oyster *Crassostrea gigas*. Journal of Invertebrate Pathology 109, 274-286.

Cotter, E., O'Riordan, R.M., Myers, A.A., 2003. Recruitment patterns of serpulids (Annelida: Polychaeta) in Bantry Bay, Ireland. Journal of the Marine Biological Association of the United Kingdom 83, 41-48.

Coughlan, J., 1969. The estimation of filtering rate from the clearance of suspensions. Marine Biology 2, 356–358.

Craft, C., Sacco, J., 2003. Long-term succession of benthic infauna communities on constructed *Spartina alterniflora* marshes. Marine Ecology Progress Series 257, 45-48.

Cranford, P.J., Hargrave, B.T., 1994. *In situ* time-series measurement of ingestion and absorption rates of suspension-feeding bivalves: *Placopecten magellanicus*. Limnology and Oceanography 39, 730–738.

Crisp, D.J., 1965. The Ecology of Marine Fouling. In (ed) Goodman, G.T., Edwards R.W., Lambert J.M., Ecology and the Industrial Society, 5th Symposium of the British Ecological Society, London, UK, pp 99-117.

Culloty, S.C., Mulcahy, M.F., 1996. Season-, age-, and sex-related variations in the prevalence of bonamiasis in flat oyster (*Ostrea edulis* L.) on the south coast of Ireland. Aquaculture 144, 53-63.

Culloty, S.C., Novoa, B., Pernas, M., Longshaw, M., Mulcahy, M.F., Feist, S.W., Figueras, A., 1999. Susceptibility of a number of bivalve species to the protozoan parasite *Bonamia ostreae* and their ability to act as a vector for this parasite. Diseases of Aquatic Organisms 37, 73-80.

Cusson, M., Tremblay, R., Daigle, G., Roussy, M., 2005. Modeling the depuration potential of blue mussels (*Mytilus* spp.) in response to thermal shock. Aquaculture 250,183 -193.

Dame, R.F., 1975. Day degree growth models for intertidal oysters. Contributions in Marine Science 19, 107-112.

Dame, R.F., 1979. The abundance, diversity, and biomass of macrobenthos on North Inlet, South Caralina, intertidal oyster reefs. Proceedings of the National Shellfish Association 69, 6-10.

Dame, R.F., 1996. Ecology of marine bivalves: an ecosystem approach. CRC Marine Science Series, Boca Raton. 254 pp.

Dame, R.F., Bushek, D., Allen, D., Edwards, D., Gregory, L., Lewitus, A., Crawford, A., Koepfler, E., Corbett, C., Kjerfve, B., Prins, T., 2000. The experimental analysis of tidal creeks dominated by oyster reefs: the premanipulation year. Journal of Shellfish Research 19, 361–370.

Dame, R.F., Spurrier, J.D., Zingmark, R.G., 1992. *In situ* metabolism of an oyster reef. Journal of Experimental Marine Biology and Ecology 164, 147-159.

Dame, R.F., Zingmark, R.G., Haskins, E., 1984. Oyster reefs as processors of estuarine materials. Journal of Experimental Marine Biology and Ecology 83, 239-247.

da Silva, E.R., Silva M.F., Fischer, H., Mortara, R.A., Mayer, M.G., Framesqui, K., Silber, A.M., Floeter-Winter, L.M., 2008. Biochemical and biophysical properties of a highly active recombinant arginase from *Leishmania* (*Leishmania*) *amazonensis* and subcellular localization of native enzyme. Molecular and Biochemical Parasitology 159, 104-111.

da Silva, P.M.D., Fuentes, J., Villalba, A., 2009. Difference in gamatogenic cycle among strains of the European flat oyster *Ostrea edulis* and relationship between gametogenesis and bonamiosis. Aquaculture 287, 253-265.

Davis, H.C., Calabrese, A., 1969. Survival and growth of larvae of European oyster (*Ostrea edulis*) at different temperatures. Biological Bulletin 136, 193–199.

Degen, J. and Nussberger, F.E., 1956. Notes on the determination of suspended solids. Sewage Industrial Wastes 28, 237-245.

Delgado, G.A., Bartels, C.T., Glazer, R.A., Brown-Peterson, N.J., McCarthy, K.J., 2004. Translocation as a strategy to rehabilitate the queen conch (*Strombus gigas*) population in the Florida Keys. Bulletin of the United States Bureau of Fisheries 102, 278-288.

Denis, L., Alliot, E., Grzebyk, D., 1999. Clearance rate responses of Mediterranean mussels, *Mytilus galloprovincialis*, to variations in the flow, water temperature, food quality and quantity. Aquatic Living Resources 12, 279–288.

Doak, D.F., Bigger, D., Harding, E.K., 1998. The statistical inevitability of stability- diversity relationships in community ecology. The American Naturalist 151, 264-276.

Dodd, J. M., McCloy, J., Davies, H.C., Edmonds, F.B.J., Orton, J.H., 1937. Maturity and Fecundity of one year old English native oysters, *O. edulis*. Nature 136, 108-109.

Eagling, L., 2012. Reproductive Success of the Re-laid Native Oyster *Ostrea edulis* in Chichester harbour. Master's Thesis. University of Southampton, UK. 63 pp.

Edwards, E., 1997. Molluscan Fisheries in Britain. National Oceanic and Atmospheric Admistration and U.S. Department of Commerce, Washington. USA. 129 pp.

Elmqvist, T., Folke, C., Nyström, M., Peterson, G., Bengtsson, J., Walker, B., Norberg J., 2003. Response diversity, ecosystem change, and resilience. Frontiers in Ecology and the Environment 1, 488–494.

Engelsma, M.Y., Kerkhoff, S., Roozenburg, I., Haenen, O.L.M., van Gool, A., Sistermans, W., Wijnhoven, S., Hummel, H., 2010. Epidemiology of *Bonamia ostreae* infecting European flat oyster *Ostrea edulis* from Lake Grevelingen, The Netherlands. Marine Ecology Progress Series 409, 131-142.

Enríquez-Díaz, M., Pouvreau, S., Chávez-Villalba, J., Le Pennec, M., 2009. Gametogenesis, reproductive investment, and spawning behavior of the Pacific giant oyster *Crassostrea gigas*: evidence of an environment-dependant strategy. Aquaculture International 17, 491-506.

Ezgeta-Balic, D., Rinaldi, A., Peharda, M., Prusina, I., Montalto, V., Niceta, N., Sara, G., 2011. An energy budget for the subtidal bivalve *Modiolus barbatus* (Mollusca) at different temperatures. Marine Environmental Research 71, 79-85.

Fabioux, C., Huvet, A., Le Souchu, P., Le Pennec, M., Pouvreau, S., 2005. Temperature and photoperiod drive *Crassostrea gigas* reproductive internal clock. Aquaculture 250, 458– 470.

Fanelli, E., Papiol, V., Cartes, J. E., Rodriguez-Romeu, O., 2014. Trophic ecology of *Lampanyctus crocodilus* on north-west Mediterranean Sea slopes in relation to reproductive cycle and environmental variables. Journal of Fish Biology 84, 1654-1688.

Fariñas-Franco, J.M., Allcock, L., Smyth, D., Robersts, D., 2013. Community convergence and recruitment of keystone species as performance indicators of artificial reefs. Journal of Sea Research 78, 50–74.

Fauchald, K., Jumars, P.A., 1979. The diet of worms: a study of Polychaete feeding guilds. Oceanography and Marine Biology: An Annual Review 17, 193-284.

Fearman, J., Moltschaniwskyj, N.A., 2010. Warmer temperatures reduce rates of gametogenesis in temperate mussels, *Mytilus galloprovincialis*. Aquaculture 305, 20-25.

Fernandez-Reiriz, M.J., Labarta, U., Babarro, J.M.F., 1996. Comparative allometries in growth and chemical composition of mussel (*Mytilus galloprovincialis* Lmk) cultured in two zones in the Ria Sada (Galicia, NW Spain). Journal of Shellfish Research 15, 349-353.

Field, J.G., Clarke, K.R., Warwick, R.M., 1982. A practical strategy for analysing multispecies distribution patterns. Marine Ecology Progress Series 8, 37-52.

Fikes, R., 2013. Artificial Reefs of the Gulf of Mexico: A review of Gulf state programs and key considerations. Gulf restoration campaign, National Wildlife Federation. USA. 22 pp.

Filgueira, R., Comeau, L.A., Landry, T., Grant, J., Guyondet, T., Mallet, A., 2013. Bivalve condition index as an indicator of aquaculture intensity: A meta-analysis. Ecological Indicators 25, 215-229.

Finke, D.L., Denno, R.F., 2002. Intraguild predation diminished in complex-structured vegetation: implications for prey suppression. Ecology 83, 643–652.

Fisher, W.S., 1987. Response of European flat oyster (*Ostrea edulis*) hemocytes to acute salinity and temperature change. Aquaculture 67, 179-190.

Fisher, W.S., 1988. Environmental influence on bivalve hemocyte function. American Fisheries Society Special Publications 18, 225-37.

Fisher, W.S., Newell, R.I.E., 1986. Salinity effects on the activity of granular hemocytes of American oysters, *Crassostrea virginica*. Biological Bulletin 170, 122-134.

Fisher, W.S., Oliver, L.M., Edwards, P., 1996. Hematologic and serologic variability of eastern oysters from Apalachicola bay, Florida. Journal of Shellfish Research 19, 555-564.

Fitt, W.K., Coon, S.L., Walch, M., Weiner, R.M., Colwell, R. R., Bonar, D. B., 1990. Settlement behavior and metamorphosis of oyster larvae (*Crassostrea gigas*) in response to bacterial supematants. Marine Biology 106, 389-394.

Ford, S.E., 1986. Comparison of haemolymph proteins from resistant and susceptible oysters, *Crassostrea virginica*, exposed to the parasite *Haplosporidium nelsoni* (MSX). Journal of Invertebrate Pathology 47, 283-294.

Ford, S.E., Ashton-Alcox, K.A., 1993. *In vitro* interactions between bivalve haemocytes and the oyster pathogen *Haplosporidium nelsoni* (MSX). Journal of Parasitology 79, 255-265.

Fox, H.M., Wingfield, C.A., 1937. The oxygen consumption of ephemerid nymphs from flowing and from still waters in relation to the concentration of oxygen in the water. The British Journal of Experimental Biology 14, 210-218.

Frechette, M., Butman, C.A., Geyer, W.R., 1989. The importance of boundary-layer flows in supplying phytoplankton to the benthic suspension feeder, *Mytilus edulis* L. Limnology and Oceanography 34, 19-36.

Fretter, V., Graham, A., 1981. The prosobranch molluscs of Britain and Denmark, part 6. Journal of Molluscan Studies 9, 285-363.

Fretter, V., Manly, R., 1977. Algal associations of *Tricolia pullus*, *Lacuna vincta* and *Cerithiopsis tubercularis* (Gastropoda) with special reference to the settlement of their larvae. Journal of the Marine Biological Association of the United Kingdom 57, 999-1017.

Fujita, T., Kitagawa, D., Okuyama, Y., Jin, Y., Ishito, Y., Inada, T., 1996. Comparison of fish assemblages among an artificial reef, a natural reef and a sandy-mud bottom site on the shelf off Iwate, northern Japan. Environmental Biology of Fishes 46, 351-364.

Fulford, R.S., Breitburg, D.L., Luckenbach, M., Newell, R.I.E., 2010. Evaluating ecosystem response to oyster restoration and nutrient load reduction with a multispecies bioenergetics model. Ecological Applications 20, 915-934.

Fulford, R.S., Breitburg, D.L., Newell, R.I.E., Kemp, W.M., Luckenbach, M., 2007. Effects of oyster population restoration strategies on phytoplankton biomass in Chesapeake Bay: a flexible modeling approach. Marine Ecology Progress Series 336, 43-61.

Furlong, J.N., 2007. Artificial Oyster Reefs in the Northern Gulf of Mexico: Management, Materials and Faunal Effects. Master's thesis. University of Northern Iowa. USA. 109 pp.

Gagné, F., Blaise, C., Pellerin, J., Gauthier-Clerc, S., 2002. Alteration of the biochemical properties of female gonads and vitellins in the clam *Mya arenaria* at contaminated sites in the Saguenay Fjord. Marine Environmental Research 53, 295-310.

Gascoigne, J., Lipcius, R.N., 2004. Conserving populations at low abundance: delayed functional mortality and Allee effects in reproductive behaviour of the queen conch *Strombus gigas*. Marine Ecology Progress Series 284, 185-194.

Gonzalez-Araya, R., Mingant, C., Petton, B., Robert, R., 2012. Influence of diet assemblage on *Ostrea edulis* broodstock conditioning and subsequent larval development. Aquaculture 364, 272-280.

Gosling, E., 2003. Bivalve Mollusca: Biology, Ecology and Culture. Fishing News Books, Wiley-Blackwell Science, West Sussex, UK. 443 pp.

Goulletquer, P., Heral, M., Deslouspaoli, J.M., Prou, J., Garnier, J., Razet, D., Boromthanarat, W., 1999. Ecophysiology and energy balance of the Manila clam, *Ruditapes philippinaram*. Journal of Experimental Marine Biology and Ecology 132, 85-108.

Grabowski, J.H., 2004. Habitat complexity disrupts predator-prey interactions but not the trophic cascade on oyster reefs. Ecology 84, 995-1004.

Grabowski, J.H., Brumbaugh, R., Conrad, R., Keeler, A., Opaluch, J., Peterson, C., Piehler, M., Powers, S., Smyth, A., 2012. Economic valuation of ecosystem services provided by oyster reefs. BioScience 62, 900-909.

Grabowski, J.H., Hughes, A.R., Kimbro, D. L., Dolan, M.A., 2005. How habitat setting influences restored oyster reef communities. Ecology 86, 1926-1935.

Grabowski, J.H., Peterson, C.H., 2007. Restoring Oyster Reefs to Recover Ecosystem Services. In: Cuddington, K., Byers, J.E., Wilson, W.G., Hastings, A. (eds) Ecosystem Engineers: Concepts, Theory and Applications. Elsevier-Academic Press, Amsterdam, Netherlands, pp 281-298.

Grant, J., Enright, C.T., Griswold, A., 1990. Resuspension and growth of *Ostrea edulis*: a field experiment. Marine Biology 104, 51-59.

Gravestock, V., James, F., Goulden, M., 2014. Solent native oyster (*Ostrea edulis*) Restoration: literature review and feasibility study. MacAlister, Elliott and Partners, UK. 77 pp. (cited15/05/15):*http://www.bluemarinefoundation.com /wp-content/uploads/2015/04/MEP-report_Restoration-of-the-Solent-oyster-fishery_FINAL1.pdf*.

Greene, C.H., Schoener, A., 1982. Succession on marine hard substrata. A fixed lottery. Oecologia 55, 289-29.

Greene, J., Grizzle, R., 2005. Oyster (*Crassostrea virginica*) restoration studies in the Great Bay estuary, NOAA award no. NA03NOS4200060. New Hampshire, UK. 23 pp.

Gristina, M., D'Anna, G., Parisi, R., Riggio, S., Toccaceli, M., 1996. Epibiosis on *Ostrea edulis*. Biologia Marina Mediterranea 3, 474-475.

Grizel, H., Comps, M., Raguennes, D., Leborgne, Y., Tige, G., Martin, A.G., 1983. Bilan des essais d'acclirnation d' *Ostrea chilensis* sur les cites de Bretagne. Revue des Travaux de l'Institut des Peches Maritimes 46, 209-225.

Grizel, H., Heral, H., 1991. Introduction into France of the Japanese oyster (*Crassostrea gigas*). Journal du Conseil 47, 399-403.

Grizzle, R.E, Adams, J., Walters, L., 2002. Historical changes in intertidal oyster (*Crassostrea virginica*) reefs in a Florida lagoon potentially related to boating activities. Journal of Shellfish Research 21, 749-756.

Guerra-García, J.M., De Figueroa J.M., Navarro-Barranco, C., Ros M., Sánchez-Moyano, J.E., Moreira, J., 2014. Dietary analysis of the marine Amphipoda (Crustacea: Peracarida) from the Iberian Peninsula. Journal of Sea Research 85, 508-517.

Gunther, R., 1897. The oyster culture of the ancient Romans. Journal of the Marine Biological Association of the United Kingdom 4, 360-365.

Gustaffson, L., Nordling, D., Anderson, M.S., Sheldon, B.C., Quarstrom, D.A., 1994. Infectious disease, reproductive effort, and the cost of reproduction in birds. Philosophical Transactions of the Royal Society B 346, 323–331.

Hagerman, L., 1983. Haemocyanin concentration of juvenile lobsters (*Homarus gammarus*) in relation to moulting cycle and feeding conditions. Marine Biology 77, 11-17.

Hand, S.C., Hardewig, I., 1996. Downregulation of cellular metabolism during environmental stress: mechanisms and implications. Annual Review of Physiology 58, 539-563.

Harding, J.M., Mann, R., 1999. Fish species richness in relation to restored oyster reefs, Piankatank River, Virginia. Bulletin of Marine Science 65, 289-300.

Harding, J.M., Mann, R., 2001. Oyster reef habitat use by estuarine fishes: Optimal or essential fish habitat? Journal of Shellfish Research 20, 951-959.

Harding, T., 1996. Reintroduction of the Native Oyster (*Ostrea edulis*) into the Firth of Forth, Scotland: a feasibility study. Master's thesis, Napier University, UK. 102 pp.

Harrison, P.A., Berry, P.M., Simpson, G., Haslett, J.R., Blicharska, M., Bucur, M., Dunford, R., Egoh, B., Garcia-Llorente, M., Geamănă, N., Geertsema, W., Lommelen, E., Meiresonne, L., Turkelboom, F., 2014. Linkages between biodiversity attributes and ecosystem services: A systematic review. Ecosystem Services 9, 191–203.

Haure, J., Penisson, C., Bougrier, S., Baud, J.P., 1998. Influence of temperature on clearance and oxygen consumption rates of the flat oyster *Ostrea edulis*: determination of allometric coefficients. Aquaculture 169, 211-224.

Hauton, C., Hawkins, L.E., Hutchinson, S., 1998. The use of the neutral redretention assay to examine the effects of temperature and salinity on haemocytes of the European flat oyster *Ostrea edulis* (L). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 119, 619-623.

Hauton, C., Hawkins, L.E., Hutchinson, S., 2000. The effects of salinity on the interaction between a pathogen (*Listonella anguillarum*) and components of a host (*Ostrea edulis*) immune system. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 127, 203-212.

Hawkins, A.S.J., Bayne, B.L., Bougier, S., Heral, M., Iglesias, J.I.P., Navarro, E., Smith, R.F.M., Urrutia, M.B., 1998. Some general relationships in comparing the feeding physiology of suspension-feeding bivalve molluscs. Journal of Experimental Marine Biology and Ecology 219, 87-103.

Hawkins, L., Hutchinson, S., Brooks, J.D., Bucke, D., 1993. Differential responses of three groups of *Ostrea edulis* to environmental stress. In: Aldrich, J.C. (Ed.). Proceedings of 27th European Marine Biology Symposium, Aldrich Press, Ashford, Ireland, pp 207-214.

Hayward, P.J., Ryland, J.S., 1996. Handbook of the Marine Fauna of North-West Europe. Oxford University Press, Oxford, UK. 812 pp.

Hedgecock, D., Launey, S., Pudovkin, A.I., Naciri, Y., Lapègue, S., Bonhomme, F., 2007. Small effective number of parents inferred for a naturally spawned cohort of juvenile European flat oysters *Ostrea edulis*. Marine Biology 150, 1173-1182.

Helson, J.G., Pledger, S., Gardner, J.P.A., 2007. Does different particulate food supply explain the presence of mussel in Wellington Harbour (New Zealand) and their absence on neighbouring Cook Strait shores? Estuarine, Coastal and Science 71, 223-234.

Herbert, R.J.H., Short, H., Burnard, D., Pegg, J., Ross, K., Britton, R., 2013. An initial investigation into the contribution of a novel artificial surf reef to sustainable fisheries. Report to the Marine Management Organisation Project FES 250. Dorset, UK. 36 pp. (cited 07/08/14): *https://www.gov.uk/government/uploads /system/uploads/attachment_ data/file/313001/fcf-asr.pdf*.

Hickman, R.W., Waite, R.P., Illingworth, J., Meredyth-Young, J.L., Payne, G., 1991. The relationship between farmed mussels, *Perna canaliculus*, and available food in Pelorus-Kenepuru Sound, NewZealand, 1983-1985. Aquaculture 99, 49-68.

Hilbish, T.J., Zimmerman, K.M., 1988. Genetic and nutritional control of the gametogenic cycle in *Mytilus edulis*. Marine Biology 98, 223-228.

Holland, D.A., 1978. Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates. In: Malins, D.C., Sargent, J.R. (Eds.), Biochemistry and biophysical perspectives in marine biology, vol. 4. Academic Press, UK, pp 85-123.

Honkoop, P.J.C., Beukema, J.J., 1997. Loss of body mass in winter in three intertidal bivalve species: An experimental and observational study of the interacting effects between water temperature, feeding time and feeding behaviour. Journal of Experimental Marine Biology and Ecology 212, 277-297.

Honkoop, P.J.C., van der Meer J., 1998. Experimentally induced effects of water temperature and immersion time on reproductive output of bivalves in the Wadden Sea. Journal of Experimental Marine Biology and Ecology 220, 227-246.

Hooper, D.U., Adair, E.C., Cardinale, B.J., Byrnes, J.E.K., Hungate, B.A., Matulich, K.L., Gonzalez, A., Duffy, J.E., Gamfeldt, L., O'Connor, M.I., 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature 486, 105-108.

Houziaux J.S., Kerckhof F., Degrendele, K., Roche, M., Norro, A., 2008. The Hinder banks: yet an important area for the Belgian marine biodiversity? Belgian Science Policy, Scientific support plan for as sustainable development policy (SPSD II), Final Report, Research contract n° EV/36/45. Belgium. 249 pp. (cited 09/09/13):*http://www.belspo.be/belspo/home/publ/pub_ostc/EV/rappEV45_en.pdf*.

Howard, D.A., Smith, C.S., 1983. Histological techniques for marine bivalve molluscs, NOAA Technical Memorandum NMFS-F/NEC.25. Technical report. Woods Hole, Massachusetts. USA. 298 pp.

Howland, K.H., Cheng, T.C., 1982. Identification of bacterial chemoattractants for oyster (*Crassostrea virginica*) hemocytes. Journal of Invertebrate Pathology 39, 123-132.

Howard K.W., Smithers H.A., Walker S., Wyatt T., 1994. Evaluating the impact of drought management measures within complex water resource systems in northwest England. In: Advances in Water Resources Technology and Management (ed. by Tsakiris, G., Santos, M.A.). Balkema, Rotterdam, Netherlands, pp 155-162.

Huang, S.C., Newell, R.I.E., 2002. Seasonal variations in the rates of aquatic and aerial respiration and ammonium excretion of the ribbed mussel, *Geukensia demissa* (Dillwyn). Journal of Experimental Marine Biology and Ecology 270, 241-255.

Hugh-Jones, T., 2003. The Loch Ryan native oyster fishery. Shellfish News 15, 17–18.

Humphries, N.E., Weimerskirch, H., Queiroz, N., Southalla, E.J., Sims, D.W., 2012. Foraging success of biological Lévy flights recorded *in situ*. Proceedings of the National Academy of Sciences109, 7169–7174.

Hutchinson, S., Hawkins, L.E., 1992. Quantification of the physiological responses of the European flat oyster *Ostrea edulis* to temperature and salinity. Journal of Molluscan Studies 58, 215-216.

Jackson, A., Wilding, C., 2007. *Ostrea edulis*. Native oyster. Marine Life Information Network: Biology and Sensitivity Key Information Sub-programme. Plymouth: Marine Biological Association of the United Kingdom (cited 08/02/2012): *http://www.marlin.ac.uk/speciesfullreview.php?speciesID=3997*.

Jackson, J.B., Kirby, M.X., Berger, W.H., Bjorndal, K.A., Botsford, L.W., Bourque, B.J., Bradbury, R.H., Cooke, R., Erlandson, J., Estes, J.A., Hughes, T.P., Kidwell, S., Lange, C.B., Lenihan, H.S., Pandolfi, J.M., Peterson, C.H., Steneck, R.S., Tegner, M.J., Warner, R.R., 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science 293, 629-638.

Jensen A.C., 2002. Artificial reefs in Europe: Perspective and future. ICES Journal of Marine Science 59, 3-13.

Jensen, A.C., Collins, K.J., Smith, P., 2000. The Poole Bay artificial reef project. In: Jensen A.C., Collins K.J., Lockwood A.P.M. (eds) Artificial reefs in European Seas. Kluwer Academic Publishers, London, UK, pp 319-329.

Jo, P.G., Choi, Y.K., Choi, C.Y., 2008. Cloning and mRNA expression of antioxidant enzymes in the Pacific oyster, *Crassostrea gigas*, in response to cadmium exposure. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 147, 460–469.

Johannesson, K., Rödstörm, E.M., Aase, H., 1989. Low genetic variability in Scandinavian populations of *Ostrea edulis* L.: possible causes and implications. Journal of Experimental Marine Biology and Ecology 128, 177-190.

Jordan-Cooley, W.C., Lipcius, R.N., Shaw, L.B., Shen, J., Shi, J., 2011. Bistability in a differential equation model of oyster reef height and sediment accumulation. Journal of Theoretical Biology 289, 1-11.

Kamphausen, L., 2012. The reproductive processes of a wild population of the European flat oyster *Ostrea edulis* in the Solent, UK. PhD thesis, University of Southampton, UK. 139 pp.

Kamphausen, L., Jensen, A., Hawkins, L., 2011. Unusually high proportion of males in a collapsing population of commercially fished oysters (*Ostrea edulis*) in the Solent, United Kingdom. Journal of Shellfish Research 30, 217-222.

Kang, C.K., Lee, Y., Choy, E., Shin, J., Seo, I., Hong, J., 2006. Microphytobenthos seasonality determines growth and reproduction in intertidal bivalves. Marine Ecology Progress Series 315, 113–127.

Kang, C.K., Park, M.S., Lee, P.Y., Choi, W.J., Lee, W.C., 2000. Seasonal variations in condition, reproductive activity and biochemical composition of the Pacific oyster *Crassostrea gigas* (Thunberg), in suspended culture in two coastal bays of Korea. Journal of Shellfish Research 19, 771-778.

Katayama, K., Tahara, M., Tsumura, K., Kakimoto, H., 2004. Shell nurseries: artificial reefs using waste shells. Developments in Food Science 42, 263-271.

Keck. R., Maurer, D., Kauer, J.C., Sheppard, W.A., 1971. Chemical stimulants affecting larval settlement in the American oysters. Proceedings of the National Shellfisheries Association 61, 24-28.

Kennedy, R. J., Roberts, D. A., 1999. Survey of the current status of the flat oyster *Ostrea edulis* in Strangford lough, Northern Ireland, with a view to the restoration of its oyster beds. Biology and Environment: Proceedings of the Royal Irish Academy 99, 79-88.

Kennedy, R.J., Roberts, D.A., 2001. Restoration of native of native oyster beds in Strangford Lough, Northern Island. Shellfish News 12, 6-7.

Keough, M.J., Downes, B.J., 1982. Recruitment of marine invertebrates: the role of active larval choices and early mortality. Oecologia 54, 348–352.

Key, D., Davidson, P.E., 1981. A Review of Development of the Solent Oyster Fishery, 1971-80. Laboratory Leaflet, No. 52. Lowestoft, UK. 40 pp.

Klimant, I., Wolfbeis, O.S., 1995. Oxygen-sensitive luminescent materials based on silicone-soluble ruthenium diimine complexes. Analytical Chemistry 67, 3160-3166.

Knights, A. M., Walters, K., 2010. Recruit-recruit interactions, density dependent processes and population persistence in the eastern oyster *Crassostrea virginica*. Marine Ecology Progress Series 404, 79–90.

Korringa, P., 1941. Experiments and observations on swarming, pelagic life and setting in the European at oyster, *Ostrea edulis* L. Archives Néerlandaises de Zoologie 5, 1-249.

Korringa, P., 1946. A revival of natural oyster beds? Nature 158, 586-587.

Korringa, P., 1951. The shell of *Ostrea edulis* as a habitat. Archives Néerlandaises de Zoologie 10, 33-152.

Korringa, P., 1952. Recent advances in oyster biology. Quarterly Review of Biology 27, 339-365.

Korringa, P., 1957. Water temperature and breeding throughout the geographical range of *Ostrea edulis*. L'Année Biologique 33, 109-116.

Kuchel, R.P., Aladaileh, S., Birch, D., Vella, N., Raftos, D.A., 2010. Phagocytosis of the protozoan parasite, *Marteilia sydneyi*, by Sydney rock oyster (*Saccostrea glomerata*) hemocytes. Journal of Invertebrate Pathology 104, 97-104.

Laing, I., Walker, P., Areal, F., 2005. A feasibility study of native oyster (*Ostrea edulis*) stock regeneration in the United Kingdom. CEFAS. UK. 94 pp.

Laing, I., Walker, P., Areal, F., 2006. Return of the native-is European oyster (*Ostrea edulis*) stock restoration in the UK feasible? Aquatic Living Resources 19, 283-287.

Lallias, D.A., 2008. *Bonamia ostreae* induced mortalities in one-year old European flat oysters *Ostrea edulis*: experimental infection by cohabitation challenge. Aquatic Living Resources 21, 423-439.

Lallias, D.A., Lapegue, S., Haley, C., Heurtebise, S., Boudry, P., Beaumont, A., 2007. A preliminary genetic linkage map for the European flat oyster *Ostrea edulis*. Aquaculture 272, 281-282.

Lambert, C., Soudant, P., Choquet, G., Paillard, C., 2003. Measurement of *Crassostrea gigas* hemocyte oxidative metabolism by flow cytometry and the inhibiting capacity of pathogenic vibrios. Fish and Shellfish Immunology 15, 225-240.

Laureau, C.N., Auffret, M., Renault, T., Langlade, A., 2003. Changes in circulating and tissue-infiltrating hemocyte parameters of European flat oysters, *Ostrea edulis*, naturally infected with *Bonamia ostreae*. Journal of Invertebrate Pathology 83, 23-30.

Lenihan, H.S., 1999. Physical-biological coupling on oyster reefs: how habitat structure influences individual performance. Ecological Monographs 69, 251-275.

Lenihan, H.S., Micheli, F., Shelton, S.W., Peterson, C.H., 1999. The influence of multiple environmental stressors on susceptibility to parasites: an experimental determination with oysters. Limnology and Oceanography 44, 910–924.

Lenihan, H. S., Peterson, C.H., 1998. How habitat degradation through fishery disturbance enhances impacts of hypoxia on oyster reefs. Ecological Applications 8, 128-140.

Lenihan, H.S., Peterson, C.H., Byers, J.E., Grabowski, J.H., Thayer, G.W., Colby, D.R., 2001. Cascading of habitat degradation: oyster reefs invaded by refugee fishes escaping stress. Ecological Applications 11, 764-782.

Lehnert, R.L., Allen, D.M., 2002. Nekton use of subtidal oyster shell habitat in a southeastern U.S. estuary. Estuaries 25, 1015-1024.

Li, Y., Qin, J.G., Li, X., Benkendorff, K., 2009. Monthly variation of condition index, energy reserves and antibacterial activity in Pacific oysters, *Crassostrea gigas*, in Stansbury (South Australia). Aquaculture 286, 64-71.

Li, Y., Siddiqui, G., Wikfors, G.H., 2010. Non-lethal determination of sex and reproductive condition of Eastern oysters *Crassostrea virginica* Gmelin using protein profiles of hemolymph by Proteinchip and SELDI-TOF_MS technology. Aquaculture 309, 258-264.

Lindberg W.J., Seaman W.Jr., 2011. Guidelines and management practices for artificial reef siting, use, construction, and anchoring in Southeast Florida. Florida Department of Environmental Protection. Miami, FL. 150 pp. (cited 12/11/12): *https://www.dep.state.fl.us/coastal/programs/coral/reports/MICCI/MICCI_18_19.pdf.*

Linehan, L.G., ÓConnor, T.P., Burnell, G., 1999. Seasonal variation in the chemical composition and fatty acid profile of Pacific oysters (*Crassostrea gigas*). Food Chemistry 64, 211–214.

Lipcius, R.N., Burke, R., 2006. Abundance, biomass and size structure of eastern oyster and hooked mussel on a modular artificial reef in the Rappahannock River, Chesapeake Bay. VIMS Special Report in Applied Marine Science and Ocean Engineering, USA. 19 pp. (cited 21/10/14): *http://www.vims.edu/ GreyLit/VIMS/sramsoe390.pdf*.

Loosanoff, V.L., 1962. Gametogenesis and Spawning of the European Oyster, *O. edulis*, in Waters of Maine. The Biological Bulletin 112, 86-94.

Loosanoff, V.L., 1965. Gonad development and discharge of spawn in oysters of Long Island Sound. The Biological Bulletin 129, 546-651.

Loosanoff, V.L., Engle, J.B., 1947. Effect of different concentrations of micro-organisms on the feeding of oysters (*C. virginica*). Fishery Bulletin 42, 31-57.

Lopez, C., Carballal, M.J., Azevedo, C., Villalba, A., 1997. Differential phagocytic ability of the circulating haemocyte types of the carpet shell clam *Ruditapes decussatus* (Mollusca: Bivalvia). Diseases of Aquatic Organisms 30, 209-215.

Lorenzen, C.J., 1966. A method for the continuous measurement of in vivo chlorophyll concentration. Deep-Sea Research 13, 223-227.

Lorenzon, S., Giulianini, P.G., Libralato, S., Martinis, M., Ferrero, E.A., 2008. Stress effect of two different transport systems on the physiological profiles of the crab *Cancer pagurus*. Aquaculture 278, 156–163.

Lorenzon, S., Giulianini, P.G., Martinis M., Ferrero, E.A., 2007. Stress effect of different temperatures and air exposure during transport on physiological

profiles in the American lobster *Homarus americanus*. Comparative Biochemistry and Physiology A: Molecular and Integrative Physiology 147, 94–102.

Lorenzon, S., Martinis, M., Ferrero, E.A., 2011. Ecological relevance of hemolymph total protein concentration in seven unrelated crustacean species from different habitats measured predictively by a density-salinity refractometer. Journal of Marine Biology 2011, 1-7.

Lucas, A., Beninger, P.G., 1985. The use of physiological condition indices in marine bivalve aquaculture. Aquaculture 44, 187–220.

Luckenbach, M.W., Coen, L.D., Ross J.R., Stephen, J.A., 2005. Oyster reef habitat restoration: relationships between oyster abundance and community development based on two studies in Virginia and South Carolina. Journal of Coastal Research I40, 64–78.

Luna-González, A., María, R.G., Ángel, C.C., Javier, O.R., Rubén, V.J., César, R.V., 2008. Seasonal variations in the immunological and physiological parameters of the Pacific oyster *Crassostrea gigas* cultured in Bahía de Macapule (Sinaloa, Mexico). Aquaculture Research 39, 1488–1497.

Lynch, S.A., Armitage, D.V., Coughlan, J., Mulcahy, M.F., Culloty, S.C., 2007. Investigating the possible role of benthic macroinvertebrates and zooplankton in the life cycle of the haplosporidian *Bonamia ostreae*. Experimental Parasitology 115, 359-368.

MacDonald, B.A., Bourne, N.F., 1987. Growth, reproductive output, and energy partitioning in weathervane scallops, *Patinopecten caurinus*, from British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 44, 152–160.

MacDonald, B.A., Thompson, R.J., 1985. Influence of temperature and food availability on the ecological energetics of the giant scallop *Placopecten magellanicus*. II. Reproductive output and total production. Marine Ecology Progress Series 25, 295-303.

Mackenzie, C.L.J., 1970. Causes of oyster spat mortality, conditions of oyster setting beds, and recommendations for oyster bed management. Proceeding of the National Shellfisheries Association 60, 59-67.

Mackenzie, C. L. J., Burrell, V.G., Rosenfield, A., Hobart, W.L., 1997. The History, Present Condition, and Future of the Molluscan Fisheries of North America and Europe. National Oceanic and Atmospheric Administration and U.S. Department of Commerce, Washington, USA. 240 pp. (cited 05/07/12): *http://spo.nwr.noaa.gov/tr129opt.pdf.*

Malham, S.K., Cotter, E., O'Keeffe, S., Lynch, S., Culloty, S.C., King, J.W., 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, 128-138.

Mallinson, J.J., Collins, K.J., Jensen, A.C., 1999. Species recorded on artificial and natural reefs, Poole Bay, 1989-1996. Proceedings of the Dorset Natural History and Archaeological Society 121, 113-122.

Mangum, C.P., 1963. Studies on speciation in malanid polychaetes of the north Atlantic coast – III. Intraspecific and interspecific divergence in oxygen consumption. Comparative and Biochemical Physiology 10, 335–349.

Mann, R., 1979. Some biochemical and physiological aspects of growth and gametogenesis in *Crassostrea gigas* and *Ostrea edulis* at sustained elevated temperatures. Journal of the Marine Biological Association of the United Kingdom 59, 95-110.

Markus, A.W., Jorg, S., Teschke, K., 2014. Artificial structures in sediment-dominanted estuaries and their possible influences on the ecosystem. Marine Environmental Research 99, 125-135.

Martınez, G., Aguilera, C., Mettifogo, L., 2000. Interactive effects of diet and temperature on reproductive conditioning of *Argopecten purpuratus* broodstock. Aquaculture 183, 149–159.

Marsden, I.D., 2004. Effects of reduced salinity and seston availability on growth of the New Zealand little-neck clam Austrovenus stutchburyi. Marine Ecology Progress Series 266, 157-171.

Massapina, C., Joaquim, S., Matias, D., Devauchelle, N., 1999. Oocyte and embryo quality in *Crassostrea gigas* (Portuguese strain) during a spawning period in Algarve, South Portugal. Aquatic Living Resources 12, 327-333.

Mathers, N.F., 1974. Some comparative aspects of filter feeding in *Ostrea edulis*, and *Crassostrea angulata* (Mollusca: Bivalvia). Proceedings of the Malacological Society of London 41, 89-97.

Matozzo, V., Marin, M.G., 2008. Can17-β estradiol induce vitellogenin-like proteins in the clam *Tapes philippinarum*? Environmental Toxicology and Pharmacology 26, 38-44.

Matthiessen, G.C., 2001. Oyster Culture. Fishing News Books, Blackwell Science Ltd. Great Britain. UK. 172 pp.

Mazouni, N., Gaertner, J.C., Deslou-Paoli, J.M., 2001. Composition of fouling communities on suspended oyster cultures: an *in situ* study of their interactions with the water column. Marine Ecology Progress Series 214, 93-102.

Mccormick-Ray, M.G., Howard, T., 1991. Morphology and mobility of oyster hemocytes: evidence for seasonal variations. Journal of Invertebrate Pathology 58, 219-230.

Mesias-Gansbiller, C., Silva, A., Maneiro, V., Pazos, A., Sanchez, L., Perez-Pralle, M.L., 2013. Effects of chemical cues on larval settlement of the flat oyster (*Ostrea edulis* L.): a hatchery approach. Aquaculture 376, 85-89.

Meyer, D.L., Townsend, E.C., 2000. Faunal utilization of created intertidal Eastern oyster (*Crassostrea virginica*) reefs in the south-eastern United States. Estuaries 23, 34–45.

Meyer, D.L., Townsend, E.C., Thayer, G.W., 1997. Stabilization and erosion control value of oyster clutch for intertidal marsh. Restoration Ecology 5, 93-99.

Micu, D., Todorova, V., Wolff, W., 2008. Newly discovered oyster reefs of the Black Sea: In time for restoration. 10th ICSR Vlissingen conference. (cited 10/02/2012):*https://www.academia.edu/239263/Newly_discovered_oyster_reefs_of_the_Black_Sea_in_time_for_restoration*.

Millar, R.H., 1961. Scottish oyster investigations, 1946-1958. Journal of Marine Research 3, 76-82.

Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-Being: Synthesis. Island Press, Washington, DC. USA. 155 pp. (cited 15/02/2013): *http://www.millenniumassessment.org/documents/document.356.aspx.pdf*.

Millican, P.F., Helm, M.M., 1994. Effects of nutrition on larvae production in the European flat oyster, *Ostrea edulis*. Aquaculture 23, 83-94.

Mistakidis, M.N., 1951. Quantitative studies of the bottom fauna of Essex oyster grounds. Fishery Investigations 17, 1-48.

Mohlenberg, F., Riisgard, H., 1978. Efficiency of particle retention in 13 species of suspension feeding bivalves. Ophelia 17, 239-246.

Moore, L.E., Smith, D.M., Loneragan, N.R., 2000. Blood refractive index and whole-body lipid content as indicators of nutritional condition for penaeid prawns (Decapoda: Penaeidae). Journal of Experimental Marine Biology and Ecology 244, 131-143.

Moore, P.G., 1977. Inorganic particulate suspensions in the sea and their effects on marine animals. Oceanography and Marine Biology 15, 225-363.

Montaudouin de X., 1996. Factors involved in growth plasticity of cockles *Cerastoderma edule* (L.), identified by field survey and transplant experiments. Journal of Sea Research 36, 251-265.

Montes, J.F., Anadon, R., Azevedo, C., 1994. A possible life cycle for *Bonamia ostreae* on the basis of electron microscopy studies. Journal of Invertebrate Pathology 63, 1-6.

Montes, J.F., Durfort, M., Garcı´a-Valero, J., 1995. Cellular defence mechanism of the clam *Tapes semidecussatus* against infection by the protozoan *Perkinsus* sp. Cell and Tissue Research 279, 529-538.

Morthorst, J.E., Holbech, H., Jeppesen, M., Kinnberg, K.L., Pedersen, K.L., Bjerregaard, P., 2014. Evaluation of York protein level as estrogenic biomarker in bivalves; comparison of the alkali-labile phosphate method (ALP) and a species-specific immunoassay (ELISA). Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 166, 88-95.

Mulvey. M., Feng, S.Y., 1981. Hemolymph constituents of normal and *Proctoeces maculatus* infected *Mytilus edulis*. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 70, 119-125.

Nagler, J.J., Ruby, S., Idler, D.R., So, Y.P., 1987. Serum phosphoprotein phosphorus and calcium levels as reproductive indicators of vitellogenin in highly vitellogenic mature female and estradiol-injected immature rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Zoology 65, 2421-2425.

Nascimento, I.A., Pereira, A.S., 1980. Changes in the condition index for mangrove oysters (*Crassostrea rhizophorae*) from Todosos Santos Bay, Salvador, Brazil. Aqualculture 20, 9-15.

Navarro-Barranco, C., Guerra-García, J.M., Sánchez-Tocino, L., García-Gómez, J.C., 2012. Soft-bottom crustacean assemblages in Mediterranean marine caves: the cave of Cerro Gordo (Granada, Spain) as case study. Helgoland Marine Research 66, 567-576.

Navarro, J.M., Thompson, R.J., 1995. Seasonal fluctuations in the size spectra, biochemical composition and nutritive value of the seston available to a suspension-feeding bivalve in a subarctic environment. Marine Ecology Progress Series 125, 95-106.

Nelson, T., 1928. Relation of spawning of the oyster to temperature. Ecology 9, 145-154.

Newell, R.I.E. 1988. Ecological changes in Chesapeake Bay: are they the result of overharvesting the eastern oyster (*Crassostrea virginica*)? In: Lynch, M.P., Krome, E.C. (eds) Understanding the estuary. Advances in Chesapeake Bay research. Chesapeake Research Consortium Publ 129, Gloucester Point, VA, USA, pp 536-546.

Newell, R.I.E., Cornwell, J.C., Owens, M.S., 2002. Influence of simulated bivalve biodeposition and microphyrobenthos on sediment nitrogen dynamics: A laboratory study. Limnology and Oceanography 47, 1367–1379.

Newell, R.I.E., Jordan, S.J., 1983. Preferential ingestion of organic material by the American oyster *Crassostrea virginica*. Marine Ecology Progress Series 13, 47–53.

Newell, R.I.E., Koch, E.W., 2004. Modelling seagrass density and distribution in response to changes in turbidity stemming from bivalve filtration and seagrass sediment stabilization. Estuaries 27, 793–806.

Newkirk, G.F., Haley, L.E., 1982. Phenotypic analysis of the European oyster, *Ostrea edulis* L.: relationship between length of larval period and post setting growth rate. Journal of Experimental Marine Biology and Ecology 59, 177-184.

Ngo, T.T.T., Berthe, F.C.J., Choi K.S., 2003. Prevalence and infection intensity of the ovarian parasite *Marteilioides chungmuensis* during an annual reproductive cycle of the oyster *Crassostrea gigas*. Diseases of Aquatic Organisms 56, 259-267.

Ngo, T.T.T., Kang, S.G., Kang, D.H., Sorgeloos, P., Choi, K.S., 2006. Effect of culture depth on the proximate composition and reproduction of the Pacific oyster, *Crassostrea gigas* from Gosung bay, Korea. Aquaculture 253, 712-720.

Norkko, J., Thrush, S.F., 2006. Ecophysiology in environmental impact assessment: implications of spatial differences in seasonal variability of bivalve condition. Marine Ecology Progress Series 326, 175-186.

World Organisation for Animal Health, 2012. Manual of Diagnostic Tests for Aquatic Animals. World Organisation for Animal Health, Paris, France. 474 pp. (cited 10/11/13): *http://www.oie.int/international-standard-setting/aquatic-manual/access-online/*.

Okumus, I., Stirling, H.P., 1998. Seasonal variations in the meat weight, condition index and biochemical composition of mussels (*Mytilus edulis* L.) in suspended culture in two Scottish sea lochs. Aquaculture 159,249-261.

Oliver, L.M., Fisher, W.S., 1995. Comparative form and function of oyster *Crassostrea virginica* hemocytes from Apalachicola Bay, Florida and Chesapeake Bay (Virginia). Diseases of Aquatic Organisms 22, 217-225.

Olsen, O.T., 1883. The Piscatorial Atlas of the North Sea and St. George's Channels. Taylor and Francis, London, UK. 8 pp.

Orban, E., Di Lena, G., Nevigato, T., Casini, I., Marzetti, A., Caproni, A., 2002. Seasonal chances in meat continent and chemical composition of mussels (*Mytilus galloprovincialis*) cultured in two different Italian sites. Food Chemistry 77, 57-65.

Organisation for Economic Co-operation and Development, 1967. Catalogue of Main Marine Fouling Organisms: *Serpulids*. Organisation for Economic Co-operation and Development, Paris, France. 79 pp.

Orton, J.H., 1920. Sea-temperature, breeding and distribution in marine animals. Journal of the Marine Biological Association of the United Kingdom 12, 339-366.

Orton, J.H., 1927. Observations and experiments on sex-change in the European oyster (*O. edulis*) Part I. The change from female to male. Journal of the Marine Biological Association of the United Kingdom 14, 967-1046.

Orton, J.H., 1937. Oyster Biology and Oyster Culture. Butler and Tanner Ltd, London. UK. 211 pp.

Osenberg, C.W., Mary, C.M., Wilson, J.A., Lindberg, W.J., 2002. A quantitative framework to evaluate the attraction–production controversy. Journal of Marine Science 59, 214–221.

OSPAR, 2009. Background document for *Ostrea edulis* and *Ostrea edulis* beds. OSPAR Biodiversity: Series 428/2009. OSPAR, UK. 22 pp. (cited 22/10/12): *http://www.ospar.org/site/assets/files/1505/p00428_ostrea_edulis_and_beds.pdf*.

Pampanin, D.M., Maragon, I., Volpato, E., Campesan, G., Nasci, C., 2005. Stress biomarkers and alkali-labile phosphate level in mussels (*Mytilus galloprovincialis*) collected in the urban area of Venice (Venice Lagoon, Italy) Environmental Pollution 136, 103–107.

Parsons, T.R., Maita, Y., and Lalli, C.M. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford, UK. 173 pp.

Pascual, M.S., Zampatti, E., 1995. Chemically mediated adult-larval interaction triggers settlement in *Ostrea puelchana*: application in hatchery production. Aquaculture 133, 33-34.

Pennec, L.M., Beninger, P.G., Herry, A., 1995. Feeding and digestive adaptions of bivalve mollusc to sulphide-rich habitats. Comparative Biochemistry and Physiology 111, 183-189.

Pérez-Camacho, A., Labarta, U., Beiras, R., 1995. Growth of mussels (*Mytilus edulis galloprovincialis*) on cultivation rafts: influence of seed source, cultivation site and phytoplankton availability. Aquaculture 138, 349-362.

Pérez-Camacho, A., Delgado, M., Fernández-Reiriz, M.J., Labarta, U., 2003. Energy balance, gonad development and biochemical composition in the clam *Ruditapes decussatus*. Marine Ecology Progress Series 258, 133-145.

Peterson, C.H., Grabowski, J.H., Powers, S.P., 2003. Estimated enhancement of fish production resulting from restoring oyster reef habitat: Quantitative valuation. Marine Ecology Progress Series 264, 249–264.

Pilditch, C.A., and Grant, J., 1999. Effect of variations in flow velocity and phytoplankton concentration on sea scallop (*Placopecten magellanicus*) grazing rates. Journal of Experimental Marine Biology and Ecology 240, 111-136.

Pipe, R.K., 1985. Seasonal cycles in and effects of starvation on egg development in *Mytilus edulis*. Marine Ecology 24, 121–128.

Pogoda, B., Buck, B.H., Hagen, W., 2011. Growth performance and condition of oysters (*Crassostrea gigas* and *Ostrea edulis*) farmed in an offshore environment (North Sea, Germany). Aquaculture 319, 484-492.

Posey, M.H., Alphin, T.D., Powell, C.M., Townshend, E., 1999. Use of oyster reefs as a habitat for epibenthic fish and decapods. In Luckenbach, M.W., Mann, R. and J.A. Wesson, (eds.). Oyster Reef Habitat Restoration: A Synopsis and Synthesis. Virginia Institute of Marine Science Press. USA, pp 229-238.

Pouvreau, S., Bourles, Y., Lefebvre, S., Gangnery, A., Bruscia, M.A., 2006. Application of a dynamic energy budget model to the Pacific oyster, *Crassostrea gigas*, reared under various environmental conditions. Journal of Sea Research 56, 156-167.

Price, J.H., Irvine, D.E., Farnham, W.F., 1980. The Shore Environment. Volume 2: Ecosystems. London Academic Press. UK. 914 pp.

Quinn, B., Gagne, F., Weber, J.P., Blaise, C., 2005. Ecotoxicological effects of semi-submerged municipal dump (Castle Harbour, Bermuda) on the calico scallop *Argopecten gibbus*. Marine Pollution Bulletin 51, 534-544.

Raj, P.J., 2008. Oysters in a new classification of keystone species. Resonance 13, 648-654.

Rameshkumar, G., Ravichandran, S., Kaliyavarathan, G., Ajithkumar, T.T., 2009. Comparison of protein content in the haemolymph of brachyuran crabs. Middle-East Journal of Scientific Research 4, 32-35.

Reidenbach, M.A., Berg, P., Hume, A., Hansen, J.C.R., Whitman, B.R., 2013. Hydrodynamic of intertidal oyster reefs: the influence of boundary layer flow processes on sediment and oxygen change. Limnology and Oceanography: Fluids and Environment 3, 225-239.

Risinger, J.D., 2012. Biologically dominated engineered coastal breakwaters. PhD thesis, Louisiana State University, USA. 128 pp. (cited 10/12/13): *http://etd. lsu.edu/docs/available/etd-07032012-120846/unrestricted /Risinger_ Dissertation.pdf*.

Rodhouse, P.G., 1977. Ecological energetics of the oyster *Ostrea edulis* in a temperate estuary. PhD thesis, University of Southampton, UK. 132 pp.

Rodney, W.S., Paynter, K.T., 2006. Comparisons of macrofaunal assemblages on restored and non-restored oyster reefs in mesohaline regions of Chesapeake Bayin Maryland. Journal of Experimental Marine Biology and Ecology 335, 39-51.

Ronquillo, J.D., Fraser, J., McConkey, A., 2012. Effect of mixed microalgal diets on growth and polynsaturated fatty acid profile of European oyster (*Ostrea edulis*) juveniles. Aquaculture 361, 64-68.

Roberts, C., 2007. The Unnatural History of the Sea. Octopus Publishing Group Ltd, London, UK. 464 pp.

Robert, M., Garcia, C., Chollet, B., Lopez-Flores, I., Ferrand, S., François, C., Joly, J.P., Arzul, I., 2009. Molecular detection and quantification of the protozoan *Bonamia ostreae* in the flat oyster, *Ostrea edulis*. Molecular and Cellular Probes 23, 264-271.

Rosas, C., Cooper, E.L., Pascual, C., Brito, R., Gelabert, R., Moreno, T., Miranda, G., Sanchez, A., 2004. Indicators of physiological and immunological status of *Litopenaeus setiferus* wild populations (Crustacea, Penaeidae). Marine Biology 145, 401-413.

Rosa, M., Ward, J.E., Shumway, S.E., Wikfors, G.H., Espinosa, E.P., Allam, B., 2013. Effects of particle surface properties on feeding selectivity in the eastern oyster *Crassostrea viginica* and the blue mussel *Mytilus edulis*. Journal of Experimental Marine Biology and Ecology 466, 320-327.

Rothschild, B.J., Ault, J.S., Goulletquer, P., Héral, M., 1994. Decline of the Chesapeake Bay oyster population: a century of habitat destruction and overfishing. Marine Ecology Progress Series 111, 29– 39.

Rowley, A., Cross, M., Culloty, S., Lynch, S., Mackenzie, C., Morgan, E., 2014. The potential impact of climate change on the infectious diseases of commercially important shellfish populations in the Irish Sea - a review. ICES Journal of Marine Science 71, 741-759.

Ruiz, C., Abad, M., Sedano, F., Garcia-Martin, L.O., Sànchez Lòpez, J.L., 1992. Influence of seasonal environmental changes on the gamete production and biochemical composition of *Crassostrea gigas* (Thunberg) in suspended culture in El Grove, Galicia, Spain. Journal of Experimental Marine Biology and Ecology 155, 249-262.

Sasikumar, G., Krishnakumar, P.K., 2011. Aquaculture planning for suspended bivalve farming systems: The integration of physiological response of green mussel with environmental variability in site selection. Ecological Indicators 11, 734-740.

Saucedo, P.E., Ocampo, L., Monteforte, M., Bervera, H., 2004. Effect of temperature on oxygen consumption and ammonia excretion in the Calafia mother-of-pearl oyster, *Pinctada mazatlanica* (Hanley, 1856). Aquaculture 229, 377-387.

Sawusdee, A., Jensen, A.C., Collins, K.J., Hauton, C., 2015. Improvement in the physiological performance of European flat oysters *Ostrea edulis* (Linnaeus, 1758) cultured on elevated reef structures: Implications for oyster restoration. Aquaculture 444, 41-48.

Schlieper, C., 1972. Research Methods in Marine Biology. Sidgwick & Jackson, London. 356 pp.

Schratzberger, M., Warr, K., Rogers, S.I., 2006. Patterns of nematode populations in the southwestern North Sea and their link to other components of the benthic fauna. Journal of Sea Research 55, 113–127.

Schulte, D.M., Burke, R.P., Lipcius, R.N., 2009. Unprecedented restoration of a native oyster metapopulation. Science 325, 1124–1128.

Scott, K., 2009. Epifaunal communities on Solent oysters. Master's thesis, University of Southampton, UK. 82 pp.

Scyphers, S.B., Powers, S.P., Heck, K.L., Byron, D., 2011. Oyster reefs as natural breakwaters mitigate shoreline loss and facilitate fisheries. PLoS ONE 6, 1-12.

Shipp, R.L., Bortone, S.A., 2009. A perspective of the importance of artificial habitat on the management of red snapper in the Gulf of Mexico. Reviews in Fisheries Science 17, 41-47.

Shpigel, M., 1989. Gametogenesis of the European flat oyster (*Ostrea edulis*) and Pacific oyster (*Crassostrea gigas*) in warm water in Israel. Aquaculture 80, 343-349.

Shumway, S.E., 1982. Oxygen consumption in oysters: an overview. Marine Biology Letters 3, 1-23.

Shumway, S.E., Cucci, T.L., Newell, R.C., Selvin, R., Guillard, R.R.L., Yentsch, C.M., 1985. Flow cytometry: a new method for characterization of differential ingestion, digestion and egestion by suspension feeders. Marine Ecology Progress Series 24, 201-204.

Shumway, S.E., Davis, C., Downey R., Karney, R., Kraeuter, J., Parsons, J., Rheault, R., Wikfors, G., 2003. Shellfish aquaculture- In praise of sustainable economies and environments. World Aquaculture 34, 15-18.

Smyth, D., Roberts, D., 2010. The European oyster (*Ostrea edulis*) and its epibiotic succession. Hydrobiologia 655, 25–36.

Smyth, D., Roberts, D., Browne, L., 2009. Impacts of unregulated harvesting on a recovering stock of native oysters (*Ostrea edulis*). Marine Pollution Bulletin 58, 916-922.

Smith, I.P., Low, P.J., Moore, P.G., 2006. Legal aspects of conserving native oysters in Scotland. Marine Pollution Bulletin 52, 479-483.

Sobolewska, H., Beaumont, A.R., 2005. Genetic variation at microsatellite loci in northern populations of the European flat oyster (*Ostrea edulis*). Journal of the Marine Biological Association of the United Kingdom 8, 955–960.

Soletchnik, P., Faury, N., Goulletquer, P., 2006. Seasonal changes in carbohydrate metabolism and its relationship with summer mortality of Pacific oyster *Crassostrea gigas* (Thunberg) in Marennes-Oléron bay (France). Aquaculture 252, 328-338.

Sommer, A.M., Pörtner, H.O., 2002. Metabolic cold adaptation in the lugworm Arenicola marina (L.): comparison of a White Sea and a North Sea population. Marine Ecology Progress Series 240, 171-182.

Soudant, P., Chu, F.L.E., Volety, A., 2013. Host-parasite interaction: Marine bivalve molluscs and protozoan parasites. *Perkinsus* species. Journal of Invertebrate Pathology 114, 196-216.

Sparck, R., 1951. Fluctuations in the stock of oyster (*Ostrea edulis*) in the Limfjord in recent time'. Rapports et Proces-verbaux des Reunions. Conseil Permanent International pour L' Exploration de la Mer 128, 27-29.

Stanton M.G., 1968. Colorimetric determination of inorganic phosphate in the presence of biological material and adenosine triphosphate. Analytical Biochemistry 22, 27-34.

Steimle, F.W., Zetlin, C., 2000. Reef habitats in the Middle Atlantic Bight: abundance, distribution, associated biological communities, and fishery resource use. Marine Fisheries Review 62, 24-42.

Summerhayes, S.A., Bishop, M.J., Leight, A., Kelaher, B.P., 2009. Effects of oyster death and shell disarticulation on associated communities of epibiota. Journal of Experimental Marine Biology and Ecology 379, 60–67.

Sunderlin, J.B., Tobias, W.J., Roels, O.A., 1976. Growth of the European oyster, *Ostrea edulis* in the St. Croix artificial upwelling mariculture system and in natural waters. Proceedings of the National Shellfisheries Association 65, 43-48.

Suzuki, T., Hara, A., Yamaguchi, K., Mori, K., 1992. Purification and immunolocalization of a vitellin-like protein from the Pacific oyster *Crassostrea gigas*. Marine Biology 113, 239–245.

Swartz, R.C., 1978. Techniques for sampling and analysing the marine macrobenthos. U.S. Environmental Protection Agency, Corvallis Environmental Research Laboratory, Oregon, USA. 26 pp.

Sytniki, N.A., Zolotnitskiy, A.P., 2014. On relation of filtration and respiration in the flat oyster (*Ostrea edulis*) at different water temperature. Hydrobioloigia 50, 93-99.

Tallmark, B., 1980. Population dynamics of *Nassarius reticulatus* (Gastropoda, Prosobranchia) in Gullmar Fjord. Marine Ecology Progress Series 3, 51-62.

Tamburri, M.N., Zimmer-Faust, R.K., Tamplm, M.L., 1996. Natural sources and properties of chemical inducers mediating settlement of oyster larvae: A re-examination. Biological Bulletin 183, 327-338.

Thayer, G.W., Stuart, H.H., Kenworthy, W.J., Ustach, J.F., Hall, A.B., 1978. Habitat values of salt marshes, mangroves, and seagrasses for aquatic organisms. In Greeson PE, Clark, J.R., Clark, J.E., eds. Wetland Functions and Values: The State of Our Understanding. American Water Resource Association. USA, pp 235-247.

The Marine Biological Association, 1957. Plymouth Marine Fauna. Plymouth: Marine Biological Association of the United Kingdom. UK. 457 pp.

The UK Biodiversity Action Plan, 1999. Native oyster (*Ostrea edulis*). Species Action Plan. In UK Biodiversity Group. Tranche 2 Action Plans. Vol. V. Maritime Species and Habitats. English Nature for the UK Biodiversity Group, Peterborough. UK. 244 pp. (cited 12/10/12): *http://jncc.defra.gov.uk /PDF/UKBAP_Tranche2-ActionPlans-Vol5-1999.pdf*.

Thomsen, M.S., Mcglathery, K.J., 2006. Effects of accumulations of sediments and drift algae on recruitment of sessile organisms associated with oyster reefs. Journal of Experimental Marine Biology and Ecology 328, 22-34.

Thomsen, M.S., McGlathery, K.J., Silliman, B.R., 2007. Spatial variation in recruitment of native and invasive sessile species onto oyster reefs in a temperate soft-bottom lagoon. Estuarine Coastal and Shelf Science 72, 89-101.

Thompson, R.J., 1977. Blood chemistry, biochemical composition, and the annual reproductive cycle in the glant scallop, *Placopecten magellanlcus*, from southeast New-found land. Journal of the Fisheries Research Board of Canada 34, 2104-2116.

Thrush, S., Halliday, J., Hewitt, J.E., Lohrer, A.M., 2008. The effect of habitat loss, fragmentation, and community homogenization on resilience in estuaries. Ecological Application 18, 12-21.

Tilman, D., Lehman, C.L., Bristow, C.E., 1998. Diversity-Stability relationships: statistical inevitability or ecological consequence? The American Naturalist 151, 277-282.

Tlili, S., Métais, I., Ayache, N., Boussetta, H., Mouneyrac, C., 2012. Is the reproduction of *Donax trunculus* affected by their sites of origin contrasted by their level of contamination? Chemosphere 84, 1362-1370.

Tolley, S.G., Volety, A.K., 2005. The role of oysters in habitat use of oyster reefs by resident fishes and decapod crustaceans. Journal of Shellfish Research 24, 1007–1012.

Tubbs, C., 1999. The Ecology, Conservation and History of the Solent. Packard Publishing Limited, Chichester. UK. 179 pp.

Tyler-Walters, H., 2001. *Ostrea edulis* beds on shallow sublittoral muddy sediment. Marine life information network: Biology and sensitivity key information: (cited 02/04/12): *http://www.marlin.ac.uk/ sensitivityrationale.php*.

University Marine Biological Station Millport, 2007. Conservation of the native oyster *Ostrea edulis* in Scotland. Scottish Natural Heritage Commissioned Report No. 251 (ROAME No. FO2A408), UK. 174 pp. (cited 17/09/12): *http://www.snh.org .uk/pdfs/publications/commissioned_reports/ Report%20No251.pdf*.

Urawa, S., 1995. Effects of rearing condition on growth and mortality of juvenile chum salmon (*Oncorhynchus keta*) infected with *Ichthyobodo necator*. Canadian Journal of Fisheries and Aquatic Sciences 52, 18–23.

Utting, S.D., Helm, M.M., Millican, P.F., 1991. Recent studies on the fecundity of the European at oyster (*Ostrea edulis*) spawning stock in the Solent. Journal of the Marine Biological Association of the United Kingdom 71, 909-911.

Utting, S.D., Millican, P.F., 1997. Techniques for the hatchery conditioning of bivalve broodstocks and the subsequent effort on egg quality and larval viability. Aquaculture 155, 45-54.

Utting, S.D., Spencer, B.E., 1992. Introductions of bivalve molluscs into the United Kingdom for commercial culture-case histories. ICES Marine Science Symposium 194, 84-91.

Valero, J., 2006. *Ostrea edulis*: growth and mortality depending on hydrodynamic parameters and food availability. Master's thesis, Goteborg University, Sweden. 47 pp. (cited 08/08/12): *http://www.tmbl.gu.se/pdf/TMBL_pdf/ Library_and_databases_pdf/ examensarbeten_pdf/JohannaValero20p.pdf*.

van Banning, P., 1985. Control of *Bonamia* in Dutch Oyster Culture. In: Ellis AE (ed) Fish and Shellfish Pathology. Academic Press, London, UK, pp 393-396.

van der Zee, E.M., Tielens, E., Holthuijsen, S., Donadi, S., Erikson, B.K., van der Veer, H.W., Piersma, T. Olff, H., van der Heide, T., 2015. Habitat modification drives benthic trophic diversity in an intertidal soft-bottom ecosystem. Journal of Experimental Marine Biology and Ecology 465, 41-48.

van Rijn, L.C., 1986. Sediment transport by currents and waves. Delft Hydraulics Report H461. The Netherlands. 15 pp. (cited 19/03/13): *http://www.leovanrijn -sediment.com/papers/P3-1990.pdf*.

Vanstaen, K., Palmer, D., 2010. Solent Regulated Fishery Oyster Stock Survey 15- 21 June 2010. Technical report. Centre for Environment, Fisheries and Aquaculture Science, Lowestoft Laboratory. UK. 29 pp.

Vause, B., 2010. Chichester Harbour Oyster Initiative. Shellfish News, 30, 5-6.

Verma, A.K., Kumar, V., Agarwal, S., Leekha, A., Tyagi, A., Moin, I. and Gupta, D., 2013. Interplay of immune response and oxidative stress induced by chitosan nanoparticles to possibly combat inflammation. World Research Journal of Bioscience 1, 28-34.

Vernberg, F.J., 1962. Comparative physiology: latitudinal effects on physiological properties of animal populations. Annual Review of Physiology 24, 517-546.

Victor, S.K., Linda, L.B., 1983. Sixteen decades of political management of the oyster fishery in Maryland's Chesapeake Bay. Journal of Environmental Management 164, 153-171.

Vismann, B., 1991. Sulfide tolerance; physiological mechanisms and ecological implications. Ophelia 34, 1-27.

Volety, A., Tolley, G., 2010. Oyster physiology and ecological distribution as an indication of environmental health and as a performance measure of restoration effectiveness of southwest Florida's estuaries. (cited 12/10/11): *http://www.docstoc.com/docs/42494627/Oyster-Physiology-and-Ecological-Distribution-as-an-Indication*.

Wallace, R.A., 1985. Vitellogenesis and oocyte growth in non-mammalian vertebrates. In: Browder, L.W. (Ed.), Developmental Biology: A Comprehensive Synthesis, New York. USA, pp 127-177.

Wall, C.C., Peterson, B.J., Gobler, C.J., 2008. Facilitation of seagrass *Zostera marina* productivity by suspension-feeding bivalves. Marine Ecology Progress Series 357, 165–174.

Walne, P.R., 1956. Experiments on rearing the larvae of *Ostrea edulis* L. in the laboratory. Fishery Investigations 20, 2-9.

Walne, P.R., 1972. The influence of current speed, body size and water temperature on the filtration rate of five species of bivalves. Journal of the Marine Biological Association of the United Kingdom 52, 345–374.

Walne, P.R., 1974. Culture of Bivalve Molluscs: 50 years' experience at Conwy. Fishing News Books, Oxford. UK. 190 pp.

Ward, J.E., Levinton, J.S., Shumway, S.E., Cucci, T., 1997. Site of particle selection in a bivalve mollusc. Nature 390, 131–132.

Ward, J.E., Shumway, S.E., 2004. Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. Journal of Experimental Marine Biology and Ecology 300, 83–130.

Warwick, R.M., Goss-Custard, J.D., Kirby, R., George, C.L., Pope, N.D., Rowden, A.A., 1991. Static and dynamic environmental factors determining the community structure of estuarine macrobenthos in SW Britain: why is the Severn Estuary different? Journal of Applied Ecology 28, 329–345.

Weiner, R.M., Walch, M., Labare, M.P., Bonar, D.B., Colwell, R.R., 1989. Effect of biofilms of the marine bacterium *Alteromonas colwelliana* (LST) on the set of the oyster *Crassostrea gigas* (Thunberg, 1793) and *C. virginica* (Gmelin, 1791). Journal of Shellfish Research 8, 117-123.

Wells, H.W., 1961. The fauna of oyster beds, with special reference to the salinity factor. Ecological Monographs 31, 239–266.

Wenner, E., Beatty, H.R., Coen, L.D., 1996. A method for quantitatively sampling nekton on intertidal oyster reefs. Journal of Shellfish Research 15, 769 –775.

Wheat, W., Ruesink, L.J., 2013. Commercially-cultured oysters (*Crassostrea gigas*) exert top-down control on intertidal pelagic resources in Wilapa Bay, Washington, USA. Journal of Sea Research 81, 33-39.

Whittingham, D.G., 1967. Light-induction of shedding of gametes in *Ciona intestinalis* and *Morgula manhattensis*. Biological Bulletin 132, 292-298.

Widdows, J., 1978. Combined effects of body size, food concentration and season on the physiology of *Mytilus edulis*. Journal of the Marine Biological Association of the United Kingdom 58, 109-124.

Wilberg M.J., Livings M.E., Barkman J.S., Morris B.T., Robinson J.M., 2011. Overfishing, disease, habitat loss, and potential extirpation of oysters in upper Chesapeake Bay. Marine Ecology Progress Series 436, 131-144.

Wilber, K.M., Owen, G., 1964. Growth. In Wilbur, K. M., Yonge, C.M., (eds.) Physiology of Mollusca, Academic Press, New York, USA, pp 211-242.

Wilson, E.Q., 1998. Biodiversity. National Academy Press, Washington DC. USA. 468 pp.

Wilson, J., Simons, J., 1985. Gametogenesis and breeding of *Ostrea edulis* on the west coast of Ireland. Aquaculture 46, 307-321.

Winter, J.E., Acevedo, M.A., Navarro, J.M., 1984. Quempil'n estuary, an experimental oyster cultivation station in Southern Chile. Energy balance in *Ostrea chilensis*. Marine Ecology Progress Series 20, 151-164.

Woollacott, R.M. 2005. Spermatozoa of *Cioma intestinalis* and analysis of ascidian fertilization. Journal of Morphology 152, 77-88.

Woolmer, A.P., Syvret, M., FitzGerald A., 2011. Restoration of Native Oyster, *Ostrea edulis*, in South Wales: Options and Approaches. CCW Contract Science Report No: 960, South Wales, UK. 88 pp. (cited 13/12/12): *http://myweb.tiscali.co. uk/andywoolmer/Reports/Restoration%20of%20Native%20Oyster%20Ostrea%20edulis%20in%20South%20Wales%20Options%20and%20Approaches%20Report%20Number%20960.pdf*.

Worm, B., Barbier, E.B., Beaumont, N., Duffy, J.E., Folke, C., Halpern, B.S., Jackson, J.B.C., Lotze, H.K., Micheli, F., Palumbi, S.R., Sala, E., Selkoe, K.A., Stachowicz, J.J., Watson, R., 2006. Impacts of biodiversity loss on ocean ecosystem services. Science 314, 787-790.

Yonge, C.M., 1926. Structure and physiology of the organs of feeding and digestion in *Ostrea edulis*. Journal of the Marine Biological Association of the United Kingdom 14, 295-386.

Yonge, C.M, 1966. Oysters. Collins, London. UK. 209 pp.

Yukihira, H., Lucas, J.S., Klump, D.W., 2006. The pearl oyster *Pinctada maxima* and *P. margaritifera*, respond to different ways to culture in dissimilar environments. Aquaculture 252, 208-224.

Zalmon, I.R., Fabricio, S.D., Neto, E.J.D., Rezende, C.E., Mota, P.M., Almeida, T.C.M., 2014. Impacts of artificial reef spatial configuration on infaunal community structure-Southeastern Brazil. Journal of Experimental Marine Biology and Ecology 454, 9-17.

Zorita, I., Ortiz-Zarragoitia, M., Soto, M., Cajaraville, M.P., 2006. Biomarker responses in mussels transplanted to a copper site gradient (Visnes, Norway). Marine Environmental Research 62, 398-398.