UNIVERSITY OF SOUTHAMPTON

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES

Ocean and Earth Science

Population structure and ecology of wild *Crassostrea gigas* (Thunberg, 1793) on the south coast of England

by

Stephanie Rachael Anne Mills

Thesis for the degree of Doctor of Philosophy

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ABSTRACT

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Crassostrea gigas (Thunberg, 1793) is native to Japan and Korea, but has achieved global distribution through human mediated dispersal pathways and natural larval dispersal. Considerable variation in recruitment to wild aggregations has been seen regionally across the globe. Wild recruitment of *C. gigas* in England has increased in frequency since the millennia however a detailed understanding of their occurrence is limited to an area within the Thames estuary. There have been no English studies to date that reveal how *C. gigas* interacts with recipient ecosystems, or what impacts winter conditions have. Furthermore conclusive evidence has yet to be presented that feral *C. gigas* in England are self-sustaining.

Intertidal surveys found substrate type and shore height to have the greatest impact on the locality and abundance of *C. gigas* recruitment. Gametogenesis initiated in *C. gigas* when water temperatures increased above 9.5 °C. Maturity was generally reached in the summer, however spawning differed between locations. Wild, intertidal *C. gigas* were found to spawn twice in a single reproductive season. Initially, spawning was triggered through tidally induced temperature shocking as water temperatures increased above 18 °C. It is thought that the second spawning was triggered by a combination of warm water (+18 °C) and an increase in phytoplankton abundance. Farmed, subtidal *C. gigas* spawned once, coinciding with the 2nd spawning of intertidal oysters. Rapid growth rates allow a size refuge from the greatest predation pressure to be achieved before growth rates decline over winter. In particular *Carcinus maenas* is capable of predating *C. gigas* with a shell length of up to at least 50 mm.

Winter conditions experienced in England are typically colder than those experienced with in the native range of *C. gigas,* and as such, were detrimental to *C. gigas.* Juvenile oysters with 4 ± 0.5 mg (dry flesh weight) had significantly higher respiration rates than 6 and 9 ± 0.5 mg juvenile oysters at water temperatures of 7 °C. Temperatures within the pallial cavity of adult *C. gigas* remained similar to the ambient environment, changing rapidly with tidal immersion/emersion. Furthermore pallial temperatures below 0 °C were recorded and found to reduce gaping activity. Juvenile oysters are the most vulnerable to cold temperatures and are likely affected most years, however only particularly cold years with extensive frosts impacted on adults.

This study expands the base-line knowledge of *C. gigas* distribution in England to include Southampton Water and Poole Harbour, and investigates the impacts that annual water temperatures and predation have on reproduction and recruitment. The accumulated knowledge and information will enable a better understanding of how establishing *C. gigas* is likely to develop in the future, and has the potential for use in predictive models and other conservation tools.

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*Author of this thesis Stephanie R A Mills previously Stephanie R A Deane.

Deane S. R. A. 2016. The Pacific Oyster: Making itself at home in the UK. PMNHS Bulletin 6: *in print*

Deane S. R. A., Sawusdee, A., Collins, K. J., Jensen, A. C. 2014. Epifaunal colonisation on shells of native European oysters, *Ostrea edulis,* and non-native Pacific oyster *Crassostrea gigas.* RECIF Conference on artificial reefs: from material to ecosystem, Cean, France, ESTIC Caen.

DECLARATION OF AUTHORSHIP

I, Stephanie Mills 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.

POPULATION STRUCTURE AND ECOLOGY OF WILD *CRASSOSTREA GIGAS* (THUNBERG, 1793) ON THE SOUTH COAST OF ENGLAND

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. [Delete as appropriate] None of this work has been published before submission [or] Parts of this work have been published as: [please list references below]:

Signed: .....

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Definitions and Abbreviations

Wild: A species that is non-native to the ecosystem under consideration.

Feral: A species that is non-native to the ecosystem under consideration.

Naturalised: A species that is non-native to the ecosystem under consideration and that is self-sustaining.

Established: A species that is non-native to the ecosystem under consideration and that is self-sustaining.

Invasive: A species that is non-native to the ecosystem under consideration and whose introduction causes or is likely to cause economic or environmental harm or harm to human health (As defined in the Executive Summary of the National Invasive Species Management Plan (NISMP)).

Spat: A juvenile oyster.

Cultchless: Juvenile and adult oysters with no or little substrate attached to the umbo (typically produced for aquaculture).

Fisheries: A unit determined by an authority or other entity that is engaged in raising and/or harvesting fish. Typically, the unit is defined in terms of some or all of the following: people involved, species or type of fish, area of water or seabed, method of fishing, class of boats and purpose of the activities (As defined by the Food and Agricultural Organisations of the United Nations (FAO)).

Aquaculture: Aquaculture is the farming of aquatic organisms in both coastal and inland areas involving interventions in the rearing process to enhance production (As defined by the Food and Agricultural Organisations of the United Nations (FAO)).

Settlement: All juvenile oysters that have successfully metamorphosed from larvae to sessile bivalve.

Recruitment: Settlement that survives to maturity and so has the opportunity to reproduce.

Post-settlement mortality: Mortality that occurs during the juvenile life stage, before the oyster reaches maturity and has the opportunity to reproduce.

Chapter 1: Introduction

1.1 The study species: *Crassostrea gigas*

1.1.1 Taxonomy and nomenclature

Crassostrea gigas (Thunberg 1793) is a filter feeding bivalve in the family Ostreidae, native to Japan and Korea. It has many common names including giant oyster, Japanese oyster and perhaps the most frequently used in the UK, Pacific oyster. A large number of synonyms have resulted from the irregular and highly variable shell shape, for example, *Gryphaea angulata* (Lamarck 1819), *Ostrea gigas* (Thunberg 1793), *Ostrea laperousii* (Schrenk 1861), *Ostrea talienwhanensis* (Crosse 1862) and *Crassostrea angulata* (Lamarck 1819) are all synonymous with *C. gigas* (Nehring 2011).

In particular the species distinction between *C. gigas* and *C. angulata* has been in question (see Humphreys et al. 2014). *C. gigas* and *C. angulata* easily hybridise producing indistinguishable larvae and adults that are both anatomically and morphometrically similar (Menzel 1974; Huvet et al. 2002), however genetic differences exist resulting in differences in shell shape, muscle scar pigmentation, susceptibility to disease and parasitic infection (Carnegie & Cochennec-Laureau 2004; Batista et al. 2008; Batista et al. 2009). Molecular comparison and DNA base sequencing have concluded that *C. angulata* is a strain of *C. gigas* originating from Taiwan and confirmed the species to be synonymous (Buroker et al. 1979; Huvet et al. 2002; Reece et al. 2008). Divergence in Asia, a few hundred thousand years ago, has been suggested as the cause for genetic differences between *C. gigas* and *C. angulata* today (Hedgecock et al. 2004).

C. angulata has been recognised as a synonym of *C. gigas* by the UK National Biodiversity Network (*Crassostrea gigas* (Thunberg 1793), NBN ID code NBNSYS0000174740). However phenotypic and genotypic differences still causes some disparity (e.g. WoRMS (World Register of Marine Species) lists *C. gigas* and *C. angulata* separately but qualifies them as very closely related and still possibly conspecific). *C. angulata* will be treated as a synonym of *C. gigas* throughout this study.

1.1.2 Morphology

The shell has a foliated structure formed predominantly of calcite and aragonite. Four layers of composite minerals form with nanoscale regularity and strength that overcomes the intrinsic brittleness of the calcium carbonate crystals. Compared to other bivalves, the shell of *C. gigas* has a relatively weak fracture strength when subjected to bending, compression and impact (Taylor & Layman 1972; Cheong-Song & Yong-Wan 2000; Yoon et al. 2003). A reduced mechanical strength is the compromise for rapid growth rates allowed by the relatively low energy cost of laying such a shell. However, *C. gigas* shells do contain cavities and chalky layers that may have evolved to act as 'crack stoppers' and so limit damage (Sarashina & Endo 1998). Shell formation is affected by substrate type, exposure and crowding (Galtsoff 1964a; Dutertre et al. 2009a; Marshall & Dunham 2013). Generally those colonising hard substrate (biogenic or anthropogenic) in sheltered water will have extensive fluting and a protruding marginal fringe, whereas softer sediments and exposed conditions result in the rounding of the shells (Pers. obvs. Figure 1.1). At high densities, crowding alters the orientation of the oysters from horizontal to vertical and causes an elongation of the shell (Figure 1.2)(Dutertre et al. 2009a; Marshall & Dunham 2013). However it should be noted that these are general trends and it is common to find a high variation in shell structure at a single site.

Figure 1.1 Example of shell morphology variations with substrate type. **Left**: The rounded shell of *Crassostrea gigas* colonising muddy sand (soft sediment)(Hill Head 30.07.2015). **Right**: Highly fluted shells on *C. gigas* colonising a yacht hull (hard sediment)(Southampton Water 10.12.14).

Figure 1.2 **Left**: *Crassostrea gigas* lay flat on the substrate at low densities (Brightlingsea 09.10.2013). **Right**: Crowding forces the orientation of *C. gigas* to become vertical (Brightlingsea 20.05.2013).

The organs associated with respiration, digestion and circulation dominate the internal structure of *C. gigas* except during the reproductive season, when gonad tissue can account for up to 50 % of the tissue mass (Cardoso et al. 2007)(see Figure 1.3 for general bauplan).

Respiration and ingestion occur primarily at the ctenidium where food particles are extracted by cilia and oxygen is taken up by the blood cells filling the gill lamellae. Active selection of particles occurs mainly at the ctenidium but also at the labial palps (Bernard 1974; Ward et al. 1998). Rejected particles are usually outside of an optimum size range or inorganic and are expelled as pseudofaeces (Newell & Langdon 1996; Ward et al. 1998). Selected particles enter the digestive system through the mouth. The digestive system of *C. gigas* contains a stomach, rich in enzymes, where extracellular digestion takes place and a system of digestive diverticula where secondary intracellular digestion takes place (Morton 1977).

The circulatory system is open, meaning that blood cells are not confined to vessels rather that they pass through and accumulate within irregular spaces of varying size known as sinuses within the tissue. A primary heart within the pericardium and 2 ancillary hearts provide systolic pressure however a large number of blood cells accumulate in the gills and mantel and are discarded through diapedesis. The direction of flow is through a venal system into branchial vessels before entering the heart (Galtsoff 1964b).

Valve movements are intrinsically linked with respiration, feeding and circulation as the pumping action they provide ensures a continuous movement of water over the ctinedae and continued blood circulation.

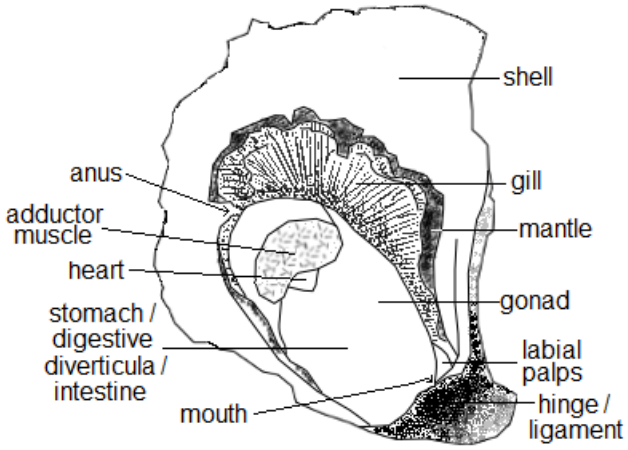


Figure 1.3 Organisation of the internal parts of *Crassostrea gigas* (drawn from a farmed *C. gigas,* Poole Harbour 29.05.2014).

1.1.3 Ecology

Crassostrea gigas colonise a range of hard substrate in the intertidal zones of estuaries and sheltered coastal marine habitats. Habitats that *C. gigas* has colonised in Europe include: littoral rock (Lejart & Hily 2011), chalky shores (McKnight 2009), boulders (Kochmann 2012) and muddy intertidal sediments (Diederich 2005b; Nehls & Büttger 2007)(see Herbert et al. 2012 for a review). *C. gigas* is typically found in the intertidal zone however distribution varies with substrate type. On a rocky shore, *C. gigas* are most likely to be found between mean high water and mean low water, and not at extreme low water or subtidally (Herbert et al. 2012). On soft intertidal habitats such as littoral mud and sand flats, *C. gigas* is generally found from approximately mean low water into the shallow subtidal (Reise 1998; Herbert et al. 2012). *C. gigas* can occasionally be found colonising subtidal habitats in large numbers. In 3 major bays in Ireland *C. gigas* has been found colonising subtidal mussel and native oyster beds (McGonigle et al. 2011; Kochmann 2012), and in the Oosterschelde (SW Netherlands) *C. gigas* colonises mud gullies and dikes down to 15 m below sea levels (Smaal et al. 2009).

C. gigas is a gregarious species known to form reefs. This happens at high densities when the orientation of oysters are forced from lying flat to standing upright and the shells of neighbouring oysters become cemented together. Over many years a consolidated and hard substrate, or reef, is formed and will persist after the oysters themselves die. In the absence of other oysters, larvae will settle and metamorphose onto a wide range of hard substrates. This includes natural substrate such as rock faces, boulders, pebbles or gravel (McKnight 2009; Kochmann 2012), biogenic substrates such as shell fragments, and anthropogenic structures such as sea defences, piers or marinas (Couzens 2006; McKnight 2009; Nehring 2011). Furthermore *C. gigas* may settle

onto other benthic fauna as epibionts (Reise 1998; Markert et al. 2010). Colonisation of mussel beds facilitated the rapid expansion of wild *C. gigas* in the Wadden Sea (Reise 1998), and by 2004 almost all mussel beds in the List tidal basin near Sylt had been colonised (Nehls et al. 2006). Densities of *C. gigas* on a mussel bed can reach up to 600 oysters per m^2 (Nehls et al. 2006). Large aggregations of wild *C. gigas* can also form on extensive mudlfats or within bays (Wehrmann 2000; Diederich et al. 2005; Dutertre et al. 2009b; Fey et al. 2010; Lejart & Hily 2011). This occurs either as a result of oysters spreading out from an initial site of hard substrate, such as in the Wadden Sea, where initial colonisation occurred on mussel beds and cockle shell ridges (Nehls et al. 2006), or through larvae metamorphosing onto small fragments of shell or rock which then in turn act as substrate to further settlement. In this way clumps of oysters form and eventually merge becoming a reef structure (Herbert et al. 2012).

Reef structures vary in area, height and thickness between locations. For example, *C. gigas* forming reefs first became noticed in the UK during the mid-2000s along the south east coast of England (Herbert et al. 2012). Small (> 5 m^2) areas of oyster reef have formed on areas of chalk shore where abundance is approximately 200 oysters per m^2, and larger reefs have established on muddy shores. However density still varies considerably along the coast and settlement has remained absent from some areas of chalk and mud (Herbert et al. 2012). In the Wadden Sea, Pacific oyster reefs developed in the mid-1990s (Reise 1998) and expanded to cover approximately 5.5 km^2 by 2005 (Dankers et al. 2006 cited in Troost 2010), in some areas the density of oysters on the reef can reach 700 - 2000 oysters per m^2 (when including live and dead oysters) (Markert et al. 2010). Typically reefs formed by *C. gigas* do not cover 100 % of the substrate. Densities > 200 live, mature oysters per m^2 generally cover most of the natural substrate they are colonising, however, there are often bare patches where soft-sediment communities are still present (Troost 2010; Herbert et al. 2016). In the Wadden Sea, shallow pools within oyster reefs contain shrimps and small fish (Troost 2010).

Crassostrea gigas filter feeds on plankton, bacteria and particulate organic matter. In high abundance *C. gigas* acts as a biological filter removing material from the water and producing biodeposits, this can result in the 'sinking' of an oyster reef where the bottom of the reef becomes covered in silt leaving only the shell fringes protruding above the surface (Markert et al. 2010). Biodeposits consist of faeces, particles that have been digested, and pseudofaeces, rejected particles that do not enter the mouth. Potential food particles are selected from the seston by the gills and labial palps (Cognie et al. 2003). Rejected particles are packaged in mucus and discarded as pseudofaeces. Oysters retain particles between 4 and 5 µm most efficiently, and actively select organic particles containing phytopigments as well as those with high lipid and protein content (Deslous-Paoli et al. 1992; Barillé et al. 1997; Cognie et al. 2003). Both processes

alter food particles to a degree and alter the chemistry of local substrate through induced sedimentation of particles ordinarily suspended in seawater. Furthermore oyster biodeposits can result in accumulation of between 2 and 100 times natural sedimentation (depending on the abundance of oysters and the concentration of seston) (Deslous-Paoli et al. 1992). Cultured *C. gigas* potentially impact on the distribution of plankton, carrying capacity and water quality of a water body (Ruesink et al. 2005; Grangeré et al. 2010; Troost 2010). However naturalised *C. gigas* feed primarily on benthic biofilms and resuspended macro algae detritus (Marchais et al. 2013). The differences in diet are thought to be the result of cultured oysters being raised off the seabed on trestle tables (Herbert et al. 2016)

Predation of *C. gigas* occurs predominantly at the juvenile life stage as vulnerability decreases with age. High growth rates result in both a size refuge from predation and a protective thickening of the shell. Cultured oysters are vulnerable to pelagic, benthic and avian predators (Table 1.1) owing to aquaculture practices largely occurring in the natural environment and the relative importance of predator types varies between countries and sites. For example in Louisiana, USA, large black drum (*Pogonias cromis*) are important predators of oysters causing mortality as high as 90 % on commercial leases (Brown et al. 2003; Brown et al. 2006), whereas in Europe, the greatest predatory pressure comes from benthic predators and in particular the European green crab (*Carcinus maenas*) (Walne & Davies 1977; Dare et al. 1983; Spencer 1992; Diederich 2005b). Furthermore, species such as *Ocinebrellus inornatus,* the Japanese oyster drill, can cause localised high mortality of oysters (Chapman & Banner 1949), with oyster beds located toward the mouth of an estuary typically suffering greater predation than those at the head of an estuary (Harding et al. 2002; Buhle & Ruesink 2009). *C. gigas* of all sizes are vulnerable to colonisation by boring sponges (*Cliona* spp.), and infestations of polychaetes (*Polydora* spp.) and copepods (*Myticola* spp.). Although these organisms are not predators, they negatively impact on condition, weakening the oyster and potentially resulting in mortality (Thomas 1979; Flimlin & Beal 1993; Miossec et al. 2009).

Table 1.1 Recorded predatory species of *Crassostrea* spp.

Predator: Phylum (order)	**Species**	**References**
Arthropoda (Decapoda)	*Carcinus maenas* (European green crab)	(This_study ; Walne & Davies 1977; Dare et al. 1983; Mascaró & Seed 2001a; Diederich 2005b; Miron et al. 2005; Miossec et al. 2009; Kochmann & Crowe 2014)
	Cancer irroratus *Cancer productus* (Rock crab)	(Miron et al. 2005; Miossec et al. 2009)
	Callinectes sapidus (Atlantic blue crab)	(Bisker & Castagna 1987b; Eggleston 1990b; Harding et al. 2002)
	Panopeus herbstii *Dyspanopeus sayi* *Eurypanopeus depressus* (Mud crabs)	(Flimlin & Beal 1993; Harding et al. 2002)
	Menippe adina (Gulf stone crab)	(Brown & Haight 1992)
Mollusca (Gastropoda)	*Rapana venosa* *Busycotypus canliculatus* *Busycon carica* (Whelks)	(Harding & Mann 1999; Harding et al. 2002; Miossec et al. 2009)
	Morula musiva *Thais clavigera* *Thais haemastoma* (Rock snails)	(Garton & Stickle 1980; Taylor 1990)
	Ocinebrellus inornatus *Ocenebra erinacea* *Urosalpinx cinerea* *Eupleura caudata* (Oyster drills)	(Flimlin & Beal 1993; Harding et al. 2002; Buhle & Ruesink 2009; Miossec et al. 2009)
	Cymatium pileare (Hairy triton)	(Littlewood 1989)
Platyhelminthes (Polycladida)	*Stylochus ellipticus* *Stylochus frontalis* *Pseudostylochus ostreophagus* (oyster flatworms)	(Littlewood & Marsbe 1990; Flimlin & Beal 1993; Miossec et al. 2009)
Echinodermata (Asteroidae)	*Asterias forbesi* *Asterias vulgaris* *Asterias rubens*	(Whittle & Blumer 1970; Flimlin & Beal 1993; Diederich 2005b; Miron et al. 2005)

Predator: Phylum (order)	Species	References
Chordata (Tetraodontiformes)	*Tetractenos* spp. *Opsanus tau* (Toadfish)	(McDermott 1964; Flimlin & Beal 1993; Anderson & Connell 1999; Harding et al. 2002)
Chordata (Charadriiformes)	*Haematopus bachmani* *Haematopus ostralegus* (Oystercatchers)	(Butler & Kirbyson 1979; Tuckwell & Nol 1997; Cadee 2008)
Chordata (Perciformes)	*Pogonias cromis* (Black drum)	(Brown et al. 2003; Brown et al. 2006; Ajemian & Powers 2012)
Chordata (Myliobatiformes)	*Rhinoptera bonascus* (Cownose ray)	(Flimlin & Beal 1993; Harding et al. 2002; Collins et al. 2007)

1.1.4 Life cycle

Crassostrea gigas is a successive and irregular protandrous hermaphrodite, reaching sexual maturity as a male one winter after settlement, before potentially switching to become female (Guo et al. 1998). Simultaneous hermaphrodites are rare and fecundity is high with females able to release up to 50 million eggs into the water column (Helm et al. 2004). Gametogenesis initiates and spawning occurs at different temperatures in different regions of the world. In their native range spawning occurs at water temperatures of 23-26 °C (Kobayashi et al. 1997), however Pacific oysters found in Europe spawn in lower water temperatures of 17-20 °C (Li & Hedgecock 1998). Fertilisation occurs in the water column and embryos develop into larvae that remain pelagic until they mature into pediveligers. Pediveligers respond to chemical cues released by mature oysters already established in the intertidal zone and initiate settling behaviours. This includes downward swimming as they become negatively phototaxic, positively geotactic and negatively buoyant to increase the chances of reaching the benthos. Upon reaching the benthos, the pediveligers crawl using their foot to find a suitable substrate (Fitt et al. 1990; Rico-Villa 2009). Once a suitable substrate has been found the larvae metamorphose into sessile oysters. At this stage the oysters are immature juveniles allocating energy primarily into growth. As early as the first summer the oysters can mature into adults and reallocate approximately 63 % of energy toward reproduction (Deslous-Pauli & Héral 1988).

1.2 *Crassostrea gigas* in aquaculture

Crassostrea gigas has been intentionally introduced to at least 25 countries outside its native range of Japan and Korea for the purpose of mariculture (Figure 1.4) (Ruesink et al. 2005; Carrasco & Barón 2010). Globally it is ranked number one in terms of aquaculture production by volume

and value. In 2014 over 625 thousand tonnes were produced worldwide with an estimated value of 1 343 million USD (FAO 2016). The popularity and success of the Pacific oyster in aquaculture is largely the result of their fast growth rates without the requirement for additional food beyond the natural supply, and their ability to adapt to a wide range of environments through tolerance to temperature, salinity and turbidity (Mann et al. 1994). Furthermore they have shown a resistance to diseases such as Bonamiosis (caused by a protozoan, *Bonamia ostrea*), which has resulted in economic losses of native oyster stocks in Europe since 1982 (Culloty et al. 1999).

Until recently it has been widely accepted that Pacific oysters were first introduced into the UK in 1965 via the hatchery and quarantine facilities at the Government Fisheries Laboratory, Conwy. A broodstock imported from British Columbia were spawned at Conwy and the resultant oysters were supplied to British hatcheries for breeding purposes (Utting & Spencer 1991). However a revision of the history of *C. gigas* in Britain now shows the first introduction of oysters to be from Arcachon, France, into Poole Harbour, Dorset, by the Poole Oyster Company as early as 1890 (Humphreys et al. 2014). During this time juvenile *C. gigas*, known as spat, were shipped into the UK and grown-on in estuaries. Oyster spat continued to be imported to boost the aquaculture industry until 1962 when imports into aquaculture ceased because shellfish imports had become a route for the inadvertent introduction of pests and diseases into local ecosystems (Spencer et al. 1994). A quarantine procedure was introduced by the Government Fisheries Laboratory, Conwy, that prevented the release of unwanted pests and disease organisms whilst imported broodstock were being conditioned for breeding. The first generation juveniles were retained in quarantine for 15 months and sea trials followed. The trials at the Government laboratories at Conwy were successful in producing oysters that remained disease free and which grew to a marketable size (75 g)(Spencer et al. 1992), whilst average summer (July-August) water temperatures remained low enough (16 – 17 °C) to inhibit reproduction and hence uncontrolled population expansion (Spencer et al. 1994).

Alongside these field trials, hatchery techniques were developed that allowed larvae to be cultivated under controlled conditions to produce spat that could be sold on to aquaculture. This meant that the full production line of *C. gigas* could now be contained within the UK and that oysters laid out in estuaries to grow-on came from a managed source. The production of Pacific oysters in the UK has increased as aquaculture sites have proliferated and expanded (Figure 1.5). This coupled with multiple years of failing recruitment of the native oyster, and continued closure of native oyster fishery grounds, has seen a shift in the industry such that farmed Pacific oysters now contribute approximately 90 % of UK oyster production (FAO 2012).

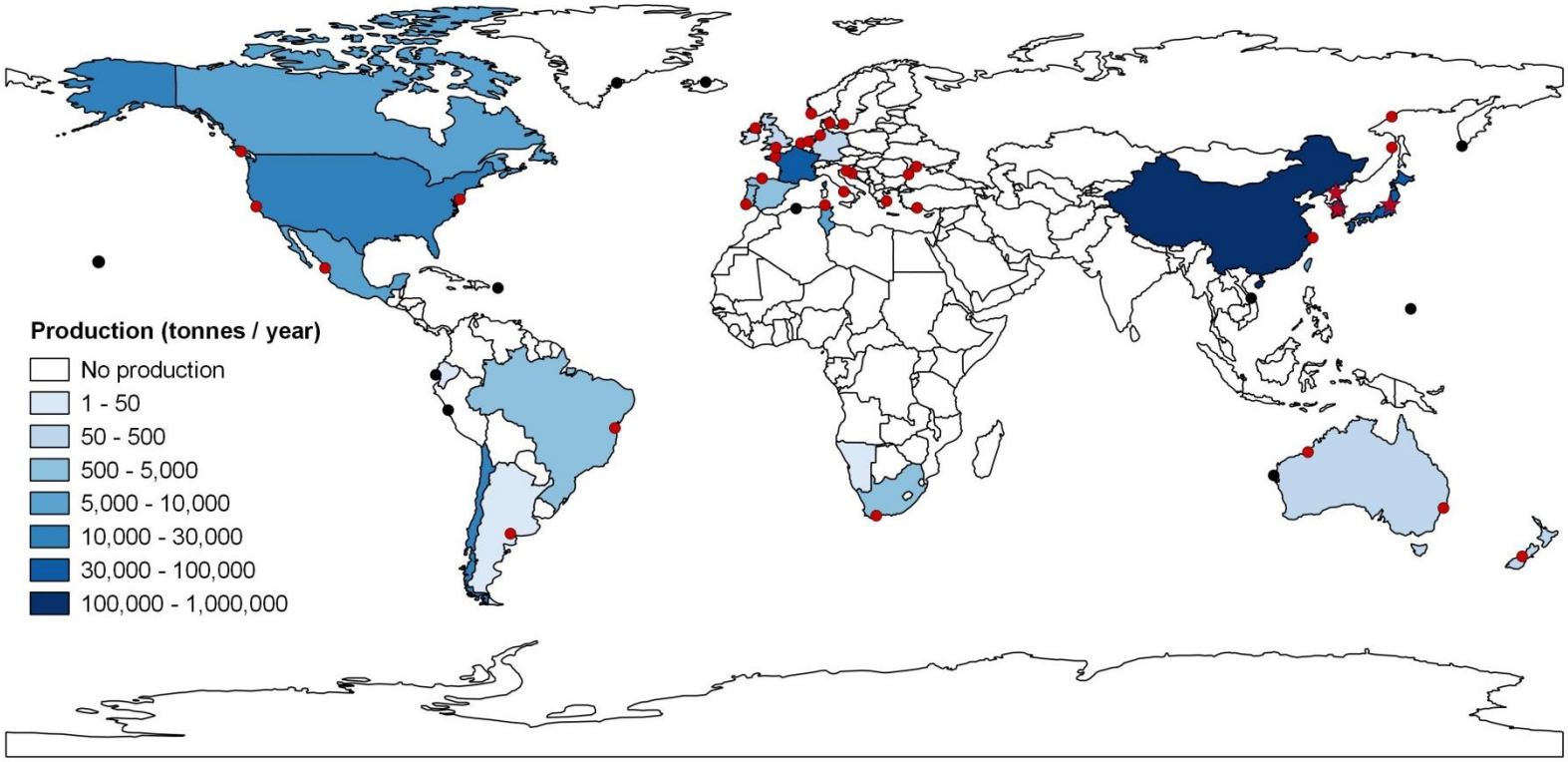


Figure 1.4 World map showing production of *Crassostrea gigas* from aquaculture by country, and geographical positions where *C. gigas* has been confirmed as established (red) or absent (black) from native biota. Production values are FAO 2012 – 2015 statistics. Presence / absence of wild *C. gigas* are published in reviewed literature, notably: Ruesink et al. 2005, Carrasco & Barón 2010 and Wrange et al. 2010.

In the UK, the greatest tonnage of farmed *C. gigas* comes from England, with the highest concentration of aquaculture beds occurring in the South east and the South west of the country (Crane & Laing 2008). There are a variety of methods for cultivating oysters that reflects the environmental requirements of the oysters, and the logistical practicalities, at different sites. Generally culture methods fall into two categories: bottom culture and off-bottom culture. Bottom culture refers to all culture methods that involve laying oysters directly onto the seabed, and off-bottom culture refers to those that require some form of structure to keep the oysters off the seabed. In England Pacific oysters are generally grown using bottom culture (Ellis et al. 2015). There are sites, however, that utilise trestle tables, baskets (Portland Oysters) or mesh bags on sinking platforms (Simpers of Suffolk). Losses to predation are notable in bottom culture and the use of dykes (low rock or cement walls to retain a pool of water), cages or mesh bags are sometimes used in conjunction with bottom culture to reduce mortalities (Dare et al. 1983; Jory et al. 1984; Héral 1991; FAO 2014a). Juvenile oysters are at the highest risk to predation and often held in mesh bags regardless of the final growing-out method. Mesh bags may be attached to infrastructure on the beach (e.g. Whitstable Oysters) or floated from pontoons (Othniel Oysters).

Bottom culture typically produces oysters with slower growth rates but thicker shells and a stronger adductor muscle than off-bottom culture (Taylor et al. 1997; Comeau 2013). Strong shells and adductor muscles are desirable to oyster growers as oysters are less likely to break during handling, or to gape open and dehydrate whilst in transit to market. However bottom culture is not suitable on very soft mud as oysters may suffer siltation or sink, or on sand that can shift and cause burial.

Off-bottom culture utilises cages, trays, racks (trestle tables), stakes, pontoons and other floating platforms (Héral 1991; Davis et al. 2013; FAO 2014a). The design differs between farms owing to local availability of materials and individual innovations. Oysters are typically contained within mesh bags and attached to the infrastructure of cages, trays and racks that require a substrate firm enough to support the structure. Oyster stakes however, can be used on very soft sediment. Oysters may be attached to the stakes directly in ‘stick culture’ or hung in bags along lines between stakes in ‘suspended culture’. Off-bottom culture is usually located in the intertidal zone in areas with sufficiently large tides to allow harvesting and maintenance to occur on foot during low tide. Aerial exposure during a tidal cycle also acts to naturally reduce biofouling (Bishop & Peterson 2006a). Culture methods that float the oysters on the surface of the water typically encase the oysters in mesh and never let them go dry. Floating structures are less constrained by

tide and substrate type. However the bags need frequent flipping to avoid biofouling and to shape the oysters.

The quality of Pacific oyster meat fluctuates over the reproductive season. The poorest meat occurs post-spawning and can prevent oysters going to market. Polyploidy oysters have been reared in hatcheries to enhance growth versus reproduction, allowing good meat condition throughout the year. Thermal or chemical shocking during early stages of development, or the cross breeding of tetraploid and diploid oysters, results in triploid larvae (Quillet & Panelay 1986; Guo et al. 1996). Triploid *C. gigas* have enhanced growth rates (Goulletquer et al. 1996; Nell & Perkins 2005) and under the correct environmental conditions, triploid oysters have the potential to grow 35 – 51 % larger than diploids within their first year (Guo et al. 1996).

The additional set of chromosomes in triploid organisms is often assumed to result in sterility (Thorgaard 1983), providing a security against the establishment of wild 'escapee' oysters from culture plots. The effect of triploidy varies between organisms; triploid *C. gigas* are neither completely sterile nor stable. They produce gametes that are fully capable of fertilisation (Guo & Allen 1994) and triploid oysters can revert back to a diploid state (see Herbert et al. 2012 and Syvret et al. 2008). The quantity of gametes produced per individual triploid oyster is fewer than in diploid *C. gigas* (Allen Jr & Downing 1990; Normand et al. 2008), and the survival of fertilised eggs to metamorphosis and settlement is considerably lower (Guo & Allen 1994). Reproductive output of triploid *C. gigas* is variable and what governs this variability is poorly understood. For example during a heat wave in France, unusually warm water was found to enhance reproduction in triploids, resulting in the spawning of mature gametes (Normand et al. 2008). However in the USA, triploids held in ambient temperatures of 8 – 15 °C underwent gametogenesis, and triploid *C. gigas* held in elevated water temperatures (30 °C) did not ripen (Shpigel et al. 1992a). It was speculated that an abundance of phytoplankton in conjunction with elevated temperatures may have favoured gametogenesis in triploid *C. gigas* in France (Normand et al. 2008).

It has been postulated that the enforced use of triploid *C. gigas* in UK aquaculture could eliminate possible environmental impacts through wild settlement (Syvret et al. 2008). However no such regulations have been passed, and despite triploid seed being available from several UK hatcheries, the majority of cultured *C. gigas* are diploid (Herbert et al. 2012). Furthermore concerns have been expressed by the UK *C. gigas* industry against triploid oysters. Suggesting that in some areas triploid *C. gigas* are difficult to cultivate because growth is not sufficient to reach market quality, whereas in other areas, growth rates are so high that the increased husbandry and maintenance required is not viable (Herbert et al. 2012). In France, triploid oyster production is increasing and there is a high demand for the 'superior' triploid hatchery spat

despite inflated prices; 80% of the 800 million spat sold by French hatcheries in 2005 were triploids (Buestel et al. 2009).

It should be noted that all *C. gigas* sampled during this thesis were diploid.

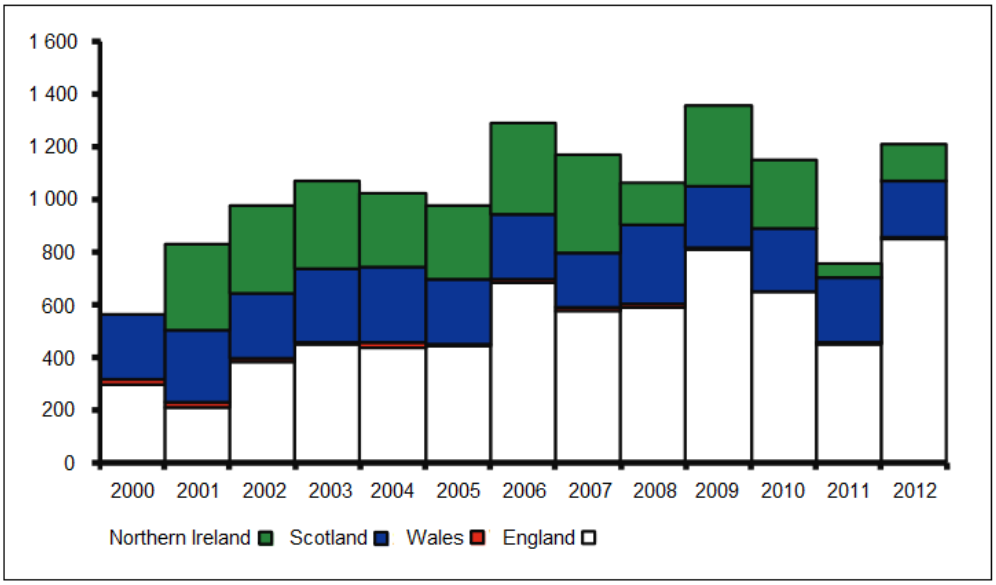


Figure 1.5 Time series of UK *Crassostrea gigas* production (tonnage), split by nation where reported. Adapted from "Aquaculture statistics for the UK, with a focus on England and Wales 2012" by Ellis et al. 2015.

1.3 *Crassostrea gigas* as an invasive species

1.3.1 Global distribution

Crassostrea gigas is considered to be a cosmopolitan species with global distribution. The introduction pathway can be traced back to aquaculture for the majority of countries (Andrews 1980; Grizel & Héral 1991; Spencer et al. 1994; Drinkwaard 1998; Orensanz et al. 2002; Ruesink et al. 2005).

Within Europe *C. gigas* was largely imported for aquaculture as a response to the over exploitation of the native oyster *Ostrea edulis* (Ruesink et al. 2005; Miossec et al. 2009; FAO 2014a). There has been a steady decline in global landings of *O. edulis* since at least the 1950s that has been exasperated by harsh winters and disease outbreak (Crisp 1964; Robert et al. 1991; Arzul et al. 2006; FAO 2014b). In particular, stocks of *O. edulis* in England and Wales were notably reduced following the severe winter of 1962/63 when the average winter water temperature (Jan-Mar 1963) in the western English Channel was 2.5°C colder than winter temperatures averaged between 1892 and 1956 (Holme 1963). Oyster landing decreased from approximately 8

million oysters per annum during the 1950s to only 3 million per annum in the 1960s (Davidson 1976). The disease Bonamiasis caused severe mortalities in *O. edulis* throughout Europe reducing production by up to 93% in the 1970s and 1980s (FAO data). This is a disease that has not been eradicated and continues to spread in the UK (Laing et al. 2014).

Trials for the introduction of *C. gigas* were carried out in Europe between 1964 and 1979, and concluded that *C. gigas* could be grown in the UK, Netherlands, Germany, Denmark, Sweden and Norway without risk of naturalisation due to low summer temperatures inhibiting reproduction (Spencer et al. 1994; Drinkwaard 1999; Kerckhof et al. 2007; Smaal et al. 2009; Wrange et al. 2010). Consequently imports began soon after. In the UK and Belgium *C. gigas* persisted after trials and imports ceased (Spencer et al. 1994; Kerckhof et al. 2007) and in the Oosterschelde Estuary the first recording of spatfall occurred in 1975 (Smaal et al. 2009). The proliferation of *C. gigas* within the Oosterschelde estuary initiated first and was perhaps the most virulent. 1976 is considered the beginning of the expansion phase and by 1980, 25 ha of wild intertidal oyster beds were present in the estuary. By 1990 this had doubled to 55 ha and in 2005, 775 ha of wild oyster beds were mapped, including an expansion into the subtidal zone on hard substrates down to 45 m (Smaal et al. 2009). The establishment and spread of *C. gigas* outside of cultured plots in the Oostershelde estuary was initially described as invasive (Reise et al. 2006), however in 1982 *C. gigas* was accepted as belonging to the fauna of The Netherlands and the commercialisation of Dutch cupped oysters (*C. gigas*) began (Smaal et al. 2009).

Notable wild recruitment coincided with summer temperatures that were a few degrees above 'normal' such as in 1982 and 1986 in the Dutch Wadden Sea (Drinkwaard 1999), and during the early 1990s when settlement was recorded outside cultured plots in the German Wadden Sea, the UK and in Belgium (Spencer et al. 1994; Reise 1998; Kerckhof et al. 2007). Unlike feral *C. gigas* in the UK which has remained at low abundances, *C. gigas* has spread considerably throughout the Wadden Sea and along the Belgian coast, rapidly establishing large intertidal beds, and in some areas reefs (Reise 1998; Drinkwaard 1999; Kerckhof et al. 2007). In the German Wadden Sea an aggregation of approximately 1 million oysters had established between the Islands of Sylt and Rømø in 1995, just 8 years after farming operations (producing approximately 25 tons per year) began and 4 years after wild recruitment was first recorded (Reise 1998). In 2006 and 2007 proliferation occurred in Scandinavia. Reef formations were observed in Denmark and Sweden with the highest densities reaching 505 oysters m^{-2} (Wrange et al. 2010). In Norway 94 *C. gigas* ranging between 48 and 123 mm and from 4 size cohorts were collected from a channel at Espevik pushing the northern boundary of distribution as far as 60°N (Wrange et al. 2010).

France is the largest producer of *C. gigas* in Europe (FAO 2014a). The introduction of *C. gigas* occurred in 2 phases and with the intention of *C. gigas* becoming a replacement species to *O. edulis.* Therefore the introductions had a different desired outcome to those occurring elsewhere in Europe. Seed oysters were intended to restock depleted beds whilst broodstock were imported to reseed those beds for future years (Grizel & Héral 1991; Miossec et al. 2009). The initial phase of introductions occurred in 1860 from Portugal. It was a successful introduction as *C. gigas* (introduced under synonym *C. angulata*) reproduced and spread and became co-cultured with *O. edulis.* In 1966 an irovirus outbreak that affected gill tissues resulted in mass mortalities and the need to import further stock. Several hundred tons of broodstock were imported from British Columbia between 1971 and 1976 alongside over 10 000 tons of spat from Japan (Grizel & Héral 1991). A total of 5 culture areas were stocked including the Arcachon and Marennes-Oleron Bays that quickly established to produce sufficient spat to supply all French production sites. By the turn of the century spat collection across France was approximately 4.5 billion, 3 billion of those spat were collected from the Marennes-Oleron Bays alone, and sustained all other French production regions through supplying spat (Miossec et al. 2009).

There are a few countries where *C. gigas* has begun to establish or has reached notable abundance and is considered invasive, where there are no registered *C. gigas* aquaculture beds (Dinamani 1971; Andrews 1980; Galil 2000). For example *C. gigas* was first noticed in New Zealand on spat collectors laid out for *C. glomerata* in 1970 (Dinamani 1971). The nearest well established population of *C. gigas* was in Australia some 1,200 miles across the Tasman Sea, consequently it was concluded that the *C. gigas* found on spat collectors must be the progeny of *C. gigas* attached to ships hulls, discarded *C. gigas* intended for food, or the larvae carried over in ballast water (Dinamani 1971). By 1990 *C. gigas* was the dominant species of oyster farmed in New Zealand and feral intertidal beds could be found on rocky forshores, amongst mangroves and within manmade harbours (Dinamani 1991). Globally, proliferation of feral *C. gigas* has largely occurred independently from dates of introduction. Coinciding instead with uncharacteristically warm summers (Minchin 2007; Sorte et al. 2010; Troost 2010).

1.3.2 UK distribution

Shipments of *Crassostrea gigas* to the UK commenced in 1890 to support the failing native oyster (*Ostrea edulis*) industry. There were no recorded incidences of wild recruitment between 1890 and 1962 when imports of *C. gigas* ceased (Spencer et al. 1994; Humphreys et al. 2014). However individuals or small clusters of *C. gigas* have been recorded in England since trials were carried out for their introduction into aquaculture in 1964 (Utting & Spencer 1991; Spencer et al. 1994). The oysters that resulted from these early trials did not persist, however multi-generational

aggregations have established in close proximity to *C. gigas* aquaculture since the 1990s. The greatest extent of settlement occurs in the south west and south east of England. Settlement is sporadic in the south west whereas regular settlement now occurs in the south east which is often used by growers as a source of seed (Syvret et al. 2008; McKnight 2009). Even within these regions there is notable variation, dense reefs have formed in the River Yealm, where anecdotally 1000 oysters can be counted in a 30 minute period (Couzens 2006), and only 100 km further east, in Portland Harbour and the Fleet, there is an apparent absence of recruitment despite cultivation in that area (Eno 1994; Herbert et al. 2012). The majority of feral oysters are the progeny of farmed adults (Lallias et al. 2015), however wild aggregations are becoming increasingly disassociated from culture plots. Distribution along the south coast in particular does not conform to the previous pattern of association with culture plots (pers. obs.).

1.4 Aquaculture as a vector for invasive species

Aquaculture has grown faster than any other food production sector over the past three decades and a total of 575 aquatic species and species groups are now (2016) registered in the Food and Agriculture Organization of the United Nations (FAO) Global Aquaculture Production statistics database. The production of unfed species contributed 31 % of the world 'food fish' aquaculture production in 2013, including 13.9 million tonnes of bivalves (FAO 2015).

Aquaculture is often carried out in the intertidal zone or inshore coastal waters to cater for the needs of the species being cultivated, whilst allowing relatively easy access for workers. However it is also the coastal waters that suffer the greatest introductions of non-indigenous species, some of which can be termed 'invasive'. In part, species that go on to become invasive are well adapted to exploit and proliferate highly disturbed areas. Therefore aquaculture of non-indigenous species provides a potentially high propagule pressure for invasive species, and promotes the spread of species outside their natural range (Carlton 1996a; Naylor et al. 2001).

In order to prevent cultured species from becoming invasive, Legislative framework has been put in place to prevent accidental introductions and to guide authorities on decisions regarding the introduction and transfers of marine organisms. The United Kingdom is a member country of the International Council for the Exploration of the Seas (ICES). Consequently all aquaculture practices are required to adhere to the recommendations procedures and practices put forth in the ICES Code of Practice on the Introductions and Transfers of Marine Organisms, with the aim of minimising the risks of detrimental effects from the international introduction and transfer of marine organisms. The code applies to public (governmental and commercial), private, and scientific interests including introductions into closed containment (ICES 2005).

1.5 Impacts of *Crassostrea gigas* establishment

The introduction of a non-native species can create an imbalance in an ecosystem through disruption of the community structure. Disruptions typically associated with introduced bivalves, include competition for resources such as space, food, and nutrients and the modification of habitat through structural engineering, bioturbation, or the alteration of sediments as a result of feeding and biodeposits (Katsanevakis et al. 2014). Furthermore non-native species often arrive free from native predators, parasites, and diseases. This can allow for rapid population expansion, particularly when combined with high fecundity and a tolerance to environmental conditions. Such characteristics are typical of species that go on to become invasive.

The impacts of naturalising *Crassostrea gigas* are wide spread and varied as they have the potential to alter ecosystems and impact upon the economy.

1.5.1 Ecological impacts

The greatest ecological concern when considering *Crassostrea gigas* is often their ability to alter habitat type. *C. gigas* are deemed as 'ecosystem engineers' because they have the ability to alter habitats through the construction of hard reef structures. Once established a reef will persist after the animals die. Perhaps the most dramatic alterations are seen when reefs form on soft substrate. The change of dominant substrate type from soft mud (or sand) to hard shell, such as that seen in the Wadden Sea (Reise 1998), the Oosterschelde (Netherlands) (Drinkwaard 1999; Smaal et al. 2009) and the Bay of Brest (France) (Lejart & Hily 2011), alters what species can survive there. Ultimately this can cause a change in species richness (Van Broekhoven 2005; Lejart & Hily 2011), biodiversity (Van Broekhoven 2005; Green & Crowe 2013; Green & Crowe 2014) and the relative proportions of functional groups (Lejart & Hily 2011). A change to the colonising species of an area will have repercussions up the food web as they represent prey species for particular predators. Therefore a change in large predatory species visiting the area would also be expected (Cranfield et al. 2001; Beaumont et al. 2007; Markert et al. 2013).

C. gigas reefs have a stable and complex structure that generates a variety of micro-habitats. The stability of the reef results from an oysters immobility and the engineering of the reef through the cementation of many shell valves to one another. Such stability favours the colonisation of sessile species such as anthazoans, hydrozoans, barnacles and algae (Beaumont et al. 2007; Markert et al. 2010). Such species further benefit from the large surface area of oyster shell, per unit area of reef, generated by the vertical orientation of the oysters and their extended shell margins (Van Broekhoven 2005; Markert et al. 2010; Troost 2010). Deep crevices between vertical shells

provides protection from predation and desiccation, a niche exploited heavily by vagile epizoic species such as the European shore crab (*Carcinus maenas*) and periwinkle (*Littorina littorea*), that combined, can contribute to 50 % of the biomass on a *C. gigas* reef (Markert et al. 2010). There can be coexistence between a number of competing predatory groups on *C. gigas* reefs as the complex structure reduces interference (Markert et al. 2010). The abundant presence of shore crabs has led to enhanced numbers of some bird species feeding at low tide, for example the Eurasian curlew (*Numenius arquata*) feeds extensively on shore crabs on reefs in the Wadden Sea (Markert et al. 2013).

The reef causes water currents to be turbulent along the shell edges and potentially non-existent deeper within the shell crevices. Low energy hydrodynamics favours sedimentation that builds up at the base of the reef and between shells, as well between the foliate shell structures of living *C. gigas* and in the empty cases of dead barnacles and bivalves. The multitude of sediment niches present allows for a variety of opportunistic, facultative filter-feeding polychaetes to inhabit the reef (Van Broekhoven 2005; Markert et al. 2010). Deposit feeding oligochaetes can be found in large numbers inhabiting the organic rich biodeposits accumulating near the base of the reef. Oligochaetes are indicators of high organic pollution and point toward an increased O_2 depletion, and H_2S production, resulting from maximum exploitation of biodeposits (Markert et al. 2010).

Larvae are able to settle and metamorphose onto an array of hard substrate and therefore interfere with an ecosystem without necessarily having to build a reef. The colonisation of other biological reefs by *C. gigas* has largely been detrimental to the existing reef-building species. Reefs of the Honeycomb worm, *Sabellaria alveolata,* have deteriorated under dense settlement of *C.gigas* in Bay St-Michel, France (Dubois et al. 2006). *C. gigas* are thought to be displacing *S. alveolata* by occupying substrate that *S. alveolata* could potentially colonise, negatively impacting *S. alveolata* condition through competition for food and as a result of physical damage caused by humans harvesting the oysters off the *S. alveolata* reef (Desroy et al. 2011; Herbert et al. 2012). In the UK, *C. gigas* has settled on reefs of Ross worm, *Sabellaria spinulosa*, and the sand mason worm, *Lanice conchilega,* overgrowing the reefs and resulting in partial displacement (McKnight 2011; McKnight 2012).

A common basibiont of *C. gigas* in Europe is the blue mussel, *Mytilus edulis* (Reise 1998; Nehls et al. 2006; Markert et al. 2010). Mussel beds in the Wadden Sea are in decline and many have become heavily colonised by *C. gigas* (Nehls et al. 2006). To what extent *C. gigas* has contributed to the decline of *M. edulis* is unclear. It has been theorised that as a result of *C. gigas* colonising shell debris associated with former mussel beds, and so occupying a potential *M. edulis* habitat, they may have interfered with *M. edulis* re-colonisation (Herbert et al. 2016). There is evidence

that oysters that attach and grow onto live mussels eventually outgrow and suffocate them (Reise 1998). The relative abundances then (1995), were approximately 8 *C. gigas* to 2000 *M. edulis* m^{-2}, and so the effect at population level was considered negligible (Reise 1998); reports of *C. gigas* numbers on *M. edulis* reefs in the Wadden Sea have since increased to 600 oysters m^{-2} (Nehls et al. 2006). *M. edulis* also colonise *C. gigas* reefs, spatfall of *M. edulis* has found to be similar across both oyster and mussel reefs (Diederich 2005) and reduced on oyster reefs (Markert et al. 2010). However settlement on an oyster reef can reduce juvenile mortality of *M. edulis* and offer some protection from bird predation for adults (Markert et al. 2013). For mussels living on an oyster reef there does, however, appear to be compromises to fitness and growth (Herbert et al. 2016).

1.5.2 Economic impacts

The main economic impacts of invasive marine species are negative impacts on human health and a decrease in economic production of fisheries, aquaculture, marine infrastructure and tourism (Bax et al. 2003).

Human health may become affected when *C. gigas* are collected and processed as a food source outside of regulated fishery and aquaculture processes. Bivalves concentrate contaminants from the water in which they grow, which in turn, may cause illness to the humans that eat them. As oysters are typically eaten raw, the risk of microbial contaminants is enhanced. In Europe there is a history of coastal areas having a high populous which has caused problems with faecal contamination of shellfish harvesting areas. This occurs both through the release of human sewage directly into the water, and animal sewage washed off the land. Ideally shellfish would be cultured in areas free from pollutants, however such sites are rare (FSA 2016), and so methods to reduce the risk of humans consuming contaminated shellfish became statutory. They include re-laying, depuration and heat treatment. Only re-laying and depuration are relevant to bivalves, such as oysters, that are eaten raw. They work through submersing bivalves in clean seawater and the resulting expulsion of intestinal contents. Re-laying bivalves means that they are moved to another area of seabed classified as Class-A. Depuration is carried out in tanks of real or artificial seawater submitted to UV or ozone cleansing and under conditions that maximise filtering activity. Both methods are effective at removing many faecal bacterial contaminants from shellfish, and so reducing the risk of illnesses such as gastroenteritis in humans that eat the treated shellfish (Lee et al. 2008).

These treatments are only suitable for the removal of light to moderate levels of microbial contaminants and cannot be used for heavily contaminated shellfish. They are limited in removal

of viral contaminants such as norovirus and hepatitis A and naturally occurring marine vibrios. Furthermore they are ineffective at removing marine biotoxins such as those causing paralytic shellfish poisoning PSP, diarrhetic shellfish poisoning DSP and amnesic shellfish poisoning ASP (Lee et al. 2008). Therefore it is critical to have an understanding of contaminants in the areas shellfish are harvested, allowing for suitable safety measures to be taken. To this end, sanitary surveys of shellfish harvesting areas are carried out and the area is classified according to EU Regulation 854/2004. Bivalve harvesting areas are classified according to the extent of contamination shown by monitoring *E coli* in shellfish flesh. The classification of an area also stipulates the necessary treatment processes required by EU legislation, to reduce the risk to human health from eating shellfish harvested from those areas (Table 1.2).

Chemical contaminants, such as heavy metals, pesticides, organochlorides and petrochemical substances are a potential hazard in some areas and so tested for under Regulation EC 854/2004. However there is little evidence that illness arising from the consumption of contaminated shellfish is a notable problem.

Table 1.2 Classification of shellfish harvesting areas and the required necessary sanitary treatments as stated by Annex II of Regulation (EC) No 654/2004.

Status	**Contamination limit**	**Necessary treatment**
Class A	≤ 230 *E. coli* / 100 g	None (can be harvested for direct human consumption)
Class B	90% of samples: $\leq$ 4600 *E. coli* / 100 g All samples: ≤ 46000 *E. coli* / 100 g	Purification in an approved plant or re-laying in an approved Class A re-laying area or EU-approved heat treatment process
Class C	≤ 46000 *E. coli* / 100 g	Re-laying in an approved Class A re-laying area for at least 2 months & (where necessary) purification in an approved plant or EU-approved heat treatment process
Prohibited	> 46000 *E. coli* / 100 g	Bivalves must not be subject to production or collected

Therefore *C. gigas* collected outside of regulated areas are more likely to be contaminated with pathogens or chemical contaminants. The risk to human health is far greater under these circumstances as treatments may not be sufficient in rendering the oysters safe for human consumption. Furthermore oysters collected outside of regulated shellfish harvesting areas are often not treated at all before consumption or resale.

A decrease in economic production of fisheries and aquaculture can be caused through the fouling of equipment by feral *C. gigas* and consequential competition for resources. Severe fouling of trestle tables by wild *C. gigas* has caused a reduction in the production of both cultivated *C. gigas* and *M. edulis* fisheries in Europe (Diederich 2005b; Cognie et al. 2006; Diederich 2006b; Wrange et al. 2010).

C. gigas can be detrimental to marine infrastructure through fouling. *C. gigas* colonises a wide range of anthropogenic substrates and appears to be more resilient than many other biofouling organisms to common antifouling treatments (Pers. obs. Appendix B). Boat hulls of all sizes can become colonised by *C. gigas* which in turn decrease the efficiency of the vessel and increases energy wastage of vessels underway. Fouling of industrial structures, such as industrial water cooling and effluent piping, results in a reduction or cessation of essential processes (Rajagopal 2005). There has been a loss of tourism to beaches in the Wadden Sea, as sharp shells pose a threat to the safety of humans and animals alike (e.g. dog walking) (Nehls et al. 2006).

The proliferation of feral *C. gigas* can however also have positive impacts on the economy. Aquaculture could benefit from a low cost supply of seed, and employment could be increased through management jobs as well as the opening of new fisheries.

1.6 Thesis structure

Research will be carried out at two case-study locations on the south coast of England to determine the extent that *Crassostrea gigas* has established. The reproductive output and recruitment of *C. gigas* will be investigated in relation to environmental conditions and biological interactions. Specifically, a baseline of feral *C. gigas* distribution and abundance will first be established at each of the study sites along with an assessment of the influence of substrate type on distribution. Subsequently investigations will then be carried out to establish whether or not *C. gigas* are reproductively active, and the relative importance of predatory pressure and winter mortality on the survival of recruitment. The results from each section will be brought together using the conceptual framework outlined in Figure 1.5. A conceptual framework is the preceding step to development of a final model (Guisan & Zimmermann 2000). It is hoped that through

illustrating the results in such a way, it will demonstrate how information, such as that gained through this study, could be practically applied in the future.

Models are a valuable tool for predicting the spread of non-native species. They allow for multiple factors to be considered simultaneously, which is particularly useful when considering ecological processes that are not fully understood. Species distribution models are widely used to estimate current and potential distributions and mechanistic models are designed to relate the presence of a species to its physiological needs. A mechanistic model predicts a geographical region, known as the functional niche, which has an appropriate set of abiotic factors for that species to survive. However restricted larval access, strong interactions with predators and competitors, and anthropogenic activities in an area (fishing, construction etc.), may restrict a species distribution from fulfilling its functional niche and being restricted to a more specific geographic area, referred to as the realised niche.

For the purpose of this study, the driving variables and constraints will be divided into 4 levels of predictors; indirect and direct gradients, resources and negative impacts (Austin 1980; Austin et al. 1985). Indirect gradients have no direct physiological importance and include elements of the habitat such as substrate type, shore width and residence time that will be addressed in chapter 3. Direct gradients are environmental factors of physiological importance including temperature, salinity and phytoplankton. The effect of temperature on reproduction will be addressed in chapter 4 and the reduction in metabolism resulting from reduced temperatures over winter will be discussed in chapter 6. Resources simply specify the matter and energy consumed by the organism. The negative impacts are not further divided into direct or indirect effects on the physiology but rather listed if they are detrimental to the health (and so condition) of *C. gigas,* such as a parasitic infection, or have a lethal impact, such as predation or fishing. Predation of *C. gigas* will be considered in chapter 5.

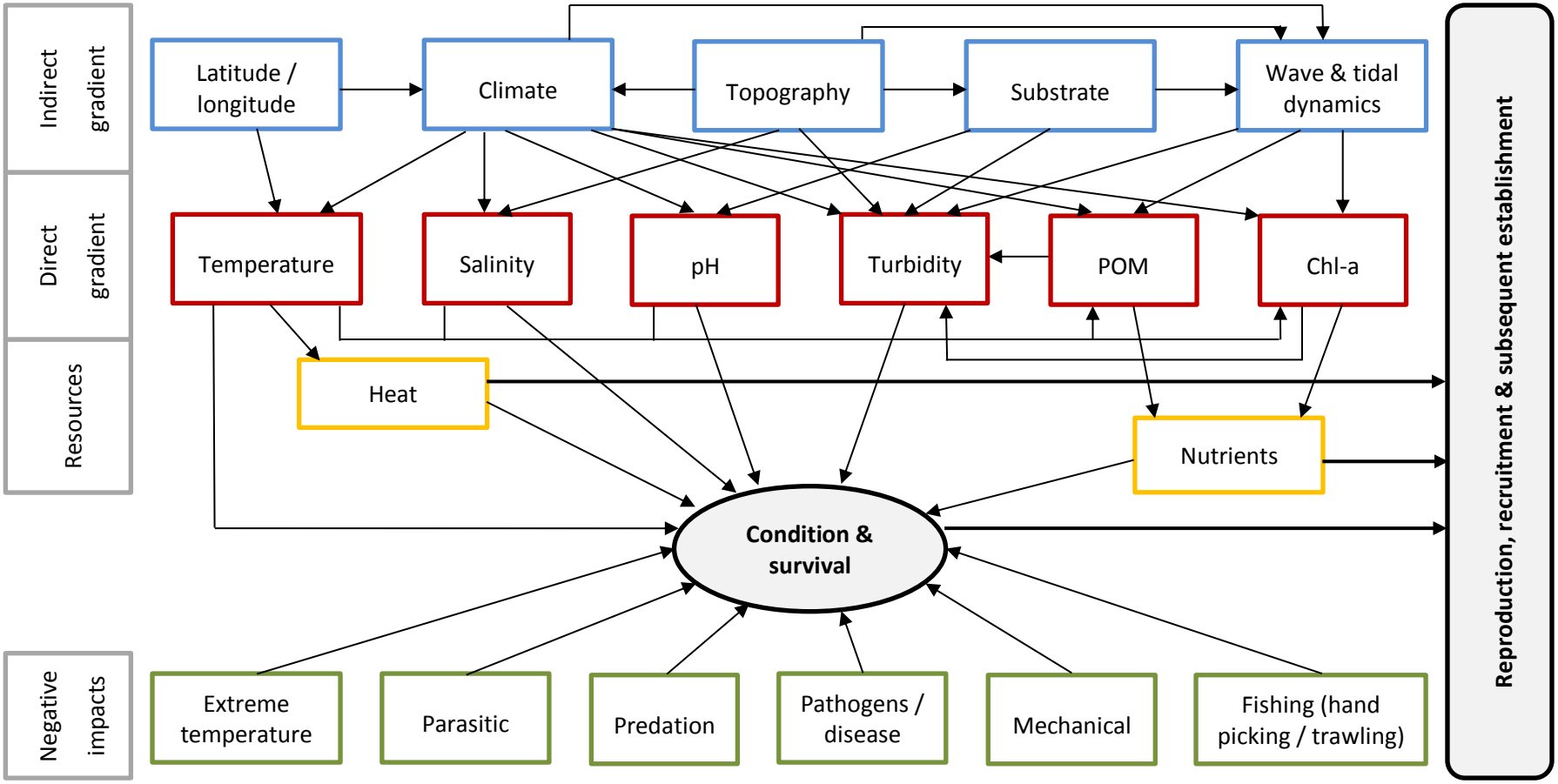


Figure 1.6 Conceptual frame work for possible use in predicting the future spread of *Crassostrea gigas*

1.7 Aims, Hypotheses and Objectives

Aims

The Pacific oyster, *Crassostrea gigas*, has a global distribution largely due to its popularity within the aquaculture industry. The spread of established feral oysters is expanding as naturalisation occurs in an increasing number of countries. The invasive nature of *C. gigas* means that there is the potential for serious and negative economic and ecological impacts as a result of naturalisation. Consequently there is great interest in understanding how reproductive output and recruitment is affected by environmental conditions both by the oyster industry which relies on a year round supply of seed, and by environmentalists who want to understand the risk to environmental degradation that wild populations pose (Syvret et al. 2008; Herbert et al. 2012).

This study will use two case-study locations with very different scenarios relating to *C. gigas* to investigate the impacts of environmental conditions and biological interactions on reproduction, recruitment and the rate of establishment. The primary aim is to first establish a baseline of feral *C. gigas* distribution and abundance at the study sites and then to establish whether or not they are reproductively active. The relative importance of predatory pressure and winter mortality on recruitment will then be addressed.

Hypotheses

The overall thesis hypothesis is that the current distribution of *Crassostrea gigas* along the south coast of England does not represent the maximum possible spread or abundance.

Hypothesis 1: *Crassostrea gigas* is present outside the designated areas of aquaculture for this species on the south coast of England, colonising natural and anthropogenic substrate in the intertidal zone.

Hypothesis 2: *Crassostrea gigas* shows evidence of gametogenesis and spawning under environmental conditions currently typical in the south of England**.**

Hypothesis 3: *Crassostrea gigas* have become part of the food web, and as such suffer losses through predation that may impede recruitment success and the rate of establishment.

Hypothesis 4: Environmental winter conditions typically experienced in the south of England negatively impact *Crassostrea gigas,* causing mortalities and potentially impeding recruitment success and slowing the rate of establishment.

Objectives

Objective 1a: Map the distribution and abundance of *Crassostrea gigas* occurring in the intertidal zone within Southampton Water and Poole Harbour.

Objective 1b: Describe population dynamics such as age, growth and mortality through the analysis of size frequency distribution.

Objective 2: Investigate the reproductive traits of wild *Crassostrea gigas* that inhabit Southampton Water and Poole Harbour, and farmed *C. gigas* from Poole Harbour, using gonad histology, Condition Indices and Gonad Somatic Indices.

Objective 3a: Establish predatory pressure on *Crassostrea gigas* inhabiting the intertidal zone using predator exclusion experiments.

Objective 3b: Investigate the vulnerability of juvenile *C. gigas* to predation by *Carcinus maenas* through a series of laboratory controlled feeding behaviour experiments.

Objective 4a: Measure the effects of cold water temperatures, typical of those experienced in Southampton Water and Poole Harbour during the winter, on the respiration of adult and juvenile *Crassostrea gigas.*

Objective 4b: Assess the impact of freezing air temperatures on the gaping activity of *Crassostrea gigas.*

Chapter 2: Study sites

This study focuses on the intertidal Osteroida bivalve *Crassostrea gigas* collected from 2 estuaries on the south coast of England (Figure 2.1). This chapter describes the study sites where samples of *C. gigas* were collected.

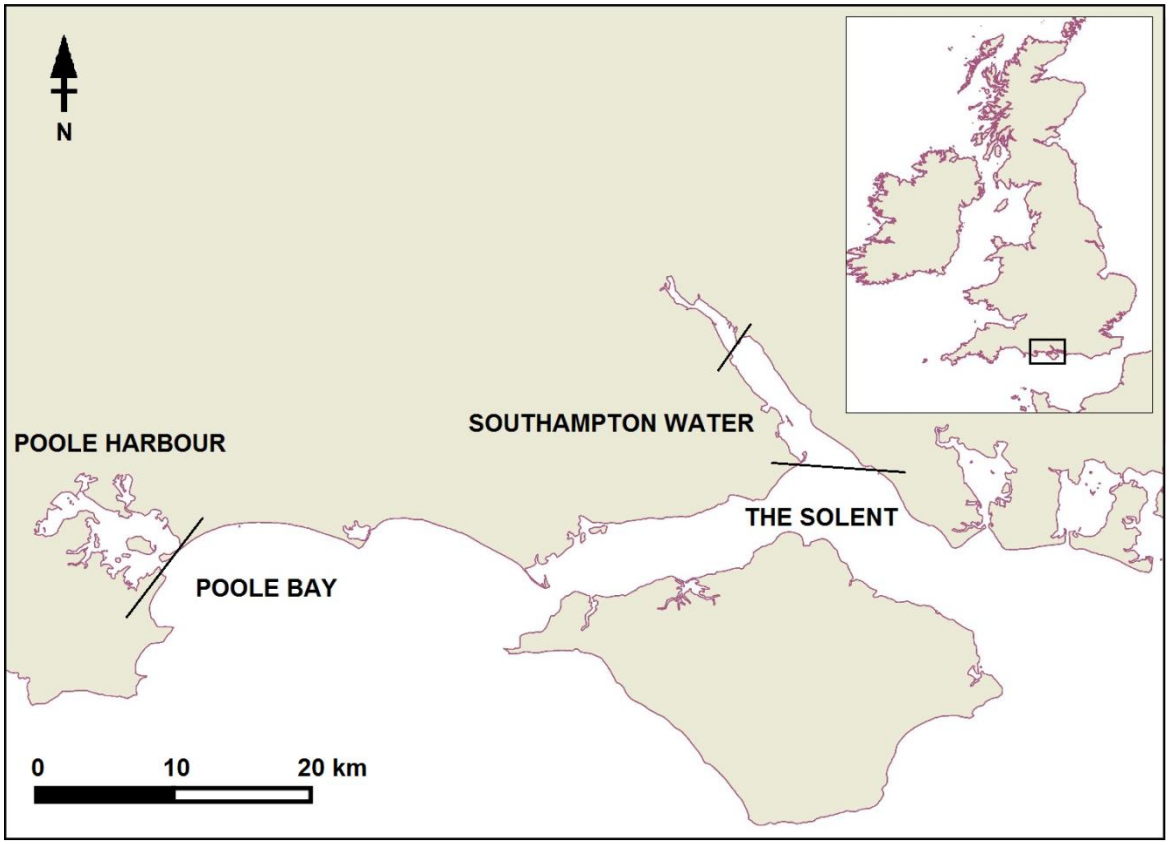


Figure 2.1 Map showing relative locations of the estuaries, Poole Harbour and Southampton Water, on the south coast of England where *Crassostrea gigas* were collected.

2.1 Poole Harbour

Poole Harbour is in the county of Dorset and was formed by the drowning of a river valley during post-glacial rise in sea level. Accretion has resulted in the narrowing of the entrance through spit growth to approximately 350 m. Poole Harbour has an average hydraulic depth of 0.45 m (Langston et al. 2003), a microtidal range of 1.8 m on springs and 0.6 m on neaps (Poole Quay) and an area of approximately 3,600 ha on a high water spring tide. Approximately 80 % of the total area of Poole Harbour is exposed on a spring low tide (Gray 1985). The tidal regime is

characterised by a prolonged “double” high water and asymmetric movement of water with the ebb tide dominating the tidal excursion and resulting in sustained water levels above mean tide level for around 16 out of every 24 hours (Humphreys 2005).

There is over 100 km of highly indented shoreline. The typical natural shoreline consists of extensive mudflats and sandflats fringed by reedbeds and saltmarsh and backed by low bluff and eroding cliff (Figure 2.2). Natural shoreline is however limited to the southern shores as the north is largely urbanised. The beaches along the northern shores are retained using groynes and sit between marinas, ports, quays and piers. The intertidal zone ranges from sand near the entrance through to a mixed muddy sediment including gravel and shell further west.

An extensive area of Poole Harbour is classified for shellfish harvesting (Figure 2.4), commercial shellfisheries consist of naturally occurring and farmed beds for the production of oysters, mussels, cockles and clams (Cefas 2009a). The double high water is of ecological significance because it extends the feeding period for many filter feeding invertebrates, such as bivalves, and conversely because limited exposure of the intertidal zone reduces the availability of feeding grounds for important bird populations (specifically waders). There are a number of statutory designations in place to protect the natural environment of Poole Harbour (Figure 2.3). These include Special Protection Areas (SPAs) that have been designated under the European Union Birds Directive (JNCC 2012), and a Ramsar site due to the notable quantity of waterfowl as well as the assemblage of rare, vulnerable and endangered species. Furthermore Poole Harbour is a designated Site of Special Scientific Interest (SSSI) for its range of estuarine habitats (including intertidal mudflats, saltmarshes, swamp and fen), recognised as part of the Poole Bay and Isle of Purbeck Sensitive Marine Area (SMA) and is an Area of Outstanding Natural Beauty (AONB). Finally Poole Harbour contains a number of non-statutory Sites of Nature Conservation Interest (SNCI) and Local and National Nature Reserves (Drake 2011).

Salinity of Poole Harbour is characterised by 2 distinct zones; the main body of the harbour is well mixed resulting in a near homogenous salinity profile. Surface salinity at the entrance to Poole Harbour, as measured throughout the year at multiple tidal stages, can range between 34.7 and 24.9. Wareham Channel (Figure 2.4) is the main fresh water flow into the harbour and as such represents a partially mixed zone where salinity stratification occurs vertically within the water column. Surface salinity at the entrance to Lytchett Bay (Figure 2.4) can range between 30.7 – 10.6, and further into Wareham Channel salinity ranges from 30.4 to 1.0 (Humphreys 2005).

Poole Harbour suffers from elevated levels of nutrients through both point and diffuse sources, including sewage treatment works and agricultural run-off (Drake 2011). Consequently it has been designated a Sensitive Area (eutrophic) and of Polluted Waters (eutrophic) under the Urban

Wastewater Treatment Directive and Nitrates Directive (EA 2010). Although microalgae blooms are less common than macroalgae blooms as a result of increased nutrient levels (Langston et al. 2003), microalgae were implicated in shellfishery closures due to the occurrence of amnesic shellfish poisoning in Poole Harbour in 1999.

Naturally occurring *Crassostrea gigas* were collected from a shallow, enclosed mudflat of approximately 12 ha known as Blue Lagoon (Figure 2.4). An aggregation of oysters had established on a patch of shell and shingle accreted on the inside of a meandering channel within the embayment. Farmed oysters were provided by Othniel Shellfish Ltd. from on-bottom culture plots located within the main body of Poole Harbour (Figure 2.4).

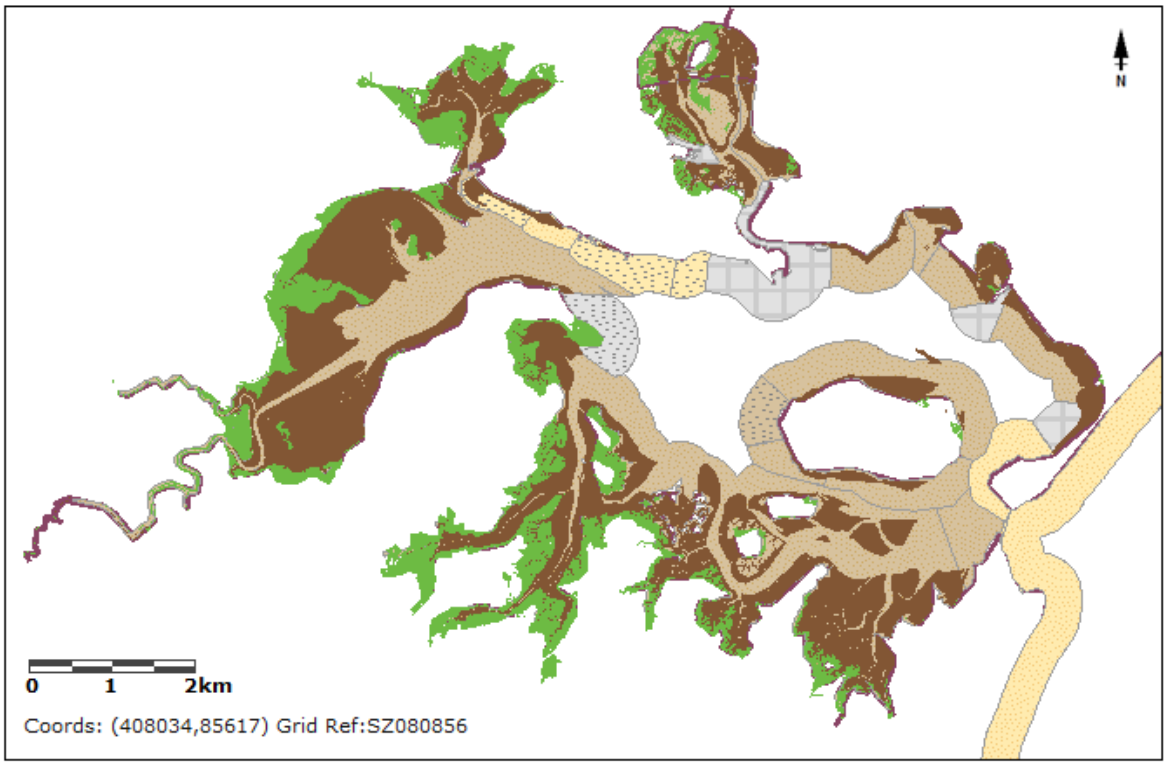


Figure 2.2 Poole Harbour habitat map: Priority Habitat Inventory; Mudflat ; Coastal Saltmarsh . Intertidal substrate foreshore; Mud ; Mud & Gravel ; Sand ; Sand & Gravel ; Rock platform with boulders/loose rock ; Man-made Ground . Generated using MAgiC Crown Copyright and databases 2016. Ordnance Survey 100022861.

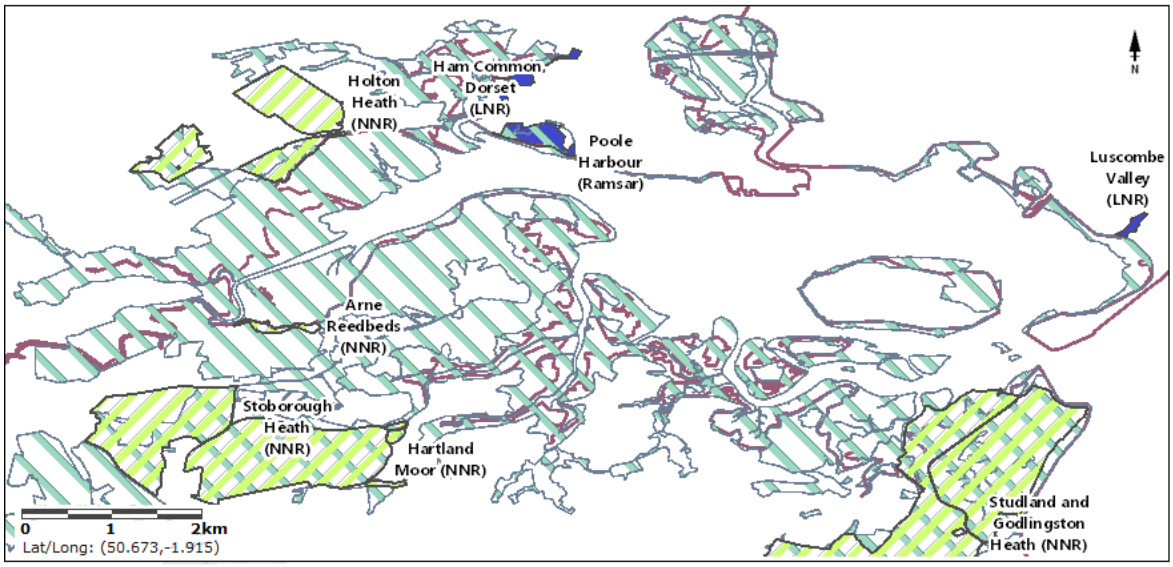


Figure 2.3 Poole Harbour statutory designations: Ramsar ; Local Nature Reserves ; National Nature Reserves . Generated using MAgiC Crown Copyright and databases 2016. Ordnance Survey 100022861.

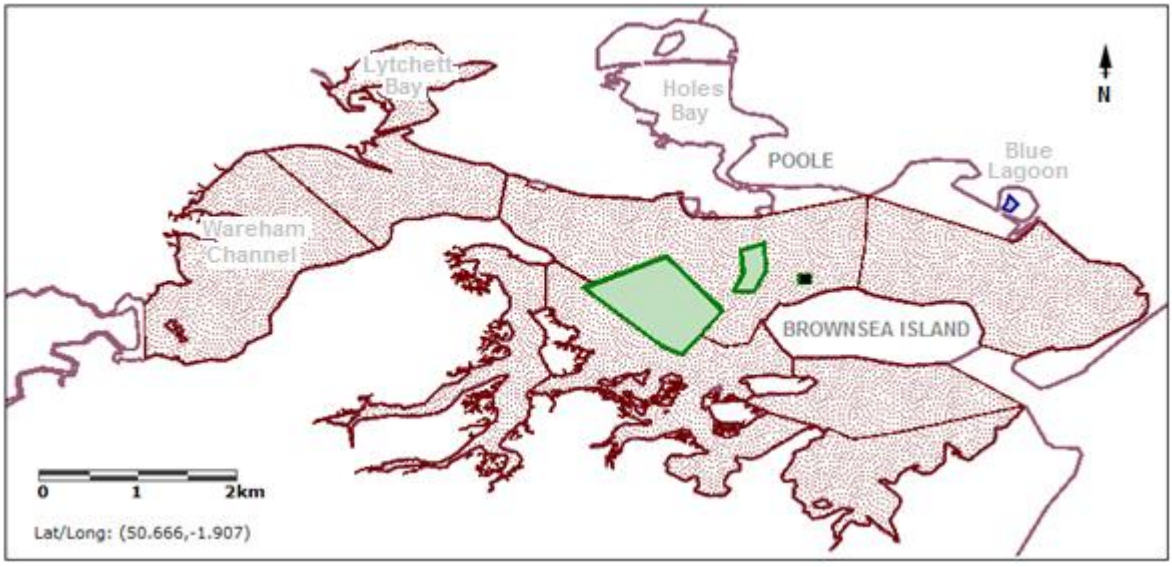


Figure 2.4 Poole Harbour showing *Crassostrea gigas* collection sites: Othniel Shellfish Ltd culture plots and processing barge ; feral aggregation in Blue Lagoon , and the Classified shellfish harvesting area . Generated using MAgiC - Crown Copyright and databases 2016. Ordnance Survey 100022861.

2.2 Southampton Water

Southampton Water is in the county of Hampshire and also formed by the drowning of a river valley during post-glacial rise in sea level. The entrance is approximately 1,960 m wide (at Calshot) as a result of several episodes of extension and breaching of the spit. Southampton Water has an average hydraulic depth of 6.2 m (ABPmer 2007), a mesotidal range of 4 m on springs and 2 m on neaps and an area of approximately 3,975 ha at high water (ABPmer 2007). Similarly to Poole Harbour, the tidal regime of Southampton Water is characterised by a prolonged "double" high water, asymmetric movement of water and an ebb tide dominating the tidal excursion.

The water column becomes stratified as fresh water from the 3 main tributaries, the Itchen, Test and Hamble, flows seaward and salt water flows into the estuary. Stratification is most pronounced at the head of the estuary, where the Test and Itchen join, and during low water. As the flooding tide reaches high water, mixing conditions dominate the estuary and salinity exhibits an almost homogenous vertical distribution (Levasseur 2008). Typical near surface salinity ranges from 18.1 – 34.2 psu at Southampton Docks to 30.8 – 34.8 at Calshot (Cefas 2009b).

There is approximately 110 km of shoreline. The western shore of Southampton Water is predominantly fringed by saltmarshes whilst the eastern shore is bordered by mudflats (Fig 2.5) which are backed by a series of low cliffs. The bed of the main channel cuts through a layer of Pleistocene gravel and into laminated silts, clays and fine-coarse sands resulting in the intertidal zone being predominantly mixed substrate (ABPmer 2007). Urbanisation has occurred mostly in the northern reaches (Figure 2.5). The lower reaches of the River Itchen and the River Test are dominated by a mix of waterside developments which has resulted in extensive areas of hard protection to the river banks. This includes Southampton Docks, the UKs most productive container port, and Europe's leading turnaround cruise port (ABP 2016). Banks along the majority of Southampton Water remain largely natural with a few developments, piers and outfall-pipes extending out into the channel. There is however, Fawley Oil Refinery and Chemical Works situated at the mouth of the Southampton Water (Figure 2.7). The refinery is the largest in the UK supplying around 14 % of all petroleum products in the UK (ExxonMobil 2011).

The variety of habitats and the abundance of fauna and flora they support have been recognised by a number of statutory designations (Figure 2.6). In particular the large assemblage of overwintering waterfowl due to the presence of saline Lagoons, saltmarshes and intertidal reefs has contributed toward the designation of Ramsar sites. Multiple SSSIs are present within Southampton Water including; 'Clashot to Hythe Marshes' which covers the most extensive area

of mudflat and saltmarsh in Southampton Water, and 'Lee-on-the-Solent to Itchen Estuary' which includes extensive intertidal mudflats, saltmarsh and vegetated shingle (Colenutt 2010).

Southampton Water has been designated a Sensitive Area (eutrophic) and Polluted Waters (eutrophic) under the Urban Wastewater Treatment Directive and Nitrates Directive (EA 2010). Elevated levels of nutrients enter through both point and diffuse sources, including sewage treatment works and agricultural run-off (Holley & Hydes 2002). In the Southampton area alone, there are 6 sewage treatment works that discharge secondary treated sewage effluent into Southampton Water (Cefas 2009b).

There is no cultivation of bivalves in Southampton Water, however much of the estuary is classified shellfish harvesting (Figure 2.7). Shellfisheries include native oysters (*Ostrea edulis*), hard-shelled clams (*Mercinaria mercinaria*), cockles (*Cerastoderma edule*) and Manila clams (*Tapes philippinarum*) (SIFCA 2013). Prohibited levels of *Escherichia coli* were found at monitoring sites within Southampton Water in 2015 that resulted in the closure of shellfisheries in the northern reaches (Cefas 2015). Additionally microalgae were implicated in shellfishery closures due to paralytic shellfish poisoning in 1999 (FSA 2000).

Wild *C. gigas* were surveyed and collected at sites distributed along the eastern shore where oysters occur naturally (Figure 2.7). Northern sites (1-3) were mud flats with areas of mixed sediment (mud/shingle/shell) and the site at the mouth of Southampton Water (4) was a sand/mud flat with intertidal shingle banks. There is no aquaculture production within Southampton Water and thus no farmed oysters were collected from this estuary.

Figure 2.5 Southampton Water map: Priority Habitat Inventory; Mudflat ; Coastal Saltmarsh . Intertidal substrate foreshore; Mud ; Mud & Gravel ; Sand & Gravel ; Rock platform with boulders/loose rock ; Man-made Ground . Generated using MAgiC Crown Copyright and databases 2016. Ordnance Survey 100022861.

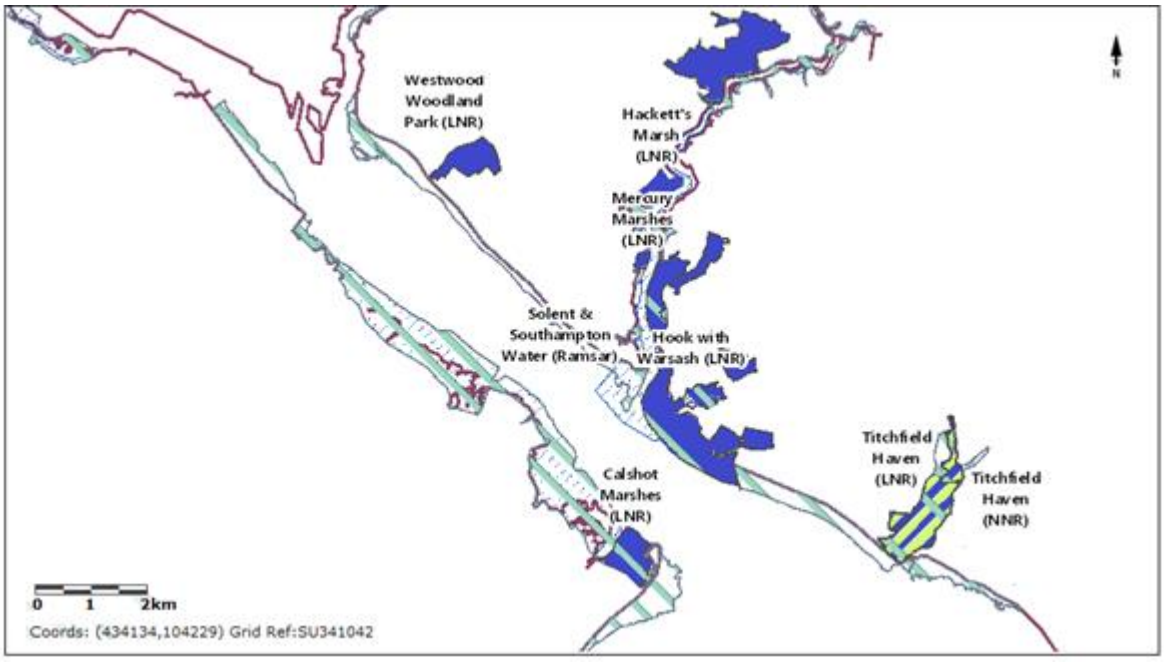


Figure 2.6 Southampton Water statutory designations: Ramsar ; Local Nature Reserves ; National Nature Reserves ; Special Area of Conservation . Generated using MAgiC - Crown Copyright and databases 2016. Ordnance Survey 100022861.

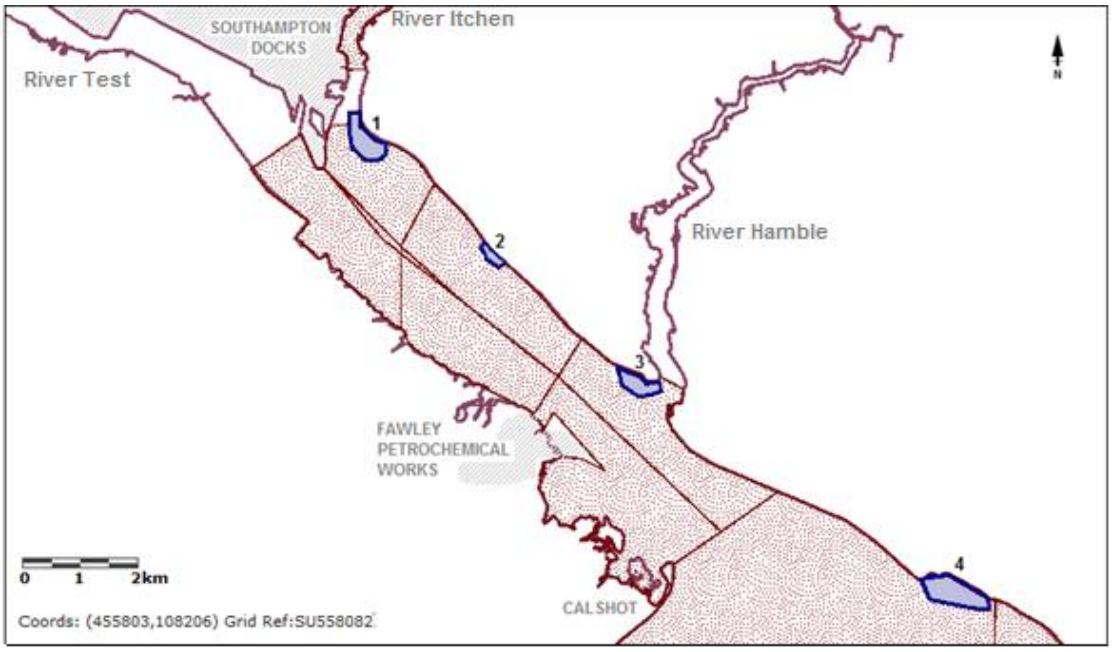


Figure 2.7 Southampton Water showing *Crassostrea gigas* collection sites: Feral aggregations 1 Woolston, 2 Netley, 3 Hamble, 4 Hill Head; and the Classified shellfish harvesting area. Generated using MAgiC - Crown Copyright and databases 2016. Ordnance Survey 100022861.

2.3 Collection site monitoring

Temperature loggers were located in Poole Harbour and Southampton Water. In Poole Harbour a temperature logger was mounted on a metal frame pushed into the sediment at Blue Lagoon (Figure 2.4) adjacent to where *C. gigas* were periodically sampled. This logger was held 5 cm above the substrate in an intertidal section of the shoreline exposed to the air on 85 % of tides. A second temperature logger was mounted on a metal rod fixed at 1 m below the Othniel Shellfish Ltd. processing barge (Figure 2.4). The processing barge moves with the tide and thus the temperature recordings are representative of 1 m below sea surface at all states of the tide. In Southampton Water a logger was attached to metal frame (as in Blue Lagoon) located at site 1 (Figure 2.7). All loggers were of the model TidbiT® v2 Temp (UTBI-001) set to record temperature to 0.001 °C every 15 minutes. Data was offloaded in the field using the HOBO® Waterproof Shuttle and ONSET Coupler2-D every 2 months between 1st May 2014 and 5th Dec 2014.

Additional data sets from 2 temperature loggers were made available; Temperatures logged at 15 minute intervals from below Othniel Shellfish Ltd. processing barge (as described above) between November 2005 and February 2013, and temperatures logged every 3 minutes from an ongoing monitoring program at Calshot (Figure 2.7) that began in March 2008. The Calshot logger was located at substrate level on a post that was only exposed on extreme spring tides.

Chapter 3: Characteristics of feral aggregations of *Crassostrea gigas* inhabiting Poole Harbour and Southampton Water

Abstract

Crassostrea gigas is a non-native aquaculture species in the UK that has begun to establish feral aggregations outside the perimeter of cultured plots. In mainland Europe feral *C. gigas* have proliferated, reaching invasive abundances that have had associated impacts on the environment and the economy. It is largely unknown to what extent *C. gigas* inhabits the south coast of England. Walking surveys, belt transects and quadrats were used to map the location of *C. gigas* in Southampton Water and Poole Harbour. Abundance, size frequency distribution and substrate type colonised were also recorded. Recruitment was estimated using a combination of Bhattacharya's method and an EM-algorithm. Further to this linear regression was used to determine the relative importance of substrate type, exposure, shore width, shore height and the residence time of the estuary in predicting *C. gigas* presence and abundance on the shore.

Recruitment has occurred over at least 4 years at both locations. The highest density of > 10 *C. gigas* m^{-2} was recorded in Poole Harbour however this was an isolated patch, typically *C. gigas* was absent or present as solitary individuals. Distribution was more uniform in Southampton Water with densities of 2 – 10 *C. gigas* m^{-2} in the upper reaches of the estuary that reduced toward the mouth. Recruitment of *C. gigas* was most abundant on man-made structures and mixed substrate in the mid to low intertidal zone. Furthermore recruitment decreased with increasing exposure. The width of the shore and residence times, in the tested estuaries, was found to have little impact on recruitment. It is evident that physical and environmental parameters in Poole Harbour and Southampton Water are conducive to *C. gigas* recruitment and in particular there is an abundance of suitable substrate. However the abundance of *C. gigas* differs substantially between the neighbouring estuaries and it is suggested that further research into hydrodynamic regimes impacting larval distribution be carried out.

3.1 Introduction

The Pacific oyster, *Crassostrea gigas,* is cultured throughout the UK. The greatest production of farmed Pacific oysters comes out of England, with the highest concentration of aquaculture beds occurring in the south east and the south west (Crane & Laing 2008). Shipments of *C. gigas* to the UK began during the 1890s to support the failing native oyster (*Ostrea edulis*) industry (Humphreys et al. 2014). Stringent legislative regimes were introduced in 1965 as a consequence of high levels of pests and disease which were associated with the imported oysters. Since the successful field trials of laboratory reared pathogen-free oysters in 1967, hatchery produced oyster seed has been used to artificially maintain populations on areas of seabed designated for aquaculture (Utting & Spencer 1991; Spencer et al. 1994). *C. gigas* was allowed to be introduced into the UK to support and broaden the shellfish industry because it required a higher annual temperature regime than normally occurred at that time in the UK in order to spawn. It therefore presented a low risk of establishment outside of designated aquaculture plots (Spencer et al. 1994). However UK water temperatures have been steadily increasing since the 1990s, attributed to global climate change (Southward et al. 1995; MCCIP 2011; IPCC 2014), and they have now exceeded reproductive thermo-limits allowing *C. gigas* to naturalise and to become established (Couzens 2006; Herbert et al. 2012).

The extent of naturalisation differs greatly within the UK and the reasons for this are not yet properly understood. The greatest magnitude of settlement occurs in the south east of England where spat (juvenile oysters) are collected and used by growers as a source of seed (Syvret et al. 2008). Settlement is sporadic in the south west of England with notable variation within the region. Dense reefs have formed in the River Yealm, where anecdotally 1000 oysters can be counted in a 30 minute period (Couzens 2006), and only 100 km further east, in Portland Harbour and the Fleet, there is an apparent absence of recruitment despite cultivation in that area (Herbert et al. 2012). Feral aggregations have established in Ireland (Kochmann 2012) and Northern Ireland (Guy & Roberts 2010). Recruitment in Ireland has been recorded both intertidally and subtidally in recent years (Kochmann 2012). In Strangford Lough and Lough Swilly up to six age classes can be found, however despite this, abundance typically remains below 1 oyster m^{-2} with localised concentrations of up to 10 oysters m^{-2} (Kochmann 2012). Wild *C. gigas* have been recorded at lower abundances in Wales and Scotland, with most cases being scattered individuals (Herbert et al. 2012; Smith et al. 2015). In Wales, occasional, scattered individuals have been found in localities in the Menai Straits and at Milford Haven (Herbert et al. 2012), many of which appear to be individuals that have persisted after Government trials for aquaculture in the Menai Straights (Spencer et al. 1994; Morgan 2007). Until recently there had been no substantiated records of wild *C. gigas* in Scotland (Maggs et al. 2010). Spencer (1994) noted that

individual *C. gigas* had been seen in Loch Sween by a third party in 1969 or 1970 but that none were seen during a survey in 1991. Aquaculture of *C. gigas* is limited to the west coast of Scotland, however 8 live *C. gigas* were recorded in 2013 on the east coast, they were aged between 3 – 6 years and located in the Firth of Forth with unknown origin (Smith et al. 2015).

Current distribution of wild *C. gigas* in the UK largely conforms to the predictions made by Syvret et. al. (2008) based on temperature profiles. Regions were allocated risk categories dependent upon the predicted likelihood of *C. gigas* being able to reproduce and recruit. The south and south east of England, including East Anglia, were predicted to be at high risk of annual recruitment. Northern Ireland, Wales and the south west of England were at moderate risk and Scotland and the northeast of England were at low risk (Syvret et al. 2008).

Genetic diversity of feral Pacific oysters largely follows patterns associated with aquaculture practices. Hatchery production has resulted in a genetic bottleneck, and genetic similarities exist between farmed oysters and those found feral on the south east coast of England and Ireland (Lallias et al. 2015). However wild oysters sampled form the south west have a greater genetic diversity than elsewhere in the UK, more similar to *C. gigas* produced in France (and Japan). The increased diversity in France is a result of French aquaculture activity based on spat from natural recruitment, however it is not known whether wild oysters are the result of human mediated movement or natural larval dispersal (Child et al. 1995; Lallias et al. 2015).

The extent of naturalisation is expanding throughout Europe with the northern boundary now extending to 60 °N in Swedish waters (Wrange et al. 2010). *C. gigas* has been introduced in many regions of the world both unintentionally and intentionally (Andrews 1980; Drinkwaard 1998; Ruesink et al. 2005). As with the UK, considerable variation in recruitment to wild aggregations has been seen regionally across the globe. In some cases, where introductions have occurred in temperate regions, rapid and expansive feral aggregations have formed. A high abundance of recruitment has resulted in large reef formations in the Oostershelde estuary, Netherlands (Reise 1998), the Dutch- (Fey et al. 2010), German- (Diederich 2005a; Diederich et al. 2005) and Danish Wadden Sea (Dolmer et al. 2014) and numerous bays along the French Atlantic coast (Cognie et al. 2006; Cardoso et al. 2007; Lejart & Hily 2011). Pockets of feral Pacific oysters have long established along the western coast of America and in British Columbia, Canada (Shatkin et al. 1997), and more recently, high density feral aggregations have been reported in southern regions of Scandinavia (Wrange et al. 2010; Dolmer et al. 2014; Laugen et al. 2016). Outside of the temperate zone reproduction has either been unsuccessful or with many years of light or failed recruitment which has resulted in a low but constant presence (Eldredge 1994; Kaufmann et al. 1994; Ruesink et al. 2005; Dridi et al. 2007). Generally, settlement has historically only been

found initially in close proximity to culture plots (Spencer et al. 1994; Shatkin et al. 1997; Ruesink et al. 2005; Cognie et al. 2006; Wrange et al. 2010), however there are now populations forming where the only possible larval inputs are from feral oysters (Wehrmann 2000; Couzens 2006).

Aim

This study aims to map the current distribution of *C. gigas* colonising the intertidal shore line of Southampton Water, Hampshire and Poole Harbour, Dorset. Distributions will be analysed against physical environmental parameters such as substrate type and site exposure, with the aim of describing the habitats most likely to be colonised by *C. gigas*. Such a description is required for models that predict future spread of *C. gigas* and allow assessments of the vulnerability of areas to colonisation and potentially invasion of *C. gigas.*

Hypotheses

H_1 = *Crassostrea gigas* naturally colonise the intertidal shoreline on the south coast of England.

H_2 = Multiple years of recruitment has occurred.

H_3 = Areas likely to experience recruitment and the years when this is most likely to happen can be predicted by physical environmental parameters.

Objectives

O_1 = Identify *Crassostrea gigas* on beach surveys throughout Southampton Water, Hampshire and Poole Harbour, Dorset.

O_2 =Measure the shell length of each *Crassostrea gigas* found and use cohort analysis to estimate how many years recruitment has occurred in a given area.

O_3 = Compare the GPS location of *Crassostrea gigas* to various physical environmental parameters using QGIS software and publicly available datasets.

3.2 Materials and methods

3.2.1 Beach surveys and mapping

Initial surveys

Intertidal walking surveys were carried out along shorelines that had some form of hard substrate. Hard substrate was chosen as the prioritising factor for intertidal surveys because as oyster larvae require a hard substrate for attachment during metamorphosis (Fitt et al. 1990; Diederich 2005a; McKnight 2009). Hard substrate included naturally occurring shingle patches and rock, as well as anthropogenic structures such as flood defences and harbours. Survey sites were selected using Google Earth, Channel Coastal Observatory aerial photography, and the knowledge of local area experts.

Surveys were shore based, and carried out within an hour either side of low water on a spring tide. Along steep sections of shore where less than 3 m of substrate was exposed, 2 surveyors walked parallel to the shore approximately a metre apart for as far as the tide/ shoreline permitted. When the intertidal zone exceeded 3 m in depth a pathway that traversed the substrate was taken. In both cases when an oyster was located, the position was recorded using a hand held GPS unit and the length of the oyster shell from umbo to the furthest peripheral (Figure 3.1) was recorded to the nearest mm using Vernier callipers. Furthermore during these initial surveys the dominant substrate type found in the upper, mid and lower intertidal zones were classified according to the SACFOR scale (Connor et al. 2004). The following classifications were used; coarse sediment (including coarse sand, gravel, pebbles, shingle and cobbles), mixed sediment (heterogeneous muddy gravelly sands often with surface shell or stones), sand and mud. Areas dominated by seagrass or reeds were labelled as 'plant' and the label, 'man' was assigned where manmade structures were present.

Abundance

A second phase of surveying was carried out in areas where *C. gigas* were found at greater densities than 1 oyster m^{-2}, and if a number of oysters occurring at a density of 1 oyster m^{-2} were found within 10 m of each other. At these sites belt transects were used to estimate population density. This was necessary at one site in Poole Harbour (Blue Lagoon: Figure 2.4) and at 4 sites in Southampton Water (Figure 2.7). Transects were laid perpendicular to the shore beginning at the strandline (mean high water spring) and finishing at mean low water spring tide level. The number of *C. gigas* and the number of *Ostrea edulis* in a 1 x 1 m quadrat either side of each transect were counted and their shell lengths (Figure 3.1) recorded. Additional transects running

perpendicular to the shore and 50 m in length were carried out in areas where *C. gigas* were present at densities of > 5 oysters m^{-2}. Repeat visits were made to each site with the number of visits depended on the size of the site being surveyed. On each return visit hand held GPS devices were used to position transects further along the shore, ensuring no duplicate surveys and a good spread of the beach was surveyed.

3.2.2 Population structure

FiSat II (version: 1.2.2; provided by the Food and Agriculture Organisation of the United Nations (FAO)) was used to analyse size cohorts within the shell length frequency data set generated from beach surveys. Size cohorts were assumed to represent recruitment from one year because mass spawning events occur only in the summer and growth is seasonal. The Bhattacharya method (Bhattacharya 1967) analyses modal progression with the assumption of normally distributed cohorts. It is a method frequently used in bivalve and *C. gigas* research (Pauly & Morgan 1987; Amin et al. 2008; Schmidt et al. 2008; Wrange et al. 2010), and so has been selected for use in this study, for comparability.

The method does, however, require some estimates and human judgement surrounding the number of cohorts present. To minimise the potential of identifying size cohorts that were not actually present, a number of precautions were taken. The separation index was always > 2, and where possible, size age groups were derived from at least 3 consecutive points (Gayanilo 1997; Amin et al. 2008). Furthermore shell length data was analysed in partitions of 4, 5 and 6 mm size classes. This was because selecting different sized classes can impact on the visualisation of the data and so the cohort selection. These size classes have been chosen following Walles (2015) thesis, in which size frequency distributions of *C. gigas* were analysed at 1, 3, 4, 5, 6 and 7 mm size classes. The Gaussian plots generated from 1 mm size classes were too scattered to obtain clear models, whereas, 7 mm intervals clearly missed some modes. Size classes in between were considered to give a reasonable number of modes and when combined indicated that certain length classes were always present (Walles 2015).

Furthermore a second method that uses different formulae to estimate cohort parameters was used to corroborate those predicted using Bhattacharya's method. An Expection/Maximisation (EM) algorithm was chosen as the second method as it estimates the parameter means and standard deviation using iterative expectation maximisation. Specifically, data was analysed using the function 'normalmixEM' from package 'mixtools' (version: 1.0.4, author: Derek Young)) in the computing environment R (version: 3.2.3). The algorithm fits mixture distributions and solves closely related modal-based clustering by alternating between the E-step and the M-step. In

brief, and using size frequency data of bivalves as an example, the E-step continually updates the likelihood of a bivalve from a given size cohort being selected, and the M-step uses the E-step outcome to update the likelihood of a bivalve of a given size coming from a particular cohort. The two steps are repeated until convergence (Benaglia et al. 2009). To ensure convergence within limited iterations, parameter start values can be manually inputted. Parameter starting values in this study were set using the Bhattacharya results. This included the number of size cohorts with the associated mean shell lengths. In the event that an equal number of modes were identified by Bhattacharya's method across all size classes (4, 5 and 6 mm), average values from across the size classes were used. When the number of modes differed between the size classes, the algorithm was run multiple times using each set of parameters and the results that best fitted the data were used.

3.2.3 Mapping

The Global Navigation Satellite System (GNSS) location (accurate to within 3 m) of each oyster, or quadrat, was collected using a hand held Garmin eTrex and entered into QGIS mapping software 2.4.0. UK coastline mapping was provided by Ordnance Survey, land height and aerial photography by the Channel Coastal Observatory and tidal height by the Environment Agency. Finally site boundaries were outlined freehand to make a polygon shape file. Site boundaries were determined by a change in substrate or physical obstruction (e.g. river channel, marina or private property) and by spring tidal limits. Site exposure levels were an index of wave fetch calculated using a wave fetch model (Burrows 2007) and averaged across each site, and finally the residence times were calculated for each estuary using the following equation:

$$Tr(days) = \frac{8.65}{TPR} - 2.45 * B_0 + 0.59 * L - 5.05$$

Where TPR is the tidal prism ratio, B_0 is the width of the estuary entrance (mouth) and L is the length of the estuary (as a straight line from the entrance to the source) (Hartnett et al. 2011).

3.2.4 Shell morphology

Crassostrea gigas shell measurements were taken using Vernier callipers accurate to 0.1 mm. Shell length was a measurement of the furthest dorsal to ventral distance from the umbo to the shell peripheral (Figure 3.1). Shell width was taken as the maximum distance anterior to posterior along a horizontal axis (Figure 3.1) and shell depth was the maximum inflation of the 2 shells when positioned as if the oyster were alive and closed.

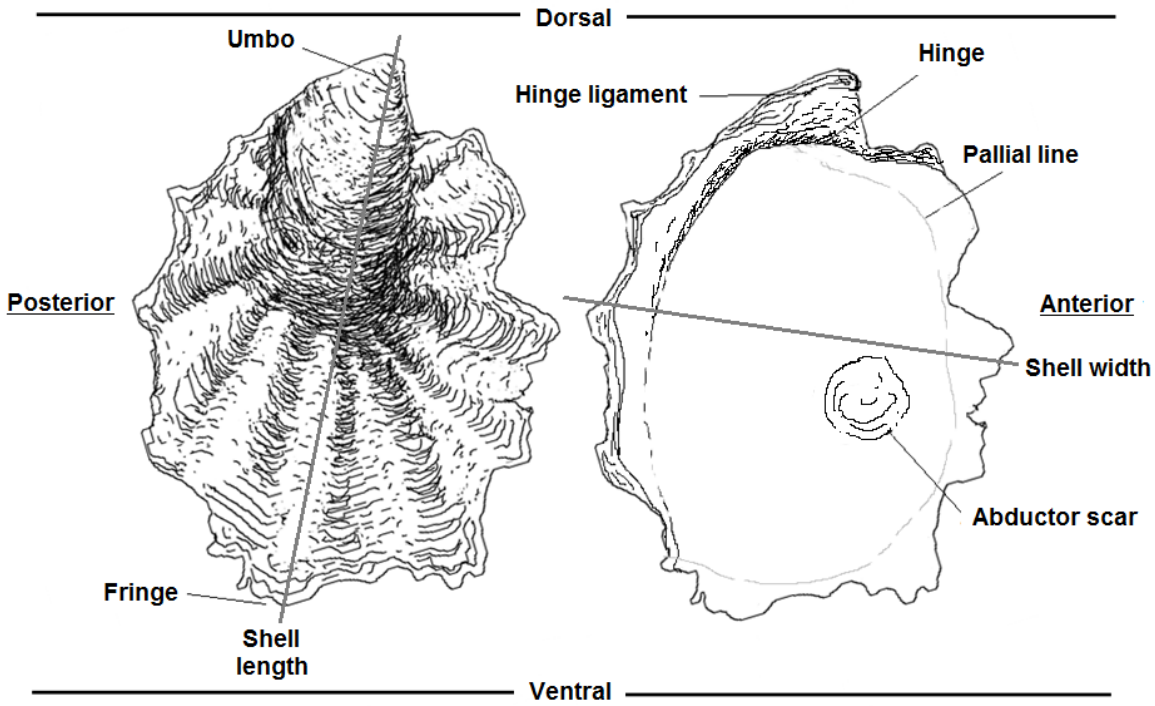


Figure 3.1 Schematic of standard bivalve shape and the measurement dimensions used (drawn from farmed *C. gigas,* Poole Harbour 29.05.2014).

3.2.5 Growth

The von Bertalanffy growth equation (Bertalanffy 1934) is routinely used when analysing bivalve growth (Shpigel et al. 1992a; Dekker & Beukema 1999; Harding & Mann 2006; Liddel 2008; Brown et al. 2010; Bagur et al. 2013). Consequently it was used in this study to determine the body size of *C. gigas* as a function of age and allow comparisons of growth parameters between studies.

Von Bertalanffy growth equation

$$L_t = L_\infty(1 - e^{-k(t-t_0)})$$

Where L_∞ is the asymptotic length, K is a curve parameter that facilitates the biological trend of declining growth rates with age, and t_0 is the initial condition parameter. Fabens method was used to estimate L_∞ and K (Fabens 1965). t_0 represents the point at which the oyster has 0 mm shell length, however larvae settle and metamorphose at approximately 300 µm (Collet et al. 1999; Ernande et al. 2003) rendering the parameter biologically meaningless.

Consequently t_0 was calculated using the following equation and assuming a mean length at metamorphosis (L_0) of 300 µm:

$$L_0 = \left(\frac{1}{K}\right) \times ln(\frac{L_\infty - L_0}{L_0})$$

Shell length was used to represent body size and data came from the beach surveys and subsequent shell length cohort analysis. Consequently it was the mean of each shell length cohort with an age estimate, which were used in calculating the parameters of the Von Bertalanffy curve. Growth performance indices were then calculated using the parameters of the von Bertalanffy curve (Pauly 1979):

$$\varphi' = 2 \times \log_{10} L_\infty + \log_{10} K$$

3.2.6 Mortality

Mortality rates were estimated from the shell length frequency data. The natural logarithm of the frequency at age data was taken, and regression between the descending right tails of each shell length cohort was calculated. The slope of the regression was interpreted as an instantaneous natural mortality rate (Ricker 1975).

3.3 Results

Initial walk-over surveys of Poole Harbour were carried out during spring tides between 14.01.2013 and 24.05.2013. The only site revisited to measure abundance was Blue Lagoon where belt transects were carried out on the 26.06.2013, 09.07.2013 and 28.08.2014. Walking surveys revealed a relatively constant distribution of *C. gigas* along the eastern shore of Southampton Water (Figure 3.3). Consequently, 4 sites that were distributed along the length of the shore were chosen to represent the spread of *C. gigas* in Southampton Water. The sites chosen were ordered from the confluence of the Rivers Itchen and Test, down to the boundary with the Solent and were as follows; Woolston, Netley, Hamble and Hill Head (Figure 2.7). Detailed descriptions of each site can be found in Appendix A. Netley was visited every 2 weeks (to coincide with spring tides) from 05.03.2014 until 07.04.2014, and Woolston, Hamble and Hill Head were visited every 2 weeks between 13.07.2014 and 12.09.2014.

3.3.1 *Crassostrea gigas* population structure

Abundance

Sites were classified according to oyster abundance using a scale drawn up for a Pacific oyster survey commissioned by Natural England (Table 3.1) (McKnight 2009). An area of approximately 430 ha was surveyed in Poole Harbour. *C. gigas* were found at 30 % of sites encompassing an area of approximately 30 ha. *C. gigas* were predominantly present in low abundances (≤ 1 oyster m^{-2}) at sites along the northern shore and solitary zones were identified from Dolphin Marina to Rockley Point. A single oyster was found along the southern shores, on a mudflat surrounding Cleavel Point. The greatest abundance found was the formation of a colony inhabiting a semi-enclosed intertidal mudflat named Blue Lagoon on the north eastern shore (Figure 3.2).

In Southampton Water groups of *C. gigas* with densities > 2 oysters m^{-2} occurred within 10 m of each other throughout the mid-intertidal shore at Woolston, Netley and Hamble. Consequently these sites were described as cluster zones. *C. gigas* were absent from the upper and mid-intertidal shore at Hill Head, however the lower intertidal could be classified as a solitary zone (Figure 3.3). The 10 km stretch of shoreline between Woolston and Hamble was covered by walking surveys and *C. gigas* were consistently present in abundances greater than 1 oyster m^{-2}. The exact abundance between the sites mentioned above is however unknown. Similarly patches of *C. gigas* were found in the River Test at Marchwood (J. Mallinson pers. coms.) and Redbridge (A. Jensen pers. coms.) however abundance was not determined.

Table 3.1 Oyster site classification (McKnight 2009), including colour coding that corresponds with the maps in Figure 3.2 & Figure 3.3.

Site classification	Map key	*C. gigas* per m^2	Description
Absent		0	Oysters absent from site.
Solitary site		1	A single oyster observed. No others in a 10 m radius.
Solitary zone		1	More than 1 solitary oyster observed within a site (site boundary determined by tide line, physical barriers such as sea defences or a change in substrate type). i.e. there is > 10 m between oysters.
Cluster site		2-10	A group of oysters, where individuals are within a 10 m range of any neighbour but density < 10 oysters m^{-2}.
Cluster zone		2-10	2 or more clusters within a site. i.e. most oysters have < 10 m between them, however there maybe areas of uncolonised substrate resulting in larger gaps. Density < 10 oysters m^{-2}.
Colony		>10	All oysters within the site have < 10 m distance between them, with areas of settlement that exceed 10 oysters m^{-2}.

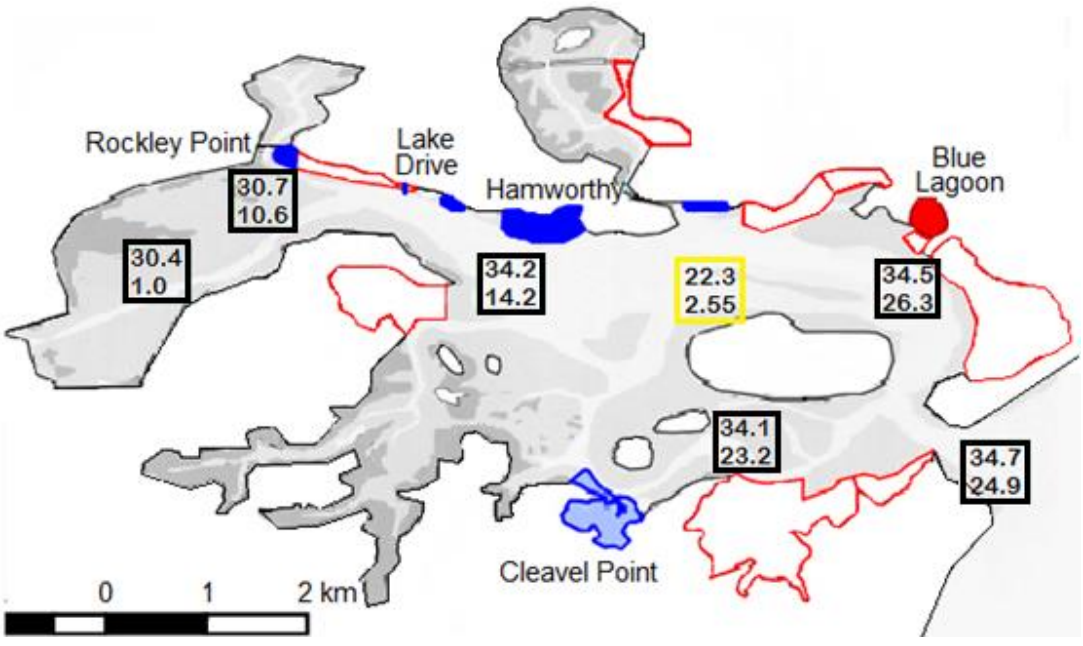


Figure 3.2 Poole Harbour *Crassostrea gigas* site classification according to abundance. See Table 3.1 for colour scale. Black boxes: Maximum and minimum near surface salinity recorded by the Environment Agency as reported by Cefas (2009). Yellow box: Maximum and minimum water temperatures 1 m below sea level in 2012 (Chapter 2.3).

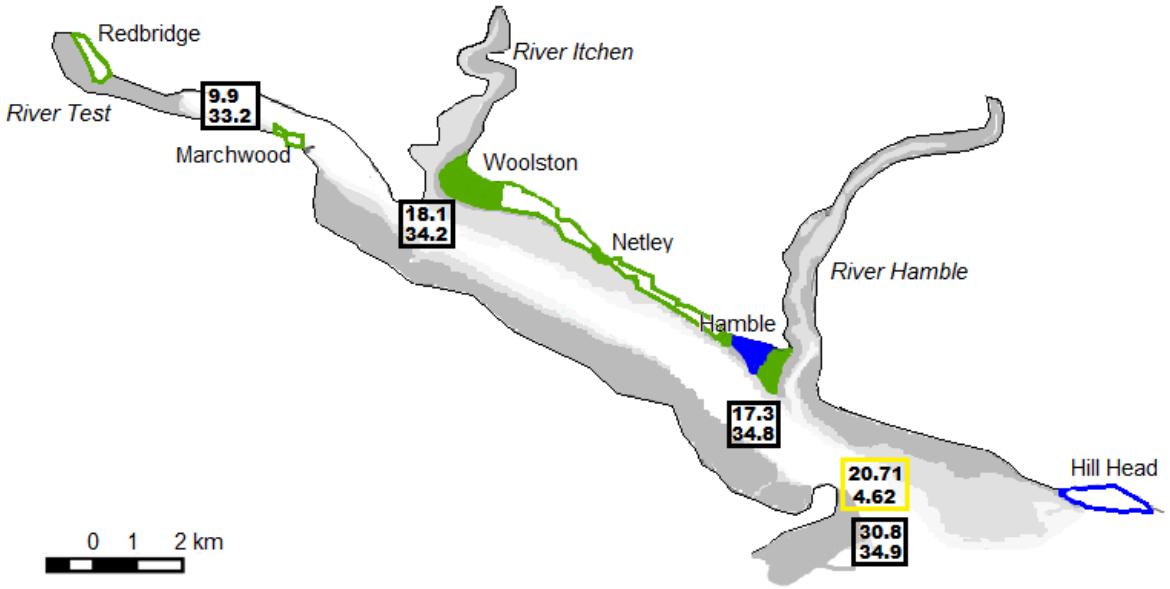


Figure 3.3 Southampton Water *Crassostrea gigas* site classification according to abundance. See Table 3.1 for colour scale, in addition areas with a green outline and no fill are where *C. gigas* was identified on walk-over surveys but the abundance was not quantified. Black boxes: Maximum and minimum near surface salinity recorded by the Environment Agency as reported by Cefas (2009). Yellow box: Maximum and minimum water temperatures of the shallow subtidal in 2012 (Chapter 2.3).

Size frequency

Bhattacharya's method and the EM algorithm assume size cohorts to have Gaussian distribution. The calculated mean shell length for each cohort determined by Bhattacharya's method was averaged across the 4, 5 and 6 mm size classes (Table 3.2 & Table 3.3). Generally the results were comparable, however on occasion a different number of cohorts were identified using the different size classes. Typically this occurred when a larger size class resulted in one age cohort with an average shell length of 2 size cohorts identified using smaller size classes. This happened when using the Bhattacharya method on shell length frequency data collected at Netley, Southampton Water; 4 and 5 mm size classes identified size cohorts with mean shell lengths of 70 and 92 mm, and 68 and 92 mm respectively, whereas a 6 mm size class identified a single size cohort with a mean shell length of 86 mm.

All four sites in Southampton Water showed multiple cohorts that decreased in abundance. The number of cohorts present at a site decreased from 6 at Woolston, to 5 at Netley, 4 at Hamble, and 2 at Hill Head (Figure 3.4). The EM-algorithm was less sensitive to cohorts with low

abundance and consequently did not identify the smallest size cohorts present at Woolston (61.7 mm), Netley (54.6 mm) and Hamble (49 mm) (Table 3.2).

C. gigas were only present at sufficient densities at one site in Poole Harbour (Blue Lagoon) to carry out size cohort analysis. Shell length frequencies were measured in Blue Lagoon during June and July 2013 and August 2014. Data collected in 2013 had 3 length cohorts and data from 2014 had 4 cohorts identified using Bhattacharya's method. Both methods provided similar results with the only notable difference occurring between year old *C. gigas* in 2014 where the EM algorithm calculated a cohort mean shell length of 100.9 mm, approximately 20 mm greater than the Bhattacharya calculation (Table 3.3). The majority of oysters measured in 2013 were dominated by those in age class 2 (Figure 3.5). These *C. gigas* are predicted to have been spawned in 2010 and continued to be the dominant size class in 2014 (Figure 3.6).

The low abundance of *C. gigas* at Hamworthy, Rockley Point and Lake Drive sampled during March and April 2013 meant cohort analysis was not possible. However those oysters found were all of similar size, with a range in shell length of 53 - 140, 135 - 155, and 70 - 110 mm respectively (Figure 3.5).

Table 3.2 Gaussian distributions calculated for *Crassostrea gigas* sampled from Southampton Water 2014. Bhattacharya's method used to determine age cohorts using different size intervals (4, 5, & 6 mm shell length).

Site	Modes per mm interval			Average length	EM algorithm	Age class
	4	5	6			
Woolston (14.07.2014, 28.07.2014, 11.08.2014, 31.08.2014)	65.5		57.9	61.7		0
	87.52	81.53	85.24	84.8	82.1	1
	109.12	110.18	117.3	112.2	114.8	2
	132.93	137.5	135.41	135.3	134.1	3
					157.6	4
	180.9	183.9		182.4	178.3	5
Netley (05.03.2014)	53		56.2	54.6		0
	70.1	68.39		69.2	62.7	1
	91.7	92.05	85.64	89.8	96.6	2
	113		113.5	113.2	125.5	3
	133	140		136.5		4
Hamble (15.07.2014, 29.07.2014, 12.08.2014, 30.08.2014)	42.6	53.65	50.84	49.1		0
	78.5	84.3	81.0	81.26	78.7	1
	104.5	109.0	105.0	106.2	116.2	2
	162.3	165.0	152.8	160.1	158.2	3
Hill Head (13.07.2014, 30.07.2014, 10.08.2014, 29.08.2014)	65	61.7		63.3	56.6	0
	81.7		82.2	81.9		1
	97	92.7	103.3	97.7	93.8	2

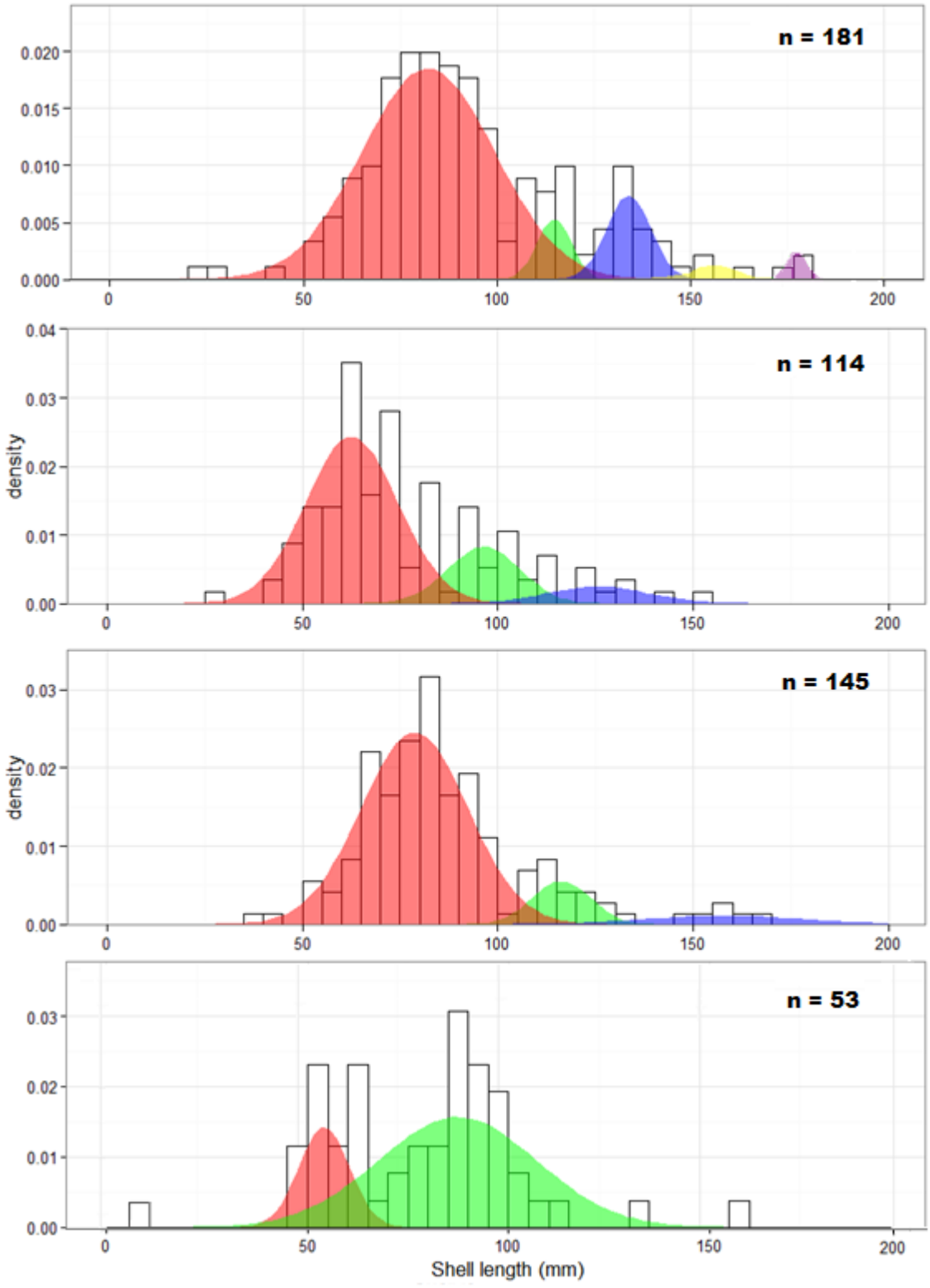


Figure 3.4 Size frequency distribution of *Crassostrea gigas* at several sites in Southampton Water. **Top**; Woolston . **Middle-top**; Netley. **Middle-bottom**; Hamble. **Bottom**; Hill Head. Year classes illustrated by overlaid Gaussian distributions with parameters determined using Bhattacharya's method and an iterative expectation maximisation algorithm (Table 3.2).

Table 3.3 Gaussian distributions for *Crassostrea gigas* collected from Blue Lagoon, Poole Harbour. Calculated for different size intervals (4, 5, & 6 mm shell length) using Bhattacharya's method to determine age cohorts.

Site	Bhattacharya				EM algorithm	Age class
	Modes per mm interval			Average length	Cohort mean mm	
	4	5	6			
Blue Lagoon (26.06.2013, 09.07.2013)	92.9	92.5	93.0	92.8	85.3	1
	144.3	144.3	145.6	144.7	147.4	2
	198.7	190.0	197.0	195.2	194.8	3
Blue Lagoon (28.08.2014)	50.0	47.5	51.0	49.5	49.0	0
		85.9	78.0	81.95	100.9	1
	169.3	156.7	160.2	162.1	155.7	2
	180.0	176.9	186.2	181.0	188.9	3

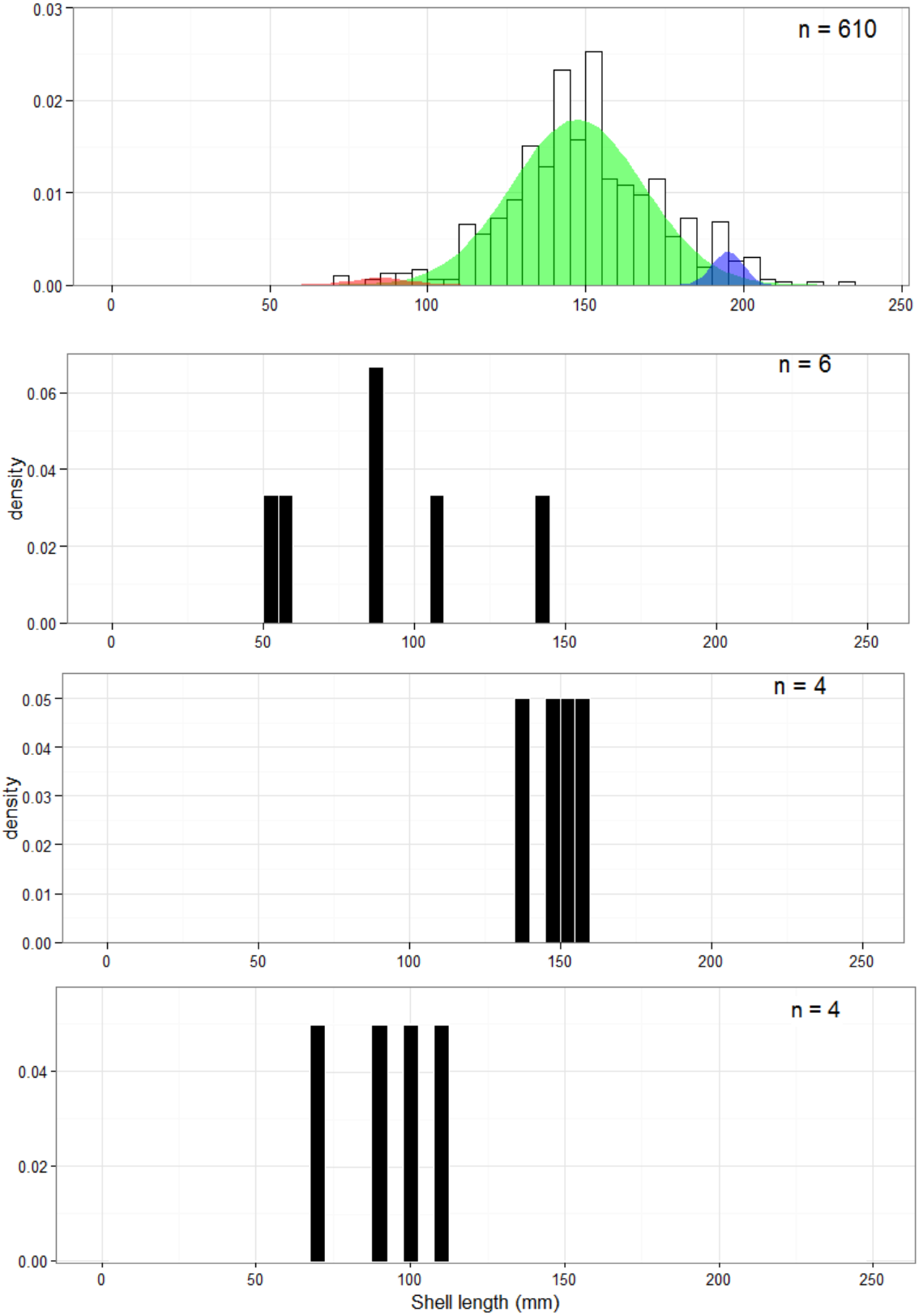


Figure 3.5 Size frequency distribution of *Crassostrea gigas* at several sites in Poole Harbour (2013 sampling only). **Top**; Blue Lagoon. M**iddle-top**; Hamworthy. M**iddle-bottom**; Rockley Point. B**ottom**; Lake Drive. Blue Lagoon was the only site where enough oysters were collected to overlay Gaussian distributions with parameters determined using Bhattacharya's method.

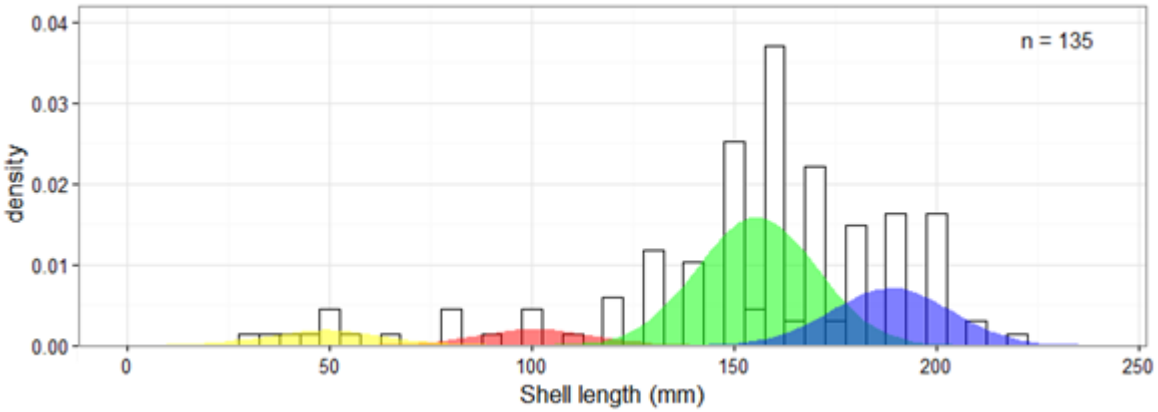


Figure 3.6 Size frequency distribution of *Crassostrea gigas* in Blue Lagoon, Poole Harbour 2014.

A von Bertalanffy curve was fitted to sites that had 4 or more size cohorts. The cohort means calculated by Bhattacharya's method and an EM algorithm were averaged (it was assumed that recruitment was annual).

FiSat II software was used to calculate K and L_∞ using Fabens' method and T_0 was calculated assuming a metamorphosis size of 300 µm (Collet et al. 1999; Ernande et al. 2003). The asymptotic shell lengths calculated using Fabens method were all in excess of 300 mm and 40 – 60 % greater than the maximum shell length measured at each site. Consequently the maximum length (L_{max}) was estimated using the extreme value theory and the K was re-calculated using a forced L_∞ equal to L_{max}. The highest curvature parameter, K = 0.419, was calculated for Blue Lagoon, Poole Harbour, where L_{max} was 218.4 mm and longevity was estimated at 7.2 years.

Table 3.4 Growth parameters for *Crassostrea gigas* in Southampton Water and Poole Harbour

		Von Bertalanffy parameters			L_{max}	φ'	Longevity (years)
		K	L_∞	t_0			
Southampton Water	**Woolston**	0.408	336.7	0.0058	175	4.0967	7.3
	Netley	0.386	348.2	0.0058	168.5	4.0398	7.8
	Hamble	0.36	358.6	0.0058	178.25	4.0584	8.3
Poole Harbour	**Blue Lagoon**	0.419	426.6	0.0058	218.4	4.3008	7.2

Longevity of *C. gigas* was predicted to be similar between sites and estuaries (Figure 3.7 & Table 3.4). However *C. gigas* from Poole Harbour grew more quickly than those inhabiting Southampton Water and had a notably greater estimated maximum size of 218.4 mm shell length in comparison to 168.5 – 178. 25 mm shell lengths (Table 3.4).

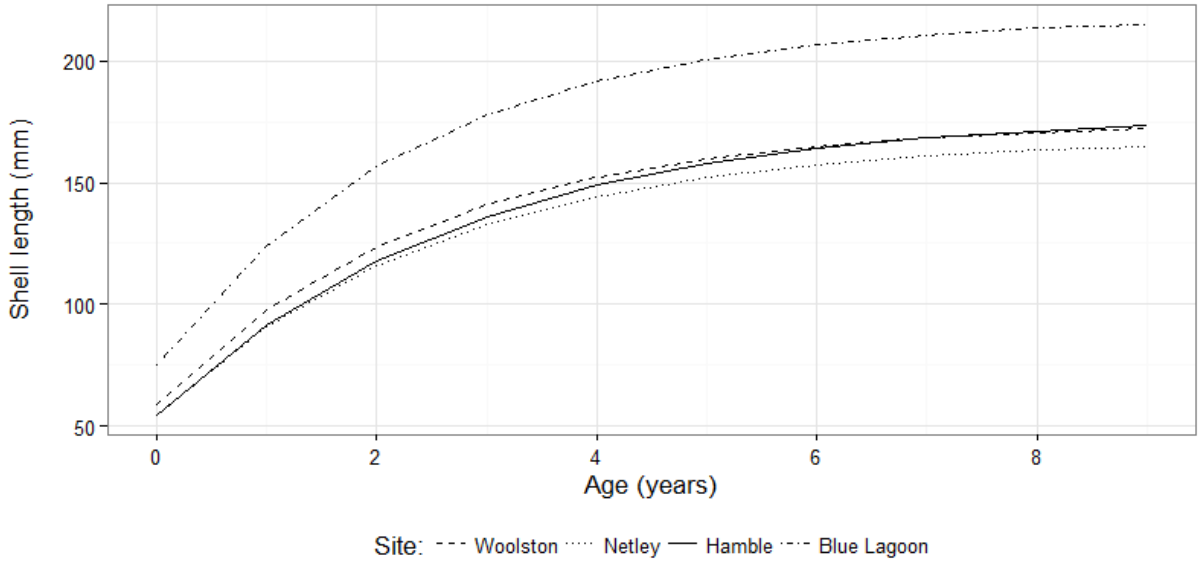


Figure 3.7 Von Bertalanffy curves for *Crassostrea gigas* for sites in Southampton Water (Woolston, Netley & Hamble) and Poole Harbour (Blue Lagoon) where 4 or more size cohorts were present.

Only sampling sites Woolston, Netley and Hamble in Southampton Water contained enough size cohorts of oysters to make them suitable for analysis of mortality rates. Analysis of covariance was used to test for differences between the regression slopes and intercepts of mortality rates. There was not a significant difference among the slopes (ANCOVA: $F(2) = 1.746$, $P = 0.1935$) indicating a similar rate of mortality between sites. The intercepts did differ significantly (ANCOVA: $F(2) = 16.038$, $P < 0.001$), specifically Netley had a significantly lower intercept ($t = 0.268$, $p < 0.001$) than the similar intercepts of Hamble and Woolston ($t = 1.053$, $p = 0.301$) (Figure 3.8). This suggests that *C. gigas* inhabiting the shore at Netley generally incur mortality at a smaller size (shell length).

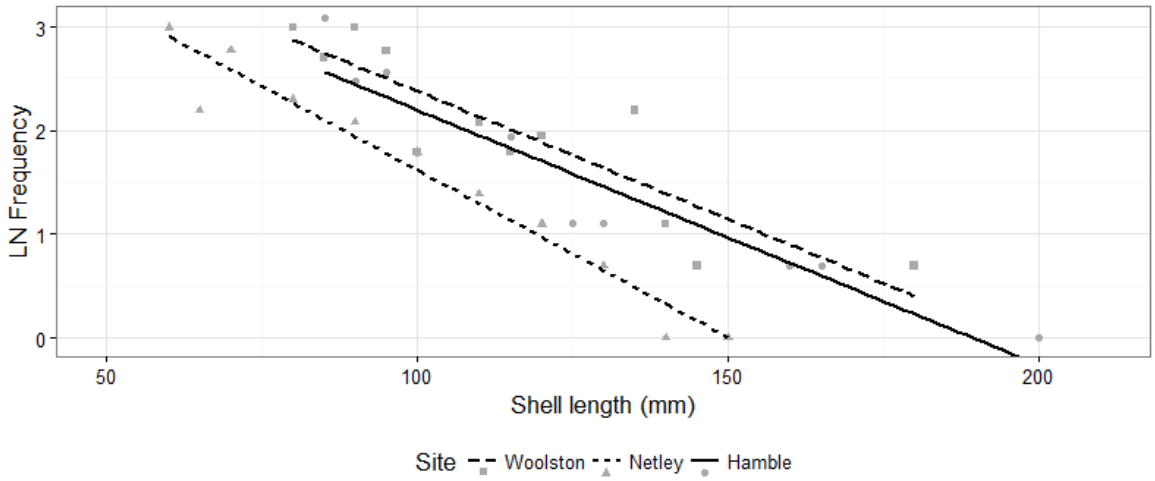


Figure 3.8 Mortality rates of *Crassostrea gigas* at various sites in Southampton Water.

3.3.2 Physical environmental factors

Predicting oyster occurrence

A proportional odds model was constructed (function: polr, package: MASS) in R using the physical parameters for each site as predictors of oyster abundance. The oyster abundance scale ordered site classifications (Table 3.1) from 0 = Absent to 6 = Colony. The starting model had the following equation:

$$\underline{\text{Abundance}} \sim \underline{\text{Width}} + \underline{\text{Height}} + \underline{\text{Substrate}} + \underline{\text{Exposure}} + \underline{\text{Residence}} + \underline{\text{Width}} * \underline{\text{Exposure}} + \underline{\text{Height}} * \underline{\text{Substrate}}$$

Width is the width of the intertidal zone (factor). Height refers to the height on the shore *C. gigas* was found on, and is split into 3 factors; lower intertidal, middle intertidal and upper intertidal. Substrate is the dominant substrate type of a site at each height level (factor). Exposure is the level of site exposure as an index of wave fetch (factor) (Burrows 2007). Residence is the residence time calculated for the estuary (numerical) (See Methods for further details). * indicates an interaction term. An interaction term was included between Width and Exposure because shore width is not included in the calculation of wave fetch (Exposure) despite its impact in shores over 100 m wide (Harley & Helmuth 2003; Burrows et al. 2008). An interaction term was included between Substrate and Height because substrate type impacts on colonising fauna dependent on where it occurs tidally (Harley & Helmuth 2003; Connor et al. 2004). A total of 6 sites within Southampton Water and 17 within Poole Harbour were used to calculate linear regression (Appendix A).

Goodness of fit tests (Chi squared and AIC) were used to reduce the model to the necessary parameters. The final model included Exposure, Substrate and Height. When a comparison of fit

was run between models with and without an interaction term between Height and Substrate, the AIC value was unchanged (154.5) and the Chi squared values were 1 and 0.999 respectively. Likewise the interaction term between Exposure and Width made no significant difference to the fit of the model and consequently the interaction terms were removed to simplify the model. The final equation is given below:

$$\underline{\text{Abundance}} \sim \underline{\text{Height}} + \underline{\text{Substrate}} + \underline{\text{Exposure}}$$

A Type II ANOVA showed the type of substrate present to be a highly significant factor in predicting the abundance of *C. gigas* ($\text{Chisq}(5) = 31.8$, $p < 0.001$). From the regression model it can be seen that manmade structures are the most likely substrate to be colonised followed closely by mixed substrate. Colonisation by *C. gigas* was least likely when the shore was dominated by reeds or seagrass (Plant), sand or larger coarse sediments such as pebbles and shingle (Table 3.4). Colonisation was most likely to occur in the low- and middle-intertidal zone (Table 3.4) with tidal height having a significant impact on the likely recruitment abundance ($\text{Chisq}(3) = 10.78$, $p = 0.013$). Finally the impact of exposure on recruitment abundance was not found to be significant ($\text{Chisq}(3) = 4.99$, $p = 0.173$), however there was a decreasing trend of *C. gigas* abundance seen at sites with increasing exposure (Table 3.4).

Table 3.4 A summary of results for the prediction of *Crassostrea gigas* abundance from tidal height, substrate type and wave exposure in a proportional odds model.

Coefficients		**Value**	**Std. Error**	**t value**
Height	Lower			
	Middle	-0.6034	6.967e-01	-8.661e-01
	Upper	-3.2226	1.177e+00	-2.739e+00
	Other (Marina)	-3.1276	1.432e+00	-2.184e+00
Substrate	Man			
	Mixed	-1.1048	8.428e-01	-1.311e+00
	Mud	-3.8704	9.616e-01	-4.025e+00
	Sand	-18.5503	3.743e-07	-4.957e+07
	Plant	-21.8010	5.377e-08	-4.054e+08
	Pebbles / Shingle	-16.6908	5.963e-07	-2.799e+07
Exposure	< 30			
	30 – 60	-1.3106	9.260e-01	-1.415e+00
	60 – 90	-1.1726	9.557e-01	-1.227e+00
	90 +	-2.0740	9.637e-01	-2.152e+00

3.4 Discussion

Crassostrea gigas were generally more abundant in Southampton Water than Poole Harbour. The distribution in Southampton Water was spread over a greater area with multiple age classes present (max: 5) in cluster zones (2 - 10 oysters m^{-2}) throughout the estuary and into at least 2 of the tributaries, the Test and the Hamble. Conversely Poole Harbour was characterised by sparsely distributed solitary zones (1 oyster m^{-2}) containing oysters of a limited size range. The abundance and irregular distribution are similar to those recorded by Spencer et al (1994) in estuaries in the south west of England in 1992, however resampling of a number of those sites by Couzens (2006) in 2006 found *C. gigas* to be absent. Additional estuaries in the south west of England (Noss Mayo and the Plym) were surveyed by Couzens and found to have higher densities of *C. gigas* than was recorded during this study (anecdotally 1000 oysters can be counted in a 30 minute period) (Couzens 2006). In the east of England, *C. gigas* abundance recorded during 2009 was typically present in cluster zones (2 - 10 oysters m^{-2}) on natural substrate (similar to Southampton Water in 2013), although areas of overlaid recruitment was present and considered to be in the advanced stages of reef building (McKnight 2009). Furthermore, sea defence structures were heavily colonised, generally with oyster density in excess of 10 oysters m^{-2} (greater than that recorded during this study). The current distribution and abundance of *C. gigas* recorded during this study are similar to those recorded in previous years along the east and west of England's south coast (Figure 3.9). The accumulation of multiple years recruitment shows the potential for this population to expand further, however fluctuations in abundance, and variability in the location of recruitment, show an unstable and dynamic population.

Dynamic fluxes in populations make future predictions difficult, and the rate at which wild *C. gigas* are increasing in the UK is unclear. The average abundance of *C. gigas* in Southampton Water was 1.7 oysters m^{-2} in the mid-intertidal zone, which is a similar abundance to that recorded on mussel beds in the Hörnum Basin of the Wadden Sea (Diederich et al. 2005). *C. gigas* were first recorded outside of aquaculture plots in the Wadden Sea in 1990, in the List Basin abundance has increased from 3.6 oysters m^{-2} in 1995 (Reise 1998), to 3.7 oysters m^{-2} in 1999 and 125.8 oysters m^{-2} in 2003 (Diederich et al. 2005). However to the south of Sylt Island, in the Hörnum Basin, abundance has remained relatively consistent at 1.1 oysters m^{-2} between 1995 and 2003 (Diederich et al. 2005). It is unknown what causes such irregularities in recruitment distribution, however, the rate of establishment has been attributed to recruitment only occurring when late summer conditions are above average (August > 18.2 °C) (Diederich et al. 2005). Conversely in France, *C. gigas* has expanded more rapidly as recruitment occurs under natural environmental conditions, only failing during years with abnormally low temperatures (Grizel & Héral 1991).

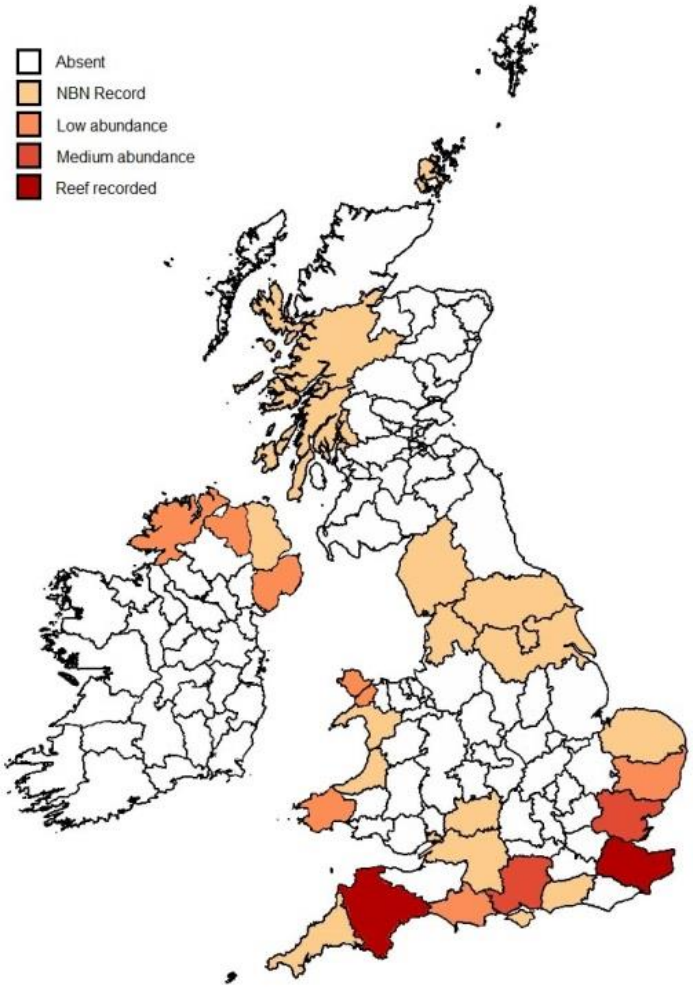


Figure 3.9 UK map of wild *Crassostrea gigas* establishment. NBN records are from the National Biodiversity Network's Gateway 2017 abundance unknown. Abundances are taken from literature where low refers to studies that found majority solitary oysters, medium refers to 2 – 10 oysters m2 and reefs are multiple years of recruitment cemented in clusters. Notable literature included: Couzens 2006, McKnight 2009, Herbert et al. 2012 and Kochmann 2012. Findings from this study are also included. nb All occurrences of *C. gigas* in Devon and Cornwall are on the south coast.

Tidal height and substrate were found to be the main determinants of recruitment dispersal. Substrate type was also considered an important factor in the proliferation of *C. gigas* in the Thames estuary (McKnight 2009), and the Wadden Sea (Diederich et al. 2005). Much like in this study, McKnight (2009) concluded anthropogenic substrate to be the most readily colonised and a potential source of larvae to further dispersal. In the Wadden Sea, it was the presence of mussel beds that provided initial recruitment substrate (Diederich et al. 2005). *C. gigas* is considered an intertidal species and so the majority of recruitment occurring in the lower and mid intertidal

zone is not a surprise and correlates with findings in other UK estuaries (Spencer et al. 1994; Couzens 2006; McKnight 2009), and further afield (Reise 1998; Drinkwaard 1999; Diederich et al. 2005; Lejart & Hily 2011).

Substrate type is an important determinant factor for where *C. gigas* establish because of the metamorphosis requirements of the larvae (Arakawa 1990; Markert et al. 2010). When larvae develop to the pediveliger stage settling behaviours are exhibited. For *C. gigas* this includes downward swimming as they become negatively phototaxic, positively geotactic and negatively buoyant to increase the chances of reaching the benthos, and crawling using the foot (Fitt et al. 1990; Rico-Villa 2009). These behaviours are designed to locate a particular substrate to metamorphose upon and metamorphosis can be delayed for up to 3 days if necessary (Coon et al. 1990; Ernande et al. 2003). Settlement is triggered by physical, chemical and biological cues associated with the bottom (Fitt et al. 1990; Metaxas 2001). *C. gigas* are typically gregarious (Arakawa 1990), however they are also commonly found attached to other shelled molluscs, shell fragments, gravel and concrete (Diederich et al. 2005; McKnight 2009). Colonisation was concentrated on man-made structures such as beach groynes, outfall-pipes and harbour walls and pontoons at both study sites. Non-native species are prominent constituents of fouling communities and often dominate communities on novel, hard substrate (Diederich et al. 2005; Wasson et al. 2005). Consequently the colonisation of anthropogenic structures by non-native species is thought to have facilitated introductions globally (Tyrrell & Byers 2007), and enabled their spread (Cohen & Carlton 1998). The ability of exotic species to occupy and maintain substantial amounts of space on artificial substrates likely contributes to their success.

Therefore when considering substrate, larval behaviour determines the ultimate distribution of adult aggregations. However, following metamorphosis, environmental parameters as well as predation can shape intertidal communities. The upper limit of the now sessile juveniles is often determined by their susceptibility to thermal stress and desiccation from the tide (Roegner & Mann 1995; Somero 2002; Harley & Helmuth 2003). Aquatic predators often determine lower boundaries of dispersal as they require submersion to predate and drive sessile organisms into protected bands above the tide line (Paine 1974; Seed & Suchanek 1992; Robles & Desharnais 2002). In this study the majority of recruitment was recorded at a tidal height that averaged 5 % aerial exposure in Poole Harbour and 8 % in Southampton Water with an upper limit of 25 %, this is comparable to the tidal zonation of *Crassostrea virginica* in Canada (Roegner & Mann 1995). However a more recent study in the Wadden Sea showed that recruitment patterns determine final distribution and not post-settlement mortality, and that growth and survival were comparatively similar in the intertidal and shallow subtidal zones (Diederich 2006b).

Abundance of *C. gigas* was negatively correlated with exposure, although the effect of exposure on determining where recruitment would occur was less than that of substrate or tidal height. Wave exposure is particularly influential on the distribution of sessile marine species because there are trade-offs between the increased risks of dislodgement, and the increase in benefits from nutrient supply and elevated larval dispersal (Steffani & Branch 2003). Wave exposure has been shown to cause particularly high juvenile mortality (Wall et al. 2005). Water movement also indirectly effects species distribution through levels of siltation (Eriksson & Bergstrom 2005; Westerbom & Jattu 2006). Reported oyster success in high silt load environments varies from; reefs surviving complete burial (Galtsoff 1964a; Comeau 2014) and higher recruitment abundance in sheltered locations (O'Breirn et al. 1995), to a sensitivity of gills to clogging (Ortega & Sutherland 1992) and a strong reduction of recruitment within sediment traps (Thomsen & McGlathery 2006). Aquaculture practices commonly include the use of racks to elevate oysters away from the sediment as this has been shown to decrease mortality and increase the production of somatic tissue (Goulletquer et al. 1998; Soletchnik et al. 1999). Recruitment seen throughout this study has favoured flats of mixed sediment where resuspension and silting is expected to be high. In particular, the colony that has formed in Poole Harbour is in a sheltered area of high siltation. Many of the larger (and so older) oysters were found positioned vertically below the surface with just the shell fringe protruding above the substrate.

Residence times did not significantly impact on the abundance of *C. gigas* in this study. However this is most likely because of the similar values calculated for Poole Harbour and Southampton Water. Residence is the time which will be taken for a particle to reach the outlet of the water body in question (Takeoka 1984). For this study residence was calculated from the length of the water body, width of the entrance and the tidal prism providing an average residence time for each body of water (Hartnett et al. 2011). Flushing rates, which can be described by certain characteristics such as residence times, can be an important factor in determining whether an estuary is likely to retain larvae (Dyer & Orth 1994). Poole Harbour had the lower residence time of 7.3 days compared to Southampton Water that had a residence of 12.9 days. Both estimates are lower than the planktonic larval phase of *C. gigas* larvae (Rico-Villa 2009). When recruitment has been compared between estuaries that have notably different residence times, specifically larger and smaller than the expected larval duration, it has strongly impacted on distribution patterns (Gaines & Bertness 1992; Kim et al. 2010; Kochmann 2012). Having a lower residence time than planktonic duration suggests larvae are flushed out of the estuaries before they have the opportunity to settle. Although this cannot be entirely true, as both estuaries have received varying amounts of recruitment, it may help to explain the greater abundance of recruitment in Southampton Water where the residence time is longer.

Herbert et al. (2011) modelled larval distribution patterns within Poole Harbour using a fine-resolution hydrodynamic model. Over 50 % of larvae released from the aquaculture site left the harbour within 2 days via the channel south of Brownsea Island. Some larvae were sucked back into the Harbour during a flood tide and permeated the convoluted shoreline to the north and south of Brownsea Island, however this refers to only a small proportion of the larvae (0.5 %) (Herbert et al. 2011). The aquaculture site used in the model is also assumed to be the major propagule pressure for *C. gigas* larvae in Poole Harbour. Both the *C. gigas* on-bottom culture beds and the suspended wracks on Othneil Oysters Ltd. processing barge are located to the west and north of Brownsea Island, and below the dredged channel that directs ships from the entrance of Poole Harbour as far as the ferry terminal, via the north of Brownsea Island (Figure 2.4). Consequently the shipping channel is a notable hydrographic barrier to the northern reaches for *C. gigas* larvae released from the aquaculture plots and the cause of a large quantity of larvae being flushed from the harbour. However it should be noted that the hydrodynamic model was coupled with a behavioural model specifically for manila clam (*Venerupis philipinnarum*) larvae, and as a consequence of species specific larval swimming behaviours effecting dispersal distance (North et al. 2008), the modelled results cannot be assumed to apply to the larvae of any other species.

As a result of natural mortality, most naturally occurring populations will have the greatest abundance of individuals in the smallest size cohort and abundance will successively decrease with the age. Oysters making up the smallest cohorts (~ 50 mm) appeared to be less abundant than larger oysters at all sites monitored, a phenomenon also recorded in Essex by McKnight (2009). The impact of human error during sampling cannot be ruled out here as smaller oysters are harder to see in the field, and so less likely to be counted than larger oysters. However unusual demographic patterns may also occur as a result of varying recruitment success. For example a greater abundance of oysters would be expected during a year when *C. gigas* recruitment has been high in comparison to a year with low recruitment. Inter-annual water temperature and salinity may impact recruitment as well as the abundance of plankton, pollutants and predators (His 1989; Ruiz et al. 1992; Steele & Mulcahy 1999; Chávez-Villalba et al. 2003; Delaporte et al. 2006). Furthermore post-settlement mortality caused by factors, such as predation and illegal harvesting, can also influence distributional patterns of sessile epifauna (Paine 1974; Keough et al. 1993; Diederich 2006b; Aswani et al. 2015). Both predation and illegal harvesting typically target a certain sized shellfish, potentially reducing abundance within individual size cohorts. Harvesting of wild *C. gigas* is prolific in Southampton Water (Appendix C).

Fluctuations in the quantity of larvae settling will have little impact on the overall level of abundance of adults if the shoreline habitat is already saturated. Changes in settlement success

will therefore be more apparent in sparsely populated areas. None of the sites monitored in the UK to date have been saturated (This_study ; Spencer et al. 1994; Couzens 2006; Herbert et al. 2012). An extreme example would be Poole Harbour, where there is evidence of multiple years of spawning, as seen by the multiple cohorts in Blue Lagoon, but for most of the harbour distribution is sparse, falling into the solitary zone category of abundance. It is most likely that during one year when conditions allowed for particularly high recruitment, oysters populating the most abundant cohort in Blue Lagoon (145 ± 15 mm) settled and a wider dispersal of larvae lead to recruitment in other parts of the harbour. The variability in shell length between sites can be explained by the differing conditions. Aerial exposure and disturbance retards growth (Griffiths 1981; Roegner & Mann 1995; McQuaid et al. 2000) and the sites that had the smallest oysters (Hamworthy and Lake Drive) experienced the greatest disturbances from activities such as bait digging and the launching of sea going vessels (pers. obs.). Furthermore the majority of oysters at these sites were found high up on wooden groynes, increasing their exposure time.

Feral oysters have consistently recruited over a large area in Southampton Water indicating a healthy supply of larvae despite the lack of aquaculture in the estuary. If an area acts as a larval sink it is possible for aggregations of non-native species to form where environmental conditions allow for growth but are suboptimal for reproduction (Aiken et al. 2007; Herbert et al. 2012). This is unlikely to be the case in Southampton Water where larval drift from an inoculation at the head of Southampton Water, or even further upstream in the Test or the Itchen, is most likely. Salinity abruptly decreases below 20, 3 – 4 km upstream (Shi 2000), restricting the source location (Fabioux et al. 2005). Settlement progressed downstream at a rate similar to that predicted by Brandt *et al* (2008) for the German Wadden Sea; reaching sites (Netley and Hamble) within 10 km of Woolston before reaching Hill Head approximately 15 km downstream. How *C. gigas* arrived in Southampton Water is currently unknown and so the progeny of naturalisation is also unknown. Larval input additional to that from wild oysters could still be impacting on recruitment abundance and population genetics (Moehler et al. 2011; Lallias et al. 2015). If the original larval source(s) is still valid today, the continued impact on recruitment remains unquantified.

The association of feral *C. gigas* with aquaculture plots has frequently led to the assumption that aquaculture has been the source (Spencer et al. 1994; Reise 1998; Ruesink et al. 2005). However relatively few studies have actually proved wild oysters to be the progeny of aquaculture (Moehler et al. 2011; Lallias et al. 2015). Aquaculture plots provide a notable propagule pressure of *C. gigas* larvae to estuaries that contain this practice. However there is currently no aquaculture of *C. gigas* in Southampton Water, where they have established throughout the estuary in notable abundance. Similar situations have arisen elsewhere globally (Dinamani 1971; Galil 2000; Smith et al. 2015). Spawning of adult *C. gigas* attached to the hulls of ships as they

enter warmer waters, the presence of *C. gigas* larvae in discharged ballast water and the discarding of live oysters from visiting ships were all suggested as potential sources into New Zealand (Dinamani 1971). Whereas in Scandinavian countries, initial colonisation was thought to have been enabled by aquaculture, and warming summer temperatures have facilitated a northward spread into estuaries and fjords where aquaculture is absent (Wrange et al. 2010).

C. gigas remain as larvae for between 2 and 6 weeks subject to environmental conditions (Rico-Villa 2009) and depending on hydrodynamic regimes, larvae have been shown to travel up to 50 km (Brandt et al. 2008). The majority of larvae travel less than 25 km with most settling within 10 km of their source (Brandt et al. 2008). This makes settlement within Poole Harbour likely to be progeny of aquaculture within the harbour. However it is unlikely that *C. gigas* larvae enter Southampton Water from aquaculture as the nearest sites are Poole Harbour, which lies at the limits of larval dispersal approximately 50 km to the west, and the Thames estuary > 200 km to the east (Herbert et al. 2012).

C. gigas has been found colonising yacht hulls in Southampton Water (Appendix B), perhaps making hull fouling a more likely source of larvae into the estuary. Southampton Water and Poole Harbour both contain ferry terminals and multiple leisure craft marinas. Furthermore the UKs most productive container port and Europe's leading turnaround cruise port are located at Southampton (ABP 2016). Large commercial vessels have ample area of hull to colonise and travel great distances between ports regularly that may expand the diversity and abundance of a biofouling community. However slower speeds and prolonged moorings result in fouling being a greater problem for leisure craft and a higher potential to spread invasive hull fouling organisms between marinas and increase coastal transfer (Davidson et al. 2010; Zabin et al. 2011). The vast majority of marinas are at the head of Southampton Water, located in the River Itchen and the River Hamble (Towler & Fishwick 2016). In one reported example, *C. gigas* represented the majority of fouling cover on a yacht hull (Appendix B). The oysters were all of a similar size (shell length approximately 30 mm) and had colonised a clean hull that had recently received a new coat of anti-fouling paint (pers. coms.). It is thought that the oysters colonised the yacht in France where it had spent the summer. This particular boat is lifted out of the water annually for cleaning and therefore it is unlikely that these oysters had the opportunity to spawn in Southampton Water, however dry-docking boats is costly and many receive less regular maintenance, allowing biofouling to potentially mature and spawn. Other introductory sources such as ballast water, disposal from restaurants and cruise liners and unauthorised fisheries venture cannot be discounted, however there is a lack of evidence to support them. For ballast water in particular, regulations have been put in place by the International Maritime Organisation to mitigate the release of live organisms (IMO 2004a; IMO 2004b).

Bivalve growth rates are highly variable and depend greatly on environmental conditions (Brown 1988; Vakily 1992; Amin et al. 2008). Growth rates calculated using the von Bertalanffy growth curve (ie. the K value) for *C. gigas* vary from 2.35 yr^{-1} in Korea (Vakily 1992), to 0.68 yr^{-1} in China (Harding & Mann 2006) and fluctuates between 0.36 yr^{-1} and 0.75 yr^{-1} at sites surrounding Vancouver Island . Growth rates in this study are comparable to those of *C. gigas* inhabiting Vancouver Island. Furthermore shell length at age data is comparable for 1 - 2 year old *C. gigas* inhabiting bays in Ireland (Kochmann 2012), the Wadden Sea (Reise 1998; Schmidt et al. 2008), Portugal (Almeida et al. 1997), Japan (Kobayashi et al. 1997), Mexico (Arizpe 1996), Washington, Oregon and California (Langdon & Robinson 1996). This conformity reflects the similar environmental conditions and bi-annual phytoplankton blooms typically experienced at temperate latitudes. Water temperature and food supply are responsible for monthly fluctuations in growth, in addition salinity and food availability have both been attributed to causing growth differences between sites (Brown 1988). Generally in locations where a high growth rate was reported it was coupled with a lower asymptotic length, consequently the calculated Growth Performance Index (GPI) was approximately 4 at all locations globally (This_study ; Brown 1988; Vakily 1992; Harding & Mann 2006). The GPI uses the von Bertalanffy parameters to measure how well an organism grows and allows overall growth performance to become comparable (Pauly 1979). A similar GPI shows *C. gigas* to be growing equally well under a variety of conditions despite the variations in growing rate and asymptotic size. This emphasises the adaptable nature of this species of oyster and also shows plasticity in shell length and growth rate according to environmental conditions.

Enhanced growth conditions experienced by *C. gigas* inhabiting Poole Harbour conflict with the lack of recruitment seen. Environmental factors that augment growth, such as warmer water temperatures (Mann 1979; Brown 1988; Roegner & Mann 1995) and greater abundance and quality of phytoplankton (Brown 1988; Amin et al. 2008), are typically conducive to gametogenesis (Ren et al. 2003; Normand et al. 2008), spawning (Ruiz et al. 1992; Cardoso et al. 2007; Dutertre et al. 2009b), larval development and metamorphosis (Chávez-Villalba et al. 2003; Rico-Villa 2009). However larvae and juvenile oysters have much narrower tolerances (Rumrill 1990; Roegner & Mann 1995; Li & Hedgecock 1998; Rico-Villa 2009) than adults (Korringa 1952; Grizel & Héral 1991; Shatkin et al. 1997; Diederich 2006a) and consequently suboptimal conditions experienced during a short period of the oysters lifecycle could be detrimental to recruitment but have limited impacts on the growth of established oysters. Therefore the conditions experienced in Poole Harbour when larvae are developing and metamorphosing may be detrimental to recruitment, however for those oysters that do survive through to adulthood the ambient conditions are conducive to high growth rates. Conversely for Southampton Water,

conditions during the *C. gigas* reproductive season are favourable for recruitment however growing conditions are less favourable than those in Poole Harbour. A potential explanation could be spawning occurring at different times of year, a concept discussed in detail in chapter 4.

Oysters can potentially live in excess of 10 years (Harding & Mann 2006), however it is rare to find *C. gigas* 6 years + (Diederich et al. 2005; Guy & Roberts 2010). The longevity estimates for this study, that range between 7 and 8 years, are comparable. Mortality rates were assumed to represent natural mortality because there is not a commercial fishery for *C. gigas* in the UK. However there is strong evidence that the oysters are being hand-picked from the shores (Appendix C) and recruitment success appears to cause large annual fluctuations. Consequently mortality estimates from this study should be treated with caution.

Summary

Characteristics of feral aggregations of *Crassostrea gigas* inhabiting Poole Harbour and Southampton Water currently reflect that seen during initial phases of establishment in the east (Thames Estuary) and west (Plymouth Sound) of the UK and elsewhere globally. In particular, multiple recruitment events have given rise to widely spread, patchy aggregations with a low abundance that resemble those seen in the Wadden Sea (1990 - 1995), pre-population expansion (2003) (Reise 1998; Diederich et al. 2005).

C. gigas adaptation to colonise anthropogenic structures and tolerance for a wide range of environmental parameters has allowed the exploitation of disturbed environments such as the busy ports of Southampton Water and Poole Harbour. Thus far recruitment has predominantly occurred on anthropogenic structures and in areas of natural, mixed substrates of mud, gravel and shell. The majority of adult oysters currently reside in the low- and mid- intertidal zones at an average abundance of 1.7 oysters m^{-2}. Clumping of 2 – 3 oysters have been found, however despite occasional high density patches accumulating, there is currently no evidence of reefs establishing.

The distribution of *C. gigas* on the shore may be the result of environmental and biological pressures shaping communities post-metamorphosis, or a result of larval selection. It has been suggested that hydrodynamic regimes, at least within Poole Harbour, might be having an overriding impact on larval delivery. The initial source of feral oysters is unknown, and although it is likely that aquaculture provides the propagule pressure within Poole Harbour, this cannot be proved without further genetic analysis. There is no aquaculture within Southampton Water and possible genesis of wild *C. gigas* could include hull fouling, dumping of live animals or an unauthorised fisheries venture.

Both sites experience growth rates and mortality estimates that are similar to *C. gigas* within their native range (Kobayashi et al. 1997), and where nonindigenous populations have reached invasive abundances (Reise 1998; Schmidt et al. 2008). In Southampton Water *C. gigas* have formed a wide spread, self-sustaining aggregation of low abundance throughout the estuary. Longevity estimates and regular recruitment suggest that the distribution will continue to spread and the abundance will increase, making it likely that reefs will form over time.

It appears that in Poole Harbour strong tidal currents act as hydrographic barriers to the northern shore and result in the majority of larvae (assumed to be produced by the aquaculture plot) being flushed out of the Harbour. The south of the harbour may receive larvae however there is a lack of suitable settlement substrate. The establishment of a colony within Poole Harbour may have been the result of stochastic flows and turbulence allowing larvae from the aquaculture plot to become entrained within the semi-enclosed embayment of Blue Lagoon (Byers & Pringle 2006), or perhaps they are the progeny of hull fouling from the yachts moored within Blue Lagoon and the result of localised retention of larvae (Herbert et al. 2011).

Regular recruitment events and longevity of adults are indicative of a self-sustaining population that has the potential to expand. Whether the UK population of *C. gigas* will increase to the scale seen in other European countries is unclear. Dynamic fluctuations, such as their presence in an estuary one year and absence in another year (Spencer et al. 1994; Couzens 2006), adds uncertainty to the populations stability.

Chapter 4: Gametogenic development of wild and farmed Pacific oysters on the south coast of England

Abstract

The establishment of wild *Crassostrea gigas* along the south coast of England suggests a regular supply of larvae. However it is currently unknown whether wild oysters are reproductively active or whether larvae is supplied from an external source such as aquaculture, hull fouling or ballast water. Twenty *C. gigas* were collected each month between and including September 2013 and September 2014 from the intertidal shore of Southampton Water (wild) and subtidally from aquaculture plots in Poole Harbour. In addition to this 20 *C. gigas* were collected quarterly from the intertidal shores of Poole Harbour (wild). Sex ratio and gametogenic development as a measure of oocyte Feret diameter and sperm characteristics were recorded from histological analysis of gonadal tissue. Condition and reproductive indices were calculated using dry weights.

A seasonal trend was seen in biometric indices and reproduction, along with a synchronous initiation of gametogenesis between sexes at both sites. A greater proportion of oysters inhabiting the intertidal site in Southampton Water had initiated gametogenesis in early spring than those oysters from the aquaculture plot in Poole Harbour. The significant growth of oocytes throughout spring ($p < 0.001$) signified gametogenesis and a plateau in oocyte growth over the summer was indicative of sexual maturity having been reached. A single spawning event in Poole Harbour occurred during August whereas Southampton Water was characterised by 2 spawning events, initially in June and then again in August. *C. gigas* inhabiting Poole Harbour developed larger oocytes and spawned at the end of summer, it is postulated that a phytoplankton bloom triggered spawning. In Southampton Water, the initial spawning event occurred as water temperatures increase above 18 °C, and a second spawning event coincided with spawning in Poole Harbour, also thought to be triggered by a phytoplankton bloom.

4.1 Introduction

Among benthic marine invertebrates two principle forms of development are found; the production of planktonic larvae that undergo metamorphosis in conjunction with settlement, and offspring with direct development to adult without a planktonic stage (Vance 1973; Christiansen & Fenchel 1979; Llodra 2002). Both life-histories can be found in marine bivalves (Vance 1973; Martel & Chia 1991; Kasyanov 2001) with the mode of development that results in the greatest reproductive output for a species in a given environment prevailing (Vance 1973). Reproductive output is a measure of the efficiency of energy uptake by an organism into the greatest number of offspring surviving to reproduce. Reproductive methods are influenced by selection pressures on larvae survival, metamorphosis success, and survival to sexual maturity. The presence of a larval stage is the most common form of development in marine benthic invertebrates despite the high levels of natural mortality to larvae (Thorson 1950; Mileikovsky 1971; Rumrill 1990). Planktotrophic larvae are found in more species than both non-feeding lecithotrophic larvae and direct development combined (Thorson 1966). A planktotrophic larval stage is considered advantageous as it provides a greater dispersal potential (Caley et al. 1996; Todd 1998). Increased larval dispersal reduces competition for resources among siblings and between parents and offspring (Economou 1991; Havenhand 1995), reduces the likelihood of inbreeding (Morgan 1995), increases geographical spread and reduces the probability of extinction (Pechenik 1999; Shanks et al. 2003). Advantages to species that brood larvae and/or produce short lived lecithotrophic larvae are that juveniles colonise favourable parental habitat, so increasing the opportunity for local adaptation (Todd 1998; Pechenik 1999; Sotka & Palumbi 2006).

There is evidence that a planktonic larval stage is a derived condition that has been lost one or more times from within many groups (Wray 1995; Pechenik 1999), and that the loss of a planktonic stage is unlikely to be regained as it is typically accompanied by substantial morphological simplification during embryogenesis (McEdward & Janies 1997). The life history exhibited by any species is reflected in all stages of the reproductive cycle from gametogenesis through to metamorphosis. Lecithotrophic development requires the production of large yolky eggs to sustain the larvae, whereas planktotrophic development produces smaller eggs. The fecundity of a species is defined as the number of offspring produced by a female in a determined time period, and is a highly plastic character limited by the bioenergetics and life history of an individual (Llodra 2002). Consequently lecothotrophic species have a lower fecundity than planktotrophic species (Vance 1973, Giangrande 1999, Llora 2002). The amount of energy allocated into each larva is reflected further in the size at metamorphosis. Lecothotrophic larvae metamorphose at a larger size than planktotrophic larvae (Vance 1973; Christiansen & Fenchel 1979).

Environmental conditions broadly affect reproduction resulting in seasonal germinal development; under cooler winter conditions metabolism slows down and reproduction enters a quiescent phase. As temperatures increase gametogenesis initiates and spawning typically coincides with peak summer temperatures (Ruiz et al. 1992; Ren et al. 2003; Cardoso et al. 2007; Dutertre et al. 2009b). The duration of the various phases of reproduction vary between species and annual, semi-annual or a continuous gametogenic cycle are known within marine bivalves. Furthermore bivalves exhibit plasticity in many reproductive traits resulting in differing reproductive strategies exhibited by the same species between locations (Newell et al. 1982; Steele & Mulcahy 1999; Fabioux et al. 2005; Cardoso et al. 2007). Consequently it has been suggested that reproductive strategies are not a result of adaptation in a species but may simply be a manifestation of variation in exogenous factors (Newell et al. 1982). In addition to temperature, reproduction is further effected by more local scale exogenous factors such as food availability (His 1989; Chávez-Villalba et al. 2003; Rico-Villa 2009), salinity (His 1989), photoperiod (Chávez-Villalba et al. 2002) and pollutants (Steele & Mulcahy 1999), interacting with endogenous factors, such as nutrient reserves (Saout et al. 1999) and hormonal cycles (Croll & Wang 2007). In particular the effect of food availability has been documented to have a pronounced effect on the fecundity condition and spawning in marine bivalves (Newell et al. 1982; Mathieu & Lubet 1993; Beekey & Karlson 2003; Delgado & Camacho 2005; Delaport et al. 2006). For instance a phytoplankton bloom has been suggested as an alternative to a threshold temperature to trigger a spawning event (Starr et al. 1990; Ruiz et al. 1992), and the quality of gamete production has been shown to be ultimately dependent on the nutrients available for gametogenesis, either in terms of nutrient reserve or food recently ingested (Newell et al. 1982; Hendriks et al. 2003; Uriarte et al. 2004; Cardoso et al. 2007).

Gender in the case of hermaphroditic bivalves is influenced by inherited genes and environmental parameters (Kennedy 1983; Chew 1991; Guo et al. 1998; Chávez-Villalba et al. 2011). The sex ratio of a population can become male biased under less favourable conditions, or in populations that experience high stress (eg. high pollutants), as male gametes are less energetically costly (Kennedy 1983; Gagné et al. 2006; Lango-Reynoso et al. 2006; Kamphausen et al. 2011). *Crassostrea gigas* is a successive and irregular protandrous hermaphrodite (Guo et al. 1998). The reproductive period includes variable characteristics, such as a dependence on seasonal phytoplankton blooms and minimal specific temperatures for both the initiation of gametogenesis and the release of gametes (Mann 1979; Ruiz et al. 1992; Ren et al. 2003). Rates of gametogenic development are correlated to temperature however initiation of gametogenesis and spawning occur at different temperatures in different regions of the World. This has been shown through controlled environmental manipulation (Mann 1979; Fabioux et al. 2005), histological analysis of

oysters *in-situ* (Ruiz et al. 1992; Lango-Reynoso et al. 2000; Cardoso et al. 2007; Dutertre et al. 2009b) and is evident from wild recruitment (Thomson 1952; Le Borgne et al. 1973; Bourne 1979; Drinkwaard 1998; Reise 1998; Diederich et al. 2005; Lavoie 2005; Kerckhof et al. 2007). Previous studies into the reproduction of *C. gigas* generally fall into one of two categories. Those that predict the future spread outside of the native range, or those that aim to benefit the oyster industry. However both directions share a focus on better understanding the reproductive capacity of *C. gigas* and how environmental conditions affect recruitment. The many factors affecting reproductive success and the plasticity of reproductive traits results in an unreliable source of seed for the industry and presents many uncertainties around the ability of *C. gigas* to spread and cause environmental degradation.

There have been many advances in hatchery culture however the demand from the industry is greater than the supply from hatcheries in many countries (Robert & Gérard 1999; Lavoie 2005; Buestel et al. 2009; Herbert et al. 2012). The uncoupling between supply and demand is further exasperated during years of low or failed recruitment in countries that rely heavily on wild seed (Buestel et al. 2009). Predicting possible impacts of wild recruitment has been aided by the increasing number of case studies focused on emerging populations but complicated by the degree of variability witnessed between locations. This varies from the rapid and expansive formation of dense aggregations and even reef structures (Markert et al. 2010; McKnight 2012; Walles 2015), to many years of light or failed recruitment resulting in a low but constant presence (Troost 2010; Wrange et al. 2010).

Aims

Emerging 'populations' of *C. gigas* in England have been mapped on several occasions (Spencer et al. 1994; Couzens 2006; McKnight 2010; McKnight 2011; McKnight 2012), however there has been no comprehensive study into the reproduction of feral *Crassostrea gigas* under the environmental conditions experienced in England. This study aims to record gametogenesis of *C. gigas* from the south coast of England to establish whether environmental conditions are conducive to the spawning of viable gametes. Histological analysis of the gonad tissue taken from monthly samples of cultivated and wild *Crassostrea gigas* over the course of a year, as well as reproductive and condition indices will allow for conclusions to be drawn over the relative propagule pressure generated from aquaculture plots versus establishing wild aggregations. Furthermore the effect on gametogenesis of localised environmental conditions experienced between estuaries will be addressed. Information gained, and in particular the water temperature required for gametogenesis and spawning, will have possible practical applications in aquaculture practices

and conservation. Specifically such information could feed into models used to predict the spread of *C. gigas.*

Hypotheses

H_1 = Gametogenesis is seasonal in *Crassostrea gigas* and viable gametes are spawned under environmental conditions experienced on the south coast of England.

H_2 = Condition and reproduction indices reflect the histological findings.

H_3 = Reproduction contains plastic traits that differ between estuaries.

Objectives

O_1 = Measure gametogenesis monthly using quantitative (oocyte Feret diameter) and qualitative (descriptive) methods.

O_2 = Compare histological findings to Condition Indices (CI) and Gamete Somatic Indices (GSI).

O_3 = Compare the histological, GSI and CI results between *Crassostrea gigas* cultivated subtidally in Poole Harbour with wild *C. gigas* established intertidally in Poole Harbour and Southampton Water. Considering also temperature data collected using loggers positioned at each site.

4.2 Materials and Methods

A total of 175 farmed and 78 wild *Crassostrea gigas* from Poole Harbour, and 175 wild *C. gigas* from Southampton Water were used for histological analysis. It was the aim to sample 20 individuals per visit per site however the number sampled actually fluctuated between 18 and 22 (Table 4.1).

4.2.1 Sample collection

Wild *Crassostrea gigas*

Collection took place within 1 hour either side of spring low water. Oysters between shell lengths of 60–100 mm were haphazardly collected (Castanos et al. 2005) between September 2013 and September 2014 from Blue Lagoon, Poole Harbour (quarterly), and Woolston, Southampton Water (monthly). Oysters that were heavily fouled or those that could not be separated from the substrate were not collected. All samples were cleaned in seawater and epifauna were scraped off using a blunt metal blade.

Aquaculture *Crassostrea gigas*

Diploid oyster larvae were reared to 4-6 week old spat at the Seasalter Shellfish (Whitstable) Ltd. hatchery located in Reculver, Kent, before being transported to Poole Harbour where they spent a further 6 weeks in upwellers on board Othniel Shellfish Ltd. processing barge (Figure 2.4). Finally the oysters were laid directly onto the muddy seafloor in Poole Harbour (Figure 2.4) to grow-on and reach a marketable size (typically 18 months). *C. gigas* with shell lengths between 80 mm and 120 mm were taken monthly during harvesting from September 2013 until September 2014 (Table 4.1). Only culture plots containing diploid oysters in Poole Harbour were sampled.

Table 4.1 Dates that *Crassostrea gigas* were collected and preserved for histological gonad analysis with the number of individuals collected indicated by ().

	Poole Harbour		**Southampton Water (wild)**
	Blue Lagoon (wild)	**Othniel Shellfish Ltd. (farmed)**	
Sep-13	21.09.2013 (19)	21.09.2013 (20)	NS
Oct-13	NS	20.10.2013 (20)	NS
Nov-13	NS	NS	NS
Dec-13	NS	16.12.2013 (20)	18.12.2013 (20)
Jan-14	NS	NS	20.01.2014 (20)
Feb-14	NS	NS	NS
Mar-14	NS	19.03.2014 (20)	22.03.2014 (19)
Apr-14	30.04.2014 (20)	30.04.2014 (19)	20.04.2014 (19)
May-14	NS	29.05.2014 (20)	29.05.2014 (18)
Jun-14	NS	NS	13.06.2014 (18)
Jul-14	29.07.2014 (20)	11.07.2014 (22)	14.07.2014 (20)
Aug-14	NS	08.08.2014 (22)	11.08.2014 (20)
Sep-14	26.09.2014 (19)	26.09.2014 (22)	25.09.2014 (21)

4.2.2 Histology

Crassostrea gigas were submerged in 10 % phosphate buffered formalin (NMNH 2016) within 3 hours of collection and left for 2 weeks to ensure proper fixation of tissue.

Embedding

A section of gonad tissue approximately 5 x 5 x 5 mm was dissected from just below the pericardial cavity between the digestive gland and the gill (Figure 4.1). Tissue was immediately placed into 50 % isopropan2ol and subsequent dehydration through alcohol concentrations of 70 %, 80 % and 90 % was carried out at 12 hour intervals. The final concentration change to 100 % isopropan2ol was repeated twice with 6 hour intervals. Following dehydration, tissue was cleared in Histoclear (CellPath UK) overnight before being dabbed dry and placed into molten paraffin wax to embed. Embedding was carried out in an oven at 70 °C for 6 hours with a wax change after 3 hours. Finally the tissue was removed from the oven and placed into individual moulds with fresh wax and left overnight to cool and set.

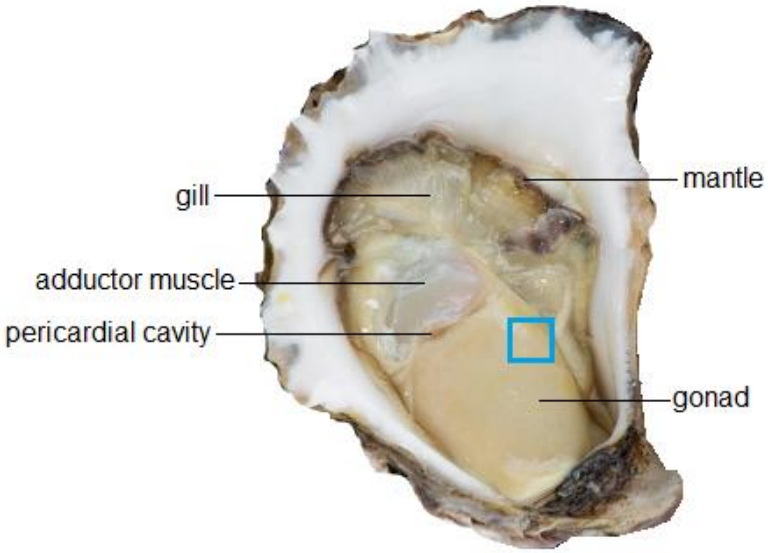


Figure 4.1 Anatomy of *Crassostrea gigas* with the section of gonad dissected for histological analysis highlighted by the blue box (photograph of farmed *C. gigas,* Poole Harbour 29.05.2014).

Slicing

Sections were sliced to a thickness of 6 µm and mounted onto glass slides. A single glass slide could hold 16 sections of gonad and 3 slides were prepared per oyster. The gonad tissue was orientated within the mould so that the outside of the gonad was sliced first and the gut was set to the back of the mould. The first slide contained tissue from the outside of the gonad, the 2nd slide was from gonad tissue taken 500 µm further toward the gut and the 3rd slide a further 500 µm again.

Staining

Slides were stained using Hematoxylin 'z' and Eosin 'y' following the protocol laid out in Table 4.2. Hematoxylin is a positive violet stain that binds to basophilic substances such as DNA and RNA, and Eosin is a negative pink stain that binds to acidophilic substances such as protein. Immediately following the staining protocol slides were mounted with a cover slip using DPX (CellPath UK) and allowed to harden for 48 hours before microscopic analysis.

Table 4.2 Tissue staining protocol, listing the chemicals used and the duration that the tissue was submerged.

Chemical	Time (minutes)
Histoclear	5
Isopropanol 100 %	2
Isopropanol 100 %	2
Hematoxylin 'z'	1.5
Running water	15
Eosin 'y'	2
Running water	2
Isopropanol 100 %	2
Isopropanol 100 %	2
Histoclear	5

Image analysis

Each slide was photographed using a Nikon Coolpix 4500. A sufficient number of photographs were taken so that 100 oocytes could be measured per female sampled. Feret diameter (Grange et al. 2007) of oocytes were measured using ImageJ 1.48s image analysis software from calibrated micrographs. Only oocytes that were near circular and had a visible nucleus were measured in order to control the cross section being measured. Images were also used to categorise the reproductive state of the oyster using a scale first suggested by Lango-Reynoso et al. (2000). The scale was altered following observations made during this study; 'Early gametogenesis' and 'Growing phase' were both divided into 2 sub categories (numerical scale 1 & 2, and 3 & 4 respectively) (Table 4.2).

Table 4.3 Reproductive scale adapted on phases suggested by Lango-Reynoso et al. (2000) and added to using observations from this study

0	Quiescent period	No evidence of gonadal development. Connective tissue consumes the visceral mass.
1	Early gametogenesis	Undiferentiated germ cells form in elongated bands surrounded by connective tissue.
2		Previtellogenic oocytes or spermatogonia line the walls of the acini, allowing sex to be determined.
3	Growing phase	Pedunculated oocytes or spermatocytes dominate the gonad. Gonadal acini anastomise. Interfollicular connective tissue is reduced.
4		Female: 50-75 % of oocytes are mature. Male: Spermatids dominate the gonad.
5	Mature	Female: >75 % of oocytes are mature. Male: Spermatozoids dominate the gonad.
6	Reabsorbtion	Post-spawned oysters that retain gametes within the gonad. Evidence of macrophage activity present.

Gonad Somatic Index and Condition Index

The Gonad Somatic Index (GSI) and Condition Index (CI) were calculated for all oysters used in histology. Tissue types were dissected out and separated into gonad and gut, and all other tissue including the gill, mantle, heart, abductor muscle and feeding palps. As in many bivalves, the gonad of *Crassostrea gigas* is fused with, and surrounds the gut. Making separation without causing damage extremely difficult, and not necessary for this analysis (Urban & Riascos 2001). Tissue and shells were dried in an oven set to 70 °C for 48 hours after which time the weight remained constant indicating that all water had evaporated. The tissue and shells were then moved into a Nalgene desiccator to cool down and weighed to 0.1 mg using an OHAUS Pioneer balance. The GSI was calculated for all *C. gigas* sampled however only those that had complete, undamaged shells were used for CI analysis.

There are various methods in use for calculating the CI of bivalves and multiple reviews that culminate in the recommendations of different methods for standard use (Mann 1978; Lawrence & Scott 1982; Lucas & Beninger 1985; Crosby & Gale 1990). Recommended formulae for CI are divided into those that use the internal volume of the shell (Lawrence & Scott 1982; Crosby & Gale 1990), and those that use the weight of the shell (Mann 1978; Lucas & Beninger 1985). Using the shell volume to calculate CI yields indices which assess the proportion of available internal cavity capacity utilised by an oyster's soft tissue. This method is recommended for

studies addressing the nutritive state of bivalves (Crosby & Gale 1990). However *C. gigas* varies greatly in shell shape even within a single age cohort, and in particular the relative shell depths of *C. gigas* of similar shell lengths would expect to differ notably between farmed oysters and wild oysters. Therefore it has been considered most appropriate for this study to express the condition of *C. gigas* using a ratio of ash free dry weight (flesh): dry shell weight (Walne & Mann 1975).

CI

$$\frac{Total\ flesh\ ash\ free\ dry\ weight\ g}{Dry\ shell\ weight\ g}\ x\ 100$$

There are also a variety of methods employed to calculate GSI in bivalves, once again they can be divided into formulae that use volume (Urban & Riascos 2001; Riascos et al. 2007; Çolakoğlu & Palaz 2014) or weight (Gagné et al. 2003; Kang et al. 2003; Ren et al. 2003; Cardoso et al. 2007). Gonad volume is an estimate calculated from linear measurements obtained directly on soft-body samples or using image analysis. Although this method takes into consideration the fused gut and gonad, there appears to be notable room for error whilst taking the linear measurement. The use of weight ratios remains popular in bivalve research, furthermore weight is being used in this study for CI, and so for continuity, weight shall be used to calculate GSI. GSI may be calculated as the ratio of gonad tissue (including gut tissue) weight to somatic weight (Ren et al. 2003; Cardoso et al. 2007), or the weight of total oocytes: somatic tissue (Choi et al. 1994; Kang et al. 2003). It is intended that GSI shall be calculated from males and females during this study and so tissue weight shall be used. Wet weights are widely used in the literature (Gagné et al. 2003; Enriquez-Diaz et al. 2009) as are dry weights (Ren et al. 2003; Cardoso et al. 2007). Using ash free dry weights removes the uncertainty surrounding the quantity of water in the tissue and food in the gut. Therefore the following formula has been used to calculate GSI during this study:

GSI

$$\frac{Gonad\ ash\ free\ dry\ weight\ g}{Total\ ash\ free\ dry\ weight\ g}\ x\ 100$$

Sex Ratio

The sex of an individual was determined using histology and the sex ratio was calculated using the Chi-squared goodness-of-fit test that compares the observed number of males and females with the number of each sex expected under sex ratio equality. The sex ratio was only calculated during months when over 50 % of the sampled oysters had distinguishable gonad tissue. The

total number of oysters sampled for each test was taken to be males + females only, and therefore hermaphroditic oysters and spent oysters were not considered during the analysis.

4.3 Results

Gonad features during gametogenesis

From the histological analysis the gonad was seen as a diffuse organ located between the digestive diverticulae and the mantle epithelium. The mass of the gonad varied notably during the reproductive cycle; there was an increase from narrow bands of germinal epithelium surrounded and supported by vesicular connective tissue, to an enlarged expanse of mature gonad that took up much of the outer visceral mass at the expense of the connective tissue. Prior to maturation and anastomosing of gonad acini, different features characterised the acini depending upon the position within the gonad. Those located in the centre of the visceral mass tended to be round with germinal epithelium lining the entire inner wall whereas those nearest the mantle were elongated and made up of ciliated epithelial cells on the side nearest the mantle, and germinal epithelium cells on the side closest to the body surface. Gametogenesis occurred throughout spring when germ cells were seen to differentiate into previtellogenic oocytes or spermatocytes so determining sex (Figure 4.2). In females, an increase in oocyte size and a greater ratio of cytoplasm to nucleus showed vitellogenesis to be occurring. Growing oocytes became irregular in shape, typically elongating along the acini wall before peeling away to become attached to the acini wall by only a stalk (also referred to as pedunculated) (Figure 4.3). Mature oocytes were rounder with a relatively larger nucleus. They had also become disassociated from the acini wall and so were floating in the lumen of the gonad (Figure 4.4). The male gonad contained only spermatogonia during the early stages of gametogenesis (Figure 4.2). Spermatogonia were the largest stage of male gamete and were present in 2 forms. (1) relatively large, pale cells that were oval in shape were closely associated with the acini wall. (2) smaller spermatogonia were both darker in colour and rounder in shape and located on the gonad side of the larger spermotogonia. As the male gametes developed they reduced in size and moved into the lumen toward the centre of the gonad. Spermatogonia were produced throughout the reproductive season resulting in multiple developmental stages of sperm being present in all samples taken between April and August 2014. Following on from the spermatogonia, spermatocytes dominated the gonad during the growing stage (Figure 4.3) and a mature male gonad was characterised by a high abundance of small dense spermatids and mature spermatozoids with a characteristic flagellum (Figure 4.4).

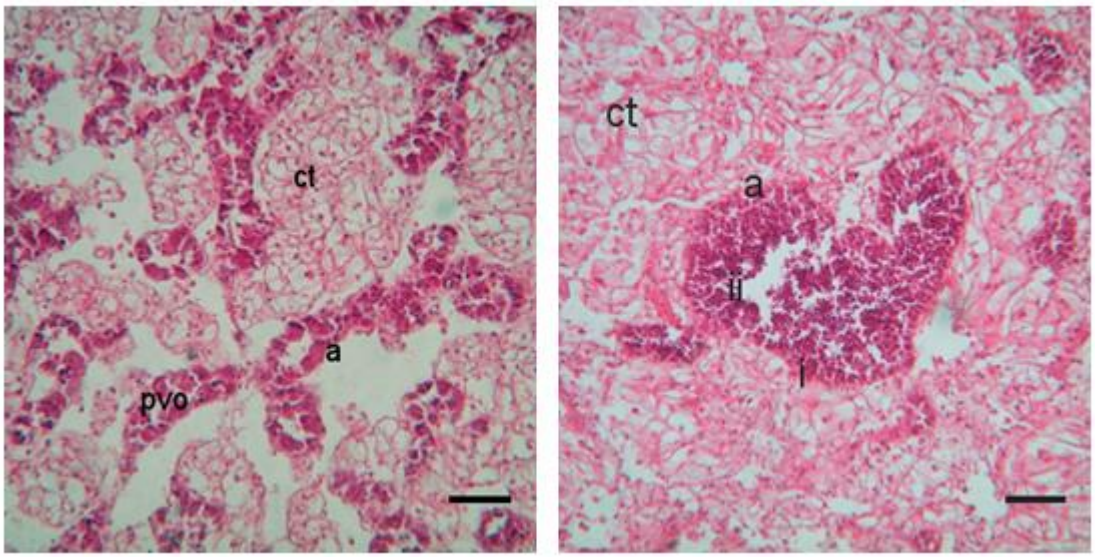


Figure 4.2 Early development of oogenesis (left) and spermatogenesis (right) in *Crassostrea gigas*. Gonad acini (a) develop within connective tissue (ct). Previtellogenic oocytes (pvo) and Type I (i) and II (ii) spermatogonia. Scale bars = 50 µm.

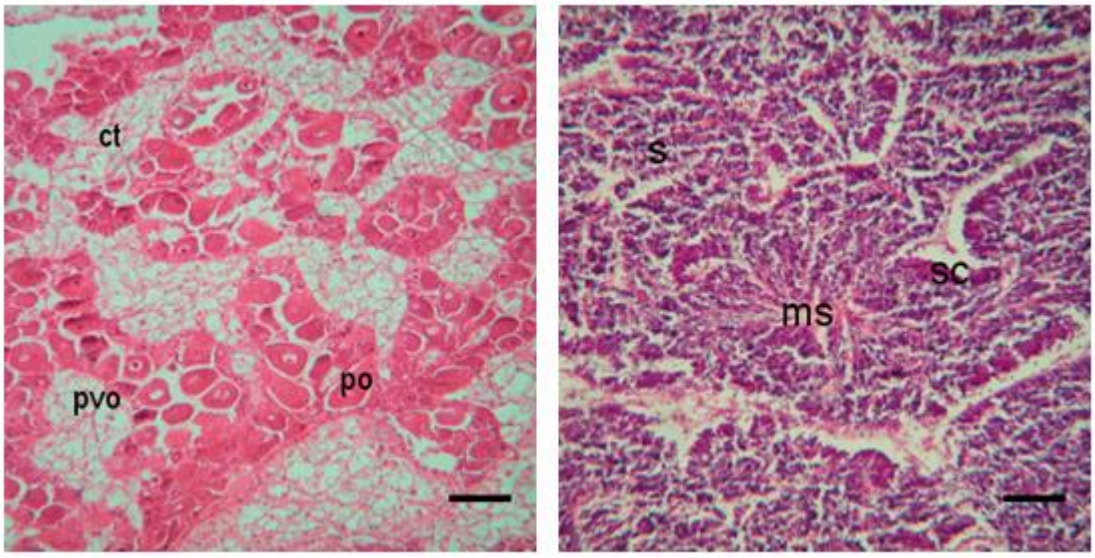


Figure 4.3 Growing stage of oogenesis (left) and spermatogenesis (right) in *Crassostrea gigas.* Oocytes have undergone vitelogenesis and become pedunculated (po) and the male gonad contains spermatocytes (sc), spermatids (s) and some spermatozoids (mature sperm-ms). Scale bars = 50 µm.

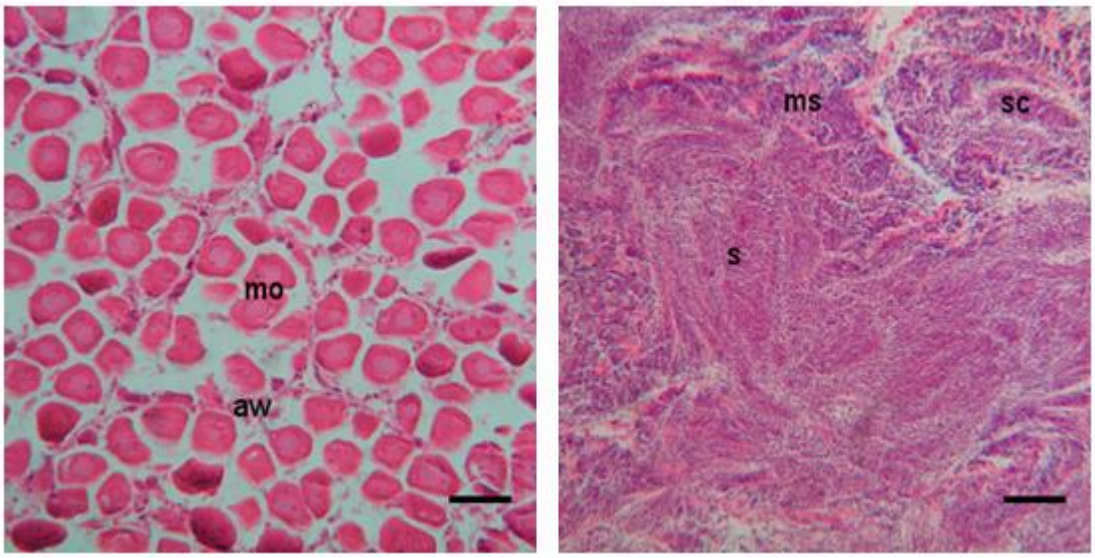


Figure 4.4 Mature gonads of female (left) and male (right) in *Crassostrea gigas* mature. Mature oocytes (mo) fill the female gonad with truncated sections of acini wall (aw) remaining. Spermatids and spermatozoids dominate the male gonad with clusters of spermatocytes present. Scale bars = 50 µm.

Gametogenic development was graded according to the reproductive scale in Table 4.3 allowing the distribution of maturity at each location to be assessed over time. There was a synchronous initiation of gametogenesis between sexes for both wild *C. gigas* inhabiting Southampton Water and farmed *C. gigas* taken from Poole Harbour. However a greater proportion of oysters inhabiting the intertidal site in Southampton Water had initiated gametogenesis in early spring than from the aquaculture plot in Poole Harbour. In general female oysters underwent a more uniform development than males, as seen by fewer stages of maturity present during any one month. Males typically reached maturity more quickly than females however a peak in maturity was achieved between sexes during June in Southampton Water and August in Poole Harbour. A spawning event followed each maturity peak. In the case of *C. gigas* in Poole Harbour the majority of oysters spawned completely, however in Southampton Water a second cohort of gametes continued to develop until they too were spawned (Figure 4.5). A small percentage of oysters retained gametes within the gonad after spawning and typically more males were seen to be reabsorbing gametes than females. There is also evidence from *C. gigas* collected from Poole Harbour that a greater percentage of oysters retained gametes following the reproductive cycle in 2013 than 2014 (Figure 4.6).

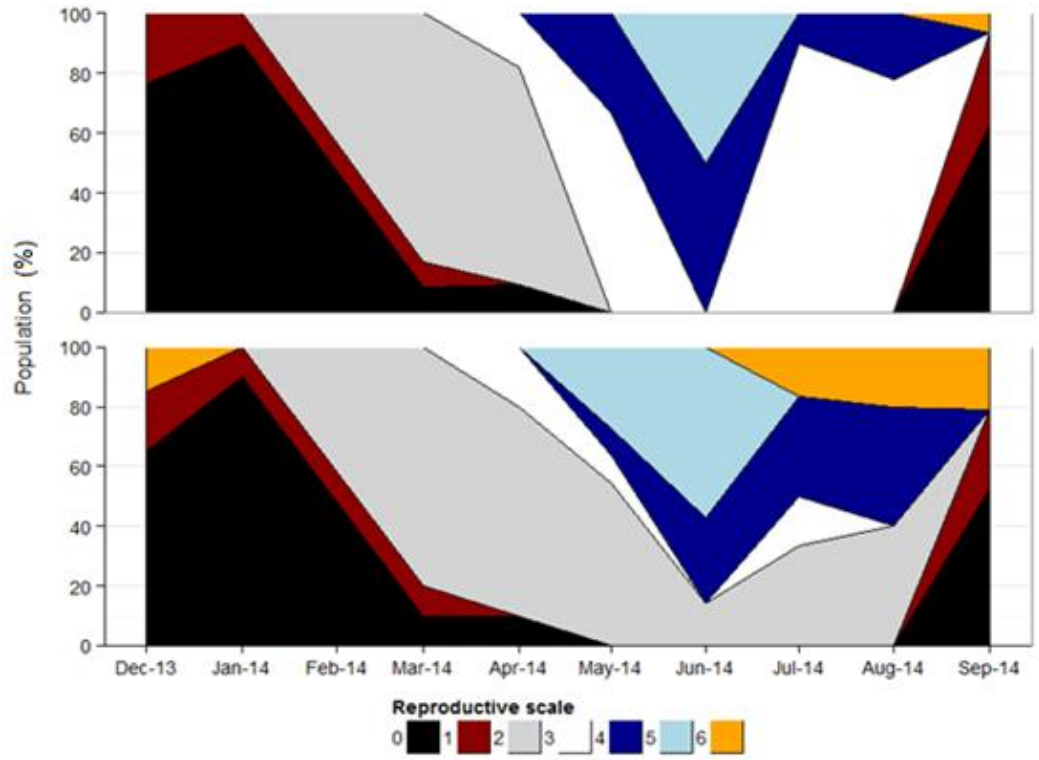


Figure 4.5 The proportion of wild *Crassostrea gigas* sampled intertidally from Southampton Water at a particular stage of gamete development. **Top**: Females. **Bottom**: Males. Reproductive scale given in Table 4.3.

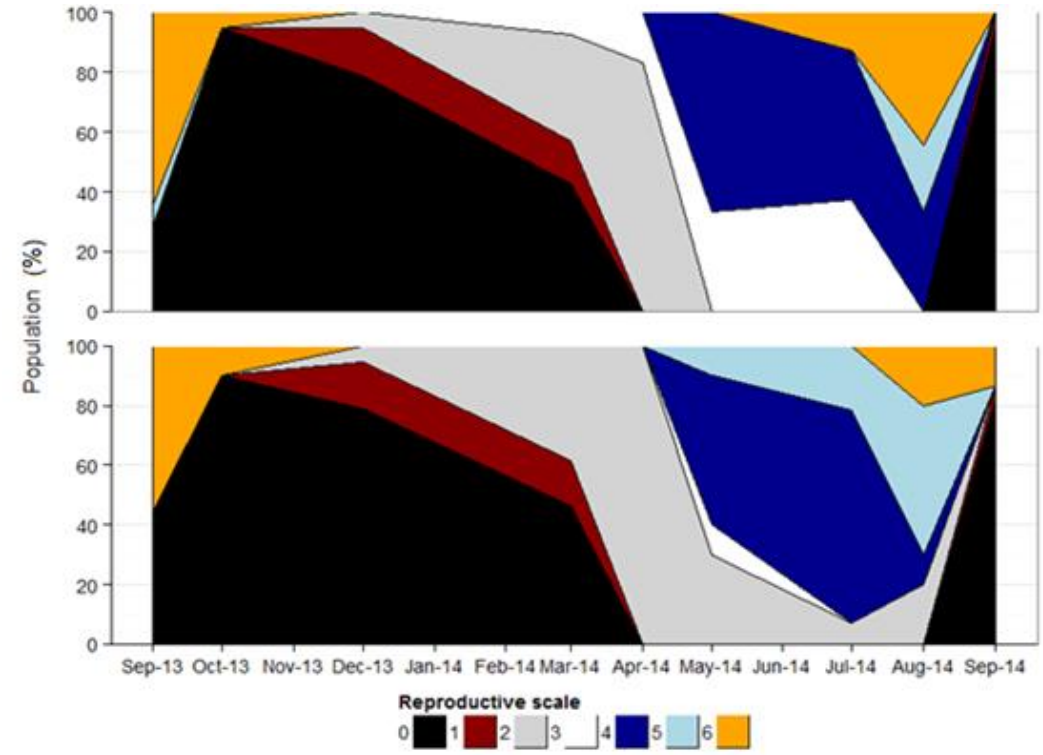


Figure 4.6 The proportion of farmed *Crassostrea gigas* sampled subtidally from Poole Harbour at a particular stage of gamete development. **Top**: Females. **Bottom:** Males. Reproductive scale given in Table 4.3.

Gametogenesis

Oocyte Feret diameter was used to quantitatively analyse gametogenesis between September 2013 and September 2014. Significant changes in oocyte size occurred over the year at all sites sampled (Table 4.4). Such evidence further demonstrates seasonal gametogenesis occurring in *C. gigas* inhabiting the south coast of England. Oocyte growth was rapid during the spring and subsequently followed by a plateau in growth throughout summer (Figure 4.7 and Table 4.4). It should be noted that oocytes measured during the months of September 2013, October 2013 and September 2014 were of retained oocytes post spawning. Excluding retained oocytes post-spawning, a maximum mean oocyte size was reached in Southampton Water in June 2014 (37.37 µm). After the maxima in June, a significant decrease in size occurred resulting in a dip in oocyte size during July (31.70 µm) that recovered in August 2014 (32.89 µm) (Table 4.4). No June sample exists for subtidal oysters from Poole Harbour, however oocyte size remained similar during July and August (Table 4.4).

Table 4.4 Statistical differences in oocyte Feret diameter of *Crassostrea gigas* between months at 3 different sites. Tukey's HSD post hoc results refers to the significance between that month and the previous consecutive calendar month. NS = no sample.

	Poole Harbour farmed subtidal		**Southampton Water wild intertidal**		**Poole Harbour wild intertidal**
	One way ANOVA	Tukey's HSD	One way ANOVA	Tukey's HSD	One way ANOVA
March	F(7) = 1889, MSE = 66818, p < 0.001	NS	F(8) = 5061, MSE = 97640, p < 0.001	NS	F(2) = 2366, MSE = 58159, p < 0.001
April		p < 0.001		p < 0.001	
May		p < 0.001		p < 0.001	
June		NS		p < 0.001	
July		NS		p < 0.001	
August		p = 0.0299		p < 0.001	
September		NS		p < 0.001	

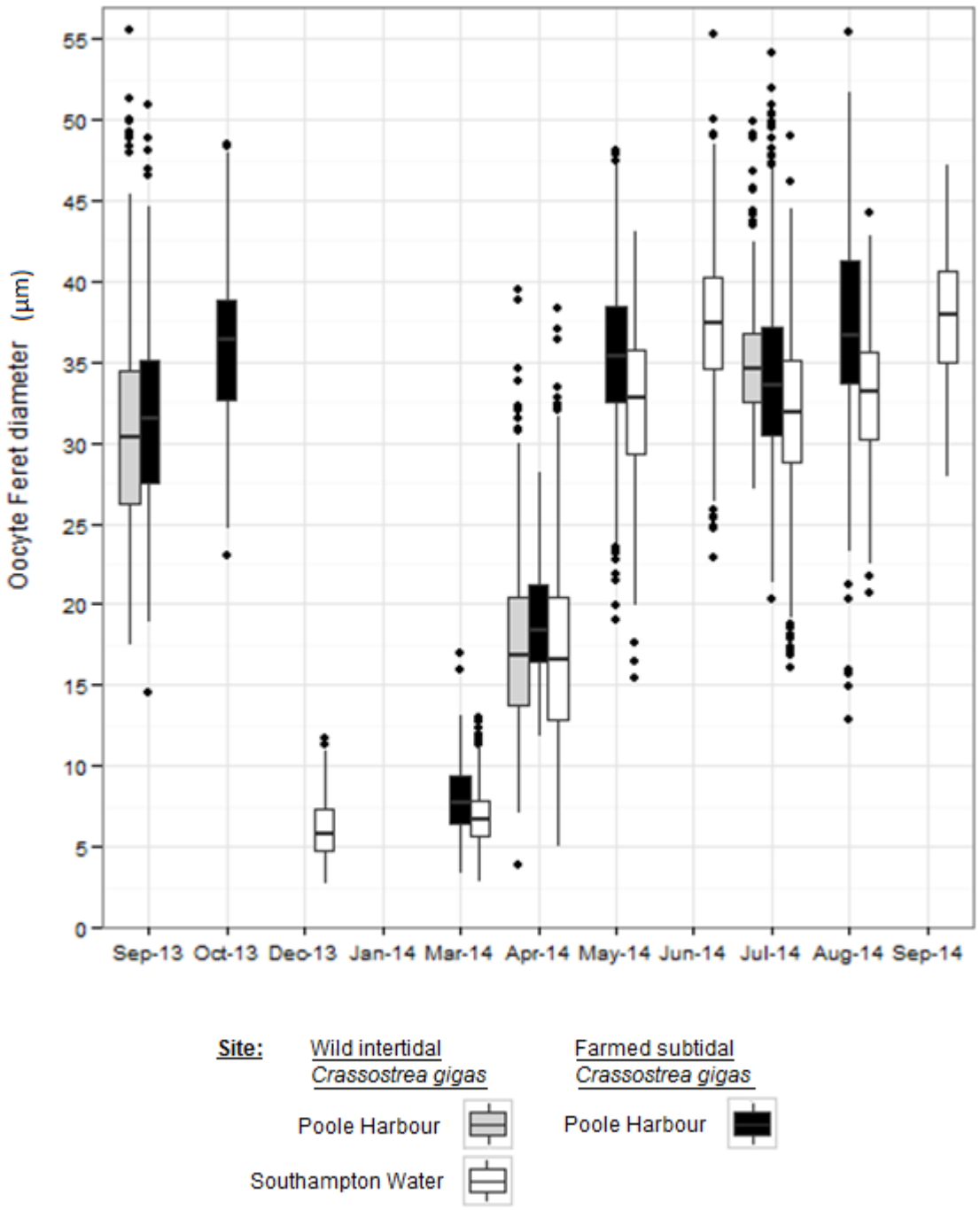


Figure 4.7 An annual gametogenic cycle of oocyte Feret diameter (µm) in *Crassostrea gigas* from Poole Harbour and Southampton Water between September 2013 and September 2014. Boxes show the mean average, first and third quartiles. Whiskers denote 1.5*inter-quartile range. Plotted points are outliers.

Oocyte size from wild intertidal oysters inhabiting Poole Harbour showed similar monthly trends, however an accurate description of the variations in oocyte size cannot be made due to the less frequent sampling at this site. To assess whether gametogenesis in *C. gigas* inhabiting the intertidal zone of Poole Harbour more closely followed that of farmed subtidal oysters in the

same estuary, or those also established in the intertidal zone but in a different estuary, oocyte size was compared between sites (Table 4.5). Oocyte size was similar in oysters sampled from wild intertidal and farmed subtidal oysters in Poole Harbour during September 2013. However oysters sampled during this month had partially spawned and so the oocytes they retained were being reabsorbed, a process known to affect size (Lango-Reynoso et al. 2000). During April farmed subtidal oysters from Poole Harbour had significantly larger oocytes than wild oysters from both intertidal sites, and intertidal oysters had oocytes of similar sizes (Table 4.5). By July all sites sampled contained oysters with different sized oocytes in decreasing order from wild intertidal oysters in Poole Harbour, to farmed subtidal Poole Harbour oysters, to wild intertidal oysters from Southampton Water (Figure 4.7, Table 4.5).

Table 4.5 Comparisons of oocyte size between subtidal farmed *Crassostrea gigas* from Poole Harbour, wild intertidal *C. gigas* from Poole Harbour and wild intertidal *C. gigas* from Southampton Water. Note that no sample was collected from Southampton Water in September 2013 (NS).

	One way ANOVA (all sites)		Tukey HSD post hoc test results	
			Southampton Water wild intertidal:	**Poole Harbour wild intertidal:**
September 2013	F(1)=1.935, p=0.165	**Poole Harbour Farmed subtidal:**	NS	p=0.165
		Poole Harbour Wild intertidal:	NS	
April 2014	F(2)=12.54, p<0.001	**Poole Harbour Farmed subtidal:**	p<0.001	
		Poole Harbour Wild intertidal:	p=0.090	p<0.001
July 2014	F(2)=112.88, p<0.001	**Poole Harbour Farmed subtidal:**	p<0.001	
		Poole Harbour Wild intertidal:	p<0.001	p<0.001

Sex Ratio

Sex ratio was calculated using samples collected between March 2014 and August 2014 as gonad was differentiated in over 50 % of oysters collected from each site during these months. Oysters collected from both sites in Poole Harbour during September 2013 were also included (no sample existed from Southampton Water). The overall sex ratio did not differ significantly from a predicted frequency of 50 % males and 50 % females (Table 4.6). The only significant deviation

from 1 male: 1 female was recorded during July in wild intertidal *C. gigas* inhabiting Poole Harbour (Table 4.6).

Table 4.6 Sex ratio (male:female) of *Crassostrea gigas* showing only months when over 50 % of the sample had identifiable gonad. The Chi squared (χ^2) value was calculated for a 1:1 male:female sex ratio with a significance of p=0.05. * denotes a sex ratio with a significantly different frequency to 1:1.

Site	Month	Sex ratio (M:F)	χ^2	N
Poole Harbour farmed subtidal	September 2013	1:1.7	1.00	16
	March 2014	1:1.2	0.09	11
	April 2014	1:0.5	2.58	19
	May 2014	1:0.8	0.20	20
	July 2014	1:0.5	2.33	21
	August 2014	1:0.8	0.22	18
	Total	**1:0.8**	**1.61**	
Poole Harbour wild intertidal	September 2013	1:1.5	0.40	18
	April 2014	1:0.8	0.20	20
	July 2014	1:0.36	4.26*	19
	Total	**1:0.7**	**1.65**	**57**
Southampton Water wild intertidal	March 2014	1:1.25	0.22	18
	April 2014	1:1.1	0.05	19
	May 2014	1:0.6	1.47	17
	June 2014	1:1.7	1.32	19
	July 2014	1:2.2	2.58	19
	August 2014	1:1.4	0.47	19
	Total	**1:1.3**	**1.52**	

Hermaphrodites

Instances of hermaphroditism were seen in Poole Harbour and Southampton Water during July and August 2014. In two examples, both taken from aquaculture plots in Poole Harbour, both the male and female gametes were at a similar stage of development, suggesting simultaneous hermaphroditism (Figure 4.8). In both cases the sperm was mature, as it was in other males at this time of year. The oocytes were pedunculated in both hermaphrodites, making them less developed and smaller than the majority of females sampled during respective months (which contained mature oocytes) (Table 4.7).

A further two hermaphrodites were sampled that contained male and female gametes at different stages of development, suggesting sequential hermaphroditism (Figure 4.9). In both instances sperm cells were clustered in the centre of the gonad surrounded by lumen, showing characteristics typical of degeneration. The visceral mass of sequential hermaphrodites contained comparatively more connective tissue in proportion to gonadal acini than single sex oysters during the same month. One of the sequential hermaphroditic oysters was sampled from Southampton Water in July. The female gametes were predominantly pedunculated and although this was the dominant reproductive stage of females at this time, the oocyte Feret diameter was significantly less in the hermaphrodite in comparison to the females (Table 4.7). The final example of hermaphroditism was in an oyster taken from aquaculture plots in Poole Harbour in August that contained few previtellogenic oocytes dispersed throughout the acini walls. Unlike in the other hermaphrodites, in this case the oocytes were at a lower density than would typically be seen in the development of a female gonad.

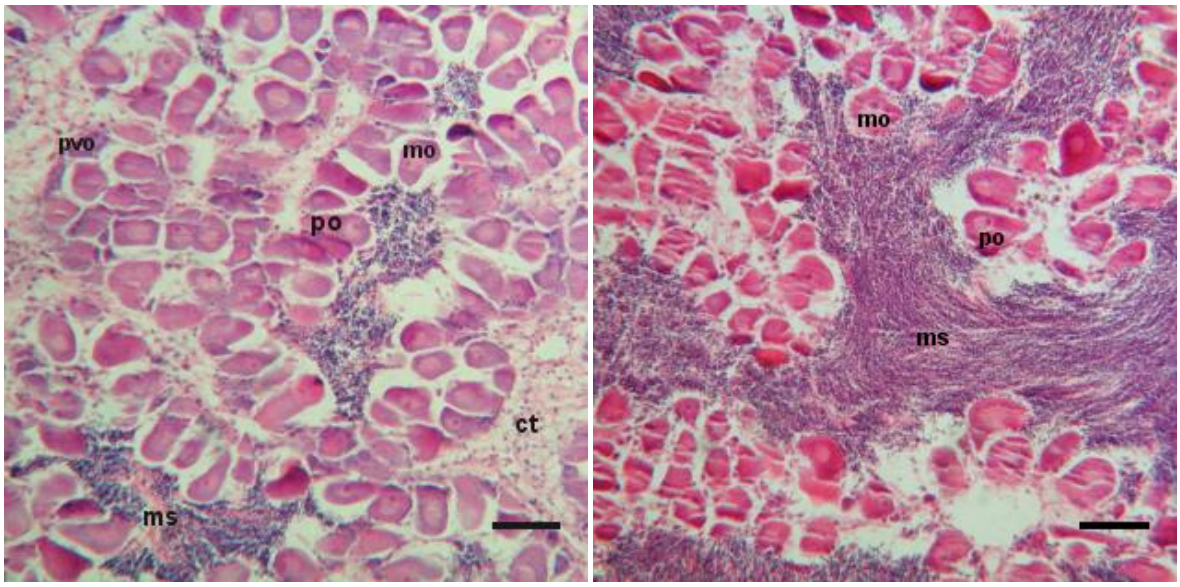


Figure 4.8 Simultaneous hermaphroditism seen in *Crassostrea gigas* sampled from aquaculture plots in Poole Harbour in July (left) and August (right) 2014. Spermatozoids (ms) and pedunculated oocytes (po) dominate the gonad with some previtellogenic (pvo) and mature oocytes (mo) also present. Scale bar 50 µm.

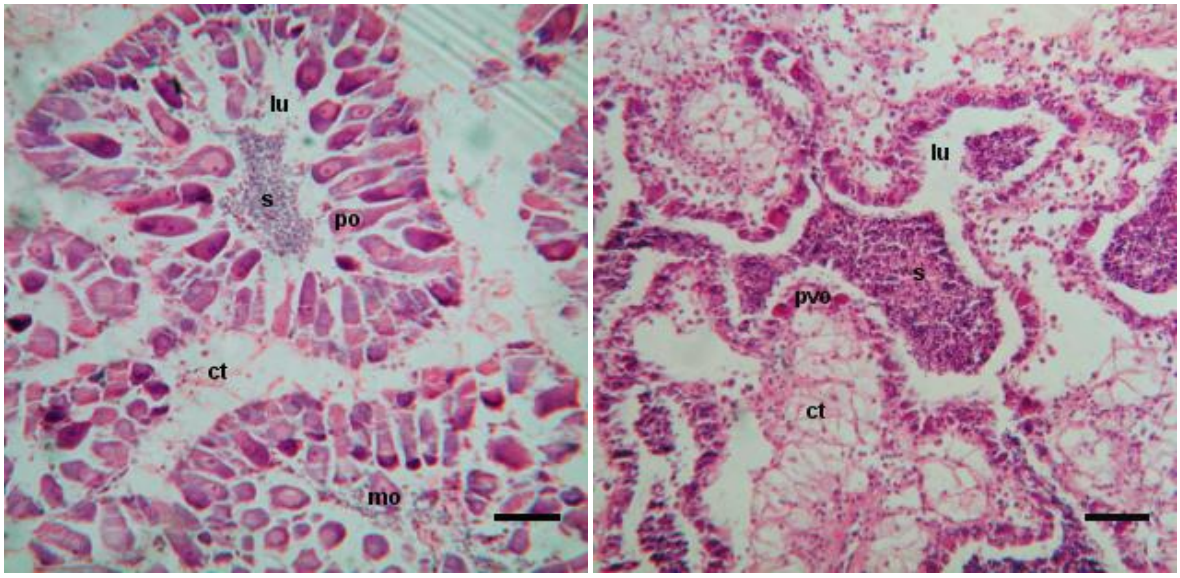


Figure 4.9 Sequential hermaphroditism seen in *Crassostrea gigas* sampled from Southampton Water in July (left) and Poole Harbour in August (right) 2014. Gonadal acini contain pedunculated (po) or previtellogenic oocytes (pvo) associated with the acini wall and separated from degenerating spermatozoa (s) by lumen (lu). The visceral mass also contains connective tissue (ct). Scale bar 50 µm.

Table 4.7 A comparison of oocyte Feret diameter between hermaphroditic and single sex *Crassostrea gigas*.

Month	**Site**	**Hermaphroditic state**	**One way ANOVA**	**Dunnett's post hoc**
July	Poole Harbour farmed subtidal	Simultaneous	$F(6) = 64.65$, $p < 0.001$	$p < 0.001$
	Southampton Water wild intertidal	Sequential	$F(7) = 51.87$, $p < 0.001$	$p < 0.001$
August	Poole Harbour farmed subtidal	Simultaneous	$F(4) = 112.5$, $p < 0.001$	$p < 0.001$

Condition and Reproductive Indices

Biometric indices showed seasonal trends at all sites sampled. However the changes in GSI and CI did not always align to, or reflect the stage of gametogenesis. GSI of wild intertidal *C. gigas* inhabiting Southampton Water showed the truest reflection of the gametogenic stage as described using gonad histology. Consistently low GSI was recorded throughout the winter and was followed by an increase during spring, from March until the peak in June (Table 4.8). After June the GSI decreased in a stepwise fashion with an initial decrease between June and July, plateaux from July to August and a second decrease between August and September (Figure 4.10, Table 4.8). CI of wild intertidal *C. gigas* inhabiting Southampton Water differed between months with a peak in CI experienced during May and June (Table 4.9). Unlike oocyte size and GSI, CI remained consistently low until May and declined significantly during August before increasing again in September (Figure 4.11, Table 4.9). GSI and CI of subtidally farmed *C. gigas* from Poole Harbour were markedly similar. Both indices increased during the spring, with notable increases between April and May (Table 4.8 and Table 4.9), and decreased from July until September (Figure 4.10 and Figure 4.11).

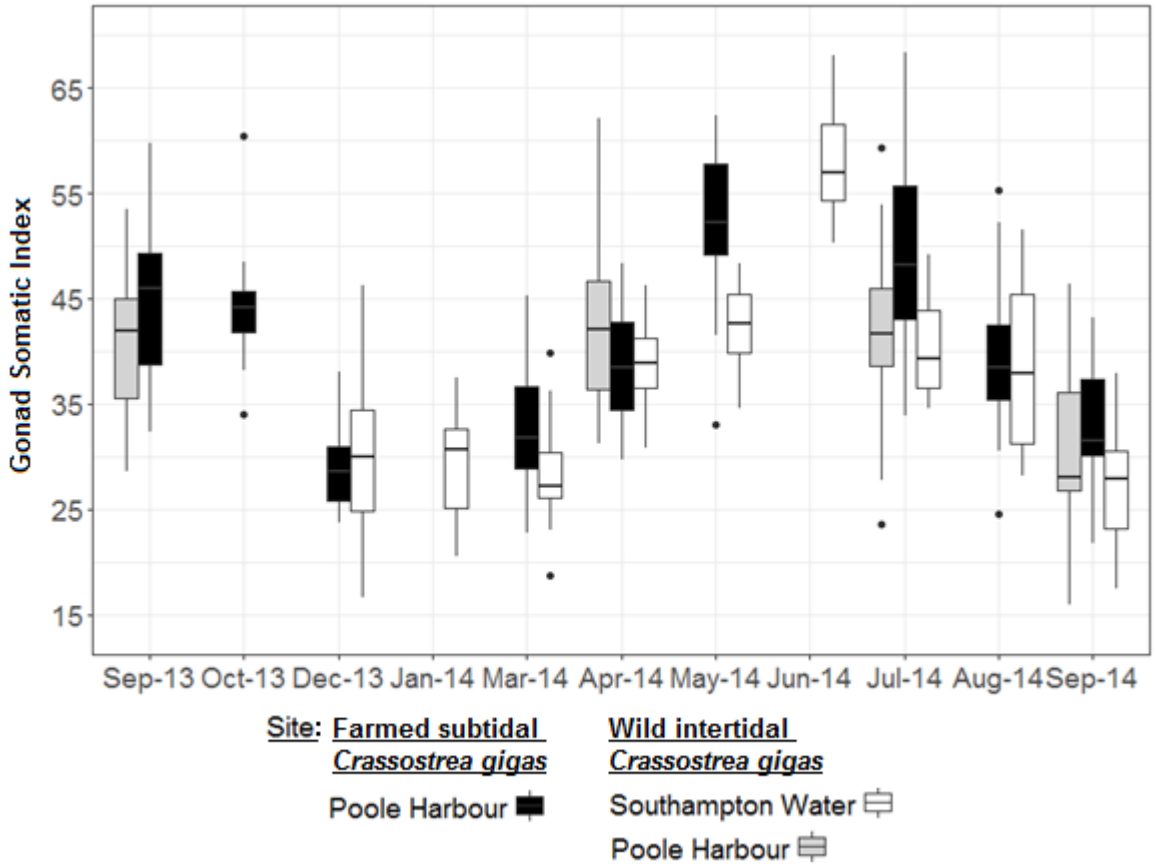


Figure 4.10 *Crassostrea gigas* from 3 locations. Monthly Gonad Somatic Index values between September 2013 and September 2014. Boxes show the mean average, first and third quartiles. Whiskers denote 1.5*inter-quartile range.

Table 4.8 A comparison of Gonad Somatic Index of *Crassostrea gigas* between March and September 2014 at 3 sites.

	Poole Harbour farmed subtidal		**Southampton Water wild intertidal**		**Poole Harbour wild intertidal**
	One way ANOVA	Tukey's HSD	One way ANOVA	Tukey's HSD	One way ANOVA
March	F(8) = 26.48, p < 0.001		F(8) = 45.97, p < 0.001		F(3) = 9.182, p < 0.001
April		0.213		p<0.001	
May		p<0.001		p<0.001	
June		NS		p<0.001	
July		NS		p<0.001	
August		p<0.001		p=0.510	
September		0.009		p<0.001	

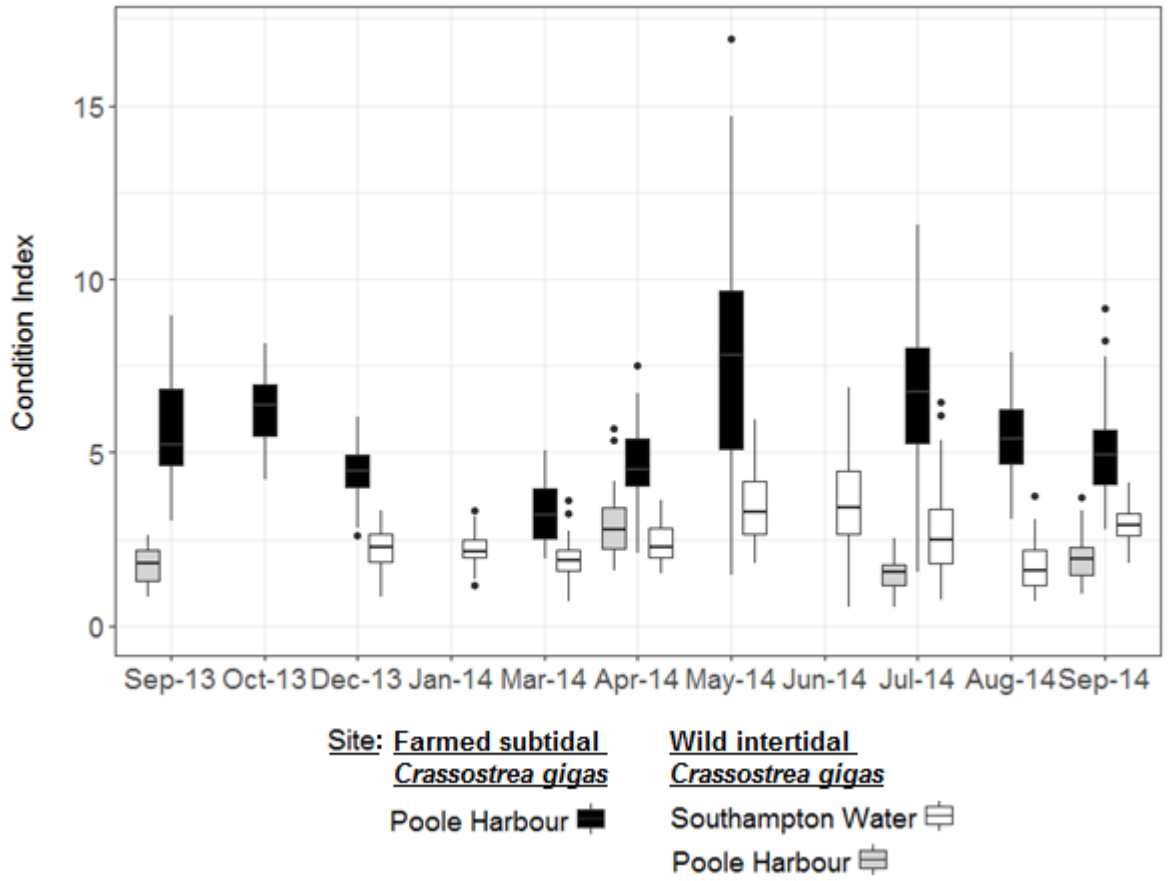


Figure 4.11 *Crassostrea gigas* from 3 locations. Monthly Condition Index values between September 2013 and September 2014. Boxes show the mean average, first and third quartiles. Whiskers denote 1.5*inter-quartile range.

Table 4.9 A comparison of *Crassostrea gigas* Condition Indices between December 2013 and September 2014 at 3 sites.

	Poole Harbour farmed subtidal		**Southampton Water wild intertidal**		**Poole Harbour wild intertidal**
	One way ANOVA	Tukey's HSD	One way ANOVA	Tukey's HSD	One way ANOVA
January	F(8) = 20.76, p < 0.001	NS	F(8) = 19.29, p < 0.001	p = 1.000	F(3) = 32.49, p < 0.001
February		NS		NS	
March		NS		NS	
April		p = 0.025		p = 0.334	
May		p<0.001		p < 0.001	
June		NS		p = 1. 000	
July		NS		p = 0.012	
August		p = 0.120		p < 0.001	
September		p = 0.998		p<0.001	

For the wild oysters collected intertidally from Poole Harbour, GSI was similar between September 2013, April ($p = 0.895$) and July 2014 ($p = 0.999$), but decreased in September 2014 ($p<0.001$) (Figure 4.10) (in correlation with histological evidence of spawning). Of the sampled months, CI of wild intertidal *C. gigas* in Poole Harbour was greatest during April, lowest during July and increased in September 2014 (Figure 4.11) (similar trend to Southampton Water).

Oysters sampled from subtidal aquaculture plots within Poole Harbour had continually greater GSI and CI than both intertidal sites during respective months ((2-way ANOVA) GSI: $F(2)=8.692$, $p<0.001$; CI: $F(2)=466.12$, $p<0.001$). Although the 2 intertidal sampling sites shared similar GSI (TukeyHSD: $p=0.993$) they were found to have significantly different CI (TukeyHSD: $p<0.001$) (Figure 4.10 and Figure 4.11).

Environment

Water temperature for the entire sampling period was only collected subtidally from Southampton Water. The average winter water temperature (December – March) was 8.3 °C, with a minimum of 7.6 °C in February, and the average summer water temperature (June – August) was 19.0 °C, with a high of 21.7 °C in July. In addition water temperatures were measured intertidally and subtidally in Poole Harbour between May and October 2014 where average summer temperatures were 19.6 °C and 19.4 °C respectively (Figure 4.12).

An intertidal temperature logger was located in Southampton Water between 14.06.2014 and 30.06.2014. Temperature fluctuations were greater intertidally than subtidally in both estuaries with Poole Harbour experiencing the greatest range; 9.4 °C daily average temperature difference. For comparison the daily average temperature difference experienced intertidally in Southampton Water was 5.6 °C, and subtidally in Poole Harbour it was 2.2 °C (Figure 4.13).

In addition to water temperatures taken during the period of study, further archived data (19.07.2004 and 26.01.2005) was made available for this study (A. Jensen pers. coms.). Surface (3 cm above sediment surface) and sediment (3 cm below sediment surface) temperatures were recorded intertidally on a mudflat in Poole Harbour (50°40’32.01”N, 2°0’17.65”W). The sediment buffered temperatures. This resulted in warmer sediment than surface in the morning and cooler in the afternoon, and a daily minimum and maximum temperature at the surface (Figure 4.14). Temperature differences were greatest during the warmest months, when the sediment remained notably cooler than the surface. The smallest temperature differences occurred during the winter months when surface waters stayed below the temperature of the sediment for most of the day (Figure 4.15).

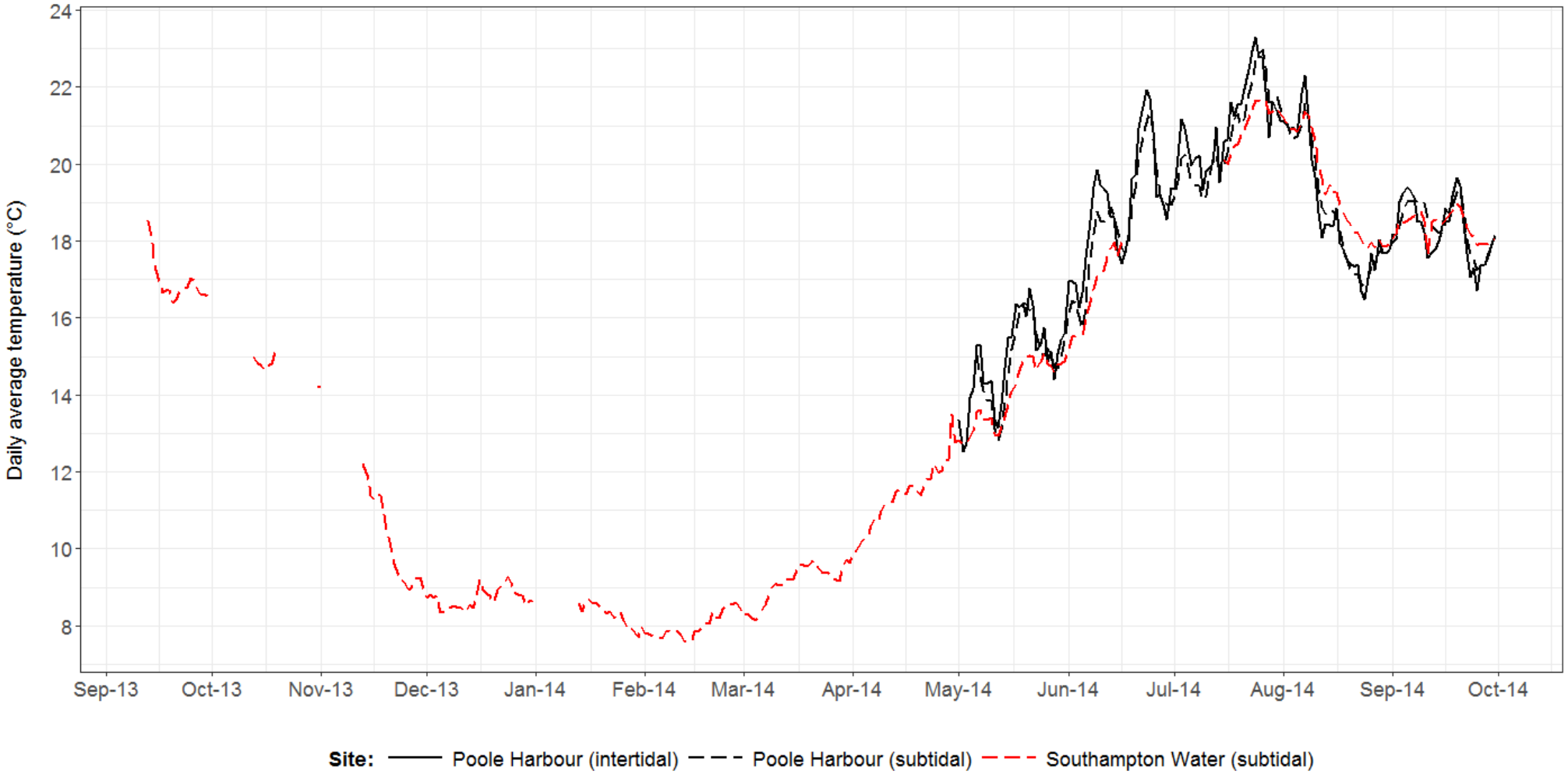


Figure 4.12 Temperature plotted as daily averages from data recorded at 15 minute intervals between September 2013 and October 2014.

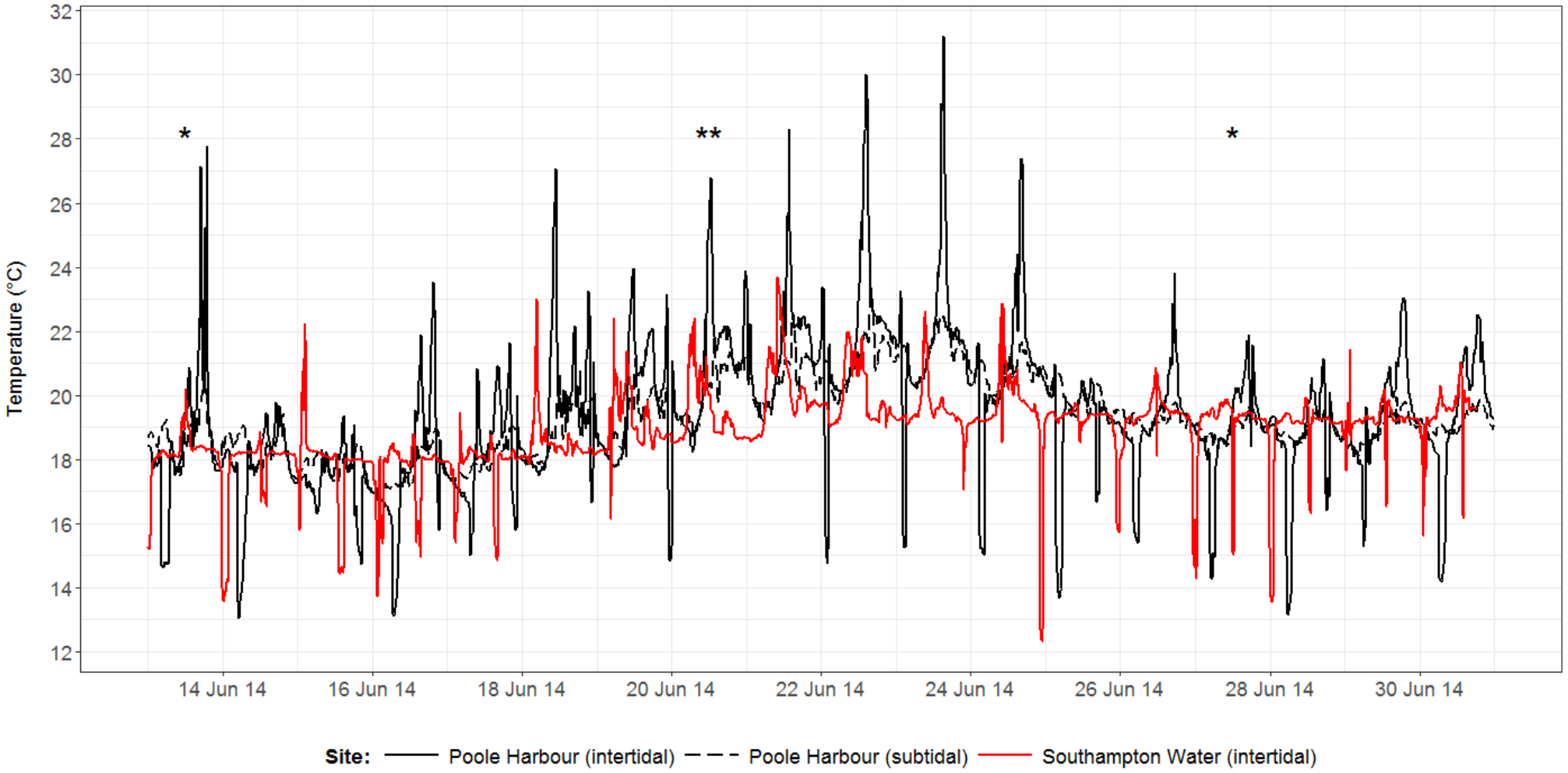


Figure 4.13 Temperature logged at 15 minute intervals, with spring (*) and neap (**) tides.

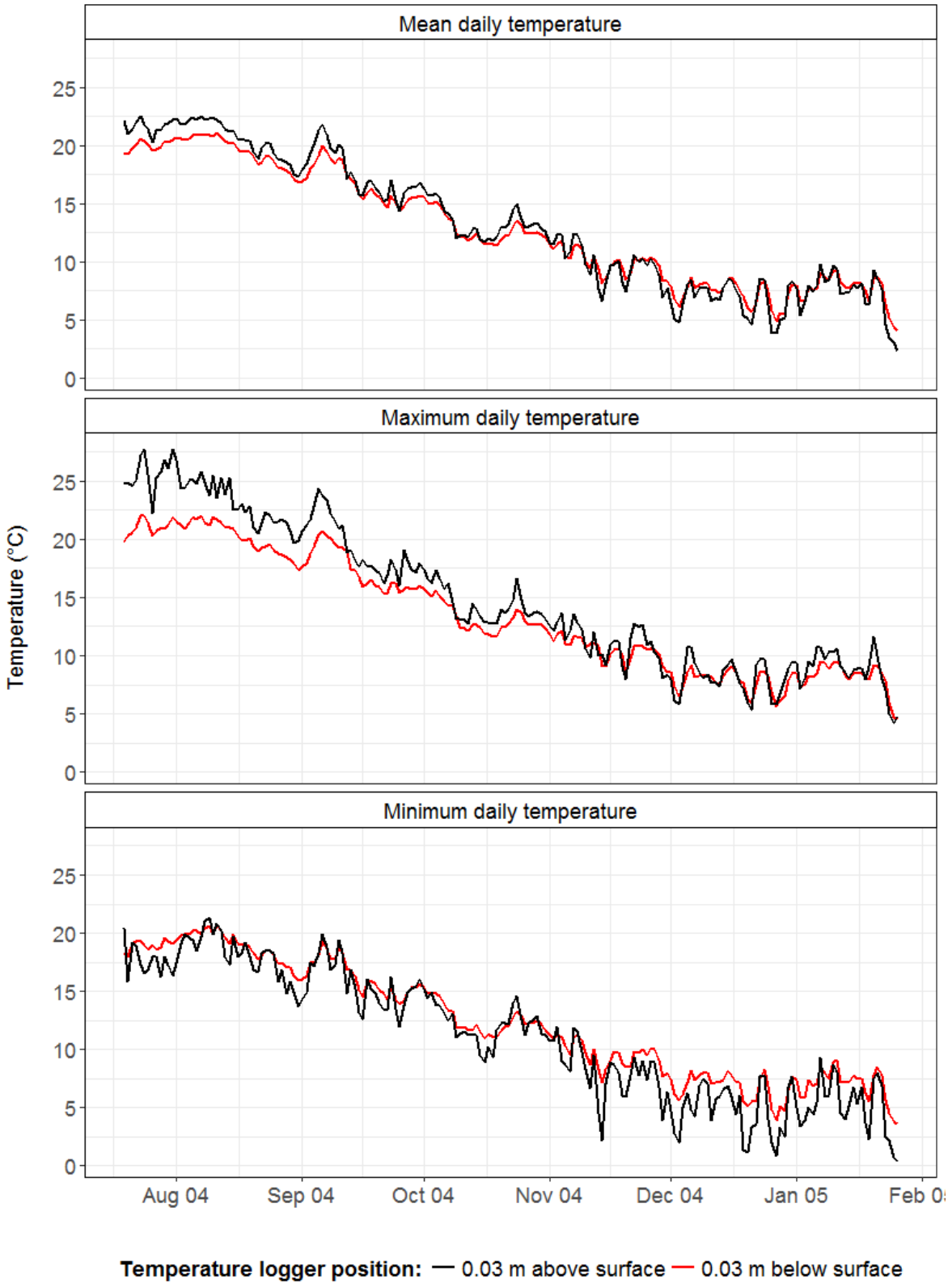


Figure 4.14 A comparison of temperature 0.03 m above and 0.03 m below the surface of a mudflat in Poole Harbour

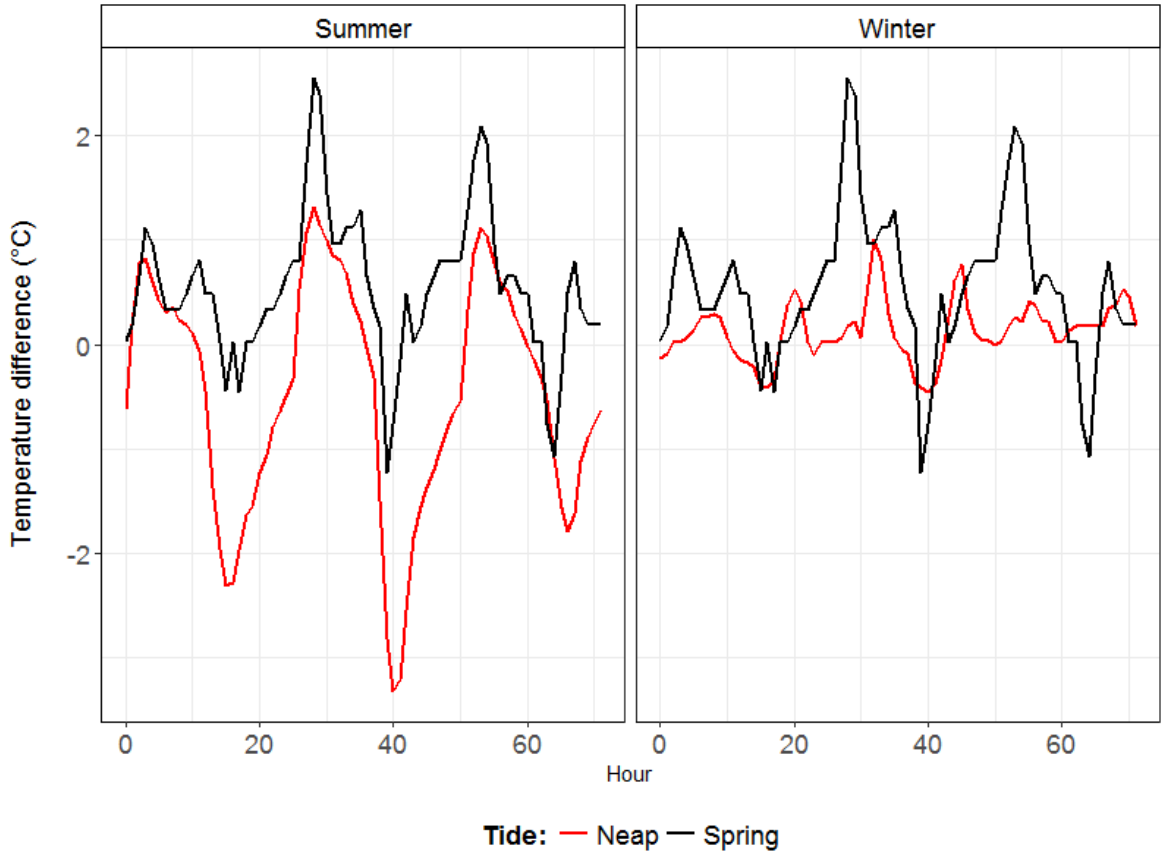


Figure 4.15 The difference in temperature between 0.03 m above and below the surface of a mudflat in Poole Harbour over a spring and neap tide ± 1 day in summer (July) and Winter (December)

4.4 Discussion

Across Europe, a variety of reproductive strategies have been observed in Pacific oysters. Sequential spawning is a feature of *C. gigas* in Portugal (Massapina et al. 1999) and Spain (Ruiz et al. 1992), whereas reproduction of *C. gigas* in France and the Wadden Sea is characterised by one spawning event during warm and extended summers (Diederich et al. 2005; Fabioux et al. 2005; Dutertre et al. 2009b; Enriquez-Diaz et al. 2009). Wild recruitment has occurred further north in Norway and Sweden, although most years maturity is reached but oocytes are reabsorbed instead of spawned (Wrange et al. 2010). During this study reproductive patterns differed between sites. Gametogenesis initiated early in the spring and rapidly reached maturity allowing for 1 or 2 spawning periods to occur in a single season. Wild oysters inhabiting the intertidal zone of Southampton Water spawned initially in June and then again in August, whereas farmed oysters in subtidal Poole Harbour spawned solely at the end of summer. Spawning during August was obvious at both sites as the gonad became replaced by connective tissue with some phagocytic activity (Mann 1979; Lango-Reynoso et al. 2000; Fabioux et al. 2005). The gonad was not completely emptied during the June spawn and pedunculated oocytes were retained within acini full of lumen.

It is unclear whether wild intertidal oysters from Poole Harbour spawned once or twice, as during the summer they were only sampled in July. Like oysters at the other sites during July, female gonads were dominated by pedunculated oocytes. Approximately 60 % of oysters had empty lumen within the gonadal acini and 40 % contained mature oocytes. GSI during July was more similar between oysters defined by tidal height than estuary however CI differed greatly between all sites. As well as the stress of tidal exposure impacting on the condition of oysters (Honkoop & Beukema 1997; Ernande et al. 2004), it is possible that the colonisation of different substrates also had an effect. Wild *C. gigas* that inhabit Southampton Water colonise a mixed substrate of shingle and mud. Larvae settle onto the shingle to metamorphose and the adults can typically be found lying flat at the surface. In Poole Harbour the intertidal collection site was a sheltered mudflat. Oysters here had sunk into the mud so that the umbo could be 100-200 mm below the surface (depending on the size of the oyster) and only the fringe of the shell protruded. The sediment acts as a buffer to temperature fluctuations, protecting the oysters from the most extreme temperatures that would otherwise cause stress detrimental to CI (Honkoop & Beukema 1997; Ernande et al. 2004). Therefore wild intertidal *C. gigas* in Poole Harbour shared characteristics with farmed subtidal *C. gigas* in Poole Harbour and wild intertidal *C. gigas* in Southampton Water, and without histological evidence of the gonad during May – June it cannot be said which reproductive pattern they underwent.

Spawning in *C. gigas* is considered to have a temperature threshold (Mann 1979; Ruiz et al. 1992; Ren et al. 2003). Both the initial spawning event (17.8 °C) and the latter (19.4 °C) occurred at water temperatures similar to spawning thresholds, of between 17 and 20 °C, reported elsewhere in Europe (Ruiz et al. 1992; Diederich 2005a; Cardoso et al. 2007; Dutertre et al. 2009b). Farmed subtidal *C. gigas* in Poole Harbour matured gradually until ripe gametes were spawned at the end of summer, following a pattern that appears to be typical of *C. gigas* both farmed and wild in temperate waters (Lango-Reynoso et al. 2000; Diederich et al. 2005; Cardoso et al. 2007; Dutertre et al. 2009b; Wrange et al. 2010). Similar reproductive patterns have been seen in both intertidal and subtidal wild *C. gigas* from the Wadden Sea (Diederich et al. 2005), fjords and estuaries in Denmark, Sweden and Norway (Wrange et al. 2010), and *C. gigas* farmed using wrack culture on intertidal mudflats of the French Atlantic (Lango-Reynoso et al. 2000; Dutertre et al. 2009b).

The sequential spawning seen in wild intertidal *C. gigas* from Southampton Water is less well documented (Ruiz et al. 1992; Li et al. 1998; Massapina et al. 1999; Enriquez-Diaz et al. 2009). Massapina et al. (1999) found 2 cohorts of oocytes developing within *C. gigas* cultured in the Ria Formosa lagoon in the south of Portugal. The main spawning event occurred first during June when water temperatures were approximately 24 °C, the second smaller spawning event took place in August after a dip in water temperatures had recovered to > 24 °C (Massapina et al. 1999). Ruiz et al. (1992) recorded two complete, successive cycles of gametogenesis in 2 year old *C. gigas* (approximately 90 mm shell length) suspended on ropes in raft culture in the Galicia region of Spain. The first spawning event coincided with peak water temperatures in June (16 – 19.5 °C) and the latter occurred in October, when water temperatures had decreased (average 16 °C). It was concluded that a substantial phytoplankton bloom was the probable cause of the second spawning event (Ruiz et al. 1992). The abundance and quality of food availability has been attributed to the rate of gametogenesis and the time of spawning (MacDonald & Thompson 1988; Starr et al. 1990; Ruiz et al. 1992; Günther et al. 1998). Starr et al. (1990) found that a metabolite released by a number of phytoplankton species was a density dependent trigger for spawning in green urchins (*Strongylocentrotus droebachiensis*) and blue mussels (*Mytilus edulis*), suggesting that such a cue allows larvae to develop under favourable conditions increasing survival (Starr et al. 1990). Interestingly, Ruiz et al. (1992) points out that phytoplankton blooms during October occur locally and that *C. gigas* cultured elsewhere in the vicinity, where water temperatures and salinity are similar but an October phytoplankton bloom does not occur, undergo a single gametogenic cycle and spawn during maximum water temperatures (Ruiz et al. 1992 and pers. coms. within). Southampton Water and Poole Harbour are both characterised by an acceleration of phytoplankton abundance in spring (typically May) and a more extensive peak or 'bloom' in the summer (typically August) with both summer blooms being dominated by the diatoms

Skeletonema and *Chaetoceros* (Leakey et al. 1992; Iriarte & Purdie 1994; Iriarte & Purdie 2004; Franklin et al. 2012). Therefore, like in Spain, the spawning events of *C. gigas* that take place later in the year coincide with greatest annual phytoplankton abundance.

In this study histological analysis revealed gametogenesis starting when water temperature increased above 9.5 °C. Cohorts of gametes were more obvious within the female gonad than the male (Massapina et al. 1999; Lango-Reynoso et al. 2000; Lango-Reynoso et al. 2006). Sperm at multiple stages of development was present within the male gonad from April through to August. Therefore the gonad was characterised by the most prolific stage present (Lango-Reynoso et al. 2000). For intertidal oysters in Southampton Water, only the June sample was made up of males characterised by mature, flagellated spermatozoa. Spawning partially emptied the gonad of 83 % of oysters before the July sample was collected. Following this initial spawning, both the male gonad and the 2nd cohort of oocytes within the female gonad, continued to mature through to August when they too were spawned. Intertidal wild females sampled from Southampton Water in June contained previtellogenic oocytes, pedunculated oocytes and mature oocytes at a ratio of approximately 1: 4: 10 and by August this had become 1: 3: 2. Consequently oocytes were being produced until gametogenesis ceased and at an increased abundance following the initial spawning. Previtellogenic oocytes produced this late in the year will not reach maturity and it is therefore unlikely that they will be spawned. Instead they are retained within the gonad and reabsorbed. Reabsorption is the process of gamete degradation and the recycling of nutrients (Berthelin et al. 2000; Chávez-Villalba et al. 2001; Dutertre et al. 2009b; Enriquez-Diaz et al. 2009). It is commonly associated with the end of the reproductive cycle (Steele & Mulcahy 1999; Ren et al. 2003; Dutertre et al. 2009b), although there is evidence that atresia of immature oocytes can occur in parallel to gametogenesis, and that nutrient cycling is the most energy efficient way to maintain a mature gonad (Frenkiel et al. 1997; Lango-Reynoso et al. 2006). The continuous production of previtellogenic oocytes seen in this study suggests intertidal females were recycling nutrients through reabsorption of previtellogenic oocytes so supporting the work of Frenkiel et al. 1997 and Lango-Reynoso et al. 2006.

As has been documented before, maturity was reached simultaneously between sexes (Massapina et al. 1999; Lango-Reynoso et al. 2000). The proportion of mature *C. gigas,* farmed subtidally in Poole Harbour, peaked in August prior to spawning however mature individuals were present from May until August. There were often multiple oocyte stages within a single female gonad and one dominant cohort of oocytes that progressed to maturity (Lango-Reynoso et al. 2000; Ren et al. 2003; Lango-Reynoso et al. 2006; Dutertre et al. 2009b). Both an extended presence of mature oysters and the continual production of gametes throughout the reproductive season is characteristic of this species (Lango-Reynoso et al. 2000 and references within), and not

dissimilar to the description for wild intertidal oysters in Southampton Water. The difference between sites comes in the reduced proportion of previtellogenic oocytes, at later stages of maturation in oysters from Poole Harbour, than Southampton Water. In May there was a ratio of 1 previtellogenic oocyte to approximately every 3 pedunculated oocytes and 3 mature oocytes, by August this had become 1: 15: 34 with previtellogenic oocytes being absent from a number of oysters sampled. Consequently it is unlikely that nutrients are recycled throughout gametogenesis in the way that was seen in intertidal oysters (Frenkiel et al. 1997; Lango-Reynoso et al. 2006).

Partially spent males and females could be found at both locations from June onwards. Partially spent oysters differed from partially spawned oysters, found at the same time of year, by the retention of mature oocytes, the presence of phagocytic activity and the intrusion of connective tissue into the acini (Figure 4.16) (Asif 1979b; Lango-Reynoso et al. 2000). Partially spent intertidal oysters were predominantly males however subtidal examples included both sexes. The presence of partially spent oysters showed that a small portion of the intertidal oysters spawned only once in June and retained gametes underwent reabsorption. For subtidal oysters that typically spawned once at the end of August, this shows that a small proportion of the population were stimulated to spawn earlier in the year. Although retention of oocytes was evident in this small number of individuals during the summer, no examples were found in September 2014, indicating reabsorption was completed in approximately one month. However gametes that were retained after the August spawning in 2013 persisted until January 2014. These observations are comparable to the slow reabsorption following incomplete spawning of *C. gigas* in France (Chávez-Villalba et al. 2001; Dutertre et al. 2009b; Enriquez-Diaz et al. 2009) and Ireland (Steele & Mulcahy 1999).

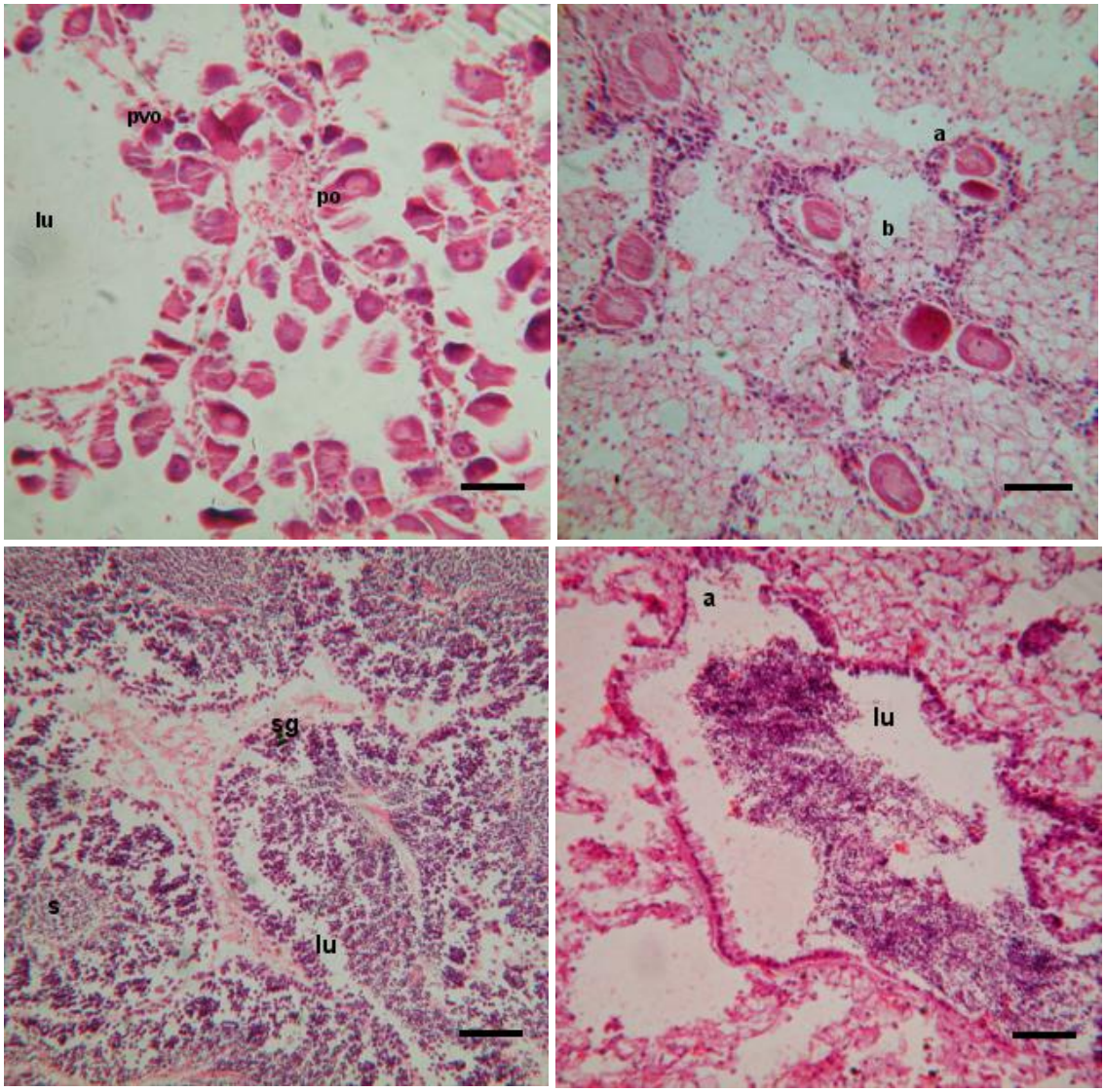


Figure 4.16 Histology of *Crassostrea gigas* gonad of partially spawned (left) female (top) and male (bottom) characterised by large amounts of lumen (lu) and the retention of pedunculated oocytes (po) and spermatids (s) with previtellogenic oocytes (pvo) and spermatogoonia also present. Partially spent (right) female (top) and male (bottom), characterised by collapsed acini (a) and the intrusion of connective tissue and macrophages into the acini (b). Scale bar = 50 µm.

The retention and subsequent reabsorption of oocytes at the end of the gametogenic cycle increases under suboptimal conditions as nutritional recycling becomes necessary in order to meet the demands of basal metabolism (Bayne et al. 1978). An increase in energy reserves, as provided by reabsorption of gametes, sustains the adult oyster throughout the winter months and may be later used in gametogenesis (Bayne et al. 1978; Pipe 1987; Steele & Mulcahy 1999). In September 2013, approximately 60 % of oysters sampled had retained gametes which were undergoing reabsorption. This was a much greater proportion than the 15 % of male oysters and

0 % of females seen one year later in September 2014. The monthly average water temperatures during August and September were very similar between years, however when considering daily averages, differences can be seen. In 2013 temperatures continually decreased following the annual maximum in late July. In 2014 the annual maximum was also experienced at the end of July, however, there was an increase in temperature from the end of August into September. Therefore temperatures in 2014 at the time of spawning were not only greater than those experienced in 2013 but they were increasing, both of which are conducive for spawning (Mann 1979; Massapina et al. 1999; Lango-Reynoso et al. 2006). Furthermore, gametogenesis is both a function of absolute temperature and exposure time (Mann 1979). Consequently in milder years or years with an unusually cold start, such as 2013, oysters will reach maturity later than seen in 2014 (Asif 1979b; Lango-Reynoso et al. 2000; Lango-Reynoso et al. 2006). This concept is known as 'day-degrees' and can be expressed by the following equation:

$$\mathrm{D} = \sum_{i=1}^{n} (t_0 - t_1)$$

This formula was adapted from Mann (1979) by Wilson & Simmons (1985). D is day-degrees, n is the number of days to reach ripeness, t_i is the temperature of the water and t_0 is the temperature below which no evidence of gametogenesis is found. Mann (1979) first described the relationship between development and temperature, calculating D = 592 and t_0 = 10.55 for *C. gigas.* Using values proposed by Mann (1979) and using subtidal Southampton Water temperatures logged during this study, maturity is approximated for mid-July 2014. This approximation is later than maturity occurred intertidally in Southampton Water and earlier than in subtidally farmed oysters from Poole Harbour. D was re-calculated taking into consideration our findings that gonadal development was seen when ambient water temperatures increased above 9.5 °C. Consequently D was calculated using t_0 = 9.5 °C and n = 73 or n = 127 for the calculation of intertidal D (Southampton Water) and subtidal D (Poole Harbour) respectively. Intertidal D = 299 and subtidal D = 859. Although no histological data exists for gametogenesis in 2013, the calculated values for D and t_0 from this study along with water temperatures logged throughout 2013 in Southampton Water can be used to predict when maturity was reached. The results suggest spawning to have occurred intertidally mid-July and subtidally mid-September. Water temperatures in mid-July were ~20 °C and the highest recorded all year. Water temperatures in mid-September were ~18 °C and decreasing. These predictions further corroborate the conclusions drawn earlier that conditions in 2013 were detrimental to spawning in farmed subtidal oysters in Poole Harbour, but suggest that conditions were conducive for spawning of the wild intertidal oysters inhabiting Southampton Water.

Sex ratio is another tool that has been used to indicate whether environmental conditions are favourable (Russell-Hunter 1979; Kennedy 1983; Kennedy et al. 1996). Gametes were only distinguishable between March and September and during 2014 the overall sex ratio did not differ significantly from the expected ratio of 1:1 at any of the sampling sites, however a bias toward males was seen during July. Fluctuating sex ratios throughout the reproductive cycle occurs in France and is attributed to the hermaphroditic nature of *C. gigas* (Lango-Reynoso et al. 1999; Lango-Reynoso et al. 2006). Males dominate French populations during the beginning of the year when conditions are suboptimal for gametogenesis and numbers of females increase with increasing temperatures and phytoplankton abundance in the spring. Consequently hermaphrodites in transition from male to female were most common in spring as environmental conditions become more favourable, allowing the higher metabolic demands of the female to be met (Lango-Reynoso et al. 1999; Lango-Reynoso et al. 2006). This study found hermaphroditic prevalence comparable to levels of <2 % recorded elsewhere (Amemiya 1929; Needler 1932; Katkansky & Sparks 1966; Berg 1969; Mann 1979; Paniaga-Chavez & Acosta-Ruiz 1995; Guo et al. 1998; Steele & Mulcahy 1999). For the majority, these studies do not discriminate between fully functional, simultaneous hermaphrodites and transitionary, sequential (otherwise known as morphological) hermaphrodites. Fully functional hermaphrodites contain mature oocytes and spermatozoa and are therefore capable of self-fertilisation (Asif 1979a). Transitionary hermaphrodites contain no mature gametes or only mature gametes of one sex (Berg 1969). *C. gigas* is a successive and irregular protandrous hermaphrodite and therefore it is possible to find transitionary hermaphrodites alternating from male to female, and also female to male. Male to female transitions are most likely to occur during spring (Lango-Reynoso et al. 1999; Lango-Reynoso et al. 2006), and in yearling oysters following their first reproductive season (Guo et al. 1998). A female to male transition is less likely to be seen and most likely to occur at the end of a year as environmental conditions deteriorate. That said, transitionary hermaphrodites are rare because a period of sexual undifferentiation typically separate gametogenic cycles (Lango-Reynoso et al. 1999). Fully functional hermaphrodites are rarer still (Needler 1932; Guo et al. 1998). All hermaphrodites seen during this study were collected during July or August. Functional hermaphrodites were only seen in farmed subtidal oysters however transitional hermaphrodites were collected from farmed subtidal and wild intertidal sites and at both locations. Functional hermaphrodites contained mature sperm and pedunculated or mature oocytes. Hermaphroditic oocyte development and growth appeared retarded in comparison to females sampled at the same time. Consequently it is unclear whether gametes of both sexes are spawned or perhaps just the spermatozoa is released. Transitional hermaphrodites contained degrading spermatozoa and immature oocytes. During the summer, gametes were reabsorbed quickly and it is unclear at what stage in development they were at. Given the time of year it is most likely that these

oysters reached maturity as males and partially spawned before developing female gametes. This would also explain the high levels of connective tissue that would not be present if the acini had remained full during a transition from an unripe (and so unspawned) male to a female.

Condition Indices (CI) of *C. gigas* collected from subtidal aquaculture plots in Poole Harbour were notably higher than those of *C. gigas* collected intertidally from wild aggregations in both Poole Harbour and Southampton Water. The increased condition of subtidal oysters is the most likely cause for the apparent lack of nutrient recycling through gamete reabsorption during gametogenesis (Bayne et al. 1978; Frenkiel et al. 1997). Growth and reproduction are successive, temperature dependent, biological processes (Mann 1978; Ernande et al. 2004). The condition of a bivalve responds quickly to food availability (Norkko et al. 2005) however during gametogenesis the majority of energy gained through feeding is channelled into maturation and under food limiting conditions this can be detrimental to growth (Moal et al. 1991; Delaporte et al. 2006). Filter feeders inhabiting lower tidal heights have greater feeding opportunities that can lead to increased condition as well as the production of larger oocytes during the reproductive period (Honkoop & Van der Meer 1997), as was seen in subtidal *C. gigas* from Poole Harbour in comparison to intertidal *C. gigas* from Southampton Water. Furthermore, the inflated CI in subtidal oysters and its close association with GSI suggests an environment where food is non-limiting as the fluctuations in visceral mass caused by maturation occur without drawing on stored energy reserves sufficiently to impact somatic growth (Mann 1979; Ruiz et al. 1992; Fabioux et al. 2005). However GSI and CI do not show the same association for intertidal oysters in Southampton Water. GSI reflects the reproductive cycle as described using gonad histology, and CI remains relatively constant with the exception of 2 short periods of increase from May-June and September. It is most likely that the peaks in CI reflect food availability (Leakey et al. 1992; Iriarte & Purdie 1994; Iriarte & Purdie 2004; Franklin et al. 2012) which suggests that during phytoplankton blooms is the only time that intertidal *C. gigas* have access to an exogenous energy supply sufficient enough to allow parallel growth of the somatic and visceral masses. Therefore during months of increased GSI and indifferent changes in CI the gonad is maturing at the expense of endogenous energy reserves (Mann 1979; Ruiz et al. 1992).

GSI and oocyte size were consistently greater in subtidal oysters than intertidal oysters during comparative months. It is not clear whether the relatively greater GSI is solely a result of larger oocytes or the combined result of larger oocytes and increased fecundity. Bivalve condition shares a positive relationship with gonad and oocyte quality (Massapina et al. 1999; Ren et al. 2003) and enhanced reproductive traits have included the production of both larger oocytes (Gallager & Mann 1986; Appeldoorn 1995; Honkoop & Van der Meer 1997) and an increase in fecundity (Honkoop & Van der Meer 1997; Chávez-Villalba et al. 2003; Cardoso et al. 2007;

Enriquez-Diaz et al. 2009). Energy investment per offspring is reflected in the volume of the oocyte and often inversely related to the incubation time (Kooijman 2000). A range in intra-specific oocyte size has been attributed to both phytoplankton availability (Honkoop & Van der Meer 1997; Le Pennec & Beninger 2000), and larval distribution (Cardoso et al. 2007). Larger oocytes, produced under more optimal environmental conditions, increase larval survival through the increased provision of lipids and protein to the developing larvae (Massapina et al. 1999; Ren et al. 2003). Conversely Cardoso et al. (2007) found smaller oocytes to be produced by *C. gigas* inhabiting conditions that produced the greatest growth rates, leading to their conclusions that under optimal environmental conditions *C. gigas* are able to produce a greater number or smaller oocytes at no cost to oocyte quality (Cardoso et al. 2007). However, without chemical analysis of oocyte quality this theory has not been proved. Therefore the larger oocytes produced by *C. gigas* in Poole Harbour is assumed to be the consequence of their subtidal location, and to result in higher quality oocytes with shorter larval duration than those produced by wild *C. gigas* inhabiting the intertidal zone of Southampton Water. Consequently abundant local recruitment should be expected to surround subtidal oysters in Poole Harbour, however recruitment remains sparse. It is most likely then, that recruitment in Poole Harbour is impacted post spawning.

Recruitment can be inhibited by a variety of environmental and biological interactions and water temperature is one of them. Successful fertilisation and metamorphosis require water temperatures above 15 °C (His 1989; Li & Hedgecock 1998; Rico-Villa 2009) and larvae require temperatures of at least 17 - 19 °C to develop (Li & Hedgecock 1998; Rico-Villa 2009). Furthermore cold winter temperatures are detrimental to the survival of juvenile oysters (Child & Laing 1998). The development period for larval *C. gigas* is temperature dependent and can vary from 2 weeks at 27 °C to over a month at 17 °C (Rico-Villa 2009). As well as a prolonged larval period at lower temperatures a reduction in settling larvae and an increase in mortality is seen (Bayne 1983; Li & Hedgecock 1998; Rico-Villa 2009). Between 2007 and 2014, water temperatures dropped below 17 °C in early to mid-September. Consequently there was a time period of approximately 4 weeks for larvae to develop and metamorphose dependent on whether spawning occurred at the beginning or end of August. It is likely that for most years autumnal water temperatures are hindering the development of larvae that have resulted from August spawning (Bayne 1983; Li & Hedgecock 1998; Rico-Villa 2009). This would be particularly detrimental to recruitment in Poole Harbour where the majority of oysters were observed spawning once, in August. However in Southampton Water it is less damaging as the August spawn is the second spawn of the year. Larvae resulting from spawning in June are developing during water temperatures that are increasing toward the annual maximum, which is typically experienced mid-late July. Therefore larval development is likely to reach metamorphosis more

quickly and with a greater settlement rate, benefiting recruitment (Bayne 1983; Li & Hedgecock 1998; Rico-Villa 2009). Metamorphosing earlier in the year also allows an increased growth of juvenile oysters prior to winter temperatures, increasing their chances of survival (Child & Laing 1998).

Summary

The reproductive cycle of *Crassostrea gigas* has adapted to environmental conditions experienced on the central English coast. Gametogenesis initiates when water temperatures increase above 9.5 °C however the timing and frequency of spawning show differences dependent upon local environmental conditions. At an intertidal site within Southampton Water spawning was triggered in wild *C. gigas* through tidally induced temperature shocking as water temperatures increased above 18 °C. *C. gigas* farmed subtidally in Poole Harbour required a combination of warm water (+18 °C) and an increase of phytoplankton abundance to stimulate spawning, which occurred later in the year. It is thought that the increased feeding opportunities of *C. gigas* in Poole Harbour also resulted in oysters with an increased condition and the production of larger oocytes.

The expression of phenotypic plasticity in reproductive strategies between sites shows an adaptability to survive in different habitats. Understanding the environmental limits in which *C. gigas* can respond successfully, is needed if wild populations are to be managed effectively. Currently in the UK wild *C. gigas* have only established in the intertidal zone, however the comparably healthier oysters and the greater reproductive output seen in the subtidal zone suggests wild *C. gigas* could spread beyond the intertidal zone. Due to the experimental design it is impossible to state with any certainty whether it was tidal height or differing environmental conditions between estuaries that had the greatest impact on reproduction. It is thought though that both phytoplankton abundance and quality, and aerial exposure play notable rolls in determining the reproductive strategy and output of *C. gigas.*

Recruitment has been successful in areas where oysters predominantly inhabit the intertidal shoreline, suggesting annual recruitment to be facilitated by the stimulation of an early spawning. Consequently wild recruitment in Southampton Water is likely to continue to increase and expand however wild oysters in Poole Harbour are likely only to recruit during either very warm years when larvae from a late spawning event have time to develop and winters are not detrimental to juveniles, or if a spring phytoplankton bloom is of sufficient size to stimulate early spawning.

Chapter 5: Top-down control on recruitment success of *Crassostrea gigas*

Abstract

Establishing wild *Crassostrea gigas* potentially provide an additional prey species for molluscivores within recipient ecosystems. Whether *C. gigas* is recognised as prey, particularly by ubiquitous native predators such as *Carcinus maenas,* and the impact top-down control has on recruitment success of establishing wild *Crassostrea gigas* were investigated.

C. gigas naturally attach to substrate whereas farmed *C. gigas* are produced to have no attachment. The reduced vulnerability of juveniles attached to substrate was investigated through manipulating the exposure or exclusion of predators to unattached or attached spat in a field based experiment. Study sites were within Southampton Water and Poole Harbour and remained *in situ* from August to December 2014. The result was dependent on site, with the highest survival rate in Poole Harbour seen in *C. gigas* that were both attached to substrate and protected from predators. In Southampton Water neither factor affected survival rates.

Carcinus maenas was chosen for further investigations into predatory feeding behaviours of *C. gigas. C. gigas* with a range of shell lengths from 16 - 50 mm were fed to male and female *C. maenas* with carapace widths between 30 mm and 60 mm. Prey density, prey attachment and water temperature were also controlled. Crab sex affected feeding behaviours depending on time of year, and the rate of predation was positively correlated to crab size and negatively correlated to oyster size. Crabs with a carapace width of 35 ± 1 mm or less (juveniles) were unable to predate *C. gigas* with a shell length of 20 mm or larger, attachment to substrate increased survival, and predation rates were enhanced in warmer water (12 °C vs 6 °C).

It is likely that juvenile *C. gigas* are being predated from both aquaculture plots and from wild aggregations forming in the intertidal zone. In particular, *C. gigas* are vulnerable to predation by *C. maenas* whilst they are juvenile and until they are at least 50 mm in shell length. Predation pressure from *C. maenas* decreases when *C. gigas* reach 20-25 mm shell length because they reach a size refuge from the most abundant size class of *C. maenas* (< 35 mm carapace width).

5.1 Introduction

Predators play an important role in sustaining biodiversity. This is achieved through the regulating of ecosystems that ensures ecological stability. Crab predation is recognized as an important factor in structuring marine benthic communities (Virnstein 1977; Jensen & Jensen 1985; Raffaelli et al. 1989; Eggleston 1990a; Ritchie & Johnson 2009), and in shallow estuarine environments has been shown to influence the biomass and diversity of prey organisms (Virnstein 1979).

The European shore crab, *Carcinus maenas* is abundant and widespread across Europe, inhabiting a wide range of substrate types and environmental conditions (Carlton & Cohen 2003; Roman & Palumbi 2004). *C. maenas* is a generalist feeder, juveniles consume a large amount of sediment to feed on the meiofauna it contains (Eriksson & Edlund 1977) and adults are essentially omnivores but also scavengers. Foods consumed include barnacles, seagrass, annelids and gastropods with a preference for bivalve prey (Crothers 1966; Ropes 1968; Elner 1981; Mascaró & Seed 2001a). The extensive predation of commercially exploited bivalve species has resulted in *C. maenas* being the subject of much research globally. Within its native range there is concern that native bivalve fisheries suffer economic losses due to crab predation, and that crab predation will also extend to introduced species of bivalve used in aquaculture, such as *Crassostrea gigas* (G. Wordsworth pers. coms.)(Dare et al. 1983; Sanchez-Salazar et al. 1987; Mascaró & Seed 2001a). *C. maenas* has become established in 5 temperate regions outside of its native Europe (Carlton & Cohen 2003). The predation pressure of widespread invasive populations on both the east and west shores of the United States of America and Canada has severely impacted commercial fisheries (Glude 1955; MacPhail et al. 1955; Ropes 1968; Elner 1981; Lafferty & Kuris 1996). The decline in commercial landings was so great for some fisheries, such as the soft shelled clam, *Mya arenaria,* in New England, that predation by *C. maenas* caused substantial annual losses (Ropes 1968). Predation by *C. maenas* was also found to be the cause for a decline in the venerid clam fisheries in Australia (Walton et al. 2002). Furthermore when *C. maenas* establishes outside of its native range it tends to become the dominant crab species, which can indirectly impact bivalve fisheries through the disruption of trophic cascades. One such case was seen in California where historically abundant Olympia oysters (*Ostreola conchaphila*) have become depleted through extensive predation by whelks. This was a result of the European shore crab being less efficient at predating whelks than the native crab species that it had displaced (Kimbro et al. 2009).

Predator-prey interactions on American oyster (*C. virginica*) reefs are well studied, oyster abundance is regulated by top-down control mostly from crabs and flatworms (Osman 1994; White & Wilson 1996; Newell et al. 2007), and the intertidal distribution is thought to be the

result of intense subtidal predation (Loosanoff & Nomejko 1946; Dame 1976; Roegner & Mann 1995), particularly during the first few months following metamorphosis (Roegner & Mann 1995). Predator-prey dynamics are regulated by prey density (Eggleston 1990b; Eggleston 1990a; Brown & Swearingen 1998), water temperature (Eggleston 1990a), relative distribution of predator and prey in time and space (Robles et al. 1990; Micheli 1997), habitat heterogeneity (Grabowski 2004) and by the size of predator and prey (Kaiser et al. 1990, Eggleston 1990, Roegner & Mann 1995). Eggleston (1990) found that the feeding functional response of blue crab *Callinectes sapidus* predating *C. virginica* was dependent on water temperatures. This is important as feeding functions can be used to describe predator-prey interactions at an ecosystem level. A type II response (Holling 1965) sees the risk of prey mortality increase with a reduction in density, thereby allowing for local or global extinction of the prey population below some low density threshold (Murdoch & Oaten 1975), unless a feeding threshold is present (displaced type II) in which case the depleted prey population would be able to recover (Valiela 2013). Eggleston (1990) found blue crabs exhibited a displaced type II during winter temperatures and a type II at peak summer temperatures. However at intermediate temperatures a type III response (Holling 1965) was seen, whereby coexistence is possible as a change occurs from an increasing to decreasing mortality risk as prey density is reduced (Lipcius & Hines 1986).

The reduction of prey abundance and biomass directly through predation is just one way in which predators can affect prey populations, feeding behaviours of predators evoke prey responses that potentially impact upon their feeding, fitness and survival (Dame & Patten 1981; Grabowski 2004; Heithaus et al. 2008). Dame & Patten (1981) conceptualized an oyster reef as an ecosystem with six components; filter feeders, detritus, microbiota, meiofauna, deposit feeders and predators. Predators were predominantly mud crabs and although they made up a small portion of the reef system they controlled 3 other reef components; deposited detritus, microbiota and meiofauna. Control was exerted directly upon deposited detritus through predation, feeding behaviours influencing filtration rates of filter feeders and the production of waste, and indirectly upon microbiota and meiofauna (Dame & Patten 1981).

Many of the examples above refer to the interaction of predatory crab species with *Crassostrea* spp. both within their native range. However when a nonindigenous species begins to establish it is unknown whether predatory species within the recipient ecosystem will control population expanse or whether the nonindigenous species will proliferate unregulated (Bishop & Peterson 2006b). The generalist feeding strategy exhibited by *C. maenas* allows the inclusion of novel prey items as they are foraged and this has both aided its success as an invasive species, and allowed the incorporation of nonindigenous species into its diet (Grosholz & Ruiz 1996; Hughes & O'Brien 2001; Baeta et al. 2006). The handling behaviour of prey seems to remain constant for all hard-

shelled prey. Small prey is crushed outright by the master chelae and weak spots in the shell of larger prey are sought out (Menzel & Nichy 1958; Elner 1978; Elner & Hughes 1978). Diet preferences of *C. maenas* from across spatially independent sites of invasion are similar to those of native crabs. Furthermore, the rank order of prey taxa based on stomach content analysis (Mollusca, Crustacea, Annelida and Chlorophyta) was consistent across eastern and western North America and South Africa (Grosholz & Ruiz 1996). The analysis of stomach content has identified 25 – 31 different organisms with many more pieces of unidentifiable food-stuff present. The relative abundance of prey in the stomach reflects what food is abundant at the time of sampling, suggesting feeding behaviour to be dependent upon prey density (Ropes 1968; Baeta et al. 2006). The size and morphology of prey are important factors effecting predation that are inherently linked (Elner 1978; Elner & Hughes 1978; Mascaró & Seed 2001a). The shell morphology affects susceptibility to predation and the size at which prey become safe from predation (Trussell 1996; Mascaró & Seed 2001a). Bivalves with differing morphologies exert different characteristics that make them more or less vulnerable to predation. The crushing resistance of a shell must be substantial enough to withstand environmental stresses as well as predation and domed shell shapes (such as that of a cockle) are intrinsically stronger with respect to crushing than flatter shell shapes. This means that a smaller proportion of bivalves within species that have domed shells, are vulnerable to predation, in comparison to those bivalves with a flatter shape, such as mussels and even more so oysters (Wainwright 1969; Sanchez-Salazar et al. 1987; Mascaró & Seed 2001a). Mascaró and Seed (2001) found *C. maenas* of a range of sizes (15 – 70 mm carapace width) to actively select small size classes of cockle (*Cerastoderma edule*) and mussel (*Mytilus edulis*) but take all sizes of oyster (*C. gigas* and *Ostrea edulis*) offered to them with no preference (10 – 40 mm shell length). Juvenile *C. maenas* (carapace width < 35 mm) predated cockles with shell length 0 – 8 mm, mussels 2 – 12 mm and oysters between 6 and 24 mm (Mascaró & Seed 2001b), whereas adult *C. maenas* (carapace width > 35 mm) predated cockles with shell lengths 5 – 20 mm, mussels 5 – 25 mm and oysters between 10 and 40 mm (Mascaró & Seed 2001a).

Previous studies of crab feeding behaviour have placed prey items loose in aquaria for laboratory testing or loose on top of the substrate in the field (Elner & Hughes 1978; Dare et al. 1983; Ameyaw-Akumfi & Hughes 1987; Mascaró & Seed 2001b; Mascaró & Seed 2001a). Such experiments have proved informative but are difficult to interpret in an ecological context as most prey species exhibit some form of defence from predation that is not taken into consideration by this experimental approach. Prey defences may be inducible phenotypically plastic traits that increase their resistance to predators, such as increased bysuss thread formation (Côté 1995; Leonard et al. 1999) or shell thickening (Palmer 1990; Trussell 1996; Leonard et al. 1999; Smith &

Jennings 2000; Newell et al. 2007). Typically the evolution of inducible defences are a result of a species experiencing a high (but unpredictable) risk of predation and the defence mechanism being energetically costly (Harvell 1990). Other defence mechanisms may be an expression of constitutive genes selected for over time. The evolution of predatory defences in bivalves is constrained by bodyplan, which differs between taxa. For example, Mytiloids possess a thick, inflexible periostracum that has prevented them from cementing to a substrate or developing structural defences such as spines, instead they form clumps (using bysuss) and exhibit behavioural traits that reduce predation (Wayne 1987; Harper & Skelton 1993).

Osteroidae shells have the weakest resistance to both crushing forces and boring of all bivalves (Taylor & Layman 1972; Gabriel 1981) due to the microstructure composition of foliated calcite and calcite prisms (Carter 1990). However calcite microstructures are comparatively lower in organic content and so less energetically costly to deposit, allowing more rapid growth rates than most bivalves and the production of a thicker shell. High growth rates result in size refuge from predation being reached more quickly, and an increased shell thickness reduces predation by boring gastropods (Kitchell et al. 1981; Kelley 1989) and crushing predators (Eggleston 1990b; Trussell 1996; Newell et al. 2007). Osteroidae shells contain 'chalky' patches within the micro structure (Lee et al. 2011) that act to prevent fractures propagating through the shell and keep damage localised (Taylor & Layman 1972; Lee et al. 2011). This may be particularly important when considering that brachyuran predators often repeatedly apply pressure in pulses to cause fracturing that breaks open the shells of prey (Elner 1978). Intraspecific differences in shell thickness influence vulnerability of individual oysters to predation (Mascaró & Seed 2001a; Newell et al. 2007), and interspecific differences in shell thickness can result in one oyster species being more vulnerable to predation than another (Newell et al. 2007).

There is growing concern that global environmental change might exacerbate predatory vulnerability of species that rely on calcium carbonate shells for protection (Green et al. 2009; Beniash et al. 2010; Waldbusser et al. 2011; Sanford et al. 2014). This is a pressing issue for estuarine species as they inhabit an environment susceptible to more pronounced changes in pH (Beniash et al. 2010; Amaral et al. 2011; Waldbusser et al. 2011). Acidification of estuaries affects shell formation and ultimately growth in bivalves through a combination of dissolution of the external shell surface and physiological changes affecting the rate of new shell deposition (Beniash et al. 2010; Waldbusser et al. 2011). Reactions of oysters to lowered pH have included reduced growth (Beniash et al. 2010; Waldbusser et al. 2011) and increased mortality (particularly in juveniles) (Dove & Sammut 2007b; Beniash et al. 2010; Amaral et al. 2011). Generally shell structure was compromised by high CO_2 levels ($pH < 7.5$) impacting biomineralisation and causing shell dissolution (Dove & Sammut 2007a; Ries et al. 2009; Beniash et al. 2010). However

Waldbusser et al. (2011) found no change in shell thickness at ~ pH 7.4 (Waldbusser et al. 2011). Vulnerability to predation is likely to increase under future climate scenarios and be exasperated for juvenile oysters as dominant predator species are known to focus predation effort on smaller, weaker prey (Mascaró & Seed 2001a; Dove & Sammut 2007b; Newell et al. 2007).

Bivalve defence mechanisms have been influenced by predatory behaviours (Harper 1991). Cementing of one valve to the substrate reduces vulnerability to predators that manipulate prey such as crustaceans and starfish (Harper 1991). 'Cultchless' oyster spat, produced for aquaculture, can suffer up to 100 % mortality from crabs as they are more easily crushed in the absence of the protection afforded by a large piece of shell substrate (Krantz & Chamberlin 1978; Bisker & Castagna 1987a; Bishop & Peterson 2006b; Newell et al. 2007). Typically oyster species naturally aggregate. Over time an oyster reef forms providing maximum protection from predation (Bishop and Peterson 2006, Newell 2007). Prior to the establishment of a reef, more vulnerable individual oysters or small groups of oysters may be found (Herbert et al. 2012). *C. gigas* is in the early stages of establishing along the south coast of England and currently individuals can be found attached to large anthropogenic structures (e.g. piers, groynes and marina walls) or attached to small fragments of shell or stone on intertidal mudflats (Chapter 3). It is unlikely that small pieces of substrate (smaller than the respective oyster) cause a hindrance to predation, but possible that being attached to a large anthropogenic structure provides protection akin to a natural reef. Understanding how substrate type can impact oyster vulnerability to predation is therefore necessary in predicting the top-down control exerted on this species and consequently its persistence in an estuary. Furthermore cultchless oyster spat are routinely used in aquaculture practices where predatory pressure causes an economic loss (G. Wordsworth pers. coms.)(Bax et al. 2003; Herbert et al. 2012), and the understanding of substrate type and spat vulnerability may be of some benefit.

Aims

This study aims to investigate the vulnerability of *Crassostrea gigas* to predation by brachyuran predator *Carcinus maenas,* in order to establish whether predation pressure is an influencing factor in distribution patterns of establishing wild oysters. The effect of cementation of spat as a defence strategy and the presence of a size refuge will be tested both under natural field conditions and under controlled conditions in the laboratory, allowing the effect of cementation on size refuge from crabs of a known sex and size to be compared. As well as informing on potential protective measures for aquaculture species, an understanding of top-down control is important for use in predictive models of the spread of *C. gigas.* Negative impacts on establishing species may influence the rate of spread and potentially present barriers to further spread.

Hypotheses

Predator exclusion and *Crassostrea gigas* survival

H_1 = Predation impacts the survival of *Crassostrea gigas* spat

H_2 = Cementation of *Crassostrea gigas* spat to substrate effects vulnerability to predation

European shore crabs (*Carcinus maenas*) as predators of *Crassostrea gigas*

H_1 = Cementation of *Crassostrea gigas* spat to substrate effects vulnerability to predation by *Carcinus maenas*

H_2 = *Crassostrea gigas* spat reach a size refuge from predation by *Carcinus maenas,* and the size refuge is dependent upon crab size, sex and moult stage

H_3 = Predation pressure on *Crassostrea gigas* spat by *Carcinus maenas* varies seasonally

Objectives

Predator exclusion and *Crassostrea gigas* survival

O_1 = Measure the survival of spat exposed to natural levels of predation and protected by cages

O_2 = Compare the survival of loose and attached *Crassostrea gigas* spat in the field

European shore crabs (*Carcinus maenas*) as predators of *Crassostrea gigas*

O_1 = Measure the proportion of *Crassostrea gigas* spat cemented to substrate that are predated by *Carcinus maenas* and compare it to the proportion of loose *C. gigas* spat predated, taking into consideration the crabs carapace width, sex and moult stage (as determined by colour).

O_2 = Measure the proportion of small *Crassostrea gigas* spat that are predated by *Carcinus maenas* and compare it to the proportion of large *C. gigas* spat predated, taking into consideration the crabs carapace width, sex and moult stage (as determined by colour).

O_3 = Compare the proportion of *Crassostrea gigas* spat predated by *Carcinus maenas* acclimated to different temperatures

5.2 Materials and Methods

Top-down control of *Crassostrea gigas* was investigated using three separate experiments; the survival of juvenile *C. gigas* attached to a substrate and cultchless was observed under field conditions using cages to control predation (Sites: Poole Harbour (27.08.2014 - 05.12.2014) and Southampton Water (30.08.2014 - 06.12.2014)). The impact of predator and prey size on the interaction between juvenile *C. gigas* and *Carcinus maenas* was investigated under laboratory conditions (21.01.2013 – 17.02.2013), and during the final experiment the effect of prey attachment to substrate was factored in (28.01.2015 - 29.03.2015).

5.2.1 *Crassostrea gigas* collection and processing

All *Crassostrea gigas* used throughout the feeding experiments were diploid juvenile spat reared to 4-6 weeks old at the Seasalter Shellfish (Whitstable) Ltd. hatchery located in Reculver, Kent. A range of sizes was provided by the hatchery. They were kept in lantern nets suspended in flowing seawater at the National Oceanography Centre aquarium until 2 weeks prior to the beginning of the experimental period, at this point they were moved into 50 L flow-through tanks and acclimated to the respective test temperature. Temperature was adjusting by 1 °C daily. Every 48 hours during this period the water flow was turned off for 1 hour and 1 litre of mixed algal culture *Chaetoceros* sp. and *Isochrysis* sp. at a concentration of approximately 2 – 4 x 10^6 cells ml^{-1} was added.

5.2.2 Predator exclusion and *Crassostrea gigas* survival

Experimental layout

Experimental cells were organised into a matrix of 4 x 5 and were spaced 25 - 30 cm apart (Figure 5.1). Before entering the field, a matrix was drawn up with letters assigned to rows and numbers to columns, each treatment was labelled 1 - 20 and using a random number generator a treatment was assigned to each cell of the matrix. This process was carried out twice so that the organisation of treatments within the matrix was different between sites.

Figure 5.1 Predator exclusion fieldwork, Blue Lagoon (Figure 2.4) (Poole Harbour 27.08.2014). Cell matrix labelled to show how treatment distribution was calculated.

Cage construction

Experimental cells were constructed from 6 mm steel rods welded into cages (exclusion) or corrals (inclusion), both enclosing 0.04 m^2 of substrate area. Plastic mesh with an aperture of 5 mm enclosed 5 sides of the cages preventing predators larger than 5 mm from accessing spat via the sides or from above. Cages stood 15 cm above the substrate ensuring birds could not access the substrate, and penetrated 5 cm into the substrate to reduce the likelihood of burrowing predators gaining access to the cage. The same mesh was also used to perimeter the corrals, however the perimeter fence sat on the substrate and stood only 5 cm tall, its purpose to prevent the spat from being washed away on the tide whilst allowing all potential predators access.

Treatment

Crassostrea gigas spat used in the experiment were hatchery reared and so were not attached to any substrate. Therefore the 'unattached' treatment required no preparation before being put into the field. Spat used in the 'attached' treatment were artificially fixed to unglazed ceramic tiles using Aryldite to glue the cupped shell to the tile. The tiles measured 200 x 50 mm and the spat were evenly spaced in 3 rows of 4. Gluing took place 2 weeks prior to the setup of field work allowing mortality and dislodging as a result of the gluing process to be discriminated from the experimental results. The spat were measured in the field and spaced evenly with the umbo lightly pushed into the substrate to generate some anchorage but without allowing sediment to cover the ventral fringe of the shell.

Sampling

Experimental sites were visited monthly during the experimental period. The visits were designed to cause minimal disturbance and consequently only the spat in open treatments were counted and photographed. The integrity of the cages was checked and any algae or debris was removed from the site. At the final visit, cages and corrals were removed from the site and the sediment from within each cell was passed through a 5 mm sieve to ensure all spat were accounted for. A hand trowel was used to dig and remove approximately 5 cm of sediment. Spat were photographed and the shell length measured using vernier calipers in the field.

Statistical analysis

The experimental design included both the fixed effect of the independent variables (exposure and spat attachment) and the random effects generated by the position of an experimental cell within the matrix and the proximity of the cell to the shore. Therefore a mixed effects linear model was fitted to the data for analysis. Both random effects were defined at 2 levels. The position of the cell within the matrix was either inside, where the cell in question was adjacent to another on all 4 sides, or outside when at least one edge was not adjacent to a further experimental cell. This factor takes into account the greater chance of a predator encountering an outside cell than an inside cell. The relative proximity of the experimental cell to the shore was either close or far depending on whether it was in the half of the matrix closest or furthest to the shoreline. This factor takes into consideration that many predators migrate with the tide and so there is a greater chance of encountering those cells that are covered by the tide first, or are orientated such that they are encountered first if approaching from the sea.

Once the model was fitted to the data, analysis of variance was run on the model using ANOVA Type II Wald Chi-squared tests to determine the impact of each independent variable on the effect of spat survival.

This field experiment is limited to the assumption that those oysters missing at the end of the experiment have been predated; additionally it is difficult to say with any certainty what the predator was. The remaining shells of predated oysters will be analysed for evidence of what type of predator caused the damage.

5.2.3 European shore crabs (*Carcinus maenas*) as predators of *Crassostrea gigas*

Crab collection and husbandry

Shore crabs, *Carcinus maenas,* were collected from Poole Harbour in January 2013 for use in the first set of feeding experiments. Shore crabs are inadvertently picked up during the harvesting of oysters from Othniel shellfish Ltd. aquaculture plots in Poole Harbour (Figure 2.4). The oyster harvester consists of an array of hydraulic jets 1 m wide that forces epifauna onto a conveyor belt, this then passes across the deck of the boat before returning the contents back into the water. Crabs were collected as they passed through the boat on the conveyor belt.

For the second set of feeding experiments *C. maenas* were caught using baited lines at Hamble Point Marina 23.01.2015 and 15.02.2015.

Carapace width was measured using vernier callipers and 10 crabs from each of the following size classes were collected for both sexes: 30±1 mm, 35±1 mm, 40±1 mm, 45±1 mm, 50±1 mm, 55±1 mm and 60±1. Only undamaged crabs were selected. Those that had missing or damaged appendages (leg or chelae), epifaunal growth on the carapace, or evidence of parasitic infection (notably a *Sacculina carcini* extrusion on the abdomen) were returned to the water. Crabs were placed into an insulated box with sea soaked towels for transportation back to the aquarium facilities at the National Oceanography Centre, Southampton.

In the aquarium, crabs were kept in flow-through tanks with fully aerated filtered sea water. Outside of the experimental period there were often multiple crabs to a tank. Crabs were target fed *Crepidula fornicatata* tissue, oily fish or squid every other evening and uneaten material was removed from the tanks the following morning. Acclimation to the testing temperature was facilitated using water baths on a thermostat regulated system accurate to 2 °C and began on arrival at the aquarium. Temperature was decreased by 1 °C per day until the desired temperature was reached. Feeding experiments were carried out at 13± 2 °C during the initial feeding experiments and at 12± 2 °C and 6± 2 °C for the second set of feeding experiments.

Experimental layout

Testing tanks (300 x 300 mm with at least 150 mm depth of seawater) were circulated with fully aerated filtered sea water and contained one crab only. The testing period was a total of 5 days. Crabs were not fed during the first 72 hours, as an allocated starvation period to standardise hunger during all experiments. Prey items were then introduced for a 48 hour feeding period.

Feeding experiment 1: Spat were divided into 3 size classes for the first study: 16 - 25 mm ($\leq$ 2.00 g), 26 - 35 mm (2.01 – 3.50 g), 36 - 50 mm (3.51 - 5.00 g). Three spat from each of the 3 size

classes were placed into the individual test tanks during the feeding period. To avoid prey density affecting predation, the shells of ingested oysters were removed at 3 hourly intervals throughout the day and replaced with a live oyster from the same size class.

Feeding experiment 2: Spat size was again used as an independent variable however only 2 size classes were used, small spat were between 6 - 16 mm and large spat were between 20 - 50 mm. Juvenile oysters were artificially set onto tiles as in the predator exclusion study. The ratio of set to unset spat varied between 12:0, 6:6 and 0:12. On tiles with 6 set and 6 unset spat, the location of the spat to be glued to the tile was randomly selected using a grid and a random number generator with spacing as before (3 rows of 4). Tile treatments were tested separately on individual crabs. Shell debris from ingested oyster spat were removed after 24 hours however no replacements were added due to the practical challenge of gluing new oysters to tiles without causing notable disruption to the experiment.

Statistical analysis

Data was analysed using general linear models with binomial distribution. One model was used for the data set collected during 2012 where the dependent variable was the number of spat eaten during a 48 hour period and the dependent variables were crab carapace width (mm), crab sex and oyster size (shell length). For the data set collected during 2014, 2 models were used for analysis due to the unequal weighting of the dependent variable resulting from experimental design. During experiments where oyster spat attached to substrate were treated independently to oyster spat loose on the aquarium floor, the number of oyster spat available for predation was 12 (n=12). When oyster spat treatments were tested simultaneously the density of spat remained constant (ie. 12 spat in a tank) however the number per treatment type was halved (n=6). Therefore data from treatments tested independently were analysed using a separate model to those tested simultaneously. Both models were run using the proportion of spat predated as the dependent variable and the following independent variables: Spat treatment (loose or attached to substrate), spat size (small or large), crab carapace width (mm), crab sex (male or female), crab colour morph (green or red) and the temperature that the experiment was conducted at (6 °C or 12 °C).

5.3 Results

5.3.1 Predator exclusion and *Crassostrea gigas* survival

In Poole Harbour, the use of predator exclusion cages ($X^2(1)=8.713$, p=0.002) and the attachment of spat to substrate ($X^2(1)=9.351$, p=0.003) both significantly reduced the mortality of *Crassostrea gigas* spat when compared to spat exposed to predation and unattached to substrate respectively. However, neither the presence of predator exclusion cages ($X^2(1)=0.997$, p=0.318) nor the attachment of spat to a substrate ($X^2(1)=0.206$, p=0.650) significantly affected the survival of spat in Southampton Water in 2014.

The estimate of variance generated by the experimental cell being in an inside or outside position within the matrix was not distinguishable from 0 and so considered not to affect the output of the model (p<0.001). Therefore it can be concluded that position of the cell within the experimental matrix had no significant effect on the survival of spat.

The estimate of variance calculated for the random effect of the cells relative proximity to the shoreline was not distinguishable from 0 in Southampton Water 2014 (p<0.001) however for the experiments carried out in Poole Harbour throughout 2014 (p=0.151) the possibility that the proximity of the cell to the shore may have affected the survival of spat during the experiment cannot be excluded.

5.3.2 European shore crabs (*Carcinus maenas*) as predators of *Crassostrea gigas*

Both an increase in the size of predating crabs and a decrease in the size of oyster spat had positive significant effects on the proportion of spat predated during all of the feeding experiments conducted.

The effect of crab size and sex on predation (2012):

Male crabs predated spat in a greater percentage of experiments than did female crabs, for all crab sizes considered (Figure 5.2). Of the 25 female crabs tested only 1 crab (carapace width 51 mm) consumed an oyster during the experimental period; the oyster was 43 mm in shell length. All size classes of male crabs predated oysters, with the percentage of crabs predating during an experiment increasing with increasing carapace width (Figure 5.2).

A single predation event of an oyster with shell length 22 mm occurred amongst small crabs (carapace width 35 ±1 mm). Crabs with carapace widths 50, 55 and 60±1 mm predated small,

medium and large oyster spat with the larger crabs (55 and 60±1 mm carapace width) most likely to predate oyster spat from the medium size class (Figure 5.2).

The remains of predated oyster spat were removed and replaced with a live oyster from the same category allowing the maximum number of oyster spat to be eaten whilst maintaining a steady prey density within the tank. Decreasing spat size (GLM: z=2.13, p=0.033) and increasing crab size (GLM: z=6.14, p<0.001) significantly increased the number of spat predated (Figure 5.3).

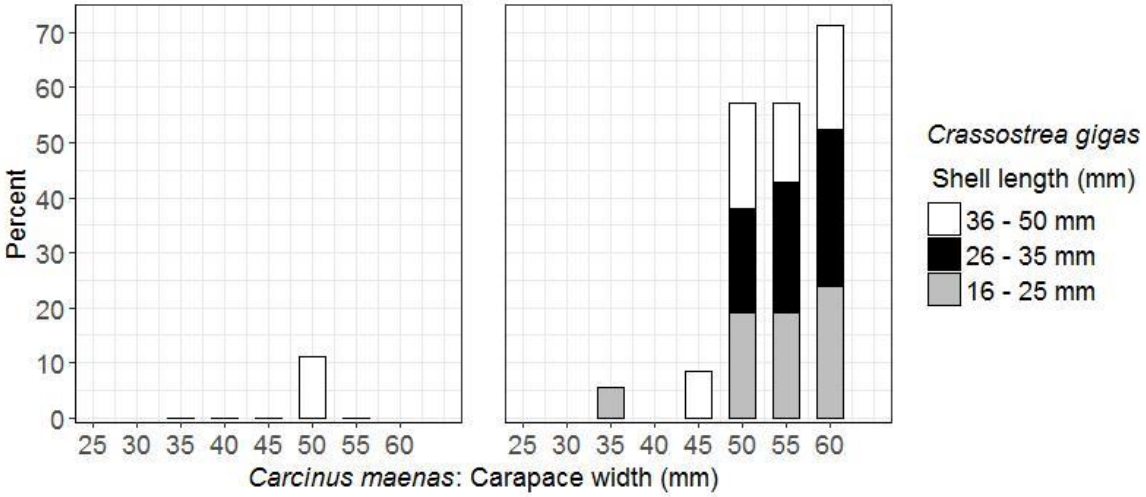


Figure 5.2 **Left:** The percentage of female *Carcinus maenas* (n = 25) and **Right:** male *C. maenas* (n = 30) that predated juvenile *Crassostrea gigas* during an experiment.

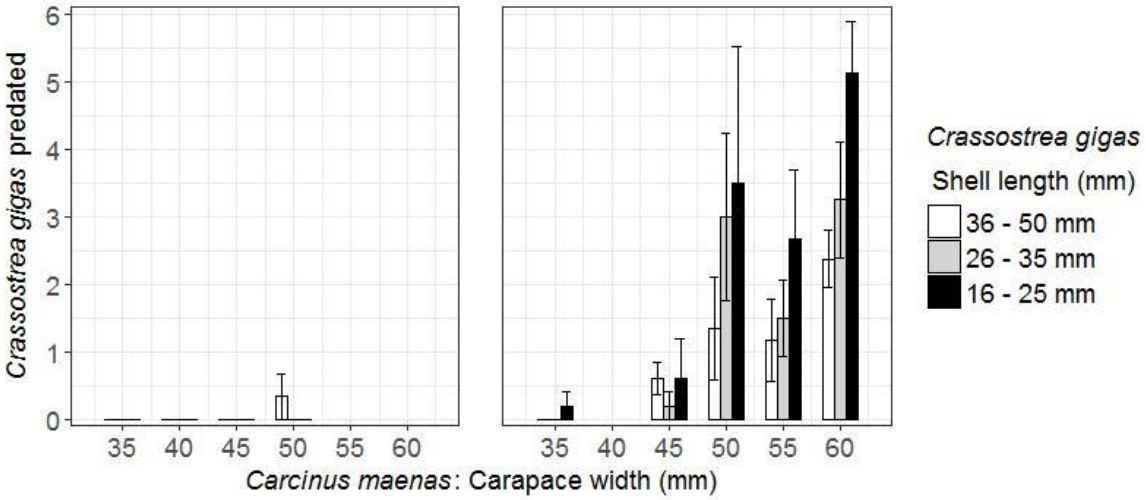


Figure 5.3 **Left**: The number of juvenile *Crassostrea gigas* predated by female *Carcinus maenas* during an experiment (n = 25) and **Right**: male crabs (n = 30) (mean ±se).

The effect of cementation to substrate of juvenile *Crassostrea gigas* on their vulnerability to predation (2015):

Juvenile *Crassostrea gigas* in the small category (shell length 6 – 16 mm) were predated by 73 % of *Carcinus maenas* tested with no clear pattern of effect seen for crab size or sex, nor the temperature the experiment was carried out at or whether the spat was attached to substrate or not (Figure 5.4). However when considering the predation of large spat (shell length 20-40 mm), at 12 °C there was an increase in the percentage of crabs tested that predated during the experiment with increasing carapace width (Figure 5.4). The feeding behaviour of crabs tested at colder temperatures was considerably more sporadic with a large proportion of crabs of all sizes not feeding (Figure 5.4). Crabs with a carapace width of 35±1 mm did not predate large spat at either 6 °C or 12 °C (Figure 5.4).

When tested independently, significantly fewer spat were predated when they were attached to substrate (GLM: $z=2.97$, $p=0.003$) (Figure 5.5), however in choice experiments being attached to substrate had no significant impact on spat vulnerability to predation (GLM: $z=1.05$, $p=0.29$) (Figure 5.5). Spat were more vulnerable to predation by larger crabs as seen by the increase in the proportion of spat eaten over a 48 hour period with increasing crab size (GLM: $z=2.99$, $p=0.003$). A significantly greater proportion of smaller spat was predated in comparison to large spat indicating the increased vulnerability of smaller spat to predation (GLM: $z=13.73$, $p<0.001$) by male and female crabs at both 6 and 12 °C (Figure 5.5). A reduction in predation was seen by crabs exhibiting the red colour morph (GLM: $z=-2.02$, $p=0.04$). At 12 °C the difference between predation by green and red crabs was negligible, however at 6 °C red crabs predated a consistently lower percentage of oyster spat during experiments (Figure 5.6).

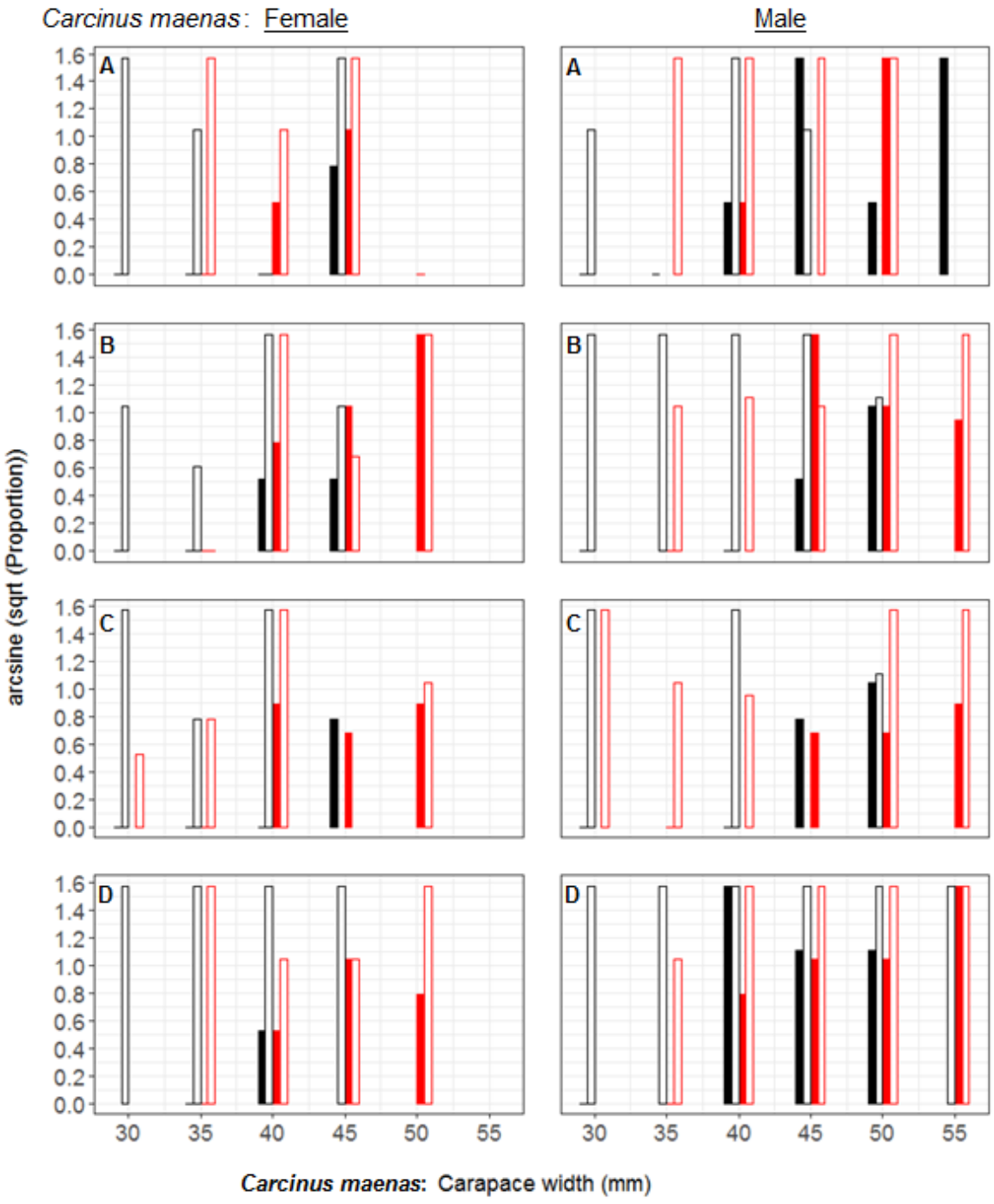


Figure 5.4 The proportion of *Carcinus maenas* that predated *Crassostrea gigas* during feeding experiments with large spat (20 – 40 mm shell length) at 6 °C ■ (n = 130) and 12 °C ■ (n = 132), and small spat (6 – 16 mm shell length) at 6 °C □ (n = 128) and 12 °C □ (n = 136). Spat attachment to substrate was a variable tested with all spat (n = 12) attached (**A**), half the spat (n = 6) attached (**B**) and half the spat (n = 6) loose (**C**), or all spat (n = 12) loose on the aquaria floor (**D**).

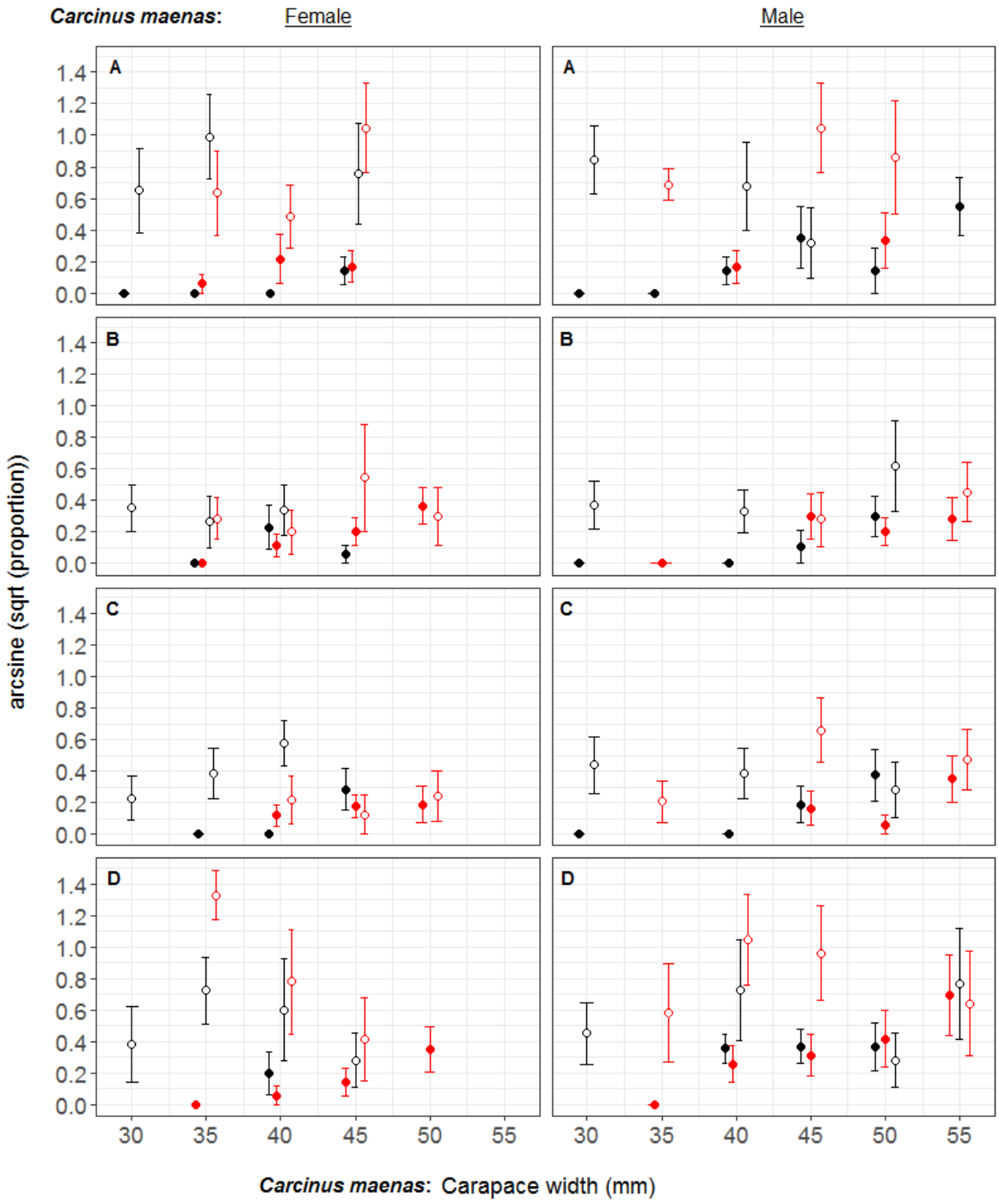


Figure 5.5 The proportion of *Crassostrea gigas* with shell lengths 6 – 16 mm predated by *Carcinus maenas* at 6 °C ○ (n = 108) and 12 °C ○ (n = 104), and *Crassostrea gigas* with shell lengths 20 – 40 mm predated at 6 °C ● (n = 112) and 12 °C ● (n = 112). Spat attachment to substrate was a variable tested with all spat (n = 12) attached (**A**), half the spat (n = 6) attached (**B**) and half the spat (n = 6) loose (**C**), or all spat (n = 12) loose on the aquaria floor (**D**). Points denote mean average of arcsine transformed data with standard error bars.

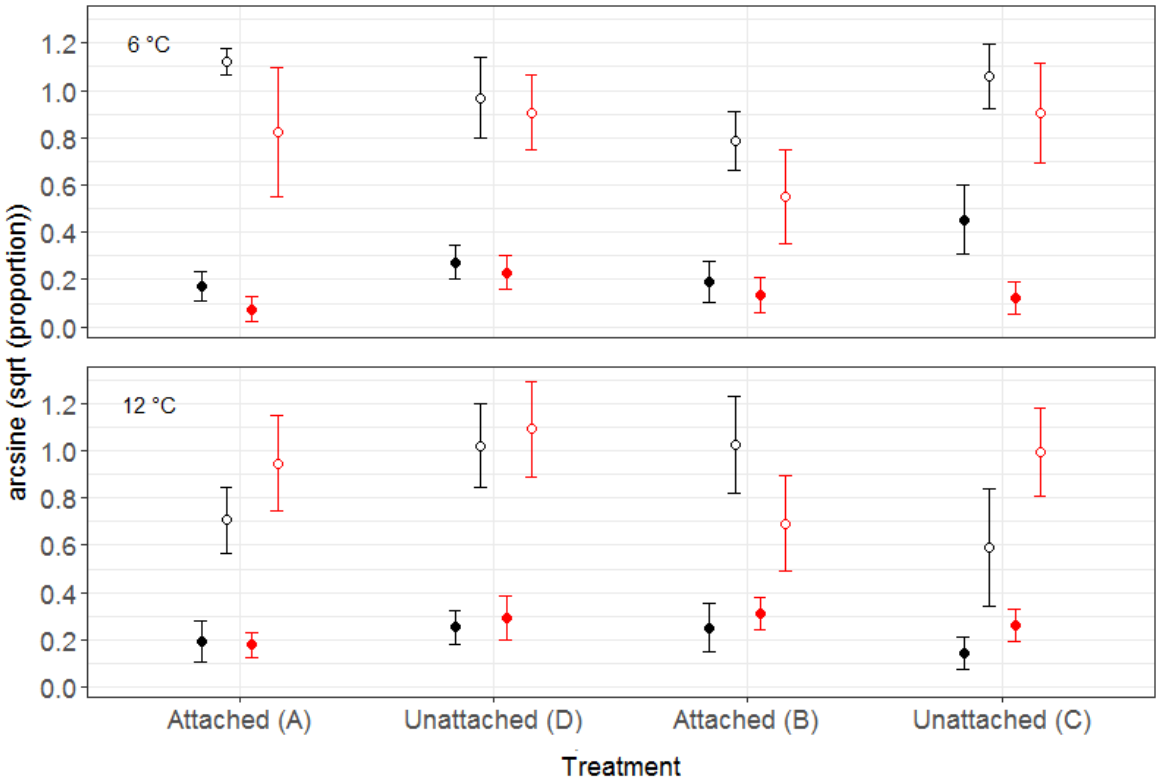


Figure 5.6 The proportion of *Crassostrea gigas* with shell lengths 6 – 16 mm predated by *Carcinus maenas* of a green colour morph ○ (n = 79) and red colour morph ○ (n = 56), and *Crassostrea gigas* with shell lengths 20 – 40 mm predated by *Carcinus maenas* of a green colour morph ● (n = 138) and red colour morph ● (n = 147). Spat attachment to substrate was a variable tested with all spat (n = 12) attached (**A**), half the spat (n = 6) attached (**B**) and half the spat (n = 6) loose (**C**), or all spat (n = 12) loose on the aquaria floor (**D**). Points denote mean average of arcsine transformed data with standard error bars.

5.4 Discussion

This study confirms that *Crassostrea gigas* naturalising along the south coast of England are subject to predation. However the impact of predation on naturalising aggregations varies locally. *C. gigas* spat protected from predation had a higher survival rate than those that were vulnerable to predation in Poole Harbour but not in Southampton Water. This suggests that *C. gigas* recruitment in Poole Harbour was/is hampered by predation and there was/is a degree of top-down control on naturalising aggregations. However *C. gigas* establishing in Southampton Water seem not to be experiencing the same controlling factors from predation. This pattern correlates with the present abundance of wild *C. gigas.* Wild *C. gigas* are absent from large expanses of substrate in Poole Harbour and in areas where they are found, it is typically as solitary individuals (meaning that there is > 10 m between individuals Chapter 3: Figure 3.2). However in Southampton Water wild *C. gigas* have established throughout the estuary with a relatively uniform distribution of 2 – 10 *C. gigas* m^{-2} (Chapter 3: Figure 3.3). This may be the result of a distribution overlap of abundant molluscivorous predators with recruiting *C. gigas* occurring in Poole Harbour but not Southampton Water. *C. gigas* in experimental cells closest to the shore, and so covered by the incoming tide first, experienced the highest levels of predation indicating that predators were migrating with the tide and not feeding at low water. This points toward marine molluscivores and away from avian predators having the greatest impact on *C. gigas* recruitment, as is the case for *C. virginica* along the Atlantic coast of America (Osman 1994; White & Wilson 1996; Newell et al. 2007). The brachyuran decapod *Carcinus maenas* has proven capable of predating substantial numbers and a range of sizes of *C. gigas* spat. This study saw *C. gigas* with shell lengths up to 25 mm being predated by juvenile *C. maenas*(< 35 mm carapace width) and up to 50 mm being predated by adult *C. maenas* (> 35 mm carapace width). A maximum of 26 spat were consumed by a single crab over 48 hours. Mascaró and Seed (2001) recorded juvenile *C. maenas* (< 35 mm carapace width) predating *C. gigas* up to 24 mm in shell length (Mascaró & Seed 2001b), and adult *C. maenas* (> 35 mm carapace width) predating *C. gigas* up to 40 mm in shell length (Mascaró & Seed 2001a). *C. maenas* was highlighted as a predator of establishing *C. gigas* in the Wadden Sea, along with seastar, *Asterias rubins,* and shrimp, *Crangon crangon* (Van der Veer et al. 1998; Diederich 2005a; Troost 2010), however their abundance was not substantial enough to dampen *C. gigas* recruitment (Troost 2010). This may also be the case for Southampton Water, however due to the high abundance of *C. maenas* inhabiting Poole Harbour it is likely that they contribute toward the top-down control of recruiting *C. gigas* (Appendix E).

The natural cementation of *C. gigas* spat to hard substrate is an effective predatory defence from predators such as brachyuran decapods that manipulate and break the shell of prey pre-ingestion

(Krantz & Chamberlin 1978; Bisker & Castagna 1987a; Bishop & Peterson 2006b; Newell et al. 2007). Impeding predatory behaviours, such as picking up the bivalve to locate weak spots in the shell or inhibiting the best angle at which to break the shell (Menzel & Nichy 1958; Elner 1978; Elner & Hughes 1978), increases handling time and can lead to prey abandonment (Ropes 1968; Vermeij 1976; Elner & Hughes 1978; Brown & Haight 1992; Bishop & Peterson 2006c). *C. gigas* attached to tiles had greater survival than cultchless *C. gigas* both in field and laboratory experiments. However site impacted survival rates and attachment to substrate only notably increased survival in Poole Harbour and not in Southampton Water. This is most likely a reflection of increased predation pressure in Poole Harbour.

The cementing of one valve to a hard substrate is an evolutionary defence mechanism in bivalves that can be found in bivalve orders Pterioida, Osteoida and Veneroida (Harper 1991; Harper & Skelton 1993). During metamorphosis oysters attach to a hard substrate along the ventral surface and develop a large, central posterior adductor muscle (Yonge 1962; Harper & Skelton 1993). Consequently oysters have a low profile, firm attachment and a strong hermetic valve closure. It is unclear however, whether such functional adaptations have evolved as a direct result of predatory pressure or have been influenced by the environment they inhabit. For example having a low profile and firm attachment may make it harder for crustacean and asteroid predators to detect and manipulate oysters however it also reduces the area exposed to wave energy and lessens the chances of being dislodged. Similarly an enlarged adductor muscle and hermetic seal have been shown to reduce predation by increasing predator handling times and so increasing the likelihood of being discarded, and reducing detection by sealing in chemical cues, however a tight hermetic valve closure also reduces thermal stress and desiccation during aerial exposure at low tide. This said, the migration of oysters into the intertidal zone is also considered an evolutionary response to predation as intertidal prey species are often better adapted to the environmental stresses than their predators and consequently the time exposed to predators and predator handling times are both reduced in comparison to subtidal prey species (Seed 1990; Taylor 1990; Harper & Skelton 1993).

The feeding mechanisms of different predators leave behind signatures on the shell of the predated bivalves (Harper & Skelton 1993; Alexander & Dietl 2003). Gastropods typically leave boreholes (Garton & Stickle 1980; Harding et al. 2002; Kelley & Hansen 2006), decapod crustaceans break the shell pre-ingestion (Elner 1978; Dare et al. 1983; Brown & Haight 1992; Van der Veer et al. 1998; Diederich 2005b; Morton & Harper 2008), asteroids and flat worms access the flesh between gaping valves (Whittle & Blumer 1970; Littlewood & Marsbe 1990; Diederich 2005b), and fish and avian predators tend to consume prey whole or leave behind the lower cemented valve (Anderson & Connell 1999; Cadée 2001; Troost 2010). The majority of spat were

removed entirely from tiles during field experiments in Poole Harbour and Southampton Water, with the lower valve occasionally remaining (Figure 5.7). The scarring of oyster valves remaining on the tile likely occurred toward the end of the experiment when oysters had, had sufficient time to grow onto the tiles, and conversely the absence of scarring is likely the result of oyster spat being removed in the early stages of the experiment. A greater absence of scarring is in accordance with greater predatory activity during warmer months (Naylor 1962; Crothers 1966; Beukema 1991; Aagaard et al. 1995). Less surface area adhered to the substrate and a smaller size would have resulted in an increased vulnerability of spat to a greater array of predators in the early stages of the experiment. Removal of spat that had cemented on to the tiles would have required dexterity and strength, most likely the result of crab or avian predation. Scarring left on tiles by *Carcinus maenas* predating *C. gigas* during laboratory experiments resembles those seen in the field, further suggesting predation by decapod crustaceans.

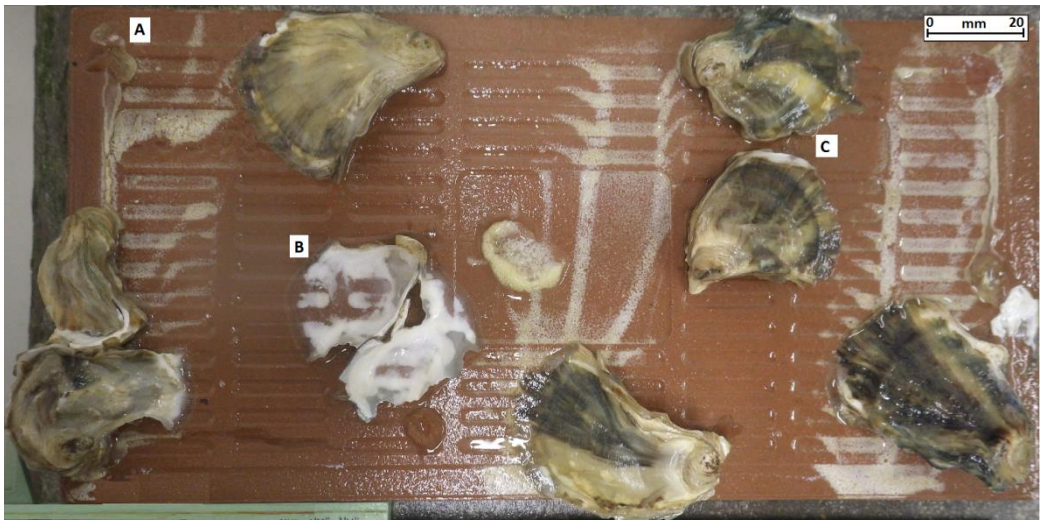


Figure 5.7 An example of a tile removed from inside of a cage in Poole Harbour after completion of predator exclusion field work. **A**: Glue residue where a juvenile *Crassostrea gigas* was attached at the beginning of the experiment. **B**: The cemented valves of 2 juvenile *C. gigas* remain. **C**: Surviving juvenile *C. gigas*.

Laboratory experiments found that small spat (6 – 16 mm shell length) were predated at a similar rate irrespective of whether they were attached to a tile or not, however large spat (20 – 40 mm shell length) suffered greater predation if they were loose on the aquaria floor. Decapod crustaceans rely on mechanosensory information gained through the maxillipeds, chelae and dactyl that are used to locate weak spots in the shell to exploit (Elner & Hughes 1978; Schmidt 1989; Garm 2005) and the umbo of bivalves is often targeted (Elner 1978; Elner & Hughes 1978; Eggleston 1990b; Brown & Haight 1992; Mondal et al. 2010). Maxillipeds are coated with dense setae, sensory hairs that are sensitive to the amplitude, velocity and direction of stimulus. They supply high resolution information on the shape, texture and hardness of prey which influences

feeding behaviours (Elner & Hughes 1978; Eggleston 1990b; Garm 2005; Hayden et al. 2007). *C. gigas* shells were collected after they were predated during laboratory feeding experiments and the damage was used to speculate on the predation strategies used by *C. maenas* (Appendix F). In accordance with previous findings damage was frequently localised to the umbo/hinge region of the shell (Elner 1978; Elner & Hughes 1978; Eggleston 1990b; Brown & Haight 1992; Mondal et al. 2010). For larger spat, the damage to the shell differed notably dependent on whether the spat was attached to the substrate or not. Cultchless spat were easily manipulated and the lateral edges were chipped away to expose the flesh and adductor muscle. The attachment to a tile impeded manipulation and instead the spat were levered off the tile. A lateral break to the lower valve and an intact upper valve suggest the cutter chelae was inserted into the gap between the glued shell and the shell that had grown onto the tile.

Naturally occurring *C. gigas* attach to the substrate along the entire ventral surface, and consequently the experimental design has influenced feeding behaviour and facilitated a behaviour that would not normally be possible. This emphasises the capability or *C. maenas* to adapt to novel experiences. Furthermore the repeated use of this behaviour by individual crabs, and the application by many crabs, implies that it is a more efficient behaviour than the alternative method of predating *C. gigas* that are fully cemented to substrate. Secondary to lateral breaks was the damage to hinges of attached large spat, it is likely that if it hadn't been possible to lever the spat from the tile, this would have become the optimal foraging strategy. The experimental design does not interfere with this behaviour making it the most likely feeding strategy to be used by *C. maenas* on naturally occurring *C. gigas*. Consequently the protection from predation rewarded by cementation to a hard substrate was most likely underestimated during these feeding experiments. This too may provide an explanation as to why the increased predation of loose spat, seen during trials when fixed and loose spat were tested independently, was not seen during choice trials when both fixed and loose spat were tested simultaneously.

Typically a hard shelled prey item will be passed to the mouthparts to be 'assessed' before attempts to open it (Elner 1978; Elner & Hughes 1978). This action is debilitated by the cementation of *C. gigas* spat to a substrate as access to part of the shell is restricted, and the prey cannot easily be probed by the maxillipeds. Therefore the different feeding behaviours exhibited between cemented and loose *C. gigas* spat were most likely a combined result of the different weak spots being exposed and the different mechanical requirements to exploit them.

Size refuge

Bivalves that experience high mortality through predation typically allocate a disproportionate amount of energy into growth, allowing a size refuge to be reached before maturity (Quale 1969;

Thompson 1979; Collet et al 1999). The evolution of size refuge against predation can also be seen in phyletic size increases, and the allocation of resources mainly to growth in small bivalves (Hallam 1978; Thompson 1979). High growth rates in *C. gigas* (Bayne 2002; Gangnery et al. 2003; Diederich 2006a) are enabled in part by the low energy requirements of shell construction (Gabriel 1981; Carter 1990; Harper & Skelton 1993), and feeding structures adapted to maximise on available food (Ward et al. 1998; Barillé et al. 2000; Honkoop et al. 2003).

Prey size refuge varies depending on the size and feeding behaviour of the predator (Elner 1980; Troost 2010). For example the maximum size of prey that a decapod crustacean can predate is correlated to the size and strength of its chelae (Elner 1980, Mascaró & Seed 2001a, Mascaró & Seed 2001b). A size refuge dictates the upper size limit of prey vulnerable to predation however for some species the size refuge is more absolute than for other species (Elner 1980; Dare et al. 1983; Mascaró & Seed 2001a). Furthermore some predators, such as *Carcinus maenas,* select prey based on profitability (Elner & Hughes 1978), which typically results in the size distribution of prey most likely to be predated being smaller than the predators capability (Elner 1978; Elner & Hughes 1978; Ameyaw-Akumfi & Hughes 1987). This was particularly evident during the 2012 feeding experiments when the smallest spat (16-25 mm shell length) consistently suffered greatest losses by all crab sizes tested, but in particular by the largest crabs tested. Profitable prey are selected to maximise the net energy gained (Elner & Hughes 1978). This is a strong driving force for predation that can result in the depletion of selected size classes from natural populations (Seed 1969; Saier 2000; Eschweiler et al. 2009). However population size frequency distribution appears to be most affected for prey, such as cockles and mussels (Elner & Hughes 1978; Seed 1969; Seed 1990; Mascaró & Seed 2001a, Mascaró & Seed 2001b) that have stronger more globular morphologies (globular periwinkle gastropod prey are also effected (Saier 2000; Eschweiler et al. 2009)). Crab predation appears to be less selective when predating flatter prey types, such as oysters, and typically a much wider size range is consumed (Dare et al. 1983; Mascaró & Seed 2001a). In agreement with these findings, this study found *C. maenas* with carapace widths < 55 $\pm$ 1 mm predated all sizes of spat offered to them without selecting for a particular size class of *C. gigas*. However if size categories are removed and each spat predated is treated independently, *C. gigas* with shell lengths between 20-30 mm experienced the greatest predation pressure (2012 only). This suggests prey were targeted according to size and the optimal value straddled the small and medium size categories. This may have been a contributing factor to the similarity in predation between spat size classes. During the second round of experiments *C. gigas* between 6-16 mm shell length experienced significantly greater predation pressure than *C. gigas* >20 mm shell length suggesting the optimal size to be < 16 mm. These results do not reveal an optimal size of *C. gigas,* rather they emphasise the lack of one, reiterating

the predation pattern mentioned earlier for the selection of a wide size range when predating oysters.

This study found size refuges of *C. gigas* from predation by a range of different sized *C. maenas* (Table 5.1). *C. maenas* reach sexual maturity in the UK after the 11th or 12th moult stage at a carapace width of 34 mm. Typically this occurs during the second summer although during a warm year maturity can be reached in the first summer (Crothers 1966; Klein-Breteler 1975b; Eriksson & Edlund 1977; Mohamedeen & Hartnoll 1989). *C. maenas* as small as 20 mm have been found to be serious predators of young mussels and other bivalves (Davis 1958; Dare et al. 1983). During this study *C. gigas* reached a size refuge from juvenile *C. maenas* at shell lengths between 20-25 mm. Sexually mature *C. maenas* were able to predate the largest size classes of *C. gigas* available and consequently no size refuge was found for *C. gigas* spat against mature *C. maenas.*

Table 5.1 The minimum size of *Carcinus maenas* able to predate *Crassostrea gigas* of given size classes

***Crassostrea gigas* shell length (mm)**	***Carcinus maenas* carapace width (mm)**
6 – 16	(<) 30
16 – 25	(<) 35
20 – 40	40
36 – 50	45

Although no size refuge against mature crabs was found, the relative vulnerability of wild *C. gigas* that have outgrown the risk of predation by juvenile *C. maenas* is likely to be far less as *C. gigas* < 25 mm are vulnerable to predation by the entire population of *C. maenas* as opposed to only part of it. Furthermore *C. maenas* are not confined to the shore and show different distribution patterns dependent on their size (Crothers 1966; Hunter & Naylor 1993; Warman et al. 1993; Baeta et al. 2005). Juveniles tend to stay on shore even when the tide goes out and their diet reflects this (Naylor 1962; Crothers 1966). As well as consuming sediment for the meiofauna it contains, juveniles also predate barnacles and juvenile *M. edulis* in order to meet the energy demands of the rapid growth phase associated with early life stages (Klein-Breteler 1975b; Eriksson & Edlund 1977; Baeta et al. 2005). It is only when the crab reaches a size when the energy requirements have increased beyond what can be sustained on this diet that they move to forage lower on the shore where larger bivalves and gastropods become their principal food source (Rangeley & Thomas 1987). Medium sized crabs with 35-50 mm carapace widths typically

migrate with the tide, spending low tide below the tide mark and moving up the shore on the flood before returning on the ebb, and the largest crabs (> 50 mm carapace width) remain below the tidemarks during all phases of the tide (Crothers 1966; Klein-Breteler 1976; Dare & Edwards 1981). *C. gigas* inhabit the mid to upper intertidal shore (0) alongside barnacles and juvenile mussels and subsequently their distribution overlaps with that of the juvenile *C. maenas.* Therefore they are constantly exposed to predation by juvenile *C. maenas,* exposed to predation from medium sized crabs for the duration of high water only and the probability of large *C. maenas* feeding on *C. gigas* is reduced to the sporadic shore migration. *C. maenas* can moult and grow when water temperatures are above 10 °C (Yamada et al. 2005) and have a maximum lifespan of approximately 4 years (Klein-Breteler 1975b; Klein-Breteler 1976). Relative abundance generally declines as carapace width increases above 40 mm, a size usually reached within their 2nd year, and crabs with a carapace width of 60 + mm (3 yrs +) are rarely encountered (Crothers 1966; Dare & Edwards 1981; Baeta et al. 2005; Yamada et al. 2005) (Appendix E). So although large crabs consumed significantly more oyster spat than small crabs during the laboratory experiments, they are far less likely to encounter *C. gigas* in the wild and consequently the greatest predatory pressure on wild *C. gigas* from *C. maenas* is likely to come from the juvenile portion of the population resulting in the *C. gigas* spat below 25 mm to be at the greatest risk of predation.

The effects of temperature, sex and intermoult stage on feeding

Environmental temperature change results in the modulation of biochemistry and physiology within ectotherms causing seasonal fluctuations in feeding patterns (Hawkins 1995). *C. maenas* experiences a suppression in feeding during the winter (Crothers 1966; Ropes 1968; Eriksson et al. 1975b; Eriksson & Edlund 1977; Elner 1980). Feeding levels remain normal until water temperatures drop to 7 °C then reduces and almost ceases at 3 – 4 °C (Ropes 1968; Eriksson et al. 1975a). However *C. maenas* has been found to feed all year round and suppresses feeding activity comparatively less during the winter than other crustaceans (Eriksson et al. 1975b; Eriksson & Edlund 1977; Tettlebach 1986). The first round of feeding experiments carried out during this study were at 13±2 °C, males fed abundantly whilst there was only one instance of a female feeding. It is likely that in this instance the *C. gigas* spat died and the female crab picked the flesh out of the gaping shells as the *C. gigas* was from the largest size class and the recovered shell had no damage to either valve and the hinge ligament remained intact. During the second round of experiments carried out at 12±2 °C and 6±2 °C, both males and females predated with no significant difference between the sexes. There was no difference in feeding rates on small *C. gigas* between 12±2 °C and 6±2 °C and although large spat were predated by a smaller percentage of crabs at 6±2 °C, the proportion of large spat predated did not differ. *C. maenas* were

acclimated to test temperatures over 2 weeks. This is ample time for the crab to adapt to the environmental change and for physiological stress responses to equilibrate (Ahsanullah & Newell 1971; Cuculescu et al. 1998; McGaw & Whiteley 2012), however biological processes such as gametogenesis may have a longer lasting effect and consequently the month during which the *C. maenas* were sampled could affect the results of the study, if for example, the feeding behaviours of female crabs in the early stages of oogenesis were compared to those carrying fully developed oocytes close to extrusion (Aagaard et al. 1995; Lardies et al. 2004). Reproduction is biannual with small females initiating oogenesis in May, late development occurring from August to September and spawning in October, and large females initiating oogenesis in November, becoming ripe between February and March and spawning during April (Lyons et al. 2012). The same breeding distinction is not present in males, and mature specimens, carrying mature sperm, can be found all year round (Lyons et al. 2012). Males do however suppress feeding when within the locality of moulting females as a result of a sex steroid released by the female to ensure that the male crabs partake in coupling (mating behaviour) instead of cannibalism (Hayden et al. 2007).

In order to minimise the difference in feeding behaviours caused by seasonal impacts the sampling was carried out in January of each year. The winter was colder and the summer shorter in 2012 resulting in a shorter growing period during the year preceding the initial feeding experiments (Eriksson & Edlund 1977). Furthermore water temperatures during the 3 months prior to sampling were much more stable during 2014/2015 showing a steady decrease of approximately 6 °C. During the same months in 2012/2013 there were two notable dips in temperature. The first occurred over two weeks mid-December and saw temperatures decrease and recover by approximately 4 °C, the second dip saw temperatures decrease from approximately 9 °C to approximately 4 °C the week prior to sampling (for details of temperature logger see Chapter 2.3) (Figure 5.8). Such temperature fluctuations may have caused stress in the *C. maenas* population which would be particularly influential to female behaviour as females would have been allocating energy reserves to oogenesis at this time (Aagaard et al. 1995; Lardies et al. 2004; Lyons et al. 2012). Consequently seasonal effects on feeding behaviours may have caused the differences seen in feeding behaviours exhibited by male and female *C. maenas* between experiments carried out during 2013 and 2015.

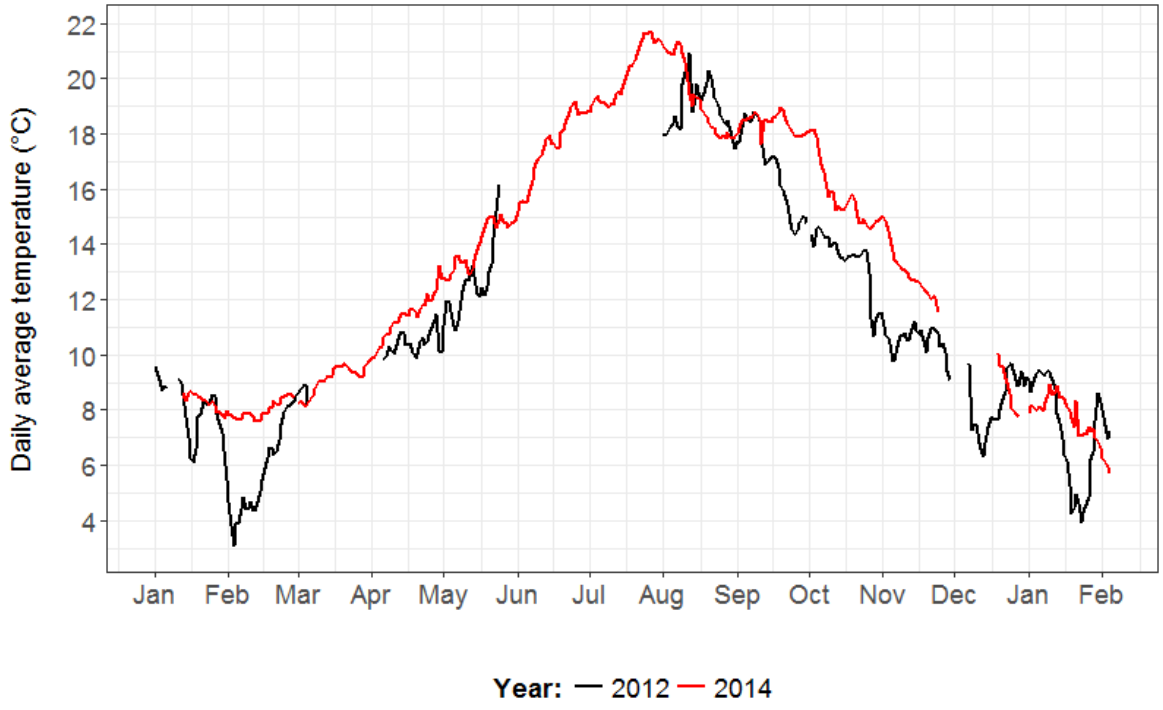


Figure 5.8 Water temperature profiles of Poole Harbour for the years preceding sampling from natural populations of *Carcinus maenas,* for feeding experiments which took place in January 2013 and January 2015.

Sampling techniques used to collect crabs differed between years, with crabs collected as bycatch from oyster beds in Poole Harbour during 2013 and using baited lines off marina pontoons during 2015. A gradual offshore migration of adult crabs begins in autumn as water temperatures decrease and during the winter few crabs continue to migrate with the tide to feed (Crothers 1966; Hunter & Naylor 1993). This is a trend and not a rule with some crabs more sensitive to environmental change and so more likely to migrate during winter to remain below the tide mark, where temperatures are warmer and more constant. This includes ovigerous females (Naylor 1962; Aagaard et al. 1995; Lardies et al. 2004) and both sexes at late stages in the moult cycle (indicated by the red colouration of the carapace) (Naylor 1962; Hunter & Naylor 1993; Warman et al. 1993; Reid et al. 1997b). Ovigerous *C. maenas* and those in moult rarely feed (Baeta et al. 2006) consequently it is plausible that the use of non-baited sampling (hydraulic pump) in deeper waters carried out during 2013 resulted in females with a suppressed diet being tested. However male crabs were actively feeding as early intermoult stages were selected for testing. The use of baited lines closer into shore, as used in 2015, would target only feeding individuals of a population and are less likely to sample females in late reproductive development, resulting in both sexes feeding during the 2015 experiments.

The experiments carried out in 2015 did however include crabs at different intermoult stages that were categorised by the colour of the carapace. Green crabs were assumed to be in early

intermoult and red crabs in prolonged intermoult, intermediate stages were not tested. All freshly moulted crabs are green in colouration and the red pigment is a result of photo-denaturation of pigments in the carapace over a long inter-moult period (Reid et al. 1997a). Not all crabs become red before moulting as crabs with a short intermoult period can moult repeatedly and remain green. Red crabs may have a lower tolerance to intertidal conditions but they have greater mating success when competing for females. Prolonger intermoult results in a thickening of the integument and strengthening of the carapace and chelae which in turn allows greater crushing force to be exerted by the chelae (Kaiser et al. 1990; McGaw et al. 1992; Reid et al. 1997b). This study found red morphs generally fed less than green colour morphs and that this pattern was exaggerated by a decrease in temperature. This is most likely a result of green crabs being more active than red crabs (Hunter & Naylor 1993; Warman et al. 1993; Aagaard et al. 1995) and so encountering prey more often during experiments and having to predate more frequently to fulfil the demands of a greater metabolism. This too would explain the exaggeration caused by a decrease in temperature as *C. maenas* is a poikilotherm in which metabolic rate is rate is proportional to temperature. During reduced temperatures red crabs can remain quiescent for long time periods however the locomotory behaviour of the green crab prevents such quiescence.

Summary

Crassostrea gigas are vulnerable to predation by brachyuran predator *Carcinus maenas.* Predation pressure varied locally and was considered substantial enough to be an influencing factor on distribution patterns of establishing wild oysters in Poole Harbour but not in Southampton Water. Natural cementation of oysters to a hard substrate make wild recruitment less likely to be predated than cultchless *C. gigas* laid on the seafloor to grow on during aquaculture processes. Feeding behaviours exhibited by *C. maenas* altered depending on whether or not *C. gigas* was attached to a substrate. This led to the conclusion that it is the exposure of the cupped valve and the ability to manipulate prey more freely that resulted in cultchless *C. gigas* being more vulnerable to predation.

A wide size range of *C. gigas* are predated by *C. maenas* and no selection of an optimal size was apparent. All sizes of *C. gigas* were consumed by both mature male and female *C. maenas* however a size refuge against juvenile crabs was reached at a shell length between 20 – 25 mm. This was considered a critical size with respect to the survival of wild *C. gigas* spat because the distribution of wild *C. gigas* overlaps that of juvenile *C. maenas* and, to a lesser degree, that of migrating adults. The differences seen between male and female feeding behaviours are thought to reflect seasonal influences as the majority of *C. maenas* migrate away from the intertidal

during the winter and suppress feeding, especially ovigerous females. The use of baited sampling selected feeding individuals from the population for testing in 2015, resulting in a contrast between the feeding behaviours exhibited by females in winter between experimental years. Therefore a smaller proportion of females than males are actively feeding during the winter but those that are, exhibit the same feeding preferences as males. The reduction in experimental temperature from 12 °C to 6 °C had little effect on feeding except to exaggerate the predation pressure of green crabs in comparison to red crabs and emphasize the selection of smaller spat in comparison to large spat.

In conclusion *C. gigas* are vulnerable to predation by *C. maenas* until they reach a shell length of at least 50 mm. However, the likelihood of being predated decreases after a size refuge from juvenile *C. maenas* is reached at a shell length of between 20 and 25 mm. *C. gigas* recruiting naturally along the southern coast of England most likely reach 20 – 25 mm shell length during their first winter, a time when predation is at its lowest as a result of cold water temperatures causing suppressed feeding in *C. maenas* and an offshore migration. Consequently it is likely that *C. gigas* will have reached a size refuge from predation by the time predatory pressure is at its maximum.

Chapter 6: The effect of winter temperatures on the health of *Crassostrea gigas* and possible implications on recruitment

Abstract

Winter temperatures can have a detrimental effect on non-native species that have evolved to inhabit warmer climates. *Crassostrea gigas* inhabiting the south coast of England is an example of such a species. It is currently unknown whether winter temperatures are detrimental to the condition of *C. gigas* or whether recruitment is impacted. Understanding such implications could advance aquaculture practices and improve predictions for the spread of wild *C. gigas.*

An assessment of oyster fitness prior to winter was carried out using respiration rates of native oyster *Ostrea edulis* and *C. gigas* from Poole Harbour and Southampton Water. Native oysters were used as a comparison because they have evolved to survive colder temperatures than *C. gigas.* Respiration rates were also analysed for juvenile oysters at a range of sizes of which would be expected during winter months.

The shell of an oyster is its primary defence and only capacity for insulation against the cold. Valve movements are closely related to vital activities such as respiration, feeding and excretion. How much protection the shell provides from cold temperatures was assessed over multiple tidal cycles using temperature probes inserted through a hole in the shell. A comparison of video footage and internal temperatures allowed an assessment of the effect of cold temperatures on valve gape activity.

Winter conditions experienced in the south of England are detrimental to juvenile *C. gigas* < 4 mg and during a cold year likely impact on all sizes of juvenile oysters as well as adults. The shell of *C. gigas* offered limited protection to the soft tissue, as freezing and rapid temperature changes were experienced during a tidal cycle, particularly during the flood tide. The exposure to freezing air temperatures were particularly detrimental as they have a prolonged effect on feeding, excretion and aerobic respiration.

6.1 Introduction

Marine bivalves have internal homeostatic mechanisms that maintain nearly uniform metabolic rates despite external changes. Optimal salinity and temperature depends upon the environment in which they have evolved, although this compensatory ability is limited. A large or rapid change may result in stress or an imbalance between food uptake and energy expenditure, affecting performance rates and reducing survival (Bernard 1983; Columbo et al. 1990). *Crassostrea gigas* inhabits the intertidal zone and experiences considerable temperature fluctuations twice daily due to aerial exposure during the tidal cycle. The different thermal properties of air and water result in notable temperature fluctuations and the risk of desiccation. Temperature fluctuation is well documented as a driver of mortality in many marine species and thermal stress and desiccation often dictates the upper distributional limit of intertidal species on the shore (Seed 1969; Paine 1974; Roegner & Mann 1995; Somero 2002).

Most studies focus on the impact of summer temperatures, however cold hardiness also impacts on a species survival and distribution (Theede 1973; Ansart & Vernon 2003; Reiss et al. 2006; Büttger et al. 2011; Strand et al. 2012). Furthermore for those species that reproduce in the autumn, such as *C. gigas,* juveniles will experience extreme cold at a smaller more vulnerable size than when they first experience extreme heat. Considering then, that an oyster's thermo-tolerance increases with size and shell thickness, a cold winter may prove more detrimental to the juvenile constituents of establishing aggregations of *C. gigas* than a subsequent hot summer.

Although potentially fatal to juvenile oysters that have just settled that autumn, a cold winter may also increase fecundity of adult oysters and so increase the settlement the following autumn (Beukema 1992; Philippart et al. 2003). This occurs as cold temperatures reduce metabolism and allow a higher reserve of carbohydrate to build up. Carbohydrates are the main respiratory substrate during gametogenesis and an increase in reserves is associated with an increase in fecundity and consequential recruitment (Ruiz et al. 1992; Cardoso et al. 2007). Colder winters also impact on growth rates. During the winter a decrease in temperature is commonly associated with a pause in growth rates (Jones 1980; Goodwin et al. 2001; Schöne et al. 2003). However, during a mild winter, bivalves remain metabolically active and growth may continue (at a reduced rate), depleting energy reserves and negatively impacting growth during the spring. Conversely a winter that is cold enough to stop growth is associated with an increased spring growth (Honkoop & Beukema 1997). Consequently winter temperatures that promote successful establishment of *C. gigas* must be a balance between temperatures mild enough for juveniles to

survive and yet cold enough to allow adults to build up sufficient reserves so as not to impede reproduction.

Negative impacts of cold weather are experienced by adults and juveniles alike when temperatures decrease below a species thermo-limit. Freezing injuries can occur as critical amounts of water become frozen in the tissue (Murphy 1983; Ansart & Vernon 2003), and reduced aerobic capacity prevents energy requirements from being met (Zielinski & Portner 1996; Sommer et al. 1997). *Crassostrea gigas* are reported to have wide thermo-limits between -1.8 – 35 °C (Leffler & Greer 1991; Bougrier et al. 1995) and aggregations persist, occasionally in high densities, in countries that typically experience winter frosts or prolonged ice formation (Diederich 2005a; Diederich 2006b; Wrange et al. 2010). Thermo-limits are typically defined by the transition from an aerobic to an anaerobic mode of metabolism (Zielinski & Portner 1996; Sommer et al. 1997) and bivalves can extend their geographic boundaries beyond their thermo-limits through physiological adaptation and the ability to respire anaerobically (Griffiths 1981; Allen & Burnett 2008; Comeau et al. 2012). However waste products, such as succinate, build up within the shell cavity during anaerobic respiration and some species of oyster maintain a narrow gape between shell valves at temperatures below the functioning threshold of feeding, and during emersion, to maintain aerobic respiration (Comeau et al. 2012; Dudognon et al. 2013). Tidal emersion accentuates the extreme temperatures experienced, increasing the risk of freezing injuries with the added risk of desiccation. Furthermore the rate of temperature change caused by the tide is considered a leading factor in winter mortality (Tokioka 1963). However *C. gigas* has established in countries where shallow seawater freezes over in winter and notable winter mortality has occurred during unusually cold and prolonged winters (Diederich 2005a; Diederich 2006b; Büttger et al. 2011).

Animals cope with freezing temperatures via two alternative strategies; freezing avoidance and freezing tolerance (Theede 1973; Ansart & Vernon 2003). Freezing avoidance is the most common strategy and refers to species that are able to supercool water and aqueous solutions within the body, allowing tissue to remain unfrozen in sub-zero temperatures. A tolerance to freezing refers to species that can survive the freezing of bodily fluids, and is limited to species that are found in the intertidal zone of temperate and arctic regions (Aarset 1982). Ice is generally formed in extracellular spaces and the rate of freezing is partially controlled by the initiation of ice formation at a relatively high temperature and forced dehydration to increase the ratio of cryoprotectants to available, freezable water (Ansart & Vernon 2003). Species that avoid being frozen typically cannot withstand as low a temperature as those that are freeze tolerant.

The evolution of wide thermo-limits has enabled *C. gigas* to become one of the most important shellfish in mariculture through successful introductions into aquaculture activities and fisheries worldwide (Mann et al. 1994; Ruesink et al. 2005). This factor has also been a primary contributing factor to the establishment of feral *C. gigas* in a large proportion of examples (Ruesink et al. 2005; Troost 2010). The impact of high temperatures on establishing *C. gigas* is more commonly considered due to the relatively narrow band of temperatures at which they can reproduce (Ruiz et al. 1992; Mann et al. 1994; Li & Hedgecock 1998; Rico-Villa 2009). Consequently there are only a few studies that consider the impact of cold hardiness on the rate of establishment (Diederich 2006b; Wrange et al. 2010; Büttger et al. 2011).

Nonindigenous species may be expected to have different tolerances to a range of environmental parameters, including temperature, salinity and desiccation, than the native taxonomically related species (McMahon 2002; Verbrugge et al. 2012; Collas et al. 2014). As a consequence of evolution the native species typically exert increased physiological tolerances to local conditions (McMahon 2002). However, tolerances are increasingly being found to be wider in non-native mollusc species than native ones (Lenz et al. 2011; Verbrugge et al. 2012). Elevated temperatures are routinely used as a stressor with decreasing temperatures often not tested (Lenz et al. 2011, Verbrujje et al. 2014). The native oyster *Ostrea edulis,* inhabits the low intertidal down to the sublittoral zone throughout the Atlantic and Mediterranean coasts of Europe (Launey et al. 2002). Generally *C. gigas* exhibits higher growth rates than *O. edulis,* however a larger size at age may be a result of *C. gigas* continued growth at lower temperatures or its ability to filter greater volumes of water (and so food) (Korringa 1952; Askew 1972; Bayne 2002). *O. edulis* are typically in better condition than *C. gigas* in low food environments (Mann 1979; Pogoda et al. 2011) and growth over winter is detrimental to the condition of *C. gigas* (Beukema 1992). Furthermore juvenile *O. edulis* have a greater tolerance for cold temperatures than *C. gigas* (Child & Laing 1998).

Valve movements are closely related to vital activities such as respiration, feeding and excretion and can be used as an indicator of vitality and circadian rhythms (Taylor 1976; Slatina 1991). Valve closure is the principle mechanism of defence in bivalves against primary stressors such as the detection of a predator (Robson et al. 2010), a deteriorating environment due to toxic tides (phytoplankton) and pollutants (Gnyubkin 2009; Tran et al. 2010), oxygen deficit (Sobral & Widdows 1997) low salinity, contact or shading (Gibson et al. 2001; Larade & Storey 2002; Wu et al. 2015). However valve closure can fail in extreme temperatures (Kennedy 1976). Some species of bivalve are better adapted to living in the littoral zone, exhibiting adaptations such as bradycardia, controlled gaping and utilisation of atmospheric oxygen during emersion (Dudognon et al. 2013).

Aims

The Pacific oyster is an important economic species to the UK that has naturalised and now poses a potential threat to local biodiversity. Understanding how winter conditions affect the health of *C. gigas,* impact on recruitment, and the rate of establishment, will aid mapping forecasts of wild oysters and is likely to be of economic importance through the improvement of cultivation methods. Furthermore by comparing respiration rates of *C. gigas* with the native taxonomically related *O. edulis,* it can be seen which species has the physiological advantage during winter conditions. This study aims to document the effects of cold temperature on the respiration and gaping behaviour of *C. gigas*. The respiration rates of adult and juvenile oysters will be compared under various winter temperatures and the gaping behaviour of adult *C. gigas* will be recorded over a 48 hour period with a simulated freezing and non-freezing tidal regime.

Hypotheses

H_1 = Winter temperatures typically experienced on the south coast of England are cold enough to impact on the respiration rates of adult *Crassostrea gigas*.

H_2 = Winter temperatures, typically experienced on the south coast of England, impact on the fitness of *Crassostrea gigas* and native taxonomic relative *Ostrea edulis* with different relative effectiveness.

H_3 = Winter temperatures typically experienced on the south coast of England are cold enough to impact on the respiration rates of juvenile *Crassostrea gigas*.

H_4 = The shell of *Crassostrea gigas* provides insulation from freezing air temperatures.

Objectives

O_1 = Measure oxygen consumption of adult *Crassostrea gigas* using flow-through respirometer chambers and standardising to dry flesh weight.

O_2 = Compare respiration rates of adult *Crassostrea gigas* with measured oxygen consumption of adult *Ostrea edulis* using flow-through respirometer chambers and standardising to dry flesh weight.

O_3 = Measure oxygen consumption of juvenile *Crassostrea gigas* using flow-through respirometer chambers and standardising to dry flesh weight.

O_4 = Measure internal temperature throughout a simulated tidal cycle with freezing air temperatures. The temperature within the shell of the oyster will be continuously monitored as will external temperatures in the testing tank.

6.2 Methods

6.2.1 Oyster collection and processing

Crassostrea gigas and *Ostrea edulis* were collected by hand during spring low tides when the full extent of the intertidal habitat could be accessed on foot. Collection took place within 1 hour either side of low water. Oysters were selected haphazardly (Castanos et al. 2005) and oysters that were heavily fouled or those that could not be separated from the substrate were not collected. All samples were cleaned in seawater and epifauna were scraped off using a blunt metal blade.

Oysters were transferred to the aquarium facilities at the National Oceanography Centre Southampton within 2-3 hours of collection. In the aquarium they were hung in lantern nets (FAO 1988) inside large holding tanks that had a constant flow of unfiltered seawater from Southampton Water. Lanterns were removed and hosed down with seawater once a week to prevent silt deposits from building up. No additional plankton food source was added to the water and therefore the nutritional supply came exclusively from the unfiltered seawater.

Juvenile *C. gigas* came directly from Seasalter Shellfish (Whitstable) Hatchery at 10 days post-settlement, and were kept in 20 L aquarium tanks with aerated, un-filtered seawater. Seawater was changed every 48 hours and 1 L of mixed algal culture *Chaetoceros* sp. and *Isochrysis* sp. at a concentration of approximately 2 – 4 x 10^6 cells ml^{-1} was added once a week.

6.2.2 Acclimation

Adult oysters were moved into 50 l aquarium tanks with a flow of filtered seawater at least 2 weeks before being used in experiments for acclimation. Water temperature was adjusted by 1 °C daily. Every 48 hours during this period the water flow was turned off for 1 hour and 1 litre of mixed algal culture *Chaetoceros* sp. and *Isochrysis* sp. at a concentration of approximately 2 – 4 x 10^6 cells ml^{-1} was added.

Juvenile *C. gigas* were acclimated for 8 months.

6.2.3 Internal temperature of *Crassostrea gigas*

The temperature inside the pallial cavity (internal temperature) of adult *C. gigas* was measured simultaneously during a laboratory experiment in a tidal tank. The internal temperature of an oyster was measured using a thermistor placed within the pallial cavity. A 7mm hole was drilled through the upper valve approximately two thirds of the total length away from the umbo and off-centre to the right; ensuring that the mantle was not damaged in the process. The thermistor was then inserted into the pallial cavity and secured in place using modelling putty. A flexible cable connected the sensor to an Arduino© Uno AT mega 328P microcontroller. Temperatures in °C were logged at 5 second intervals to a text file generated by the Arduino and saved onto an SD card.

Internal temperature was described during a tidal cycle under 2 scenarios; a mild winter using seawater temperatures of 6 °C and air temperatures that remained above freezing at 1 °C, and a cold winter where water temperature remained the same but air temperatures dropped below freezing to -3 °C. The winter scenarios are realistic of the south coast of England, where average winter water temperatures over oyster sites is between 5 and 7 °C (Cefas 2012), the number of winter air-frost days ranges between 8 and 44 (non-consecutive) and minimum winter air temperatures range from daily averages of -3 to 4 °C (MetOffice 2016). Specifically, air temperatures were monthly average aerial temperatures (December – February) from the UK Meteorological Office time series for the south and south east of England 2005 – 2015, and the water temperature was determined from data loggers subtidally in Poole Harbour and intertidally in Southampton Water (Table 6.1). The tidal cycle timings were calculated using GNSS (Global Navigation Satellite System) coordinates of oysters (hand collected), shore depth data (CCO Light Detection and Ranging (Lidar)) and tidal data (EA tidal gauge).

Table 6.1 Winter average seawater and air temperatures from 2005 - 2014. Winter includes the months of December, January and February. Water temperatures are an average of those taken 1 m below the sea surface in Poole Harbour and Southampton Water. Air temperatures are for the south of England courtesy of the Met Office.

	Temperature °C	
	Seawater	**Air**
2005	5.11	5.10
2006	7.38	4.13
2007	8.33	6.80
2008	5.94	5.93
2009	5.54	3.70
2010	6.81	2.83
2011	6.15	3.73
2012	7.60	5.40
2013		4.17
2014	8.34	6.40

6.2.4 Respiration

Respiration rate was measured using 1000 ml flow-through respirometer chambers for adult *C. gigas* and *O. edulis,* and a 50 ml flow-through respirometer chamber for juvenile *C. gigas,* with a Presens Fibox 3™ fibre-optic oxygen meter. The respirometer was calibrated for 100 % and 0 % oxygen saturation by bubbling compressed air through the seawater to produce 100 % oxygen and adding sodium thiosulphite in excess to reduce the oxygen concentration to 0 %. It was possible to run 6 chambers simultaneously, 4 chambers contained oysters and 2 were used for controls (no oyster). Oxygen saturation was measured at the beginning of the experimental period and then at hourly intervals.

The respiratory responses of 12 adult *C. gigas* and 12 *O. edulis,* from Southampton Water and Poole Harbour, to cooling water temperatures post-spawning were measured for 3 -4 hours at 14.5 °C. Previous histological analysis showed the spawning season for oysters to be August – September and consequently the respiration rate of oysters during November was used. This gave post-spawning health of the oysters and a reflection of their fitness going into winter. The experiments were run at 14.5 °C, the monthly average temperature for October between 2006 and 2014.

Respiration rates of juvenile *C. gigas* weighing 4 ± 0.5 mg, 6 ± 0.5 mg and 9 ± 0.5 mg were run at 7 °C until and including the 10th hour. This experiment was designed to monitor the health of spat when they are most susceptible to environmental conditions (due to their small size and thin shell) and at temperatures that would be typically experienced by oysters of this size. Water temperatures from Southampton Water and Poole Harbour during December, January and February, between 2005 and 2014, were averaged to give an experimental temperature of 7 °C.

Oxygen consumption was standardised using the dry weight of flesh, giving a unit of microliters of oxygen consumed per milligram of dry weight per hour (corrected against a control). To calculate dry weight, the flesh was dissected from the shell immediately after removal from the respiration chamber and dried at 60 °C until constant weight was achieved. Juvenile oysters reached a constant dry weight within 24 hours and adult oysters within 48 hours.

6.3 Results

6.3.1 Internal temperature of *Crassostrea gigas*

The internal temperature of *C. gigas* was measured using a probe inserted through the shell and into the pallial cavity. The impact of aerial exposure was tested using a two-way ANOVA comparing the pallial cavity temperature and ambient air temperature during 2 tidal cycles at temperatures simulated to represent mild and cold winter conditions on the south coast of England. Generally internal temperatures followed the pattern of ambient environmental conditions (Figure 6.1). Internal temperatures were significantly affected by which winter scenario was being tested ($F(1,24) = 368.1$, $p<0.001$) and whether the oyster was covered or exposed by the tide ($F(1,24) = 363.7$, $p<0.001$). Furthermore there was a significant interaction between the winter scenario and tidal state on pallial cavity temperature ($F(1,24) = 8.3$, $p = 0.008$). When considering the simple main effects, internal temperature was significantly colder during aerial exposure both for mild ($F(1) = 1022.9$, $p<0.001$) and cold ($F(1) = 162.1$, $p<0.001$) winter scenarios (Figure 6.2). Water temperature was kept at 6 °C for both winter conditions but the air temperature decreased from 1 °C during a mild winter, to -3 °C during a cold winter. Oysters exposed to the air in a cold winter scenario maintained an internal temperature (-1.6 ± 0.7 °C) that was greater than the ambient air temperature (-2.7 ± 0.6 °C) ($F(1) = 6.2$, $p = 0.032$) (Figure 6.2). The probe recording air temperature during the mild winter scenario failed and so similar comparisons under milder winter conditions could not be made.

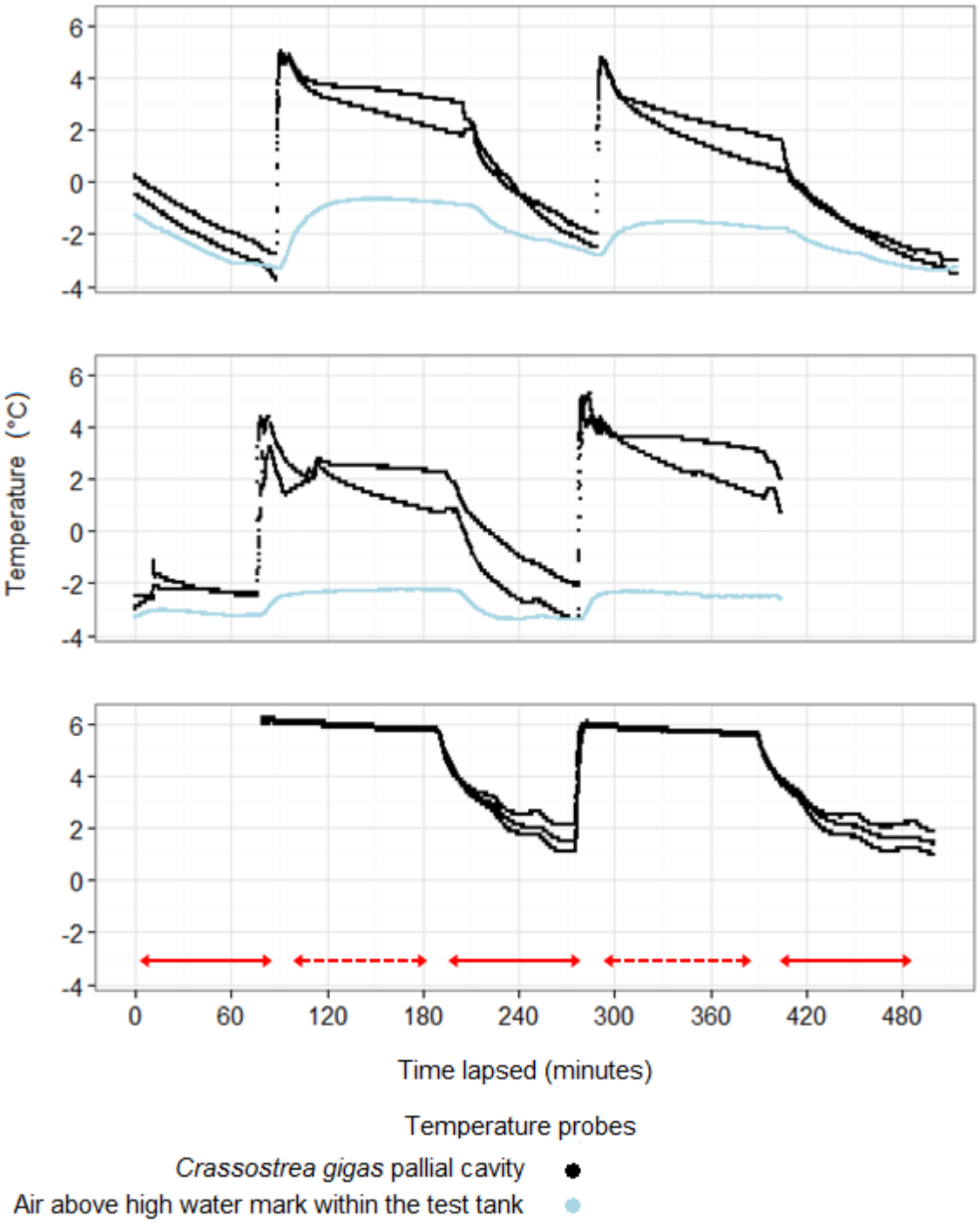


Figure 6.1 **Top & Middle:** Temperature within the pallial cavity of *Crassostrea gigas* during 2 simulated tidal cycles with aerial temperatures of -3 °C and water temperatures of 6 °C. **Bottom:** Temperature within the pallial cavity of *Crassostrea gigas* during 2 simulated tidal cycles with aerial temperatures of 1 °C and water temperatures of 6 °C. Solid red arrows indicate when test tanks were emptied of sea water and oysters were exposed to the air (ebb tide) and dashed arrows show when the test tank was filled with sea water and oysters were submerged (flood tide)

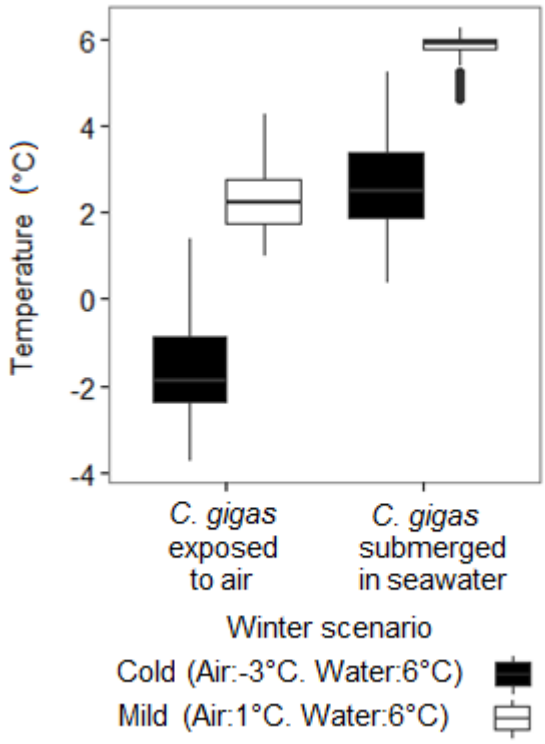


Figure 6.2 The average temperature within the pallial cavity of *Crassostrea gigas* during periods of submersion and aerial exposure under two winter scenarios (Boxes show the mean average, first and third quartiles. Whiskers denote 1.5*inter-quartile range) (Cold: n = 8, Mild: n = 6).

The greatest rate of temperature change within the pallial cavity was consistently experienced during the flood tide (Figure 6.3). On the flood tide temperatures increased exponentially under both mild and cold winter scenarios. On an ebb tide the internal temperature decreased in 2 linear stages, the former occurring immediately on emersion to the air and was more rapid than the latter which occurred as internal temperatures slowly merged with ambient air temperatures. The rate of temperature change was greater during the colder winter scenario during the flood tide and ebb tide only. Temperature change during aerial exposure was similar between both winter scenarios.

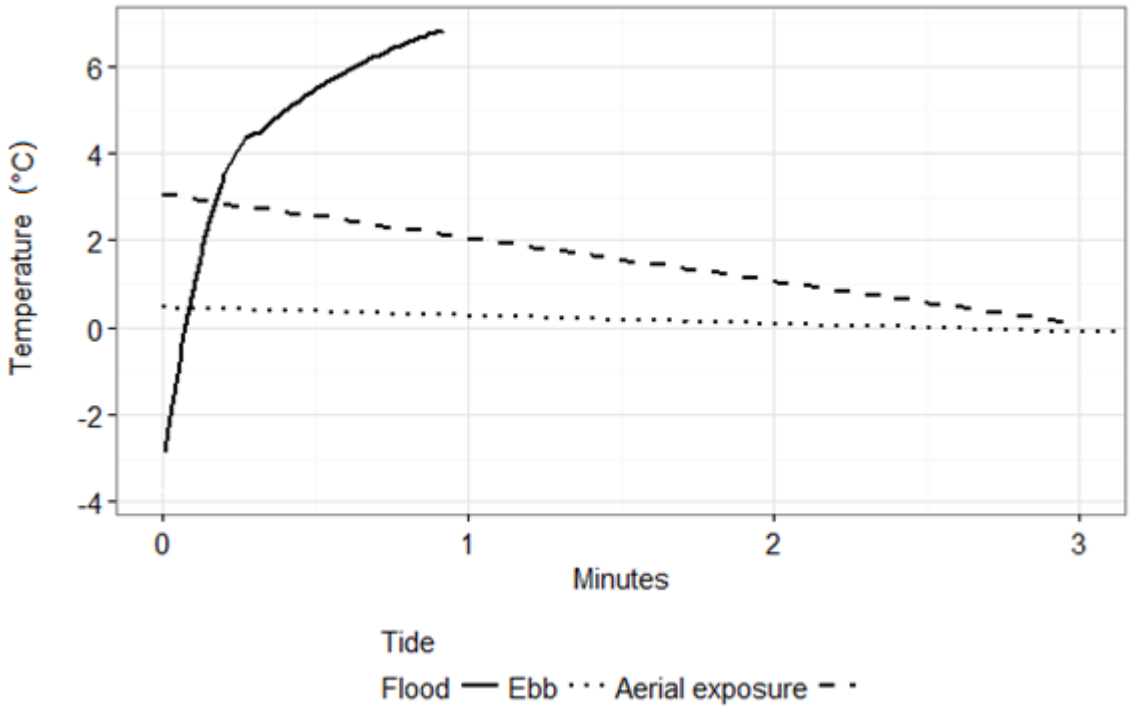


Figure 6.3 An example of typical rates of temperature change within the pallial cavity of *Crassostrea gigas* at different stages of the tidal cycle

The rate of temperature change within the pallial cavity on a flood tide and ebb tide were compared between cold and mild winter scenarios using a two-way ANOVA. The test tank took 12 minutes to fill or empty, depending on the tidal cycle. The duration with which the pallial cavity was changing temperature within the 12 minute window of a tidal exchange was compared (Figure 6.4). The state of tide (factor) was found to have a significant effect on the rate of temperature change ($F(1,29) = 34.7$, $p<0.001$), however the winter scenario did not ($F(1,29) = 1.8$, $p = 0.188$) and nor was there an interaction between the two factors ($F(1,29) = 0.4$, $p = 0.532$). Considering the main effects, the duration of temperature change was significantly less on a flood tide than on an ebb tide ($p<0.001$), and the duration of temperature change was similar between cold and mild winter scenarios at comparative tidal states ($p = 0.36$).

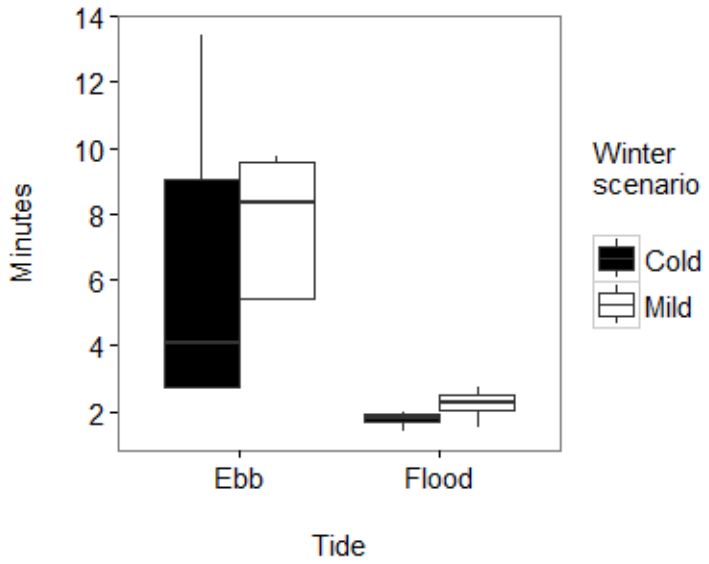


Figure 6.4 The period of rapid temperature change within the internal pallial cavity of *Crassostrea gigas* within a 12 minute window immediately after aerial exposure on an ebbing tide and submersion on a flooding tide (Boxes show the mean average, first and third quartiles. Whiskers denote 1.5*inter-quartile range) (Cold: n = 8, Mild: n = 6).

6.3.2 Respiration

Adults

Crassostrea gigas (shell length 85 ± 10 mm) and *Ostrea edulis* (shell length 60 ± 10 mm) were collected from the intertidal zone in Poole Harbour (Blue Lagoon) and Southampton Water (Hamble) (see Chapter 2 Figures 2.4 & 2.7). Collection took place during November 2014 when water temperatures in the field ranged from approximately 15 °C at the beginning of the month to 11 °C by the end of the month (Appendix D).

The objective was to compare respiration rates of *C. gigas* with those of *O. edulis*, a native species of oyster expected to be 'better physiologically adapted' to the winter conditions experienced in the UK. It was intended that a comparison between these species would be made from Poole Harbour and Southampton Water. However unregulated water temperatures in the laboratory resulted in a proportion of the data being incomparable. Water temperature within the laboratory were relatively stable during the first 2 weeks of experimentation (14.5 $\pm$ 0.25 °C) however it decreased to 13.25 $\pm$ 0.25 °C during the subsequent week and further still to < 11 °C for the final week of experiments (Figure 6.5).

C. gigas were tested only at 14.5 °C however *O. edulis* were tested at 14.5, 13.25, 11 and 9 °C. It was found that this drop in water temperature significantly affected the respiration rate of *O. edulis* ($F(1) = 9.91$, $p = 0.007$) and pairwise *post hoc* analysis showed respiration to decline notably with water temperatures below 13.25 ± 0.25 °C. Consequently experiments carried out at water temperatures <13.5 °C were excluded from further analysis. All specimens of *C. gigas* were included (Southampton Water n = 12, Poole Harbour n = 8) however only half of the sample of *O. edulis* from Poole Harbour was usable (n = 4).

C. gigas inhabiting Southampton Water had a significantly higher oxygen consumption rate than *C. gigas* inhabiting Poole Harbour ($F(1) = 16.628$, $p = 0.003$)(Figure 6.5), and within Poole Harbour the native oyster, *O. edulis* had the highest rate of oxygen consumption ($F(1) = 8.601$, $p = 0.013$). Respiration rates were calculated from measurements of hourly oxygen saturation and converted into oxygen consumption per unit of flesh (as ash free dry weight). Oxygen saturation declined linearly for both species of oyster (Figure 6.6). At the end of the experimental period oxygen saturation was consistently highest in chambers containing *O. edulis* which contradicts the above results. However this can be explained through the use of smaller individuals of *O. edulis* that allowed for the comparison of similar aged oysters between species.

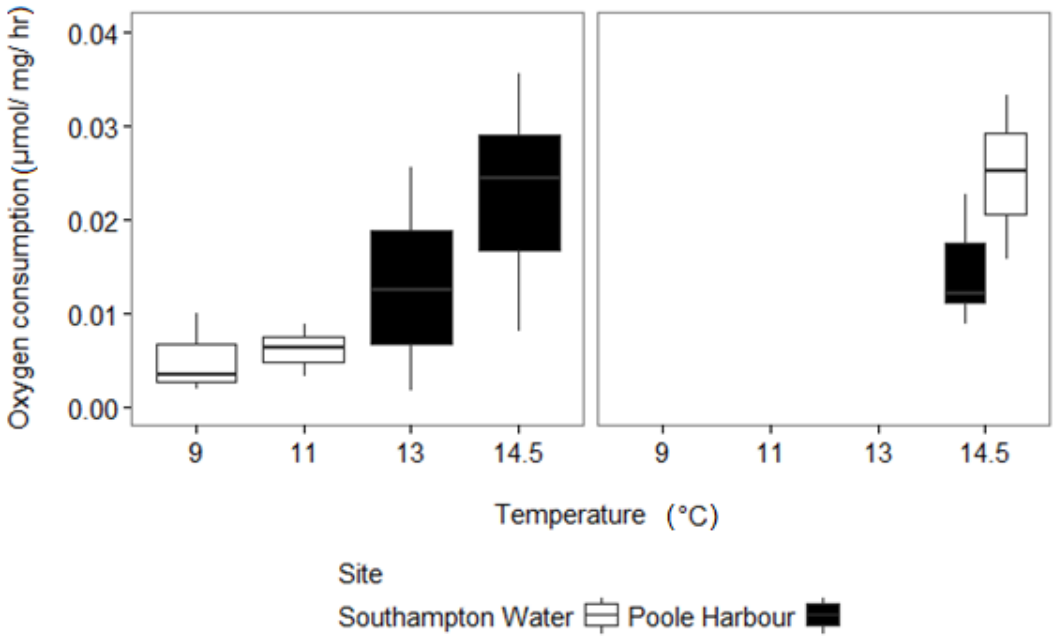


Figure 6.5 Laboratory water temperature decreased over the experimental period (03.11.2014 – 14.01.2016) affecting respiration rate of **Left:** *Ostrea edulis* (Southampton Water: n = 8, Poole Harbour: n = 8) and **Right:** *Crassostrea gigas* (Southampton Water: n = 12, Poole Harbour: n = 8) (Boxes show the mean average, first and third quartiles. Whiskers denote 1.5*inter-quartile range).

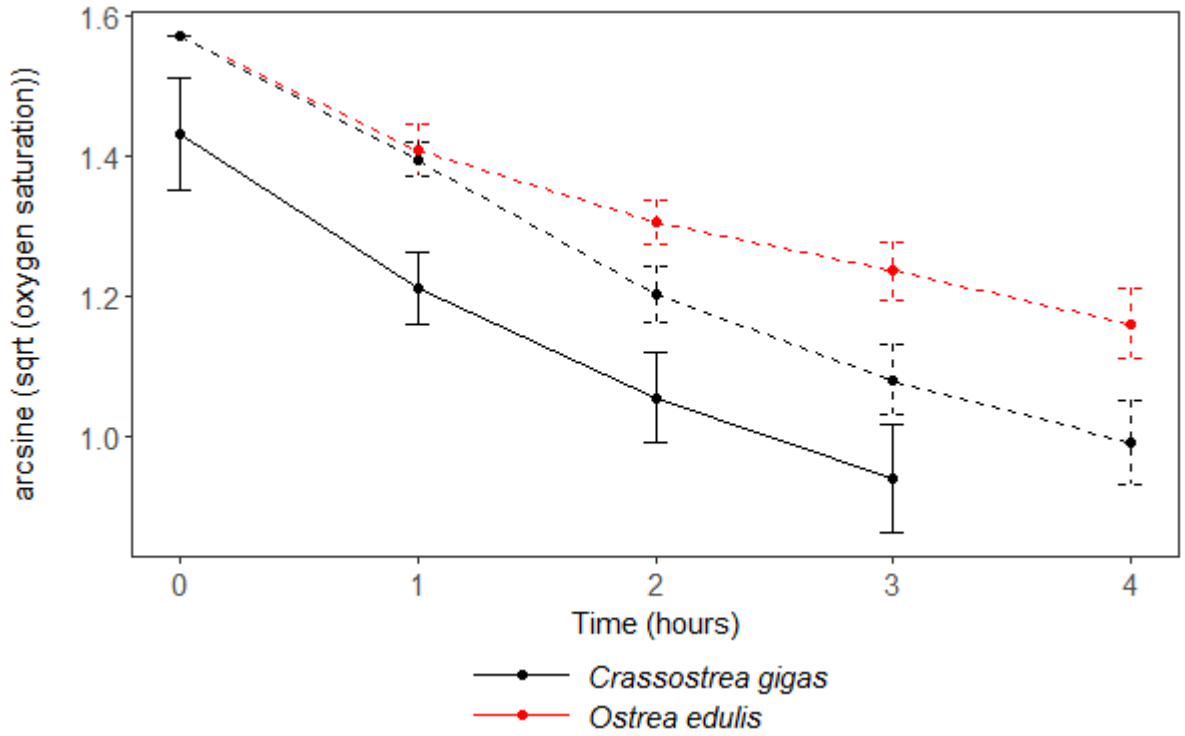


Figure 6.6 Hourly respiration rates of *Crassostrea gigas* from Poole Harbour (dashed line) and Southampton Water (solid line) (Southampton Water: n = 12, Poole Harbour: n = 8) and *Ostrea edulis* from Poole Harbour (n = 4) (mean ± se).

Juvenile

The weight of juvenile *C. gigas* as a measurement of ash free dry weight significantly effected their oxygen consumption ($F(2) = 5.645$, $p = 0.0125$) (Figure 6.7). A pairwise *posthoc* T-test showed juvenile *C. gigas* weighing 4 ± 0.5 mg had a significantly greater uptake of oxygen than 6 ± 0.5 mg *C. gigas* ($p = 0.021$) and 9 ± 0.5 mg *C. gigas* ($p = 0.021$). Furthermore oxygen consumption was similar between juvenile *C. gigas* weighing 5.5 – 9.5 mg ($p = 0.938$). As with adult oysters, uptake of oxygen by the juveniles was linear over time (Figure 6.8).

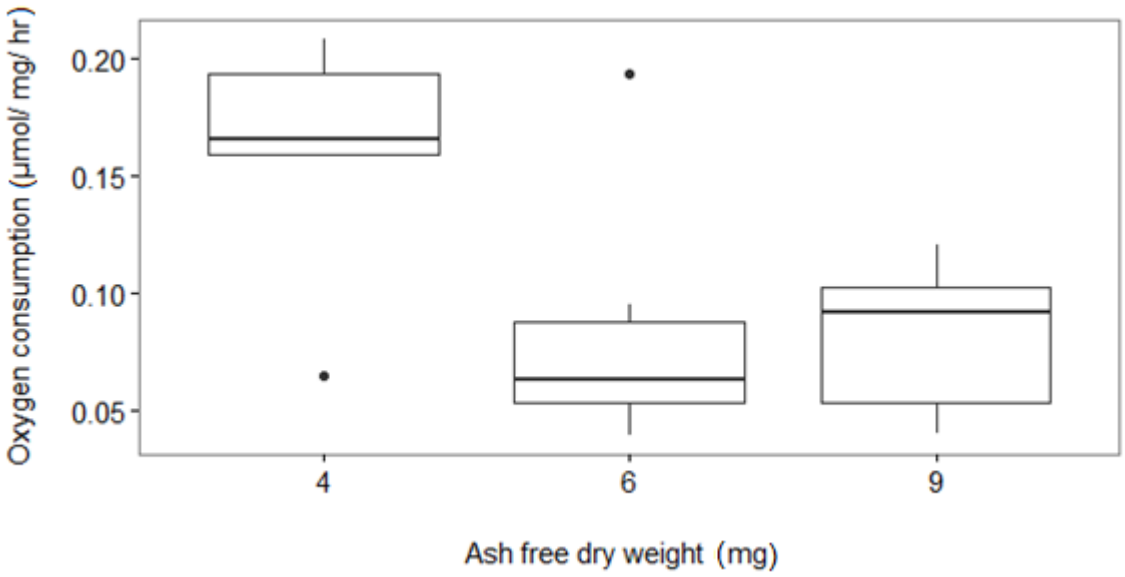


Figure 6.7 Respiration rate of juvenile *Crassostrea gigas* of different weight categories (4 mg: n = 16, 6 mg: n = 32, 9 mg: n = 32) (Boxes show the mean average, first and third quartiles. Whiskers denote 1.5*inter-quartile range).

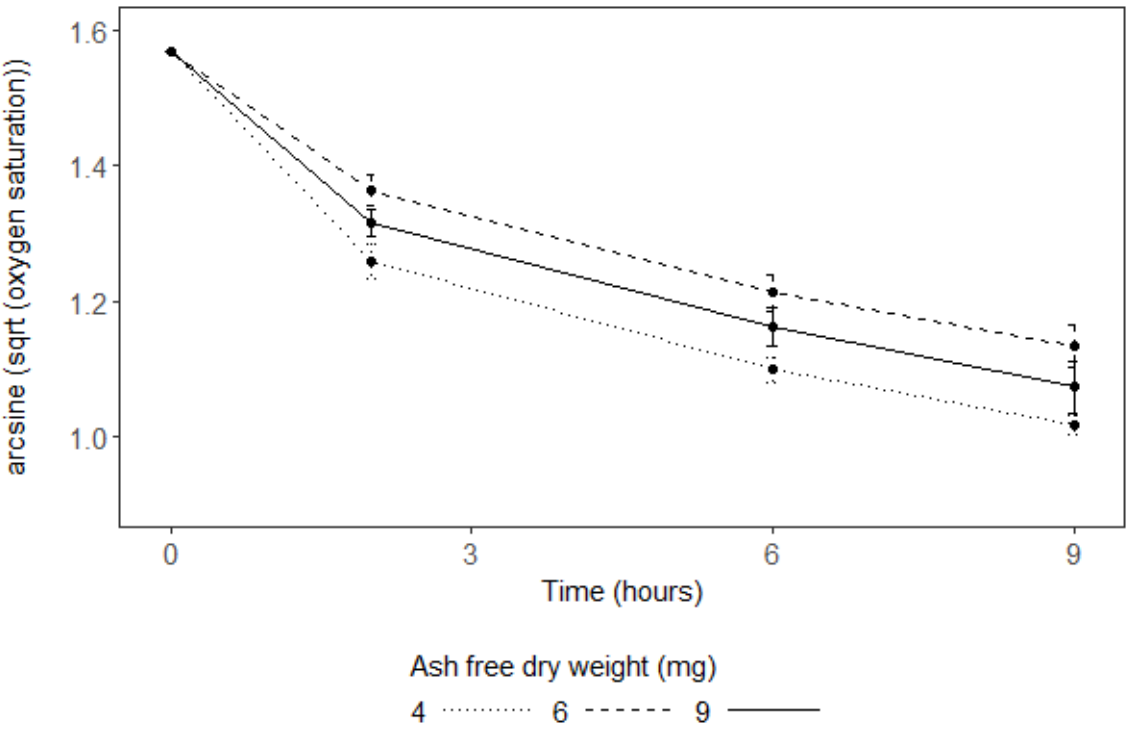


Figure 6.8 Hourly respiration rates of juvenile *Crassostrea gigas* of a range of different weights (4 mg: n = 16, 6 mg: n = 32, 9 mg: n = 32) (mean ± se).

Literature comparison: Respiration rates of *Crassostrea gigas* at various shell lengths and temperatures are summarised in the below graph and table.

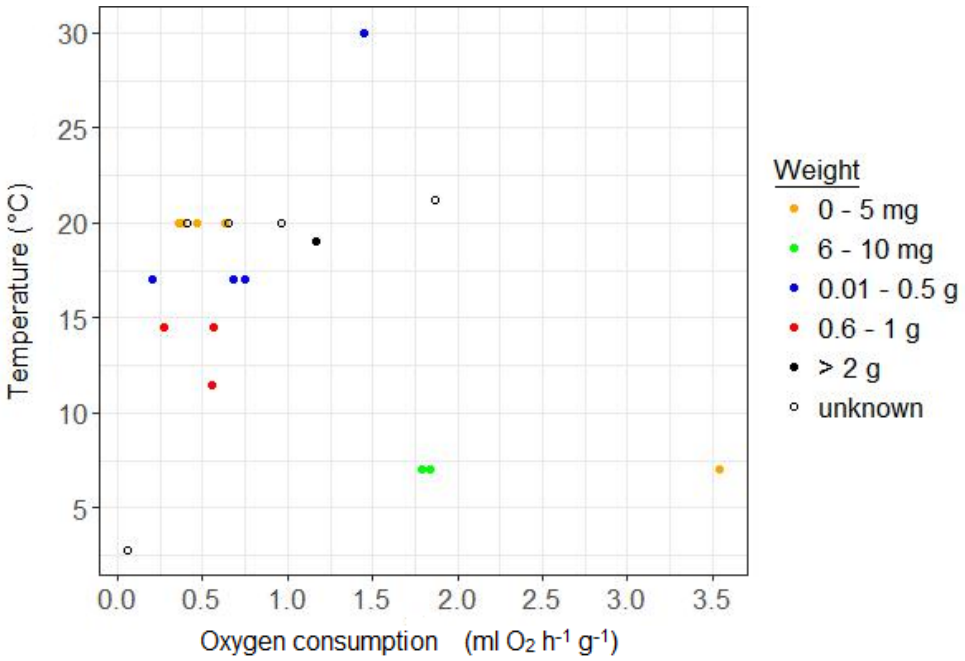


Figure 6.9 & Table 6.2 A comparison of *Crassostrea gigas* respiration rates from literature

Oxygen consumption (ml O_2 h^{-1} g^{-1})	**Temperature (°C)**	**Weight (g)**	**Reference**
0.061	2.8		Mao *et al* 2006
0.200	17	0.2	Bayne *et al* 1999
0.269	***14.5***	***0.79***	***This study***
0.359	20	0.00136	Goulletquer *et al* 1999
0.382	20	0.00179	Goulletquer *et al* 1999
0.410	20	(95 mm)	Ren *et al* 2003
0.465	20	0.00145	Goulletquer *et al* 1999
0.5-0.6	8-15	0.8	Shpigel *et al* 1992
0.560	***14.5***	0.63	***This study***
0.629	20	0.00129	Goulletquer *et al* 1999
0.650	20		Gerdes 1983
0.680	17	0.09	Bayne *et al* 1999
0.750	17	0.21	Bayne *et al* 1999
0.960	20		His 1972
1.167	19	1.4 - 3.2	Haure *et al* 2003
1.4-1.5	30	0.4	Shpigel *et al* 1992
1.792	***7***	***0.006***	***This study***
1.8368	***7***	***0.009***	***This study***
1.868	21.2		Mao *et al* 2006
3.5392	***7***	***0.004***	***This study***

6.4 Discussion

6.4.1 The effect of winter aerial exposure on internal temperature

Irrespective of environmental temperatures, sessile animals that inhabit the intertidal zone have to face variations in physical and chemical parameters during tidal cycles. During immersion, shell valves gape open allowing the filtration of food particles and an uptake of oxygen directly from the seawater as well as the excretion of pseudofaeces. During emersion, shell valves are closed to prevent desiccation reducing the amount of available dissolved oxygen to a small reserve of seawater trapped within the pallial cavity. The reduction of oxygen coupled with the increase in levels of carbon dioxide results in a decreased tissue pH which impacts upon the immune system (Allen & Burnett 2008) and energy producing mechanisms (Dudognon et al. 2013). On depletion of oxygen reserves aerobic respiration becomes anaerobic. However switching to anaerobiosis is unfavourable as it leads to a decrease in ATP levels and an accumulation of compounds at toxic levels (Zwaan & Wijsman 1976). *C. gigas* avoids anaerobic respiration through metabolic plasticity in response to fluctuating oxygen levels, bradycardia and reduced oxygen uptake (Le Moullac et al. 2007; Sussarellu et al. 2010). Furthermore behavioural adaptations such as the transfer of seawater from body cavities to the pallial cavity (Buzin et al. 2011) and controlled gaping during tidal emersion also prolong periods of aerobic respiration (Rafrafi & Uglow 2009).

When immersed by the tide, adductor muscles control the aperture of the gape. The aperture varies depending upon food availability (Newell et al. 2001; Maire et al. 2007), water quality (Nagai et al. 2006; Tran et al. 2010), light levels (Gibson et al. 2001), temperature and the time of day (Comeau et al. 2012). In this study, behaviour during a tidal cycle was interpreted through the temperature of the pallial cavity. A base line of oyster activity was previously described using video footage of *C. gigas* under controlled environmental parameters in aerated, filtered seawater (Appendix G). Base activity was characterised by the gradual increase of gape aperture through a repeated pumping action. Valves partially closed before reopening again during pumping with an incremental increase in overall gape aperture. After a short duration the shell valve aperture began to reduce with the same pumping action, however immediately before the valves reached closure, there was a succession of larger pumps. The valves then remained closed for an extended time period. Much like *C. gigas,* mussels (*Mytilus edulis* & *M. galloprovinciallis*) are sessile (or minimally motile) bivalves inhabiting the intertidal zone that depend on their hard shells for protection, but must part the shell valves in order to feed. There has been a body of work in the past 2 decades describing the considerable variance in abduction and adduction of

mussel valves (Newell et al. 2001; Maire et al. 2007; Robson et al. 2007; Robson et al. 2009; Robson et al. 2010). Mussel behaviour was found to involve complex trade-offs maximising feeding and pseudofaeces production whilst minimising predation risk (Robson et al. 2007; Robson et al. 2010). In low food or high predation environments gape aperture remained small and in high food environments (associated with low predation) aperture was maximal and valve movement speed at its greatest. The use of different muscle types was assumed to be responsible for different speeds of valve movement whilst the combination of maximising food uptake with the need to excrete more and increase diffusion of newly reoxygenated haemolymph throughout the tissue is thought to explain the increased magnitude (Robson et al. 2010). The degree of valve activity seen during this study was most likely stimulated by the presence of microbes in the seawater that were not removed by the filtration system and that can be utilised as a food source by *C. gigas* (Langdon 1983; Shpigel et al. 1993). This would mean then, that the test conditions represented low food availability with predation risk. If *C. gigas* responds to such conditions in the way seen in *Mytilus* spp., the base line activity characterised here would represent a gape magnitude that is less than the maximum. An apparent difference between mussel and oyster behaviour occurs immediately prior to prolonged valve closure. Mussels reduced the gape aperture to close slowly whereas the oysters appeared to 'cough' with a few large pumping actions before prolonged closure. Large valve movements are energetically costly (Robson et al. 2007) and so reducing energetic expenditure before closure, as with the mussels, may extend the potential duration of the closed period. However the large and presumably costly 'coughing' seen in *C. gigas* may act to flush out any remaining pseudofaeces and refresh the seawater within the pallial cavity. The pay-off in increasing the amount of oxygen available during closure and reducing the build-up of toxins is presumably greater than the energetic cost of 'coughing'. The differences in such behaviours may reflect the techniques used by each species for coping with reduced respiration and feeding opportunities that are associated with shell closure.

Considering first *C. gigas* submerged by the flood tide; during a mild winter scenario the internal temperature of the oysters in the experiment was the same as that of ambient water temperature (6 °C), however during a cold winter scenario internal temperatures between individual oysters differed. Equal temperatures under mild winter conditions were most likely the result of oysters opening their valves to pump water over their gills to feed, respire and excrete. However the variability of internal temperatures seen under cold winter conditions are more likely the result of the valves of oysters remaining closed. The pumping of water into the pallial cavity would then be inhibited and any differences seen between the temperatures within the

pallial cavities would be the result of varying insulating properties between the shells of individuals tested. This pattern continued until the water was drained out of the tank 2 hours later indicating that the valves remained closed throughout the entire simulated tidal cycle. Internal temperatures continued to vary between oysters as they chilled through exposure to the air. The simulated ebb tide was approximately 2 hours long and in this time the shell provided sufficient insulation to prevent the internal temperature reaching ambient air temperature. Internal temperatures for the cold winter scenario did however decrease below 0 °C. Decreasing below 0 °C did not appear to affect the rate of change of internal temperatures however the length of time after aerial exposure did. Under both winter scenarios temperatures decreased most rapidly immediately after aerial exposure. This is expected as it coincides with the greatest temperature differential. Under mild winter conditions it is unclear whether the rapid temperature change is amplified by the oysters becoming exposed to air before the valves are closed.

6.4.2 Winter respiration rates

Respiration rates give an estimation of the overall sum of all energy consuming processes, and can be indicative of suboptimal or stressful environments (Columbo et al. 1990; Li & Vandepeer 2004; Lannig et al. 2010). Factors that cause stress, such as adverse environmental conditions, mechanical injury, pathogens or predators (Columbo et al. 1990), interrupts energy homeostasis and typically results in escalated respiration to meet the increase in metabolic demand (Columbo et al. 1990; Li & Vandepeer 2004; Lannig et al. 2010). If it is a long term effect, energy becomes diverted away from somatic growth, reproduction and immune responses, and has the potential to impact on future population dynamics and survival (Columbo et al. 1990; Li & Vandepeer 2004; Lannig et al. 2010).

This study found oxygen consumption of adult *C. gigas* to vary between sites and that of juvenile *C. gigas* to vary depending on weight. A wide range of respiration rates have been reported for *C. gigas* with causes being attributed to different genetic strains (Shpigel et al. 1992b; Bayne et al. 1999; Goulletquer et al. 1999; Samain et al. 2007), seasonal variation in metabolic activity (Mao et al. 2006), experimental temperature (Shpigel et al. 1992b; Bougrier et al. 1995; Mao et al. 2006), acclimation time (Widdows 1976; Bayne et al. 1999), and the age/mass of the oysters being tested (Bayne et al. 1999; Ren et al. 2003). In this study, adult *C. gigas* collected from Poole Harbour had an average soft tissue dry weight of 0.8 g and consumed energy at an average rate of 0.012 µmol mg^{-1} hr^{-1}, a comparable rate to that measured by Bayne et al. (1999) and Goulletquer et al. (1999), however the oysters used in this study were considerably larger and the water

temperature was considerably cooler (Figure 6.9 & Table 6.2). It would be expected that an increase in body weight and a lower temperature would both act to reduce the comparative respiration rate (Shpigel et al. 1992b; Bougrier et al. 1995; Mao et al. 2006). *C. gigas* collected from Southampton Water had an average soft tissue dry weight of 0.6 g and consumed energy at an average rate of 0.025 µmol mg^{-1} hr^{-1}, a comparable rate to that recorded by Shpigel et al. (1992) using *C. gigas* of a similar weight and temperature (Figure 6.9 & Table 6.2). The difference in weight between the oysters sampled from Poole Harbour and Southampton Water may have contributed toward the difference in respiration rate, however the variation in weight was relatively small. It is more likely the difference in condition of the oysters between sites. Oysters from Poole Harbour had an average Condition Index (CI) of 4.8, considerably higher than the CI of oysters sampled from Southampton Water (3.6). The reduced condition of oysters in Southampton Water may reflect the higher tidal height that they inhabit. Consequently immersion times are reduced along with the potential to feed, respire and excrete (Bougrier et al. 1998; Haure et al. 2003).

The distribution and abundance of an establishing nonindigenous species may be limited by the conditions of the recipient ecosystem, and in particular temperature, differing from that of their native range (Hicks & McMahon 2005; Troost 2010). Native taxonomically related species typically have increased physiological tolerance as a consequence of evolving under such conditions (McMahon 2002). Temperature generally becomes debilitating to a species at the boundary of its geographic region or if it is establishing at a different tidal height (Theede 1973; Aarset 1982; Brown & Swearingen 1998; Firth et al. 2011). However nonindigenous species that successfully establish exert rapid growth rates, early maturity and elevated fecundity allowing population recovery in the event of an event of environmental extremes (McMahon 2002).

Ostrea edulis has a native range that includes the UK (Launey et al. 2002), however energy consumption (0.023 µmol mg^{-1} hr^{-1}) and CI (3.5) were similar to that of *C. gigas* inhabiting Southampton Water. It is thought that this has occurred as a result of *O. edulis* being sampled from the upper limits of its tidal distribution (Aarset 1982; Harley & Helmuth 2003; Allen & Burnett 2008). Furthermore it is possible that *C. gigas* sunk into the mud, experience reduced energy consumption as the substrate acts to buffer temperature fluctuations and extremes. *C. gigas* of all sizes were frequently found in Poole Harbour with the umbo sunk into the mud and only the peripheral margin exposed. Just 3 cm below the sediment surface, temperatures can differ by >3 °C (see Chapter 4 Figure 4.12).

Juvenile *C. gigas* were tested under colder conditions than the adults because the aim of this study was to conclude whether winter water temperatures are detrimental to recruitment. An extensive acclimation period ensured seasonal variability in metabolic rate and stress induced by acclimation, and the subsequent recovery, did not affect the results (Widdows 1976; Child & Laing 1998; Bayne et al. 1999). However keeping juvenile oysters in consistently cooled water supressed growth (Child & Laing 1998; Bayne et al. 1999) and considerable decreases in body mass of juvenile *C. gigas* have been observed at 6 °C and mortality at 3 °C (Child & Laing 1998). Study animals were more similar in size to 5 week old hatchery reared spat (2 – 4 mg)(Child & Laing 1998), than 5 month old juveniles (12 – 18 mg) (Goulletquer et al. 1999) as a consequence of being held at 7 °C for 8 months. Energy consumption of 6 and 9 mg oysters was similar, with an average of 0.080 µmol mg^{-1} hr^{-1} and 0.082 µmol mg^{-1} hr^{-1} respectively. However energy consumption of 4 mg oysters was notably greater at an average of 0.158 µmol mg^{-1} hr^{-1}, making it apparent that seawater temperatures of 7 °C are low enough to cause a depression in the metabolic demand of juvenile oysters and supress growth, however only detrimental to juveniles with a mass of < 6 mg.

Comparative respiration rates of 5 month old *C. gigas* were considerably lower than those recorded in this study despite their larger size (Goulletquer et al. 1999). It is possible that this is the result of the greater metabolic rate of young animals, resulting in a decreased metabolic intensity with increasing body mass (Farrell-Gray & Gotelli 2005; Seibel 2007). Maturity also effects metabolic demand as the energy needs of immature bivalves are solely allocated to somatic growth, and thereafter metabolism is mainly directed to gamete maturation and spawning. The reproductive effort is responsible for an 18 % energy loss for yearling oysters, and about 63 % loss during the second year (Deslous-Pauli & Héral 1988). However gametogenesis does not initiate at temperatures below 9.5 °C (see Chapter 4) and therefore should not have affected the metabolic demand of oysters in this study.

Summary

In summary, the winter conditions experienced in the south of England are likely to have hindered the establishment of *C. gigas.* Estuarine water temperatures in the south of England average between 5 – 8 °C during the winter. Therefore, a typical winter is likely to be detrimental to juvenile *C. gigas* < 6 mg in weight. During a cold winter however, temperatures are likely to impact the adult population as well as the juvenile recruits.

A bivalve's shell provides the only physical barrier reducing environmental temperature penetrating to the vulnerable body parts. This study found limited protection afforded by the

shell of *C. gigas,* allowing soft tissue to be subjected to rapid temperature changes during a tidal cycle and to decrease below freezing during cold winter aerial exposure. Furthermore the impact of a frost prevailed after tidal immersion as *C. gigas* remained with their shell valves closed, inhibiting feeding, excretion and aerobic respiration.

Environmental change can be considered stressful if an organism needs to increase energy expenditure on maintenance, defence or repair. The condition of *C. gigas* going into winter was similar to that of the native taxonomically related *O. edulis.* Post-spawning *C. gigas* showed no increase in metabolic demand compared with literature results, however those sampled from the limit of their tidal distribution had an elevated rate of energy consumption compared with those located more centrally. High respiration rates in juvenile oysters < 6 mg, were recorded in ambient water temperatures of 7 °C, however larger spat were unaffected.

Chapter 7: Synopsis

Non-native marine species introductions have increased globally with the expansion of dispersal corridors by activities, such as the transportation and release of species in ships ballast water and the introduction of non-native species into fisheries and aquaculture (Ruiz et al. 1997; Cohen & Carlton 1998; Carlton 1999; Ruiz et al. 2000; Naylor et al. 2001). Non-native species have the potential to become invasive and therefore detrimental to the local environment, ecology, economy and society (Ropes 1968; Carlton 1999; Bax et al. 2001; Defra 2008). Negative impacts can include the alteration of ecosystems and the exclusion of native biota, a reduction in productivity of local fisheries and aquaculture, and degradation of the overall living quality of an area. However, benefits can also accompany establishing species. Positive impacts can include an increased biodiversity through the generation of new habitat, the potential for new fishery and aquaculture opportunities, and coastline stabilisation. Whether the positive impacts outweigh the negative impacts is often dependent upon achieving a balance between the recipient ecosystem, and the establishing species.

Some species, such as *Crassostrea gigas,* have achieved extensive distribution in the temperate marine and estuarine environments. Typically such a cosmopolitan species exhibits life characteristics resulting in that species becoming invasive. These include a wide tolerance to environmental parameters such as temperature, salinity and aerial exposure, rapid growth rates, and a high fecundity. Considerable variation in recruitment to wild *C. gigas* aggregations has been seen regionally across the globe and consequently its status also differs. For example *C. gigas* was introduced into France for the purpose of replenishing fisheries and the good growth rates and success of natural setting on the French Atlantic coast were considered to be confirmation of a successful outcome (Grizel & Héral 1991). However in the Wadden Sea, *C. gigas* was introduced as an aquaculture species and has since become feral outside of aquaculture plots. Furthermore feral *C. gigas* are considered invasive (Nehls & Büttger 2007; Brandt et al. 2008; Schmidt et al. 2008) because of the establishment and spread of large aggregations that have colonised native mussel beds, and negatively impacted upon tourist attractions and local fisheries (Diederich 2005a; Diederich 2006b; Wilsman et al. 2008).

Once established, eradicating *C. gigas* has proven to be extremely difficult and potentially expensive (Pimentel et al. 2000). Maturity is reached within a year and fecundity is high. Larvae develop in the water column allowing dispersal of up to 50 km (Brandt et al. 2008), and a wide range of hard substrate can be colonised (Reise 1998; McKnight 2009; Markert et al. 2010). Furthermore evidence of simultaneous hermaphroditism was shown in chapter 4, a reproductive trait that has been suggested to improve recruitment in small or dispersed bivalve populations (Reed 2013). In this case, the presence of simultaneous hermaphrodites may improve recruitment during the initial years of establishing feral aggregations or after an attempt at eradicating the species. The success of the eradication scheme must in part, rely on the knowledge of the source of larvae into the wild, and the ability to terminate it. However, the larval source is often assumed and, as discussed in chapter 3, there may be multiple, often unmanageable sources of larvae making complete eradication unlikely. Due to the difficulties of eradicating an established non-native species, actions taken to control or manage the species are often implemented instead. A programme set up to control *C. gigas* establishing along the Thanet (UK) coastline was based on three guiding principles; containment not eradication, a targeted response based on a monitoring programme, and a long term commitment. These guidelines allowed available resources to be matched against a maximum achievable outcome. *C. gigas* were not eradicated completely, however numbers were greatly reduced in the area, and the strategy employed was economically viable and repeatable (McKnight & Chudleigh 2015).

Actions taken to prevent the introduction of non-native species are preferential to eradication or control and form the basis for many Government policies (Defra 2003; IMO 2004a; IMO 2004b; Defra 2008). Studies that assess invasion potential have in the past focused on ballast-water transport and hull-fouling transfers as primary mechanisms for aquatic introductions (Carlton & Geller 1993; Ruiz et al. 1997; Smith et al. 1999; Smith et al. 2000; Wonham et al. 2000; Wonham et al. 2001), with few studies focusing on non-shipping mechanisms (Courtenay & Williams 1992; Weigle et al. 2005). Consequently most regulatory efforts are directed toward ballast-water management despite the potential risk posed by non-shipping mechanisms (Weigle et al. 2005).

Research for this thesis aimed to provide an understanding of how *C. gigas* has become established along the south coast of England. It is hoped that through an understanding of how environmental parameters and biological interactions relate to reproductive output and recruitment, predictive models for the future geographic spread of *C. gigas* within the UK can be generated. Predictive models are a useful tool in prevention management or control of a potentially invasive species through their ability to identify likely future recruitment hotspots and so allow resources to be allocated accordingly. As well as benefiting environmentalists who want

to understand the risk to environmental degradation that wild populations pose and the government bodies responsible for deciding the outcome of aquaculture applications, such models may also have a use in the future of the oyster industry through selecting sites and dates for oyster seed collection.

7.1 Environmental and biological interactions on *Crassostrea gigas* establishment

A fine scale mechanistic model allows the inclusion of interspecific interactions and dynamics between larval sources. Therefore, it is suggested by the author, to be the most appropriate tool in predicting the spread of *C. gigas* at a local scale. The proposed conceptual framework (Guisan & Zimmermann 2000) for such a model was illustrated in Chapter 1.6 (Figure 1.5) and has provided the structure for this thesis. The proposed conceptual framework will now be revisited, and expanded upon using the knowledge gained throughout this study (Figure 7.1). Findings from this study have been included (**bold**) along with figures from the literature (*italics*). Gaps in our current knowledge have also been highlighted (<u>underlined</u>) when information could not be found via a literature review and did not form part of this research, but is considered to be important for the condition and survival of *C. gigas*, and so predicting its spread.

The research carried out for this thesis has been extensive but not complete. There are factors known to affect the condition and survival of *C. gigas,* such as salinity (Brown & Hartwick 1988; Leffler & Greer 1991; Calvo et al. 1999; Fabioux et al. 2005), nutrition (Brown & Hartwick 1988; Ruiz et al. 1992; Chávez-Villalba et al. 2003; Rico-Villa 2009) and parasitic infection (Carnegie & Cochennec-Laureau 2004; Batista et al. 2009) that have not been included. Such factors are illustrated in the conceptual framework by the filled in boxes (Figure 7.1). Furthermore where shortcomings in this study were evident, they have been discussed in the future work section later on in this chapter.

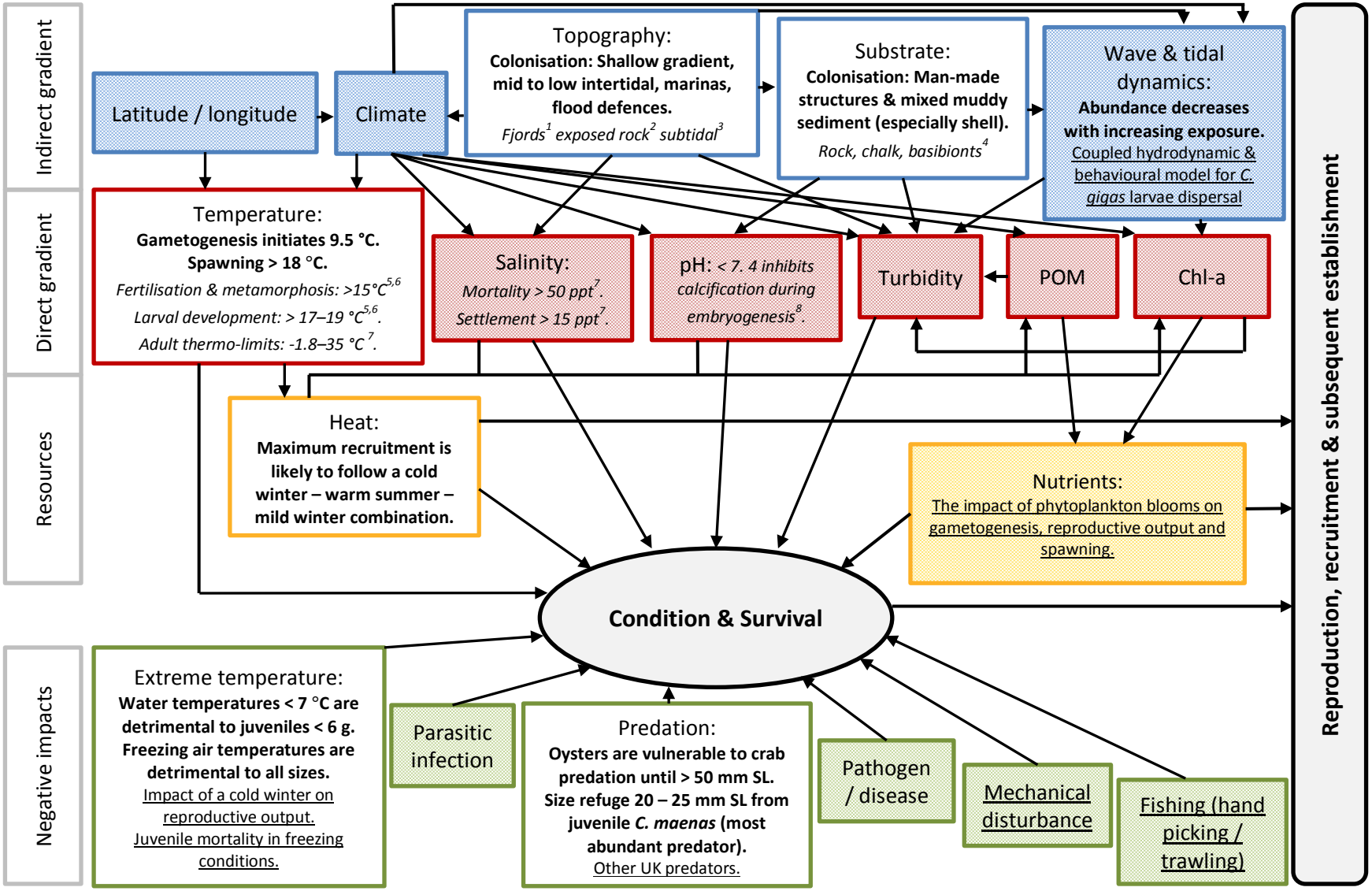


Figure 7.1 Conceptual frame work for a fine scale mechanistic model for predicting the future spread of Crassostrea gigas. **Findings from this study**, *literature* and gaps in current knowledge. [1]Smaal et al. 2009, [2]McGonie et al. 2011, [3]Kochman 2012, [4]Herbert et al. 2012, [5]Li & Hedgecock 1998, [6]Rico-Villa 2009, [7]Leffler & Greer 1991, [8]Kurbara et al. 2007.

7.1.1 Indirect gradients

This study found substrate type and the location of *C. gigas* within the tidal range to have the greatest impact on the locality and abundance of *C. gigas* recruitment. It is however, postulated that the hydrodynamic regime can have an overriding effect, determining which parts of the shore are accessible to larvae. For example, there are large areas of suitable habitat within Poole Harbour that are within relatively close proximity to aquaculture plots of *C. gigas,* and yet *C. gigas* was absent or present in isolation or small isolated groups. Recruitment success was lower than may have been expected considering the substantial propagule pressure, in this case the farmed *C. gigas*. It is suggested that the most likely reason for low recruitment is the separation of the oyster aquaculture plot from suitable habitat by strong tidal currents (Herbert et al. 2011). The tidal currents are accentuated by a dredged shipping channel resulting in the larvae being flushed from the harbour or deposited on the southern side of the harbour where recruitment potential is notably lower due to a lack of suitable substrate. Therefore an understanding of the hydrodynamics of an estuary and a map of substrate types is an essential base for a predictive model of potential *C. gigas* recruitment.

The importance of resolution and simulating all potential larval sources is highlighted by the presence of a single uncharacteristic patch of *C. gigas* within a small embayment on the northern shore (Blue Lagoon) of Poole Harbour where densities reached > 10 *C. gigas* per m^2, the highest recorded during this study. Blue Lagoon was also the only site within Poole Harbour with multiple age cohorts present suggesting a larval sink and potentially a site where *C. gigas* has established independently from the aquaculture plot. Recruitment may have initially occurred as a result of a particular combination of weather and tides that allowed larvae from the aquaculture plot to reach the north shore of Poole Harbour, or it may have been the result of hull-fouling and subsequent marina fouling that spawned and produced larvae north of the dredged channel that facilitated recruitment on the northern shore. However, without DNA analysis the larval source is speculation and epitomises the difficulties in predicting future spread of *C. gigas* without considering all potential sources of larvae (Lallias et al. 2015). Furthermore Blue Lagoon highlights the need to use a model of sufficient resolution. Blue Lagoon is a small enough embayment (12 ha) that it is often not included in map outlines or models of Poole Harbour and yet it represents a larval sink with suitable substrate and contains the most significant example of established feral *C. gigas* within Poole Harbour and possibly the highest density feral aggregation on the south coast of England (Spencer et al. 1994; McKnight 2009; Herbert et al. 2012).

Previous studies have suggested a relatively quick way of assessing whether the hydrodynamic regime of an estuary is conducive to the establishment of various benthic colonising species, including *C. gigas,* by calculating residence times (Gaines & Bertness 1992; Dyer & Orth 1994; Kim et al. 2010; Kochmann 2012). The rationale being, that if residence times are greater than the expected larval development period, then there is a much greater chance of local recruitment. However this study found recruitment in 2 estuaries, both of which had residence times less than the expected larvae development period. In particular, Southampton Water was calculated to have a residence time of approximately 13 days, which is approximately 8 to 15 days less than the expected larval duration (Rico-Villa 2009), and yet the majority of mixed substrate in the mid-low intertidal zone has been colonised by *C. gigas,* and recruitment is present from at least 5 settlement events (years) at densities between 2 and 10 *gigas* per m^2. Therefore, although an increase in residency duration may increase the abundance of annual recruitment, it is suggested that a fine-resolution hydrodynamic model such as that used by Herbert et al. (2011) is more suitable to predicting if and where recruitment might occur.

Climate is an indirect gradient that influences all direct gradients to some degree. Although the impacts of climate on *C. gigas* have not explicitly been considered in this study, the impacts of global climate change on the frequency and abundance of non-native species are well documented (Southward et al. 1995; Dukes & Mooney 1999; Harley et al. 2006; Ricciardi 2007; Rahel & Olden 2008; Syvret et al. 2008). Long-term trends show a warming of inshore waters throughout the UK since 1989, with the average summer rise being higher in the south east of England than elsewhere in the country and equating to 0.9 °C increase every 10 years (MCCIP 2011). This is of note because the inclusion of a climate change term within the model will increase the predictive power, especially when considering the direct gradients of temperature and salinity.

7.1.2 Direct gradients

Environmental factors that have a direct effect on physiological processes can be measured through the organism's response. Consequently it is often possible to determine boundaries and relationships of direct gradients. The physiological tolerance of adult *C. gigas* to environmental parameters such as temperature and salinity are fairly well documented as being wide and accommodating for introductions into new ecosystems (Korringa 1952; Mann 1979; Grizel & Héral 1991; Leffler & Greer 1991; Bougrier et al. 1995). However reproduction, larval and juvenile development, have much narrower tolerances, that can vary between countries (Steele & Mulcahy 1999; Chávez-Villalba et al. 2003; Diederich et al. 2005; Lango-Reynoso et al. 2006).

The effect of temperature on gametogenesis and spawning of *C. gigas* adapted to living on the south coast of England, were investigated through the comparison of monthly gonadal development and water temperatures logged at the site of sample collection. Through conducting this analysis the importance of nutrition on reproduction was implied however no direct measurements of phytoplankton or particulate organic matter were taken. Gametogenesis was found to initiate in *C. gigas* when water temperatures increased above 9.5 °C (approximately 1 °C than previously thought). Maturity was generally reached during the summer however spawning frequency differed between sites. Wild, intertidal *C. gigas* were found to spawn twice in a single reproduction season. Initially spawning was triggered through tidally induced temperature shocking as water temperatures increased above 18 °C. Subsequently a combination of warm water (+18 °C) and an increase of phytoplankton abundance stimulated a second spawning. Farmed, subtidal *C. gigas* spawned once, coinciding with the 2nd spawning of intertidal oysters. Site, whether the oysters were wild or farmed, and tidal height were confounded by the experimental design eliminating the opportunity to postulate further as to which variable (or combination) impacted the frequency of spawning.

Water temperature differed between Southampton Water and Poole Harbour (Appendix D). The average depth of the estuary combined with the percentage of the intertidal area will affect how readily atmospheric conditions are reflected in the water temperature. Consequently, to accurately calculate the likelihood of *C. gigas* spawning in a given estuary, the temperature profile of that estuary must be known. Furthermore, multiple years of water temperature data were taken during this study and showed that annual variability in water temperature could be sufficient to impact on reproduction. Therefore, if only one year of data is available, an understanding of whether that year represents a 'normal' year or whether or not it is colder or warmer than average, would be crucial.

7.1.3 Negative impacts

This study found juvenile life stages of *C. gigas* to be the most vulnerable to negative impacts. Consequently it is the negative impacts that effect recruitment, as opposed to adults, that have the greatest detrimental potential on the rate of wild *C. gigas* establishment.

C. gigas have become part of the food web, and as such suffer losses through predation. In particular, *C. gigas* are vulnerable to predation by the native brachyuran predator *Carcinus maenas,* whilst they are juvenile and until they are at least 50 mm in shell length. Predation pressure decreases after a size refuge from juvenile *C. maenas* (< 35 mm carapace width) is reached when *C. gigas* reach 20-25 mm shell length. Furthermore predation is reduced by

attachment to a hard substrate. This is of particular interest considering that that juvenile *C. gigas* are likely being predated from aquaculture plots where juvenile *C. gigas* are loose on the seafloor, and from wild aggregations, where juveniles are attached to hard substrate.

Environmental winter conditions typically experienced in the south of England negatively impact juvenile *C. gigas* that weigh less than 6 mg (flesh dry weight). Water temperatures of 7 °C initiated a stress mediating response. Such a response increases energy demand beyond energy supply, causing a diversion of energy away from somatic growth and immune responses, and a depletion of energy reserves (Columbo et al. 1990; Li & Vandepeer 2004; Lannig et al. 2010). During this study the juvenile *C. gigas* were acclimated for a 5 month period at 7 °C and mortality rates remained low. However this was most likely because food was not limiting and so the energy required to sustain a stress mediated response did not exceed energy supply. On the south coast of England, phytoplankton abundance is low during October through to March (Leakey et al. 1992; Iriarte & Purdie 1994; Iriarte & Purdie 2004; Franklin et al. 2012) and water temperatures are < 7 °C typically throughout December until March (Appendix D). Consequently substantial mortalities of juvenile *C. gigas* < 6 mg are likely to occur when water temperatures decrease below 7 °C.

Adult *C. gigas* are more resilient than juvenile *C. gigas* to lower water temperatures and showed comparable physiological adaptation to the taxonomically related native oyster species, *Ostrea edulis*. However this study found freezing aerial temperatures to be detrimental to adults through the freezing of water within the pallial cavity and the resulting prolonged periods of valve closure. It is suggested that freezing air temperatures subsequently result in a reduced condition of *C. gigas* as it is going into the reproductive period, which results in a decrease in reproductive output. Furthermore there is the potential for freezing injuries as a result of freezing within the pallial cavity, making extended periods of freezing air temperatures likely to result in mortalities of *C. gigas*. The internal pallial temperatures were measured for adult *C. gigas* only, however due to the increased thickness of adult shells in comparison to the juveniles shell, it can be assumed that adults have a higher tolerance. The shell of *C. gigas* was found to offer limited protection to the soft tissue. As well as the pallial cavity temperature decreasing below freezing (having a prolonged effect on feeding, excretion and aerobic respiration), rapid temperature changes were experienced during a tidal cycle that have the potential to cause shock and mortality. Between 2005 and 2015 there have been 4 severe winters, where average monthly water temperatures have been below 7 °C for 3 consecutive months, and 3 mild winters, during which average monthly water temperatures have remained above 7 °C.

This study found adult *C. maenas* to exert the greatest predatory pressure per individual. However juvenile *C. maenas* are present in greater abundance and inhabit the intertidal zone where *C. gigas* are found, unlike adult *C. maenas* that are more typically found subtidally. Therefore greater predatory pressure is likely exerted on *C. gigas* by juvenile *C. maenas* than adult *C. maenas* (Figure 7.2). Winter conditions impact upon *C. maenas* recruitment and abundance in the intertidal zone (Figure 7.2). *C. maenas* recruitment occurs intertidally during the summer (0-group) and can be delayed by up to 6 weeks following a severe winter (Beukema 1991). If the preceding winter is mild however, early recruitment is able to reach a carapace width of 20 mm by autumn at which point they feed less on meiofauna and predate more bivalve spat (Klein-Breteler 1975a; Davies et al. 1980; Dare et al. 1983). Some of the 0-group juveniles will migrate with the tide, limiting predation within the intertidal zone to high tides. These individuals often migrate further offshore staying subtidal throughout the winter. For those that remain in the intertidal zone throughout winter, it is thought that their activity decreases as water temperatures decrease below 7 °C and feeding ceases below 3 – 4 °C (Eriksson & Edlund 1977). Juvenile abundance increases within the intertidal zone in spring as last year's 0-group, now this year's 1-group, return from deeper waters. As with recruitment the return of juveniles occurs much earlier after a mild winter than a severe winter (Beukema 1991). Consequently during a mild winter when *C. gigas* recruitment is likely to be greatest, there will also be the greatest predatory pressure from *C. maenas.* Likewise, severe winter temperatures will hamper both the recruitment of *C. gigas* and *C. maenas* and predatory pressure will be greatly reduced. 1-group *C. maenas* present the greatest predation risk as a result of their size (20-35 mm carapace width) and high abundance (Klein-Breteler 1976; Dare & Edwards 1981; Walton et al. 2002; Yamada et al. 2005) and consequently predatory pressure is greatest during the spring and summer following a mild winter. However *C. gigas* are vulnerable to predation from juvenile *C. maenas* for a relative short period, outgrowing this risk within their first spring. Consequently the optimal conditions for *C. gigas* recruitment with respect to minimising predation from *C. maenas* would be a severe winter followed by a mild winter. The initial colder winter would be detrimental to recruitment of both species but allow adult *C. gigas* to build-up carbohydrate reserves. The following mild winter would promote high levels of recruitment in both species. Reproductive output of *C. gigas* would be increased as a result of the increased reserves built up the previous winter, and predation pressure from 1-group *C. maenas* would be low.

C. maenas rarely visit the intertidal zone to predate after their second year and therefore wild recruitment of *C. gigas* that primarily colonises the intertidal zone is largely unaffected by mature adults. However few adult *C. maenas* will likely continue to migrate with the tide (Dare & Edwards 1981). Males pose the greatest risk to *C. gigas* because sexual dimorphism results in

enlarged chelae and their ability to predate a wider size range of prey. Males predate all year with a dip in feeding activity occurring during the summer (Hayden et al. 2007). Females reduce feeding over the winter which then increases again in spring (Hayden et al. 2007).

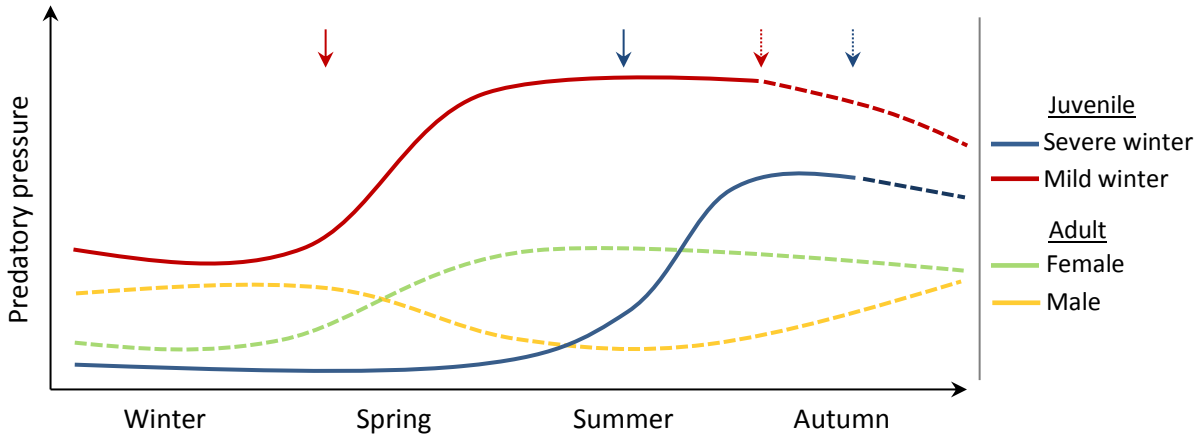


Figure 7.2 A schematic of predatory pressure of *Carcinus maenas* on *Crassostrea gigas* inhabiting the intertidal zone. Solid lines represent crabs that permanently inhabit the intertidal zone. Dashed lines represent crabs that migrate into the intertidal zone with the tide.

7.2 Future Work

The conceptual frame work suggested for the production of a model, to predict the future geographic spread of feral *C. gigas* within estuaries on the south coast of England, contains a number of variables that were not measured during this study. Some, such as the direct effect of salinity and pH on the condition of *C. gigas* have been researched previously, and consequently a sufficiently evidenced model term could be generated through a literature review (Brown & Hartwick 1988; His 1989; Calvo et al. 1999; Kurihara et al. 2007). However novel relationships have been revealed, that remain uncharacterised and therefore the only way to generate an adequate model term would be by conducting further research.

The most notable, uncharacterised relationship, was that revealed between site and reproductive strategy. The addition of a partial spawn occurring earlier in the year in Southampton Water has great potential to increase recruitment as larvae develop and metamorphose in warmer, nutrient rich, waters and results in larger juveniles going into winter. Increasing their resilience to the cold and decreasing vulnerability to predation come spring. The relative impacts of temperature shocking and aerial exposure experienced in the intertidal zone are unknown, as is the potential

importance of nutrition on triggering spawning. Farmed, subtidal *C. gigas* inhabiting Poole Harbour spawned at a later date than wild, intertidal *C. gigas* inhabiting Southampton Water, during a period of declining temperatures. Previously it has been thought that spawning in *C. gigas* is triggered by water temperatures exceeding a threshold (Ruiz et al. 1992; Ren et al. 2003; Cardoso et al. 2007). It was most likely that spawning coincided with a plankton bloom suggesting this to be the trigger. Hence research into the effects of nutrition on spawning would greatly expand the current knowledge of *C. gigas* reproduction.

Furthermore there has been much speculation throughout this project as to the impact of winter temperatures on the condition of *C. gigas* and its consequential effect on reproductive output. The condition of *C. gigas* was found to be inversely related to stress, and those with the lowest condition were sampled from intertidal, as opposed to subtidal, habitats. *C. gigas* inhabiting the intertidal zone most likely have a lower condition because of reduced opportunities to feed, respire and excrete (Bougrier et al. 1998; Haure et al. 2003), however *C. gigas* more commonly inhabit the intertidal zone than the subtidal zone, and organisms adapt compensatory abilities to their niche (Gillmor 1982). Low condition reflects low energy reserves and may be fatal to an oyster if the reserves are not sufficient to survive low food levels over the winter. Mortality through depleted reserves is most likely common during a mild winter when the metabolic rate is not depressed by cold temperatures. Furthermore repercussions of a detrimental winter are felt during the subsequent reproductive season when there would be fewer reproducing adults (as a result of mortality) and those that survived will have a much lower reproductive output (Honkoop & Beukema 1997). Consequently there is scope for further work into the relationship of water temperature and metabolic depression and in particular looking to quantify at what water temperature the metabolism becomes sufficiently depressed to allow *C. gigas* to survive extended periods of low food without a notable decrease in condition, and subsequent reduction in reproductive output.

Finally, the negative impact of fishing activities is relatively unknown. During this study all subtidal oysters were sampled from aquaculture plots that regularly undergo mechanical disturbance, whereas all intertidal oysters were sampled from feral aggregations. Although illegal fishing activities do take place in both Poole Harbour and Southampton Water, this involves hand picking select oysters and consequently there is little disturbance to unfished oysters. How much impact the mechanical disturbance has on the condition and reproductive output of *C. gigas* is unknown. The scale of unregulated fishing became evident during surveys of Southampton Water. Piles of shucked shells were routinely found in the intertidal zone during spring low water (Appendix C). Fishing activities target adult oysters and unregulated fishing activities appear to impact substantially on *C. gigas.* Consequently, a model that focuses primarily on the predicted

distribution of future recruitment has no requirement for the inclusion of a fishing term, however if the rate or degree of feral establishment is important then a fishing term is necessary. Quantifying the impact of an unregulated fishery however, is difficult as many perpetrators will be unwilling to cooperate or be forthright with information. The size and frequency along the shore of piles of shucked shells has increased throughout the period of this study and there has been a trend from fishing during the day to much of the fishing being carried out at night (pers. obvs.). This is a dangerous development as a large proportion of the intertidal zone is mudflat and so the risk of becoming stuck is high. Furthermore it renders shore observations to estimate unregulated fishing effort virtually impossible.

7.3 Application

The Pacific oyster, *Crassostrea gigas*, has a global distribution largely due to its popularity within the aquaculture industry. The spread of established feral oysters is expanding as naturalisation occurs in an increasing number of countries. The invasive nature of *C. gigas* means that there is the potential for serious and negative economic and ecological impacts as a result of naturalisation. Consequently there is great interest in understanding how reproductive output and recruitment is affected by environmental conditions both by the oyster industry which relies on a year round supply of seed, and by environmentalists who want to understand the risk to environmental degradation that wild populations pose (Syvret et al. 2008; Herbert et al. 2012).

The Pacific oyster industry is economically important to the UK and likely to persist, and grow, despite the associated risks. Therefore efforts directed toward reducing the risks of establishment and spread of *C. gigas* are pertinent. All aquaculture practices are required to adhere to the recommendations, procedures and practices put forth in the ICES Code of Practice on the Introductions and Transfers of Marine Organisms (ICES 2005). The code was created (in 1973) to provide guiding principles during the initiation of mariculture, with particular attention to three challenges that had been identified through past experiences; (I) The ecological and environmental impacts of introduced and transferred species, (II) their potential genetic impact if allowed to reproduce with native species, and (III) the inadvertent coincident movement of harmful organisms associated with the target (host) species. The same three challenges are still true today and the ICES Code of Practice 2005 now includes the ongoing introductions of transfers which have been an established part of commercial practice, and has a goal of reducing the spread of exotic species (ICES 2005).

Research for this thesis has provided an understanding of how environmental parameters and biological interactions relate to reproductive output and recruitment of *C. gigas,* and has

facilitated naturalisation along the south coast of England. Potential impacts of the results from each chapter have been demonstrated through the description of a predictive model for the future geographic spread of *C. gigas* within the UK. Such a model could prove to be a useful tool when applying for aquaculture of *C. gigas.* It is necessary to submit a prospectus detailing, amongst other things, the target area of release, a review of the biology and ecology of the species (physical, chemical and biological requirements for reproduction and growth, and natural and human-mediated dispersal mechanisms), and information on the receiving environment. Previously it has been necessary to rely, in large, on research conducted in other countries despite knowing that *C. gigas* exerts phenotypic plasticity that results in localised adaptation (particularly in reproduction, growth and condition). In particular, new figures for the initiation of gametogenesis can be used in conjunction with the concept of degree days to assess whether maturity will be reached and the potential for spawning.

If a fine scale mechanistic model to predict the spread of *C. gigas* were to be realised, it could be used as a tool in assessing the probability of establishment occurring, a fundamental step in the risk assessment. Furthermore, if the recommendations for estuary scale resolution were taken into consideration there is the potential to predict recruitment location with far greater detail and to produce an estimated abundance scale. This information would allow for an improved assessment of the consequences of establishment in the receiving environment. Mitigation factors and management issues are also reviewed as part of the application, and being able to forecast potential sites of establishment with a scale of abundance, allows a more detailed and economic plan to be put into place. For example a robust mitigation plan consuming many people hours and the use of expensive equipment may be necessary if recruitment was forecasted to be abundant and widespread, but unnecessary if it were to be sparse and irregular.

Many risk assessments for the introduction of non-native species focus heavily on reproductive potential, and whilst this is a key component for establishment to occur, this study has highlighted the importance of considering negative impacts that could disrupt and dampen recruitment success. Predation and cold intolerance were found to have the potential to impact considerably on *C. gigas* recruitment, but to differ between estuaries. Both factors are considered to be particularly relevant for intertidal species establishing in productive estuaries, where extreme temperature fluctuations can be experienced along with a considerable array of permanent and migrating predators.

The importance of using an up to date habitat type map, with resolution to shore height was highlighted by the colonisation of hard substrate predominantly in the intertidal zone. Anthropogenic structures are readily colonised by *C. gigas,* and it is therefore important to note

their presence as available potential substrate. This is particularly true in areas of otherwise unsuitable settlement substrate where they can act as stepping stones to further distribution. This study is in agreement with the previous work by Brandt et al. (2008) that larvae typically settle within 10 km of the source and emphasises the need to classify all source points of propagule pressure (e.g. multiple lay-out areas in an estuary, or the presence of a nursery). The prominence of hydrodynamic regimes and larval behaviour on dispersal has been alluded to throughout this study and is reiterated here (and proven using Manilla clam larvae by Herbert et al. (2011)). Flushing rates and tidal regimes may remove larvae from an estuary before they are able to settle, and may under some circumstances, be the overriding determining factor for that species establishing. Eno (1994) suggested this to be the case for *C. gigas* in Fleet Lagoon, and this study postulates hydrodynamics to be a leading cause for the sparse recruitment in Poole Harbour.

The majority of seed *C. gigas* used in UK aquaculture is produced in hatcheries (Syvret et al. 2008; Herbert et al. 2012), with some wild seed collected on the east coast (McKnight 2009). As water temperatures continue to rise as a result of global climate change (MCCIP 2011; IPCC 2014), it is likely that wild seed could provide for a greater number of aquaculture sites. Positioning spat collectors to maximise yield is important if it is to be a viable economic venture. Finding the best locations through trial and error can take many years, however with the use of a model, such as is being discussed, collection sites could be predicted with relative confidence. The use of wild seed could save independent oyster farms money, and potentially provide the basis for a new industry in the UK.

7.4 Conclusions

Feral aggregations of *Crassostrea gigas* are present along the south coast of England and this study set out to explain the lack of *Crassostrea gigas* recruitment in Poole Harbour, an estuary on the south coast of England with notable aquaculture production of *C. gigas.* Southampton Water was chosen as a comparable case study due to its close proximity to Poole Harbour, the lack of aquaculture (and so *C. gigas* propagule pressure) and sightings of *C. gigas* on its shore.

The location of *C. gigas* being farmed in Poole Harbour in relation to tidal height and tidal currents, the more extreme temperature regime, and the greater predatory pressure experienced have all contributed to the low occurrence of naturalisation. Conversely the speculated introductory point at the head of the estuary, the minimal predatory impact and the abundance of suitable settlement substrate accessible to larval distribution, has allowed feral *C. gigas* to

establish over a large area in Southampton Water. Furthermore the naturalisation in the intertidal has insured regular recruitment that is likely to expand the abundance over time.

This study has expanded the base-line knowledge of *C. gigas* distribution to include Southampton Water and Poole Harbour, and investigates the impacts that annual water temperatures and predation have on reproduction and recruitment. The knowledge and information gained will enable a better understanding of how establishing *C. gigas* will develop in the future, with particular applicability to England, and has the potential for use in predictive models and other conservation and aquaculture tools.

Predicting the introduction of a particular species is extremely difficult and to predict whether subsequent establishment or invasiveness will follow, requires detailed knowledge of the species biology as well as the ecology, environment and hydrodynamics of a particular site (Carlton 1996b; Maggs et al. 2010). This study culminates in the proposal of a conceptual framework for a mechanistic model with a fine resolution. Such a model includes indirect gradients that allow the potential geographic locations that *C. gigas* could establish to be identified, direct gradients that determine whether *C. gigas* are able to reproduce, the potential reproductive output and potential for recruitment, and negative impacts that act to slow the rate of establishment through dampening recruitment success, reducing reproductive output and causing mortality. It is hoped that, if realised, such a model could benefit the economy through reducing potential detrimental impacts associated with invasive aggregations of *C. gigas* and through the generation of jobs in selecting future aquaculture sites, highlighting potential recruitment hot spots for conservation management or alternatively collection of spat for the aquaculture industry.

Appendices

Appendix A

Site descriptions entered into the linear regression model used to calculate which physical environ mental factors had the greatest influence on *Crassostrea gigas* distribution and abundance (Results: Chapter 3.3.2).

Estuary	Site	General description	Substrate	Abundance
Southampton Water (Residence time: 13 days)	Woolston	Woolston is at the head of Southampton Water on the eastern shore where the River Itchen joins. The average shore width is 60 m. The channel at high water is approximately 0.5 km wide. A pier structure bisects the site. To the north of the pier public access is restricted by Woolston Wastewater Treatment Works. To the south access is promoted by the presence of paths and carparks. Recreational anglers frequent the shore as do unregulated hand pickers of shellfish. Southampton Sailing club has a concrete slipway traversing the upper to middle shore.	**Upper**: Coarse sediment **Middle:** Mixed sediment **Lower:** Mud	Upper: 1 Middle: 5 Lower: 3
	Woolston Pier	A wooden structure that houses pipes from the upper intertidal out to the dredged channel of Southampton Water. No public access.	**Upper**: Man **Middle:** Man **Lower:** Man	Upper: 5 Middle: 5 Lower: 5
	Netley	Netley is mid-way between the River Itchen and the River Hamble on the eastern shore of Southampton Water. The estuary channel at high water is approximately 2.5 km and the shore at low tide has an average width of 270 m. Manmade sea defence structures restrict high tide resulting in the whole beach being submerged. There are 2 concrete slipways on Netley shore. One reaches out into the middle shore and the other only covers the upper shore.	**Upper**: Coarse sediment **Middle:** Mixed sediment **Lower:** Mud	Upper: 1 Middle: 6 Lower: 3

Estuary	Site	General description	Substrate	Abundance
Southampton Water (Residence time: 13 days)	Hamble	Hamble shore is located where the River Hamble joins Southampton Water on the eastern shore. The channel is approximately 2 km wide at high tide and the average shore width at low tide is 440 m. The sampling site was bordered by a pier to the west and Hamble Marina and the River Hamble to the east. Hamble is opposite Fawley Oil Refinery (on the western shore) however free parking and easy access makes this shore popular with families and dog walkers.	**Upper**: Coarse sediment **Middle:** Mixed sediment **Lower:** Mud	Upper: 1 Middle: 4 Lower: 3
	Hamble Pier	A wooden structure that houses pipes from the upper intertidal out to the dredged channel of Southampton Water. No public access.	**Upper**: Man **Middle:** Man **Lower:** Man	Upper: 5 Middle: 5 Lower: 5
	Hill Head	Hill Head is located at the mouth of Southampton Water where the estuary is no longer confined and the nearest land is Calshot spit on the western shore (~5.5 km) and Cowes, Isle of Wight (~6 km). The shore is the widest sampled at an average width of 262 m. A sailing club, paved promenade and beach huts line the beach which is popular with families and dog walkers. Numerous unregulated handpickers of shellfish have been seen here.	**Upper**: Coarse sediment **Middle:** Mixed sediment **Lower:** Mixed sediment	**Upper**: 1 **Middle:** 1 **Lower**: 3
Poole Harbour (Residence time 7 days)	Blue Lagoon	Blue Lagoon is a small, shallow, enclosed mudflat on the north shore of Poole Harbour. *Crassostrea gigas* were present only on a patch of shell and shingle accreted on the inside of a meandering channel. The exposure index is 8.53 and the average shore width is 248 m.	**Upper**: Mud **Middle:** Mud **Lower:** Mixed sediment	**Upper**: 1 **Middle**: 6 **Lower**: 6

	Site	General description	Substrate	Abundance
Poole Harbour (Residence time 7 days)	Dolphin Marina	Dolphin Marina is located on the northern shore of Poole Harbour. *Crassostrea gigas* were attached to floating pontoons. The marina is sheltered, with an exposure index of 9, and the width of the marina is on average 86 m.	Man	3
	Rockley Point	Rockley Point is an accretion of mixed substrate on the inside of Rockley Channel as it leaves Lytchett Bay. There is a sailing club at the mouth of Lytchett Bay and limited access to the beach. The exposure index was 19.58, and the average shore width was 279 m. All *C. gigas* were found near the sailing club.	**Upper**: Shingle **Middle:** Mixed Sediment **Lower:** Mixed sediment	**Upper**: 1 **Middle**: 3 **Lower**: 3
	Holes Bay	Holes Bay is a semi-enclosed Bay on the north shore of Poole Harbour containing saltmarsh, mudflat and a marina. The eastern edge is protected by sea-defence boulders and the western edge is predominantly saltmarsh with a number of private pontoons. The exposure index is 28.95 and the average shore width is 800 m. The Channel in to Holes Bay is dredged and commercialised. The site is popular with bait diggers.	**Upper**: Man **Middle:** Mud **Lower:** Mud	**Upper**: 1 **Middle**: 1 **Lower**: 1
	Ower Bay	Ower Bay is on the southern shore of Poole Harbour. It is a mudflat fringed by reeds and designated a bird sensitive area. The exposure index is 30.08 and the average shore width is 299 m. Access is via private road.	**Upper**: Plant **Middle:** Mud **Lower:** Mud	**Upper**: 1 **Middle**: 2 **Lower**: 1
	Parkstone	Parkstone is an embayment on the northern shore of Poole Harbour with a number of boat yards and marinas. The banks have been reinforced with concrete sea defences and the intertidal is mudflat. The exposure index is 39.96 and the average shore width is 236 m.	**Upper**: Man **Middle:** Mud **Lower:** Mud	**Upper**: 1 **Middle**: 1 **Lower**: 1

Estuary	Site	General description	Substrate	Abundance
Poole Harbour (Residence time 7 days)	Newton Bay	Newton Bay is on the southern shore of Poole Harbour. It is a mudflat fringed by reeds and designated a bird sensitive area. The exposure index is 49.75 and the average shore width is 499 m. Access is via private road, with restrictions around Goathorn Point.	**Upper**: Mud **Middle:** Mud **Lower:** Mud	**Upper**: 1 **Middle**: 1 **Lower**: 1
	Arne Bay	Arne Bay is an embayment of Arne peninsular, a quiet zone containing an RSPB site. The peninsular is at the back of Poole Harbour separating Wareham Channel from the harbour itself. Access to the shore is through the RSPB site and so limited. The shoreline is natural low bluff to shingle before becoming mud in the intertidal. The exposure index is 56.26 and the average shore width is 481 m.	**Upper**: Shingle **Middle:** Mud **Lower:** Mud	**Upper**: 1 **Middle**: 1 **Lower**: 1
	South Haven Point	South Haven Point is at the mouth of Poole Harbour. The sandy beaches and easy access makes this area popular with tourists and anglers fish the fast waters of the channel. There is a chain ferry across the mouth of the harbour. The exposure index is 75.92 and the average shore width is 240 m.	**Upper**: Sand **Middle:** Sand **Lower:** Plant	**Upper**: 1 **Middle**: 1 **Lower**: 1
	Rockley Channel	The Rockley Channel site extends along the northern shore of Poole Harbour to the east of the Lytchett Bay opening. There are low cliffs that are protected by gabions and access to the shore is only feasible from either end at low tide. The exposure index is 83.79 and the average shore width is 163 m.	**Upper**: Man **Middle:** Shingle **Lower:** Mud	**Upper**: 1 **Middle**: 1 **Lower**: 1
	Whitley Lake	Whitley Lake is a sandy stretch containing Eelgrass that stretches from the opening to Poole Harbour round to Salterns Marina. With ample parking and a designated watersports area, this is a very popular area. The exposure index is 86.64 and the average shore width is 840 m.	**Upper**: Sand **Middle:** Sand **Lower:** Plant	**Upper**: 1 **Middle**: 1 **Lower**: 1

Estuary	Site	General description	Substrate	Abundance
Poole Harbour (Residence time 7 days)	Outer Blue Lagoon	Much of the shoreline outside the entrance to Blue Lagoon is accessed by private land only. You can however walk from Salterns Marina at low tide. The shore is fully submerged at high tide with a manmade concrete and gabion breakwater separating Blue Lagoon from the main body of Poole Harbour. The shore is mostly mixed muddy sediment however an area of compact sandy mixed sediment is present adjacent to Salterns Marina. Occasionally the muddy area is dug over by bait diggers and anglers occasionally fish off of the breakwater. The exposure index is 88.46 and average shore width is 270 m.	**Upper**: Mixed sediment **Middle:** Mixed sediment **Lower:** Mixed sediment	**Upper**: 1 **Middle**: 1 **Lower**: 1
	Lake Drive	The area of shore accessible by Lake Drive is bordered by Rockley Pier to the west and fenced off from a Marines base to the east. A number of small fishing vessels launch from this site. The exposure index is 102.76 and the average shore width 69 m.	**Upper**: Shingle **Middle:** Mud **Lower:** Mud	**Upper**: 1 **Middle**: 1 **Lower**: 1
	Lake Drive slip ways	There are 2 short concrete slipways only extending into the upper intertidal shore.	**Upper**: Man **Middle:** N/A **Lower:** N/A	**Upper**: 2 **Middle**: N/A **Lower**: N/A
	Baiter Point	Baiter Point is the main public slipway used in Poole Harbour. There is ample parking, a recreation area and facilities. Furthermore there is a footpath running parallel to the beach. The site is on the northern shore of Poole Harbour and the slipway extends to the lower intertidal, giving access at all stages of the tide into the North Channel and designated personal watercraft area. The exposure index at this site is 126.73, and the average shore width is 330 m.	**Upper**: Shingle **Middle:** Mud **Lower:** Mud	**Upper**: 1 **Middle**: 1 **Lower**: 1

Estuary	Site	General description	Substrate	Abundance
Poole Harbour (Residence time 7 days)	Hamworthy	Hamworthy is an area to the west of Poole with a large recreation area and numerous beach huts. The shore is easily accessible and consequently popular with dog walkers, beach goers and recreational anglers. The mixed sediment intertidal is frequently dug over by bait diggers making it treacherous under foot. The sites exposure index is 166.73, and the average shore width is 391 m.	**Upper**: Shingle **Middle:** Mixed sediment **Lower:** Mud	**Upper**: 1 **Middle**: 3 **Lower**: 1
	Hamworthy	A series of wooden groins maintain the beach. They are relatively short measuring approximately 25 m and reaching from the promenade above high water to the mid intertidal.	**Upper**: Man **Middle:** Man **Lower:** N/A	**Upper**: 1 **Middle**: 3 **Lower**: N/A
	Moriconium Quay	Adjacent to the entrance for Moriconium Quay Marina Village is a small area of shore that is accessed via a dead-end road. The small size and discrete access has resulted in the shore predominantly being used by local people with the majority being fishermen. The exposure index is 179.15, and the average shore width is 99 m.	**Upper**: Shingle **Middle:** Mixed sediment **Lower:** Mud	**Upper**: 1 **Middle**: 3 **Lower**: 1

Appendix B

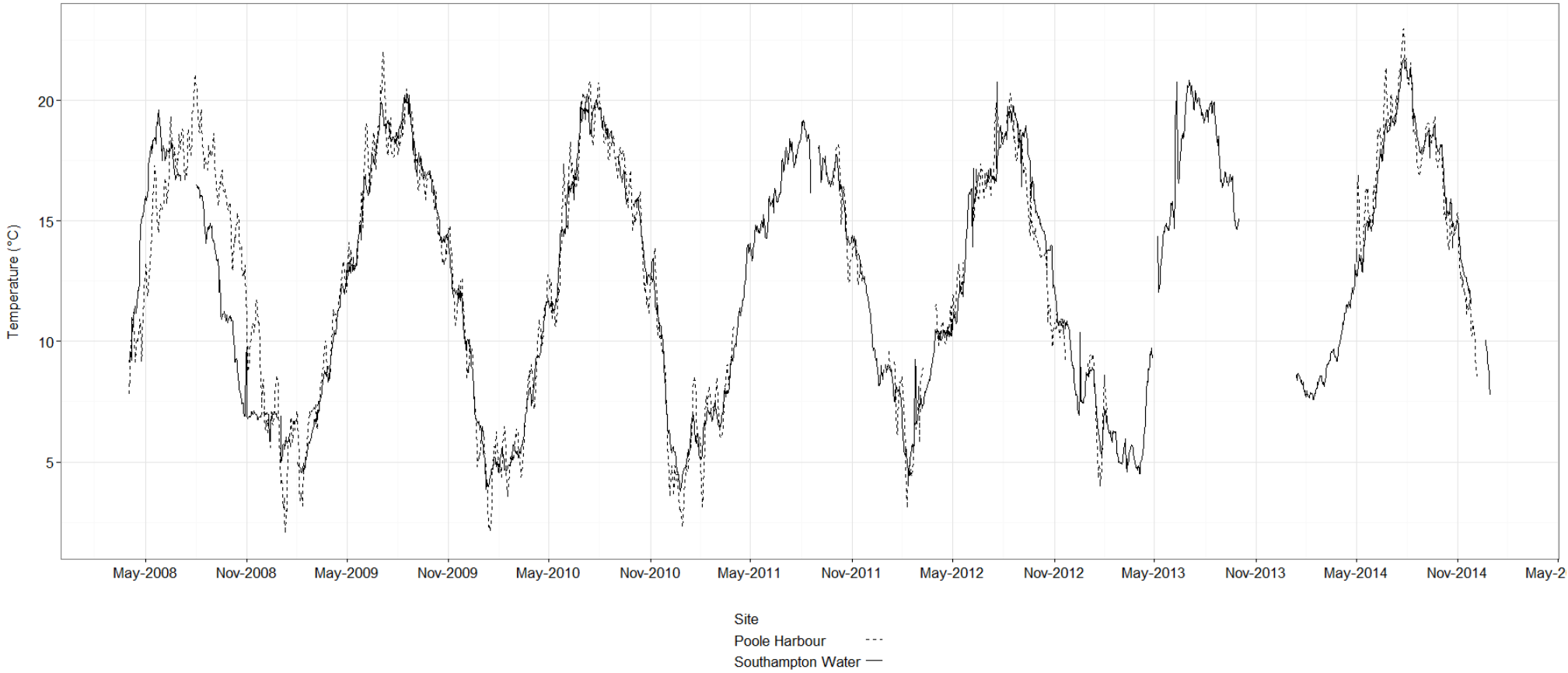
Crassostrea gigas fouling the hull of yachts lifted from Southampton Water. Yacht 1 = top image (Penny diameter = 20 mm) (10.12.2014). Yacht 2 = middle & bottom images (no scale available) (10.12.2014).

Appendix C

Large piles of shucked *Crassostrea gigas* shells along Weston shore. This pile was found between Woolston and Netley and contained > 100 oysters (18.03.2016).

Appendix D

Temperature plotted as daily averages from data recorded at 15 minute intervals.



Appendix E

A series of Mark Release Recapture experiments were carried out in Poole Harbour and Southampton Water in order to estimate the abundance of *Carcinus maenas* and to characterise the population.

Poole Harbour:

The *Carcinus maenas* population was sampled using two methods in Poole Harbour. Initially a mark, release, recapture experiment using baited pots was carried out in Dolphin Marina. Standard steel framed parlour traps 0.55 m in length were used, with 10 mm mesh, selective grill on the bottom and 2 x 80 mm fixed diameter, side entrances (Figure 1).

Figure 1: Steel framed parlour trap.

Half a mackerel was hung in a net bag immediately prior to the trap being sealed and placed in-situ. Traps were laid for 12 hours from 19:00 until 07:00. Carapace width (cw), a measurement from between the 4th and 5th anterolateral teeth, was taken using vernier calipers. Sex, colour and missing appendages were noted. One anterolateral spine was clipped to mark the animal before being released. The Jolly & Seber model was used to estimate population density.

The area of the Pacific oyster aquaculture plots exceeded the capacity of just two pots(Fisheries et al. 1978; Munch-Peterson et al. 1982), consequently the oyster harvester was used to sample *C. maenas.* An array of hydraulic jets 1 m wide forces epifauna onto a conveyor belt which is carried up onto the deck. Abundance was estimated using the speed of the boat, area of ground sampled and the duration.

Results:

A total of 234 individual crabs were caught using baited pots in Poole Quay Boat Haven. Of the animals sampled; 66 % were female, 11 % were male and 23 % were undeterminable either as a result of being immature (< 34 mm carapace width) or parasitized by *Sacculina carcini*. The median size for both males and females was 40-45 mm carapace width (Figure 2). A population density estimate of 1.11 crabs per m^2 was calculated from the Jolly & Seber method (Table 1)(Jolly 1965; Seber 1965).

Carcinus maenas were collected from aquaculture plots during 5 months between November 2012 and July 2014. 3308 crabs were measured and sexed. In all months, except March 2014, the size class 35 – 40 mm was most abundant. During March 2014 immature crabs were more abundant than mature crabs, with the most abundant size class being 25 – 30 mm (Figure 3). The density of crabs on the aquaculture plot varied over the year with the minimum density occurring in March and the maximum in September and October (Table 2), correlating with water temperature.

Table 1: Jolly-Seber calculations for the estimated population size of *Carcinus maenas* at Town Quay Boat Haven, Poole Harbour.

Sample	$\widehat{M_t}$	$\widehat{\alpha}_t$	Population estimate (crabs per hectare)
1	19.05	0.13	**146**
2	41.56	0.08	**1186**
3	185.56	0.13	**1781**
4	456.25	0.28	**1600**
5	686.00	0.29	**2429**
6	447.75	0.38	**1608**

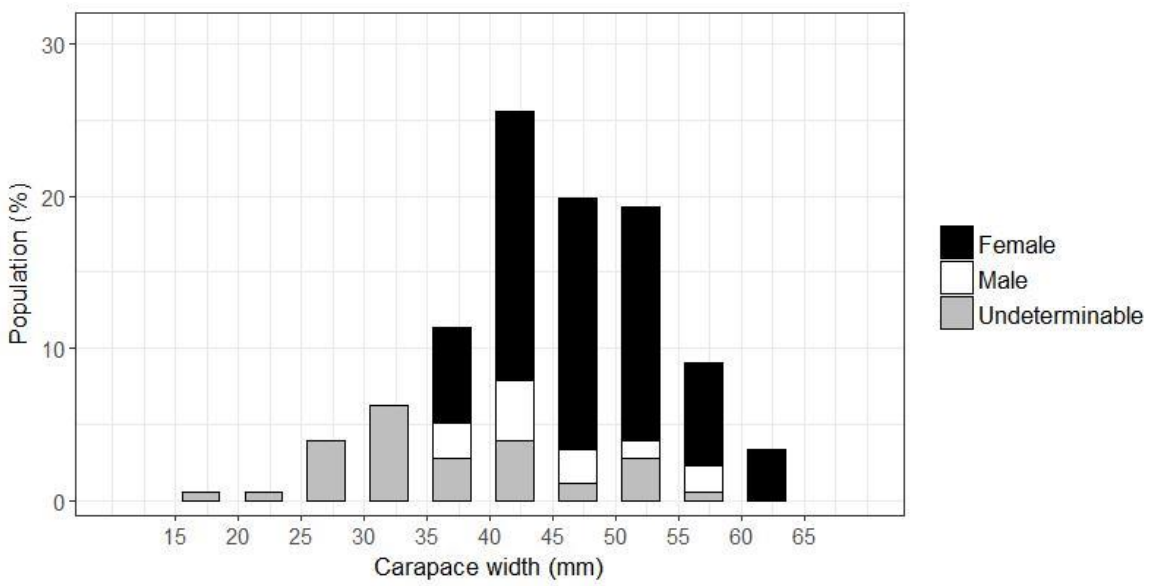


Figure 2: Size frequency distribution and relative abundance of crab sex per size class of *Carcinus maenas* sampled from Town Quay Boat Haven, Poole Harbour. n = 234.

Table 2: Population size of *Carcinus maenas* inhabiting the *Crassostrea gigas* aquaculture plots in Poole Harbour estimated using data collected using an oyster harvester.

Date	Harvesting swath (m)	Speed (knots)	Duration (minutes)	Crabs collected	Population density estimate
Nov-2012	1	1.5	45	532	260 crabs per hectare
Sept-2013	1	1.5	30, 10	638, 288	500 crabs per hectare
Oct-2013	1	1.5	30, 10	382, 395	500 crabs per hectare
Mar-2014	1	1.5	15, 15	124, 214	243 crabs per hectare
July-2014	1	1.5	20, 20	300, 435	429 crabs per hectare

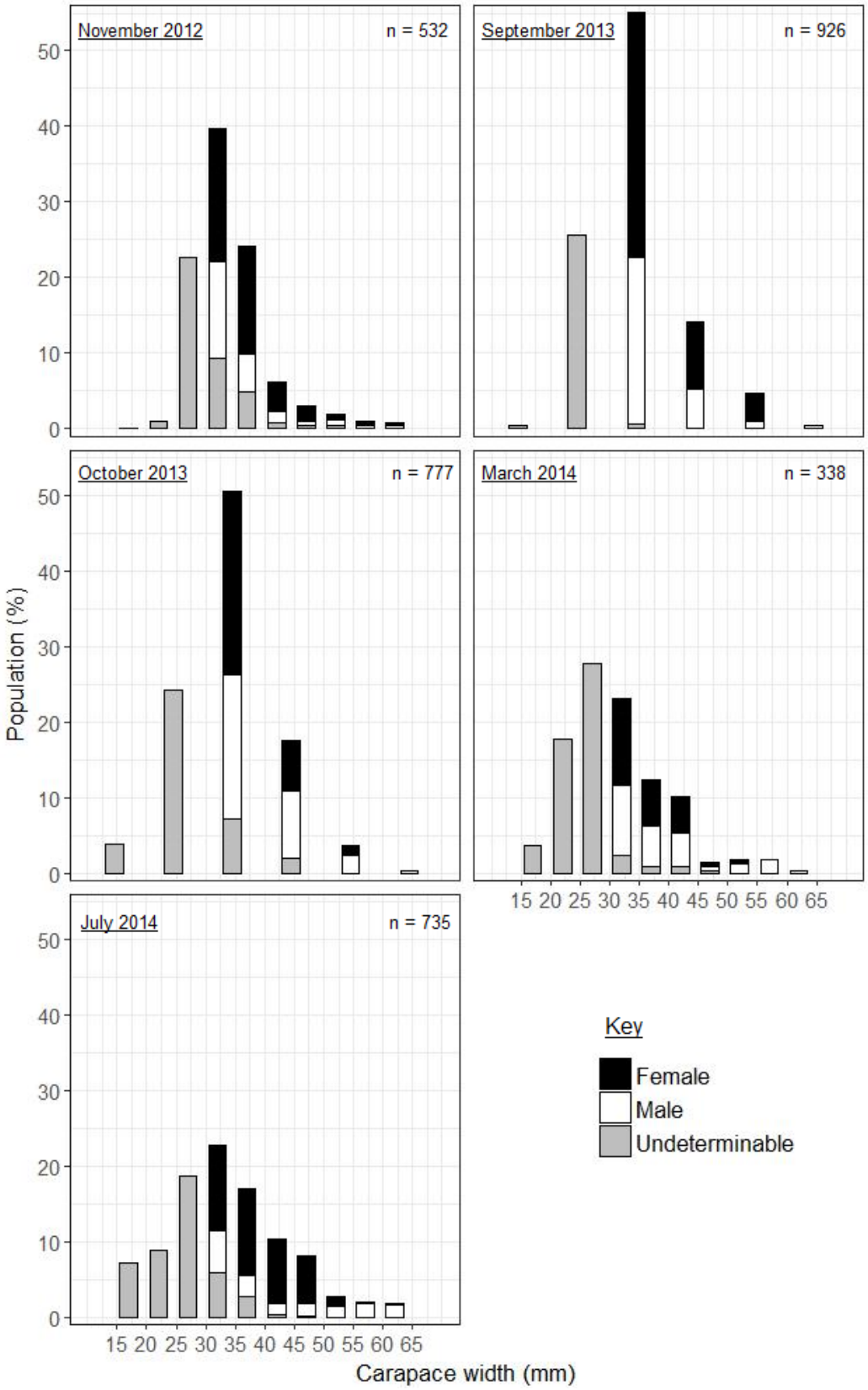


Figure 3: Size frequency distribution and relative abundance of crab sex per size class of *Carcinus maenas* sampled from aquaculture plots in Poole Harbour.

Southampton Water:

Only 33 crabs were caught using baited pots at Woolston in Southampton Water. Of the animals sampled; 19 % were female, 42 % were male and 39 % were undeterminable as a result of being immature (< 34 mm carapace width). No crabs were found with extrusions from *Sacculina carcini*. The median size for both males and females was 35-40 mm carapace width (Figure 4). Too few crabs were caught and no individuals were recaptured, therefore the Jolly & Seber method could not be used to estimate density.

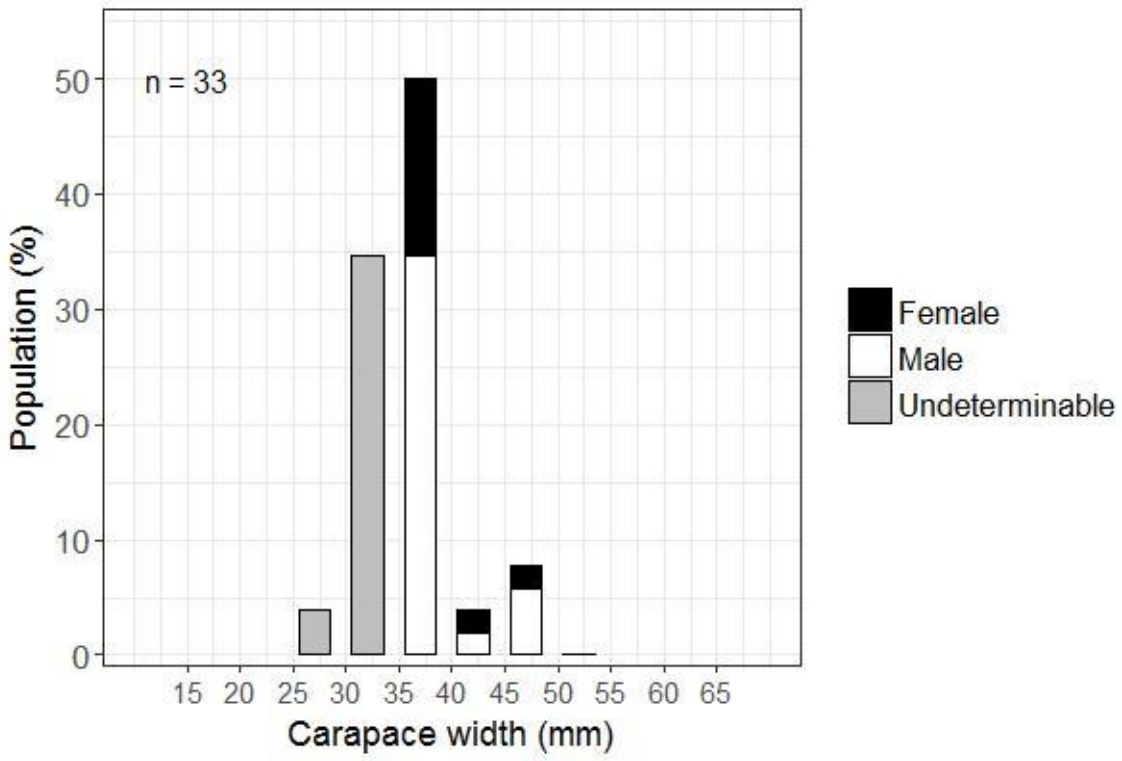


Figure 4: Size frequency distribution and relative abundance of crab sex per size class of *Carcinus maenas* sampled from aquaculture plots in Southampton Water.

Appendix F

The shells of *C. gigas* predated during laboratory feeding experiments were collected for further analysis. The damage caused during predation left scars that could be used to speculate on the feeding behaviours used by *C. maenas*.

The majority of attacks were focused on the umbo/hinge region of the spat, and in many instances the only notable damage to the spat was localised at the hinge (Figure 1).

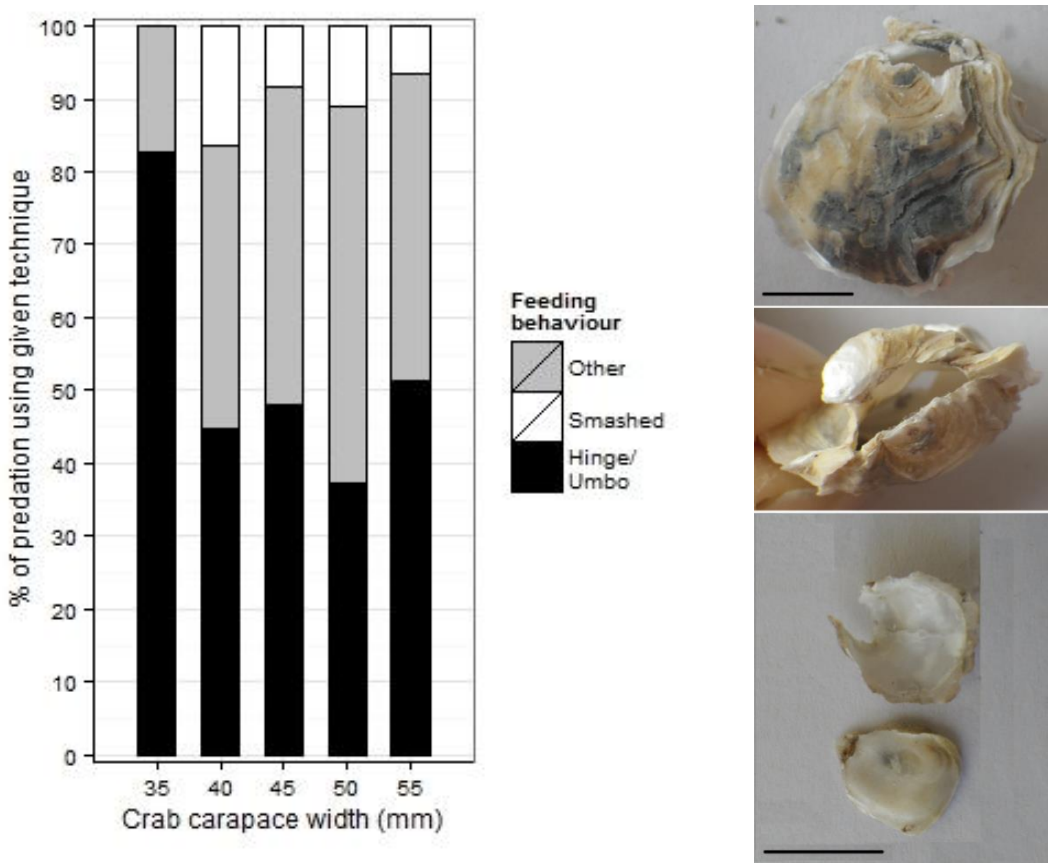


Figure 1 The percentage of predated *Crassostrea gigas* shells that had damage localised to the umbo/hinge region compared to all other regions combined (other) and shells that were crushed to the degree that no target area was definable (crushed). Images show examples of umbo/hinge localised damage. Scale bar = 10 mm.

Both the size of the spat and whether it was fixed to a tile was found to influence the locality on the shell most frequently targeted by *C. maenas*:

Small attached *C. gigas* (shell length 6-16 mm): Upper valve was removed intact (or crushed by larger crabs >45 mm) leaving the lower valve attached to the tile with evident damage to the umbo/hinge (Figure 2).

Small unattached *C. gigas* (shell length 6-16 mm): Both valves crushed. Some instances of localised damage to the umbo/hinge region, and holes made above the abductor muscle in the cupped valve (Figure 2).

Large attached *C. gigas* (shell length 20 - 40 mm): Lower valve broken in half ventrally whilst the upper valves remained relatively undamaged (Figure 4).

Large unattached *C. gigas* (shell length 20 - 40 mm): Chipping to the fringe of the shell along the anterior and posterior edges (Figure 4).

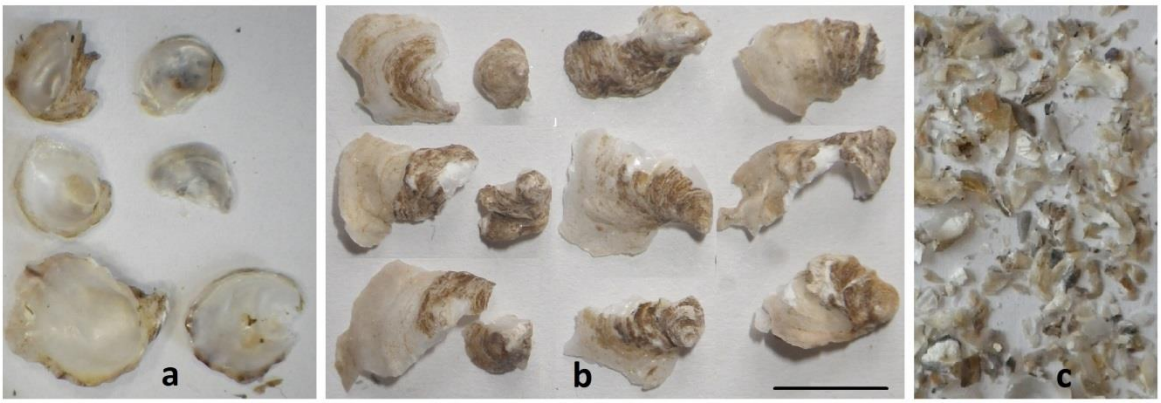


Figure 2 Examples of shell damage most frequently experienced by small juvenile *Crassostrea gigas* (6-16mm shell length). **a**: *C. gigas* that were fixed to a tile – damage minimal and localised on the cupped valve (left), flat valve (right) chipped away at the fringe (top), broken in half (middle) and missing an umbo (bottom). **b**: loose *C. gigas* – cupped valves broken in half with no recovered flat valves. **c**: fragmented shell was found in feeding experiments with fixed and loose *C. gigas.* Scale bar = 10 mm.

Particular damage was seen repeatedly throughout the experiment suggesting feeding behaviours typical of *C. maenas:*

Hole below the umbo:

A hole was consistently made in the cupped valves of small and large *C. gigas* spat by crabs of all sizes (Figure 3). This feeding behaviour was only exhibited on cultchless spat suggesting a weakness in the shell structure that cannot be exploited when spat have their cupped valve cemented to the substrate. A hole could have been made by *C. maenas* boring with the cutter chelae, however boring is associated with long handling times and high energy demands, and typically only used when the prey is too large to be crushed (Klein-Breteler 1975a; Elner 1978; Elner & Hughes 1978).

C. gigas below the crushing threshold were repeatedly found with holes in the cupped valves. This may be the result of the more common feeding behaviour of crushing, as *C. gigas* shells contain chalky patches that prevent the shell from fracturing (Taylor & Layman 1972; Cheong-Song & Yong-Wan 2000). Crabs commonly open hard-shelled prey using repeated application of pressure to the weakest point of the shell (Klein Breteler 1975; Elner 1978; Elner & Hughes 1978). Pulsing is an energy efficient way of fracturing the shell that eventually breaks, however the differing shell structure of *C. gigas* may instead, create a hole at the point of crushing. Although this was a consistent feeding behaviour used on loose spat, it only accounted for 12 % of small spat predated and 7 % of larger spat.

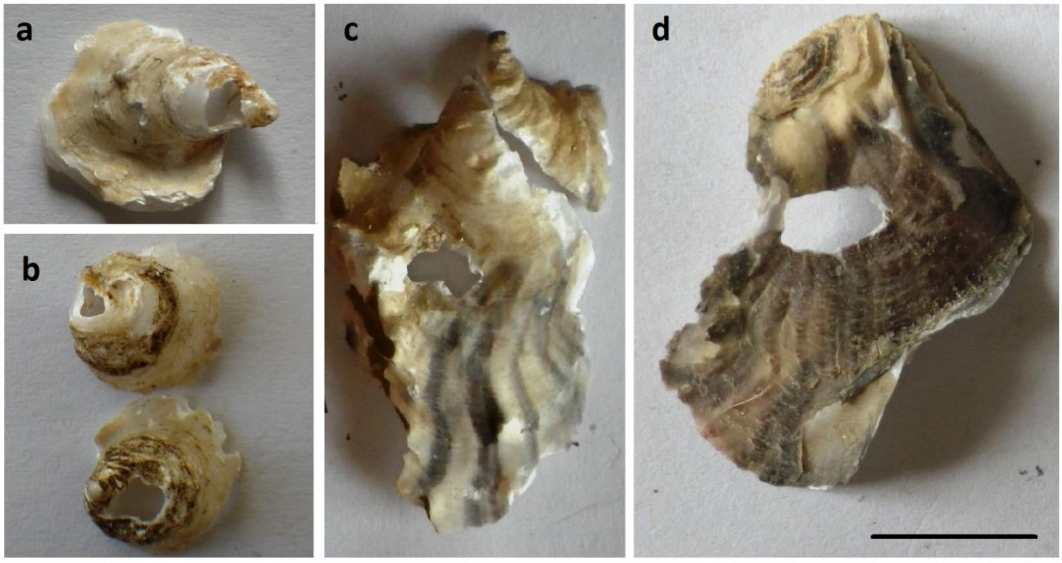


Figure 3 A feeding behaviour that resulted in a hole below the umbo on the cupped valve of small (**a** & **b**) and large (**c** & **d**) *Crassostrea gigas* spat was exhibited by male and female *Carcinus maenas* from 35 – 55 mm carapace width.

Chipping: *C. gigas* has overlapping valves, in large juveniles unattached to a substrate they were frequently chipped away (Figure 4). Such damage would give the crab access to the adductor muscle which when cut causes the oyster to gape exposing the flesh. Chipping to the lateral fringe, and in particular on the posterior valve margin by chewing the shell with the mandibles, has been previously recorded as a technique used by *C. maenas* to open mussels that are too large to be crushed by the chelae (Ameyaw-Akumfi & Hughes 1987; Morton & Harper 2008). The mandibles were used due to the strength and globular shape of the mussel shell, for *C. gigas* it is likely that the chelae could also be used. Chipping was used on unattached spat suggesting this behaviour requires manipulation impeded by additional substrate.

Lateral break: *C. gigas* spat that were fixed to a tile were often found with intact flat valves and cupped valves that had been broken in half laterally (Figure 4). In all cases the break was at the

seam between the glued part of the shell and where it had grown onto the tile. Perhaps this represented the weakest area or perhaps the join provided a gap in which the chelae could be inserted and the oyster levered off the tile. In some instances valves remained hinged together as the umbo was not attacked and the hinge ligament remained intact. Lateral crushing of bivalve prey is uncommon and usually only seen in larger crabs with chelae physically large enough to encompass the bivalve and the strength to break the shell at its strongest point. Oyster spat during this experiment were broken in half by male and female crabs with carapace widths from 45 mm, furthermore many of the flat valves remained undamaged suggesting that the spat were not crushed but levered upward and snapped off the tile.

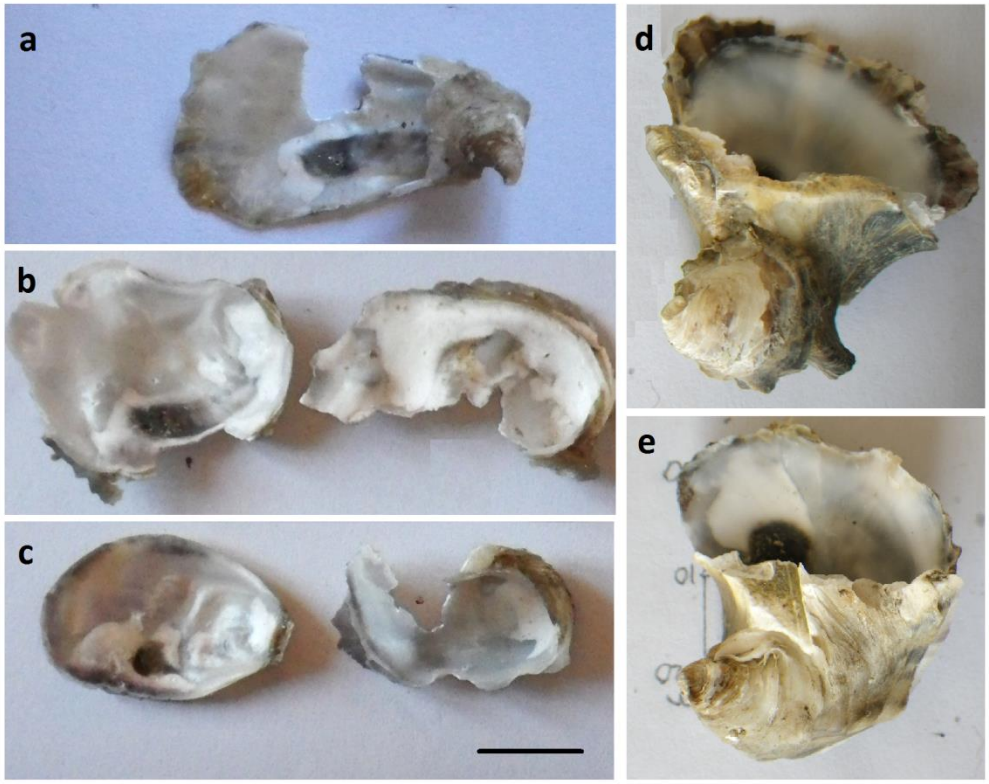


Figure 4 Examples of shell damage most frequently experienced by large juvenile *Crassostrea gigas* (20-40mm shell length). **a, b** & **c**: loose *C. gigas* – shell chipped away along the lateral edges with the most damage sustained by the cupped valve. **d** & **e**: fixed *C. gigas* – cupped valve broken at the point that the shell grew onto the tile, hinge ligament intact and no damage to flat valve. Scale bar = 10 mm.

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Appendix G

Valve gape has conventionally been measured using kymograph and strain-gauge methods (Loosanoff & Nomejko 1946; Kuwatani 1963; Fujii 1977; Higgins 1980), stimulation coils and electromyography (Lent 1968; Kramer et al. 1989). More recently image analysis from video footage (Newell et al. 2001; Maire et al. 2007) and the use of Hall sensors (Nagai et al. 2006) have been favoured. This is a result of an increased resolution through using these methods and that they are relatively unobtrusive and durable in comparison to conventional methods. Video analysis has the benefit over the use of a Hall sensor as no handling of the animal is necessary prior to the experiment and of requiring no additional weight to be added to the shell of the bivalve (although a Hall sensor typically weighs approximately that of a barnacle (Nagai et al. 2006)).

Methods: The amount of gape between the valves of the oysters was measured using a ratiometric Hall sensor and magnet. A Seimens KSY 10 Hall sensor was glued onto the peripheral edge of the upper valve using epoxy-resin, and a flexible cable connected the sensor to an Arduino© Uno AT mega 328P microcontroller. A neodymium boron magnet was glued onto the lower valve directly below the Hall sensor. It was necessary to calibrate the Hall sensor to each individual as the magnetic field strength perceived by the sensor is not linearly related to the distance between the magnet and the sensor. Therefore before tests began a calibrating pole with mm increments marked on to it was photographed next to each oyster. The proximity of the Hall sensor to the magnet results in a voltage change that is logged. Following the calibration this can be directly related to the distance between the Hall sensor and the magnet and thus the oysters gape. The voltage, as a ratio was logged at 5 second intervals to a text file generated by the Arduino and saved onto an SD card.

A series of videos were recorded in order to calibrate the Hall sensors and convert the Hall readings into more meaningful data, such as % gape or gape in mm standardised by shell length. A freeze frame of the video footage was taken every 5 seconds using the software ImageGrab (Figure 1), allowing the gape measurements to align with the Hall values that were programmed to record every 5 seconds. The freeze frames were then analysed using ImageJ where the distance between the upper and lower shell valve were measured at 3 points on each image.

Results: There was notable variability in the Hall sensor data that resulted in the 2 data sets having a weak correlation (Pearson's correlation: $t = 0.95$, $df = 299$, $p = 0.343$). From the beginning of the video the oyster could be seen gaping to varying extents, this is the case until minute 17, after which the valves closed and did not reopen. The range of Hall values recorded

over the 20 minute period ranged from 319 to 336 with a mean average of 328.1. When considering only the final 3 minutes, the valves were closed and the Hall values ranged between 322 and 330, with an average value of 327.7 (Figure 2). Smoothing algorithms of 5, 10 and 15 seconds were applied to the data in an attempt to reduce variability. The algorithms were not effective and smoothing can conceal behavioural traits in bivalves (Robson et al. 2009). Consequently it was not possible to convert the remaining Hall data into usable valve gape measurements. It is not clear whether biological noise created high variability (Griffiths 1981; Curtis et al. 2000; Wilson et al. 2005) or whether this was the fault of experimental design. The video analysis did however allow a base line of activity to be characterised and enforced the importance of appropriate sampling frequency as rapid and constant micro-movements of the shell valves required a high frequency of sampling to record accurately (Ropert-Coudert & Wilson 2004; Robson et al. 2009).

Base activity was characterised by the gradual increase of gape aperture through a repeated pumping action. Valves partially closed before reopening again during pumping with an incremental increase in overall gape aperture. After a short duration the shell valve aperture began to reduce with the same pumping action, however immediately before the valves reached closure, there was a succession of larger pumps. The valves then remained closed for an extended time period.

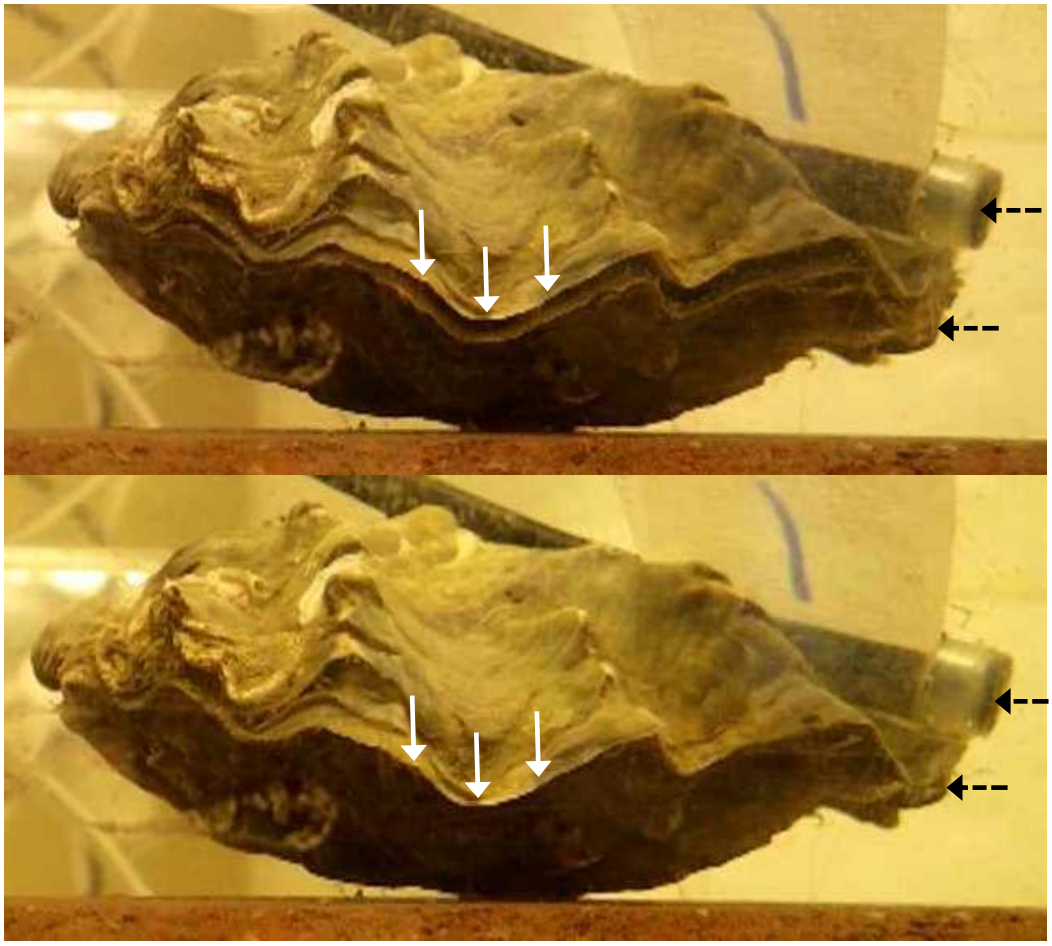

Figure 1 Top: A freeze frame of video footage showing a *Crassostrea gigas* with gaping valves. Bottom: *Crassostrea gigas* with closed valve. White arrows indicate the point at which valve gape was measured and black dashed arrows show the Hall sensor (top valve) and associated magnet (bottom magnet).

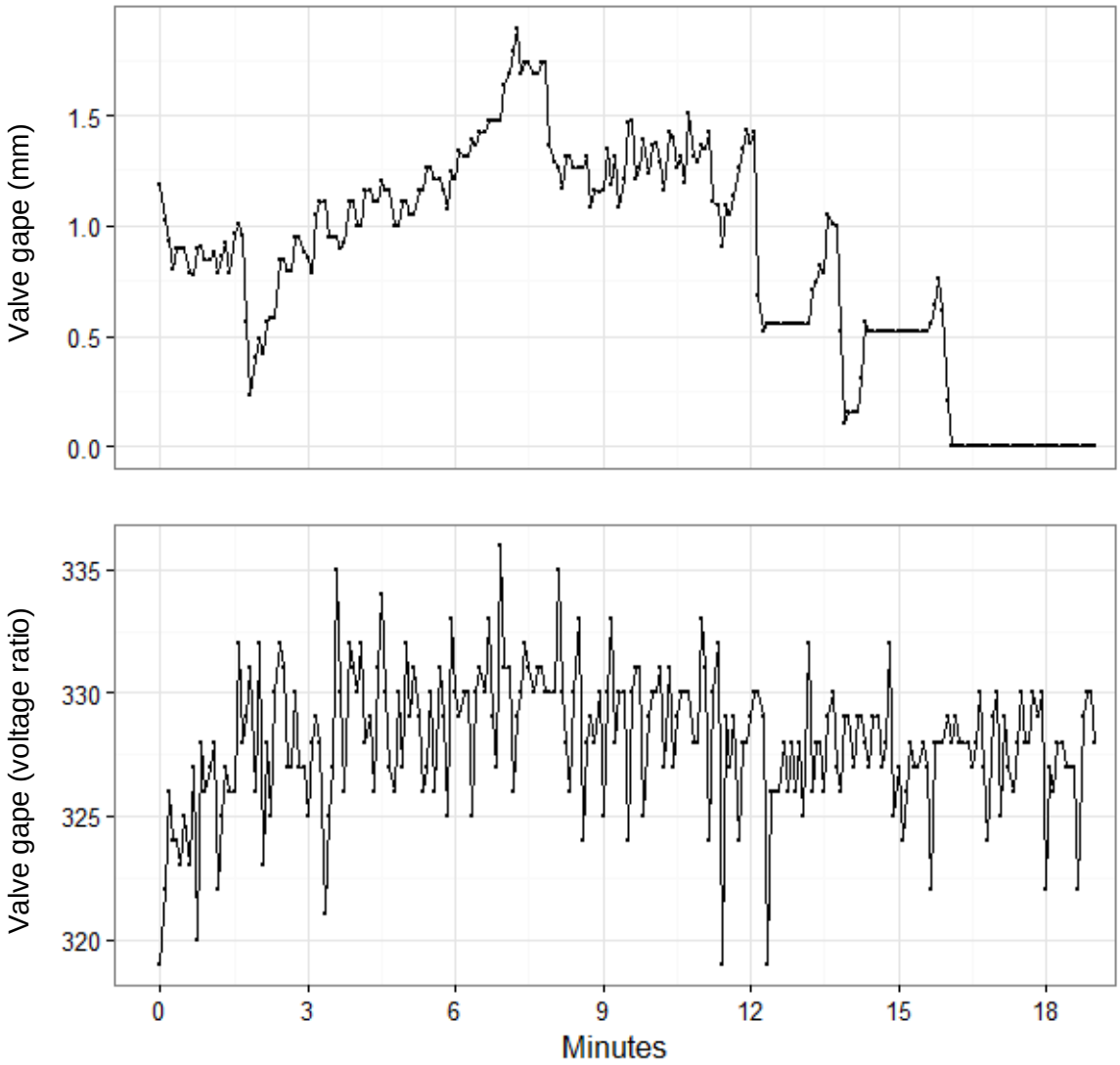

Figure2 Bottom: Highly variable data from the Hall sensor masks the Top: valve gaping behaviour of *Crassostrea gigas* captured through image analysis.

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