A study of the naturalisation and dispersal of a non-native bivalve, the Manila clam, *Ruditapes philippinarum* (Adams and Reeve 1850) in estuaries along the South coast of England.

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Abstract

The Manila clam, *Ruditapes philippinarum*, was introduced into the British Isles in the 1980s for the purpose of aquaculture in order to take advantage of the rapid growth rate and high profitability of this non-native species. The decision to import the Manila clam, was based on the findings of a report commissioned by the Ministry of Agriculture Fisheries and Food which determined that the Manila clam would be able to be grown to marketable size faster than the local species *Ruditapes decussatus,* but would not be able to reproduce due to the inclement seawater temperature regime around the British Isles. Within four years of its introduction into Poole Harbour in Dorset, the Manila clam had successfully reproduced and by 2004 it had naturalised in the Harbour. This thesis sets out to determine the factors that influence reproductive and recruitment success of the Poole Harbour population of Manila clams and to determine what factors could influence the further dispersal of the Manila clam along the South and South Eastern coastline of England.

The population of Manila clams at Holton Mere in Poole Harbour was quantitatively sampled on a monthly basis, with some omissions, from June 2009 until August 2012. For each sample month, the density and population dynamics of the population were estimated. From February 2010 until August 2012 the condition index of the population was estimated on a monthly basis. The population dynamics of the Holton Mere population were influenced by fishing pressure with the removal of the majority of clams over the minimum landing size of 35mm. Condition index followed a seasonal pattern with high condition in summer and low condition in winter.

Recruitment success in the Holton Mere population was inconsistent, with successful recruitment events coinciding with higher levels of condition index. A study to correlate environmental parameters with clam condition index, using long term monitoring with a multi-parameter sonde were undertaken in Poole Harbour between July 2011 and July 2012. Seawater temperature and food availability was found to positively correlate with condition index.

Experiments determined that predation by the European shore crab, *Carcinus maenas* has the potential to influence the success of recruitment events due to high levels of predation on newly settled and juvenile clams. Reduced salinities and low temperatures influence the rate of predation of juvenile Manila clams by crabs.

Salinity influences behaviour in both adult and larval Manila clams, with burial by the adults and swimming in the larvae ceasing at salinities below 18psu. Manila clam larvae actively swim through haloclines into areas of reduced salinity. Manila clam larvae are able to tolerate salinities as low as 10psu for 24 hours with low levels of mortality. The Manila clams' tolerance to reduced salinity allows it to colonise areas of marginal habitat where competition is low. Predicted increasing seawater temperatures will allow the Manila clam to extend its range northwards by causing cold water species to vacate ecological niches for the Manila clam to occupy and to improve the consistency of reproductive success.

The Manila clam is now established along the Southern coastline of England and is unlikely to disappear. As such it should be classed as a naturalised species and managed in the same way as native species. The Manila clam is likely to spread northwards in the future and will provide both economic and ecological benefits in the form of new fisheries and also prey for local species including wading birds. The high growth rate and versatility of the Manila clam would allow it to be used in polyculture systems and be grown in habitats that were previously deemed unsuitable for bivalve culture.

Contents[i]

Declaration

Whilst registered as a candidate for the above degree, I have not been registered for any other research award. The results and conclusions embodied in this thesis are the work of the named candidate and have not been submitted for any other academic award.

List of tables and figures

Abbreviations

Ash free dry mass.....AFDM

Blue green algae.....BGA

Condition index.....CI

Dissolved oxygen.....DO

Distance based linear model.....DistLM

Geographical information system.....GIS

Global positioning system.....GPS

General Linear Model.....GLM

Generalized Linear Model.....GLZM

Inshore Fisheries and Conservation Authority.....IFCA

Institute of Marine Sciences.....IMS

Marine Management Organisation.....MMO

Ministry of Agriculture Fisheries and Food.....MAFF

Minimum Landing Size.....MLS

Monthly increment.....MI

Modal progression analysis.....MPA

Practical salinity units.....psu

Relative fluorescence units.....RFU

Southern Inshore Fisheries & Conservation Authority.....SIFCA

Special Protected Area.....SPA

Standard error.....SE

Acknowledgments

I would like to dedicate this PhD to my late grandfather Jacques Marcel Harris who 27 years ago took a two year child to a rock pool to collect starfish and crabs and brought home a marine biologist. Without your passion, enthusiasm and willingness to sit for hours with me staring into pools of water this PhD would never have happened.

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Chapter 1: A general introduction to the biology and ecology of the Manila clam

When the decision was made to introduce the Manila clam to the British Isles in the 1980s, the scientific view was that it would be an ideal candidate for aquaculture but would not be able to reproduce and naturalise due to the low seawater temperatures (Spencer, *et al*. 1991). This proved to be incorrect and within six years of its introduction it had naturalised in Poole Harbour and supported a viable fishery (Jensen *et al.*, 2004). In the context of this thesis, the term naturalised signifies a species that has been artificially introduced but has been able to successfully reproduce and produced a self-sustaining population. A non-naturalised population would be one that relies on the introduction of individuals by human activity in order to maintain its presence.

The fact that the Manila clam did manage to naturalise in Poole Harbour raises many questions that this thesis sets out to answer. These include: Is the Manila clam more widely distributed than currently reported? How might larvae respond to environmental stimuli and would this influence dispersal? What is the current status of the Poole Harbour population? What factors influence clam health and condition? Has the introduction of the Manila clam created new predator-prey interactions?

1.1 Natural distribution, habitat and biology and ecology of the Manila clam

The Manila clam, *Ruditapes philippinarum* (Adams and Reeve 1850) is a bivalve mollusc that belongs to the family Veneridae (Humphreys *et al.*, 2007). Since it was first described by Adams and Reeve in 1850 it has been assigned a variety of scientific names, a selection of which include: *Amygdala japonica, Amygdala philippinarum, Paphia philippinarum, Ruditapes semidecussatus, Tapes japonica, Venerupis japonica* and *Venus japonica* (Goulletquer, 1997). The currently recognised taxonomic name is *Ruditapes philippinarum* and this is how it will be referred to in the rest of this thesis. As with scientific names, the Manila clam is also known under a variety of names distinct to areas in which is found, these include: The japanese littleneck clam (S. Ponurovsky &

Yakovlev, 1992), Japanese carpet shell (C Campos & Cachola, 2006) the asari clam (Tezuka et al., 2013), The baby necked clam, the short-necked clam, palourde japonaise and almeja japonesa (Goulletquer, 1997).

It is very similar to the European palourde *Ruditapes decussatus* in appearance (Hurtado *et al.*, 2011) and habitat; intertidal soft sediments (I. Laing & Child, 1996). This has made the usual method of using shell morphology to determine species more difficult when comparing Manila clams and palourdes (Hurtado *et al.*, 2011). Consequently the most accurate method of differentiating between the two species is to look at the siphons in live specimens, Manila clams have fused siphons for the majority of their length, whilst the palourde has separate siphons (see figure 1.1) (Hurtado *et al.*, 2011).

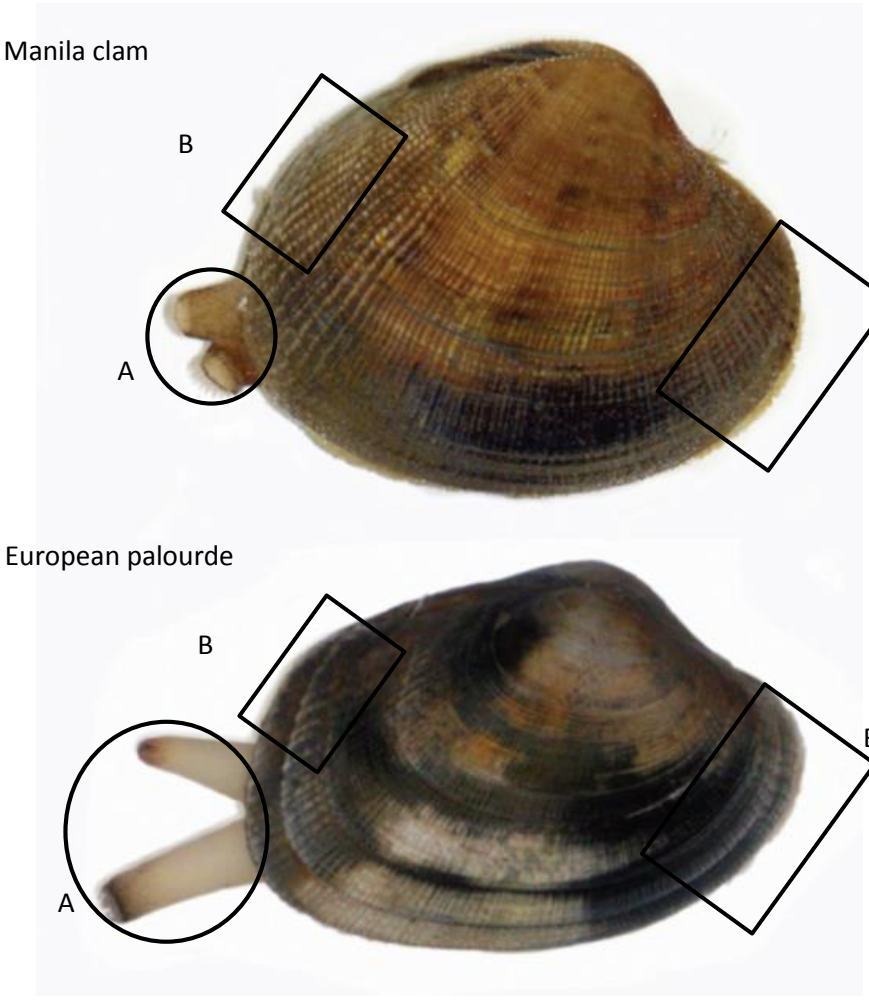

Figure 1.1 Morphological features used to differentiate between the Manila clam (top) and the European Palourde (bottom). The Features used include the siphons (A), which are fused in the Manila clam, but are separate in the Palourde. The shell morphology is also often used, with the Manila clam having a more rounded shell than the Palourde which has a more angular shell (B). (Image used with permission from Humphreys 2010).

Differentiating between the species is also more difficult because some interbreeding between Manila clams and palourdes in North-West Spain has occurred producing some hybrid clams with also some clams with intermediate shell characteristics of both Manila clams and palourdes (Hurtado *et al.,* 2011). The intermediate clams made it hard to identify whether they were Manila clams or Palourdes, so molecular techniques were required to identify the species of clam and whether they were hybrids or not (Hurtado et al., 2011).

Hurtado (2011) reported that out of a combined total of 328 Palourde and Manila clams sampled from the Ria de Vigo in NW Spain, 9 were hybrids with genetic sequences specific to both species (Hurtado et al., 2011). This accounts for 2.7% of the total clams sampled. It was hypothesised that the hybridisation was due to the partially overlapping spawning periods of the native Palourde and the introduced Manila clam (Hurtado et al., 2011).

Hybridisation between the Manila clam and the Palourde could lead to individual clams exhibiting traits that would result in a higher probability of survival. An example of a trait that could be advantageous to hybrid clams, is the production of proteins in response to thermal stress by the European palourde as opposed to a cessation of activity in Manila clams (Anacleto et al., 2014). This would present an advantage to hybrid clams over pure strain Manila clams, as it would enable them to tolerate long term global water temperature increases as the cessation of metabolic activity could lead to reduced fitness and death (Anacleto *et al.,* 2014). Manila clams exhibit a faster growth rate than Palourdes, growing to marketable size up to a year faster, so a hybrid may also have a faster growth rate than the local palourde species (Spencer *et al.*, 1991).

This suggests that Manila clam-Palourde hybrids could have the potential to adapt to local conditions and any future environmental changes more effectively than either pure-strain Manila or Palourde clams. The ability for the Manila clam to hybridise with local species may be due in part to the similarity of the habitats into which it has been introduced with those found in its natural distribution.

The Manila clam is naturally distributed along the Pacific coast of Asia, between latitudes 25° to 45°N (Bourne, 1982; Scarlato, 1981). It is found along the coast lines of Japan (

Bourne, 1982), Korea (Uddin *et al.,* 2012), The Philippines (Spencer *et al.,* 1991) and China (Ren *et al.*, 2008). The Manila clams natural habitat consists of estuarine (Cigarria & Fernandez, 2000), lagoon (Mistri, 2004), bay (Kasuya *et al.*, 2004) and tidal flat (Kasai, et al., 2004) environments characterised by low wave energy. It is an intertidal to shallow subtidal species that can be found in sandy, sandy-mud, and muddy substrates (Yasuo Nakamura, 2001; Uddin *et al.*, 2013). In its natural habitat it can experience a range of salinities from 16-36psu (Kang *et al.*, 2007). Manila clams inhabit the top 10cm in the substrate, this depth is limited by the length of their siphons (Bourne, 1982; Kasai *et al.,* 2004).

The burrowing activity of Manila clams can destabilise sediments and increase erosion rates at water velocities at which sediment erosion does not usually occur (Sgro, *et al.*, 2005). This is due to clam burial behaviour and the opening and closing of the valves in order to eject sediment and pseudo-faeces (Sgro *et al.*, 2005). The high population densities that Manila clams attain in the wild have the potential to affect sediment stability and increase erosion (Sgro *et al.*, 2005).

The presence of the Manila clam in estuarine environments may bring an advantage in terms of food availability. The Manila clam is a suspension feeder and ingests food items directly from the water column (Nakamura, 2001). It is known to feed upon: phytoplankton, cyanobacteria and particulate organic matter (Kasai *et al.*, 2004; Nakamura, 2001). Laboratory experiments have calculated that Manila clams need to ingest 0.2g of wet food biomass per day to ensure that clams do not enter metabolic debt, this equates to each Manila clam filtering 20 m^3 of seawater a year (Sorokin & Giovanardi, 1995).

The Manila clam is highly efficient at filtering food particles out of the water column, under laboratory conditions clams were able to filter out 96% of the available food within two hours (Magni *et al*., 2000). The highly efficient filtering capacity of the Manila clam means that in its natural habitat the Manila clam plays an important role in nutrient cycling by recycling the inorganic nitrogen and phosphorous for primary producers (Magni *et al.,* 2000). Feeding rate is directly linked to water temperature, with the clearance rate of particles increasing with temperature (I. Laing & Child, 1996). Respiration and excretion rates reach their maximum values at water temperatures of

20˚C, above this the rates decrease (Magni *et al.*, 2000). The population of Manila clams in the Seto Inland Sea in Japan has been calculated to have a daily ammonia excretion rate of 35.2 mmol per m^2, this high level of excretion is comparable with levels of excretion by mussel beds (Magni *et al.,* 2000).

Due to the fact that it inhabits estuarine areas, terrestrial organic matter in the form of decaying plant matter often forms a part of its diet (Kasai *et al.*, 2004). This decaying matter enters the estuary from land runoff (Kasai *et al.*, 2004) and may provide Manila clams with a food supply when the phytoplankton is at low levels in the winter months. Food availability is also known to affect reproductive success (Uddin *et al.*, 2013), so a constant year round supply of food could result in higher reproductive success.

The types of environments that the Manila clam naturally inhabits; estuaries and bays are also those where marine invasions predominately occur (Miller *et al.,* 2007). The fact that the Manila clam is able to tolerate a wide range of salinities is also important because salinity tolerance is one of the most important factors in determining the success of an invasion (Miller *et al.*, 2007). The success of an introduction is also due to the similarity between the environmental conditions found in an organism's natural environment to those where it is being introduced (Miller *et al.*, 2007).

1.2 Invasive species in the marine environment

There are five main drivers that are expected to cause change in global diversity, these are: land use, climate change, nitrogen deposition, biotic exchange and atmospheric CO_2 (Sala *et al.,* 2000). Terrestrial environments are predicted to be most affected by changes in land use and climate change, with ecosystems at higher latitudes being more affected than those around the equator and in temperate zones (Sala *et al.*, 2000). Freshwater ecosystems however are likely to be most impacted by biotic exchanges as well as land use and climate change due to the high utilisation of these resources and their close proximity to large human populations (Sala *et al.*, 2000). The impact of biotic introductions on ecosystem biodiversity is not confined to freshwater ecosystems. The

introduction of non-native species is also known to affect marine ecosystems as well and has been reported to have a variety of ecological and economic impacts (Streftaris & Zenetos, 2006).

Non-native species have been introduced into the marine environment both intentionally (i.e. for the purpose of aquaculture) and unintentionally (through the release of ballast water by ships, and alongside the intentional introduction of other species)(Miller *et al.*, 2007). Not all introductions of a non-native species result in the successful establishment of a population (Levinton, 2001, p423). For an introduction to be successful, it must result in the population of the introduced species becoming established and self-sustaining, with a steady stream of new recruits (Levinton, 2001). Without a steady source of recruits the population will decline and eventually disappear, rendering the invasion a failure (Levinton, 2001).

The species richness and diversity of an ecosystem can influence the success of an introduction, with ecosystems with a high species diversity more resistant to invasions than those with low species diversity (Stachowicz *et al.,* 1999). This often results in invasions being more successful in areas of marginal habitats, as marginal habitats often have lower species diversity than optimal habitats (Miller *et al.*, 2007). Changes in environmental conditions on a global scale as well as habitat modification as a result of increasing levels of anthropogenic activity, may increase the susceptibility of “optimal” habitats to invasion by alien species (Didham *et al.,* 2007).

The impact of the introduction of a non-native species into an ecosystem is not uniform, with some ecosystems being impacted less critically than others (Ricciardi & Atkinson, 2004). Ecosystems that already contain species of the same genera as that of the invader are less likely to suffer detrimental effects of an invasion than those without similar species already present (Ricciardi & Atkinson, 2004). This is because there is less space for the new species to inhabit (Stachowicz *et al.,* 1999) and there are already organisms fulfilling the ecological niche of the invader (Ricciardi & Atkinson, 2004).

Although not all introductions of alien species are successful or impact the ecosystem into which they have become introduced, there are many well documented examples of introduced species having a negative impacts. Introduced species can influence

ecosystems in a variety of ways including: predating upon local species (Goldschmidt, et al. 1993), outcompeting local species for resources and space (C. Griffiths et al. , 1992; E. D. Grosholz & Ruiz, 1996), modifying habitats (Kerckhof, Haelters, & Gollasch, 2007) and introducing pathogens and diseases (Wolff & Reise, 2002).

The accidental introduction of the European Shore crab, *Carcinus maenas*, along the North American coastline has led to the decline in the abundance of several bivalve populations, most notably in Bodega Harbour (E. D. Grosholz & Ruiz, 1996). This reduction in bivalves has impacted other species including several species of shorebird whose numbers have declined due to reduced food availability (E. D. Grosholz & Ruiz, 1996). The introduction of the European Shore crab has also led to the decline of a local crab species, *Hemigrapsus oregonensis* by outcompeting it for available food resources (E. D. Grosholz & Ruiz, 1996). Although predation by introduced species can have serious impacts on an ecosystem, the introduction of non-predatory species can also have impact upon the host ecosystem as well.

The introduction of the Pacific oyster, *Crassostrea gigas* into European waters for the purpose of aquaculture has had a variety of impacts. The reef forming behaviour of adult oysters has caused habitat modification in areas where they have become established, an example of reef formation can be seen in figure 1.2.

Figure 1.2: Habitat modification by the Pacific oyster, *Crassostrea gigas* in Nieupoort, Belgium. The Pacific oyster has created artificial reefs in areas where it has become established (Image taken from Kerckhof et al. 2007).

Reef formation by Oysters does create ecological niches for some species of macrofauna to exploit, however it removes habitat for infaunal species by covering the seabed. The introduction of the Pacific oyster also acted as a vector for the introduction of many pathogens and diseases into the aquaculture areas. These pathogens have spread from the aquaculture areas and have had far-reaching ecological impacts, including causing mass mortality events (Kerckhof et al., 2007; Wolff & Reise, 2002).

Not all introductions of alien species have negative ecological impacts. The introduction of the Manila clam into Poole Harbour in Dorset has resulted in benefits to shorebirds, this is due to increased food availability over the winter period (Caldow et al., 2007). Non-native species can also fill ecological roles that have been vacated due to climate change and other disturbance events, provide habitats for native species and act as catalysts for ecosystem restoration (Schlaepfer et al., 2011).

The introduction of an alien species can have economic impacts as well as ecological ones. One positive result of an introduction could be the creation of novel fisheries, an

example of this is the introduction of the Manila clam into Poole Harbour in Dorset England (Jensen et al., 2004) and the Marano Lagoon in Italy (Sladonja et al., 2011). However not all introductions have positive economic impacts. In North America the introduction of the European Shore crab has impacted the local bivalve fishery, predation by the crab upon the native bivalves has resulted in reduced yields for fisherman (E. D. Grosholz & Ruiz, 1996). The crab has had enough of an impact that local clam farmers have had to develop methods, such as laying clam spat later in the season in order to reduce predation and ensure the fishery remained financially viable (Grosholz et al., 2001). Other impacts of invasive species include the fouling or harbours and reduction in water quality due to phytoplankton blooms, both of which can affect tourism (Streftaris & Zenetos, 2006).

The importance of the impact that alien species have upon the marine environment is evident from the amount of literature available on this topic (Miller et al., 2007; Ruiz, et al. 1997; Stachowicz et al., 2002; Stachowicz et al., 1999; Streftaris & Zenetos, 2006). Areas with low levels of human activity receive fewer exotic species than areas with high levels of human activity (Sala et al., 2000). The Mediterranean Sea is a good example of a location that has a high level of human activity and has had a large number of alien species introduced, both intentionally and unintentionally. A review by Streftaris and Zenetos (2006) of the impact of invasive species in the Mediterranean rated the Manila clam in the top 100 “worst alien invasive species”. This was due to the impact of its introduction on biodiversity, fisheries and infrastructure (Streftaris & Zenetos, 2006). The status of the Manila clam in the top 100 “worst alien invasive species” suggests the importance of further investigation of this species in British waters and of potential mechanisms for further dispersal.

1.3 The current distribution of the Manila clam in European waters

The Manila clam was intentionally introduced into European waters for the purpose of aquaculture in the 1970s, with clams first being introduced into France in 1972 (Goulletquer, 1997). Since the introduction into French waters, the Manila clam has been introduced into Spanish (Cigarria & Fernandez, 2000; Hurtado et al., 2011),

Portuguese (C Campos & Cachola, 2006), Italian (Mistri, 2004; Mura et al., 2012) and UK waters (Drummond et al. 2006; Jensen et al., 2004). The location of the introductions and the reported Manila clam populations can be seen in figure 1.3.

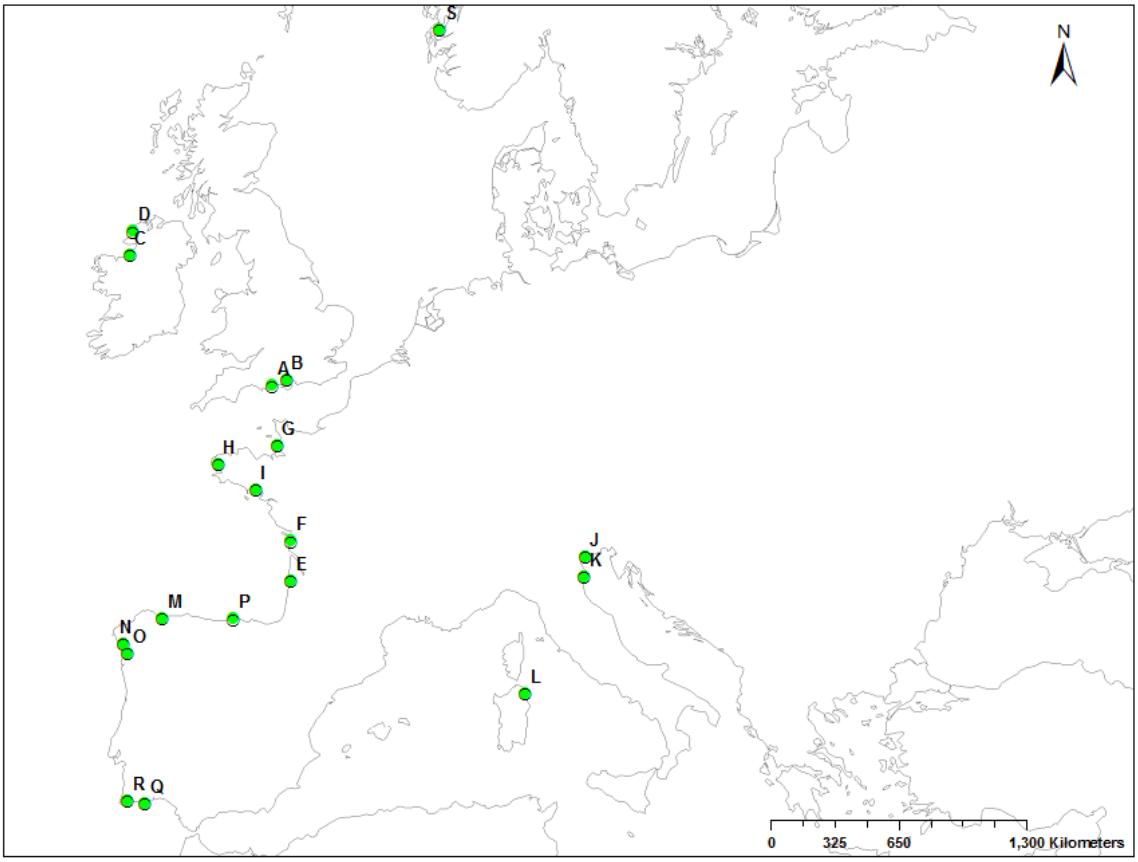

Figure 1.3: The reported distribution of the Manila clam in European coastal waters. The populations of Manila clams are denoted by green circles. All of the populations represented on the map have been reported in the scientific literature (See table 1 for further details on these locations).

Although Manila clam populations are found in a variety of geographical locations (see figure 1.3) they have not become naturalised in all of the sites in which they are found. The naturalisation status of the populations of Manila clams shown in figure 1.3 including the year of introduction are displayed in table 1.

Table 1: The location and naturalisation status of the Manila clam populations represented in figure 1.3. The year Manila clams were introduced and whether they have become successfully established is also included.

Site	Location	Year introduced	Naturalisation status	Reference
A	Poole Harbour, England	1988	Naturalised	(Jensen et al., 2004)
B	Southampton Water, England	2005	Naturalised	(Tumnoi, 2012)
C	Drumcliffe Bay, Ireland	1984	Non-naturalised	(Drummond et al., 2006)
D	Dungloe Bay, Ireland	1984	Non-naturalised	(Drummond et al., 2006)
E	Arcachon Bay, France	1972	Naturalised	(Dang et al., 2010)
F	Marennes-Oleron bay, France	1972	Naturalised	(Flye-Sainte-Marie et al., 2007)
G	Chauset Islands, France	2000	Non-naturalised	(Soudant et al., 2004)
H	Bay of Brest, France	2000	Non-naturalised	(Soudant et al., 2004)
I	Morbihan Gulf, France	2000	Non-naturalised	(Soudant et al., 2004)
J	Venice Lagoon, Italy	1983	Naturalised	(Sladonja et al., 2011)
K	Sacca Di Goro, Italy	1983	Naturalised	(Sladonja et al., 2011)
L	Gulf of Obia, Sardinia	1983	Naturalised	(Mura et al., 2012)
M	Eo Estuary, Spain	1980	Naturalised	(Cigarria & Fernandez, 2000)
N	Ria de Arousa, Spain	1980	Naturalised	(C Campos & Cachola, 2006)
O	Ria de Vigo, Spain	1980	Naturalised	(Hurtado et al., 2011)
P	Bay of Santander, Spain	1980	Naturalised	(Juanes et al., 2012)
Q	Ria Formosa, Portugal	Late 1980's	Naturalised	(Campos & Cachola, 2006)
R	Ria de Alvor, Portugal	Late 1980's	Naturalised	(Campos & Cachola, 2006)
S	Espevik, Norway	1987	Non-Naturalised	(Mortensen, 1993)

Manila clams have not successfully naturalised at all of the locations in which they have been introduced, most notably in Norway and Ireland. The populations in Drumcliffe bay and Dungloe bay in Ireland, were unable to successfully reproduce. It has been suggested that this is due to the incompatibility of the seawater temperatures with the Manila clams reproductive cycle (Drummond et al., 2006). As a consequence the clams are reliant on the introduction of juvenile clams by fisherman in order to maintain the population (Drummond et al., 2006). The Manila clams in Norway were observed to have mature gametes but were also found to be under-nourished and it is not known whether the clams successfully reproduced and have spread out of the initial sites of introduction (Mortensen, 1993). This suggests that the compatibility of the reproductive cycle with local environmental conditions is an important factor in determining whether the Manila clam becomes naturalised or not.

1.4 The reproductive cycle of the Manila clam

Before it was allowed to be cultured in the UK, extensive trials were carried out to determine the suitability of the Manila clam for aquaculture (Humphreys, 2010). These trials determined that the Manila clam would be able to grow to marketable size, but would not be able to reproduce due to the temperature regime (B. Spencer et al., 1991). This proved to be incorrect and the Manila clam managed to become naturalised in Poole Harbour in Dorset (Jensen et al., 2004). This raises the question of, did the Manila clam adapt to the environment in the UK, or were the minimum temperatures required for reproductive processes lower than reported by the trials?

Seawater temperature is known to influence the distribution and reproductive activities of the majority of marine organisms, with a species' northern limit often defined by its ability to successfully reproduce (Orton, 1920). In tropical ecosystems which experience little variation in temperature or seasonality, reproduction is often continuous, and individuals can be found at any stage of the reproductive cycle at any time of the year (Orton, 1920). In temperate ecosystems, which have more defined seasons and experience a wider range of temperatures, reproduction takes place in more discrete breeding seasons (Orton, 1920). There are also variations in the duration of the breeding season across temperate regions, with populations of the same species often having a shorter reproductive season in more northerly latitudes compared to populations in more southerly latitudes (Orton, 1920).

Organisms in temperate ecosystems require the seawater to achieve a minimum temperature in order to trigger a physiological response, otherwise breeding does not occur (Orton, 1920). Once the water has reached the minimum temperature threshold for reproduction, breeding often occurs continuously until the water drops below this temperature (Orton, 1920). The influence of seawater temperature on the reproductive cycle and distribution of marine organisms has been referred to as Orton's rule (Patel & Crisp, 1960).

Recent studies have found that the reproductive cycle of many species of bivalves is influenced by food availability as well as seawater temperature (Cardoso et al., 2007; J.

Cardoso et al. 2009; Serdar & Lök, 2009). Like other species of bivalves, the reproductive cycle of the Manila clam is temperature and food dependent, with the minimum lower temperature limits reported by Mann (1979) as 8°C for gametogenesis, 12°C for gamete ripening and 14°C for spawning (Mann, 1979). Orton's rule therefore suggests that the Manila clam will be limited to areas that exhibit a temperature regime that is compatible with the figures reported by Mann (1979) for the reproductive cycle.

In Manila clams larval growth occurs at temperatures ranging from 12-30°C, but is optimised at 25°C (Numaguchi, 1998). Manila clam larvae are able to tolerate a temperature range of between 10-25°C with low levels of mortality (Numaguchi, 1998), with larvae able to survive temperatures of up to 30°C for short periods of time (Numaguchi, 1998).

Like many other species of bivalve, the Manila clam is a broadcast spawner and fertilisation takes place in the water column (Utting et al., 1991). The larval stage can last for 1.5-2 weeks and water temperature determines the length of this stage (Herbert, et al., 2012; Ruesink et al., 2013). Within 24 hours of fertilisation, the Manila clam develops into the D veliger larva stage at 95µm shell length (see figure 1.4)(Utting et al., 1991).

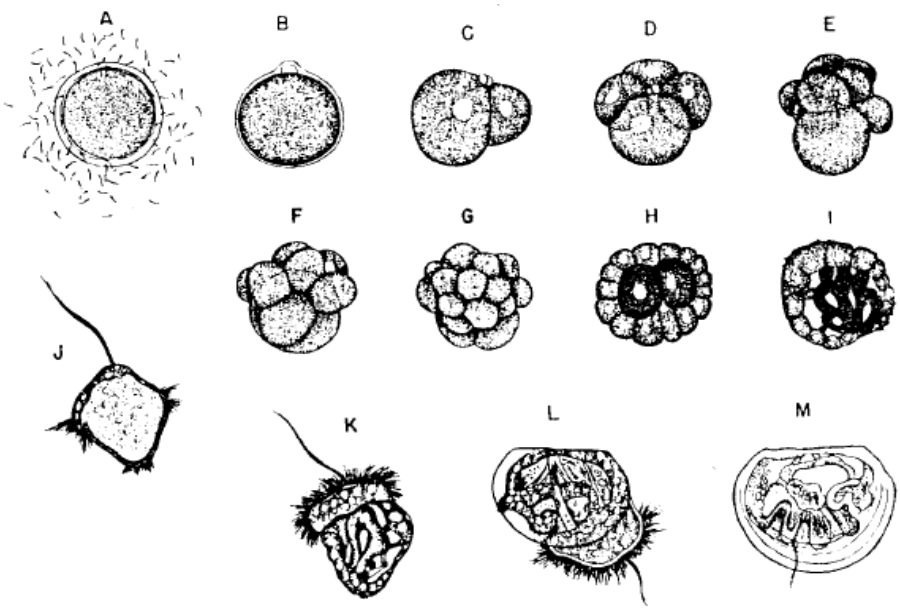

Figure 1.4: The fertilisation and development of the early stages of Manila clam larvae up until the D larvae stage (taken from Utting and Spencer 1991). A) depicts the initial fertilisation of the clam oocyte, with C) through to I) depicting cell division, J) and K) depict the trochophore stage. L) and M) depict the D veliger stage.

The D larvae stage is highly mobile and actively swims, after 4 days the D veliger larvae changes in to the veliger stage at circa 135µm shell length (Utting et al., 1991). The veliger is also an actively swimming larval stage, it lasts approximately 4 days before developing into the pediveliger stage at ca 215µm shell length (Utting et al., 1991). The pediveliger is not as active a swimmer as the veliger or D larval stages and often spends the majority of its time testing and crawling along the substrate using its foot (Utting et al., 1991). Once the pediveliger has found a suitable habitat and reached a size of circa 230µm shell length it metamorphoses into the juvenile or spat stage of clam and remains in the substrate (Utting et al., 1991).

Populations of Manila clams have often been observed to have two spawning events a year (Drummond et al., 2006; Humphreys et al., 2007; Pellizzato et al. , 2011; Uddin et al., 2012). At the end of the spawning season, any gametes that have not been released are reabsorbed by the clam and are incorporated into somatic tissue by the process of atresia (Drummond et al., 2006; Tumnoi, 2012; Uddin et al., 2012). In some countries, such as Ireland, spawning events do not always lead to a successful recruitment of juvenile clams (Drummond et al., 2006). In Ireland, the Manila clam is at the northern extreme of its range and the temperature required to trigger spawning occurs later in the year (Drummond et al., 2006). Consequently the larvae often do not survive due to a reduction in seawater temperature (Drummond et al., 2006), because below 10°C larval survival is poor in the Manila clam and low temperatures also result in a longer larval period (Ruesink et al., 2013).

1.5 Bivalve larvae locomotion and behavioural responses to environmental conditions

Many species of bivalve, including oysters, mussels and clams have a planktonic larval stage in which the majority of dispersal takes place (McQuaid & Phillips, 2000; North et al., 2008; Wood & Hargis, 1971). Although unable to control dispersal directly, larval bivalves including oysters (North et al., 2008) and clams (Herbert et al., 2012) are able to influence dispersal by modifying their position in the water column. Larvae modify their position in the water column by either: actively swimming, passively sinking or a

combination of these behaviours (Cragg, 1980; Hidu & Haskin, 1978; Wood & Hargis, 1971).

Active swimming in bivalve larvae is facilitated by a specially adapted organ, the velum (Cragg, 1980; Stanton, 2012). The velum has a band of cilia that is used for both swimming and feeding (Stanton, 2012) (see figure 1.5). Larvae actively swim by beating the cilia on the velum (Cragg, 1989; Stanton, 2012).

Figure 1.5: Pediveliger Larva of *Crassostrea gigas* with the velum extended (Image taken with permission from Stanton 2012). The velum is denoted by a), and the pre-oral cirri by b). The velum is used as a swimming organ and the pre-oral cirri are used for feeding.

When swimming actively, bivalve veliger larvae swim in a helical motion and are able to control their velocity and direction (Cragg, 1980; Hidu & Haskin, 1978; Mann et al. 1991)(see figure 1.6). Active swimming is used by larvae that are ascending or descending in the water column.

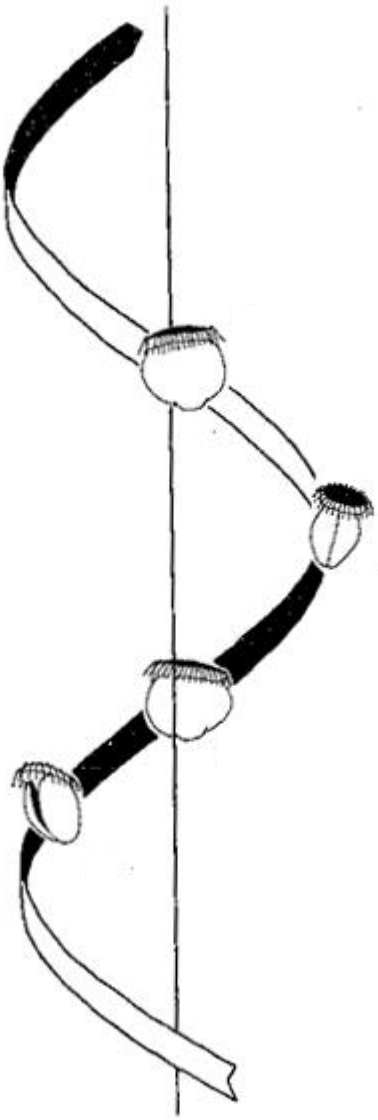

Figure 1.6: The vertically orientated helical swimming trajectory of swimming veliger larva (image taken with permission from Cragg 1980).

Passive sinking is only used by descending larvae and is achieved by withdrawing the velum and closing the valves of the shell (Cragg, 1980; Stanton, 2012; Wood & Hargis, 1971). Passive sinking in bivalve larvae is considered to be a reaction to suboptimal

conditions (Wood & Hargis, 1971) or a fright response to water turbulence (Stanton, 2012).

Once larvae have reached the pediveliger stage they often congregate in the bottom layers of the water column and begin to crawl along the substrate in order to locate areas of suitable habitat (Cragg, 1980; Stanton, 2012; Utting et al., 1991). If unsuitable conditions for settlement are encountered by the larvae, settlement does not occur and the larvae remain in the water column.

Environmental conditions such as light (Bayne, 1964), gravity (Cragg, 1980), temperature (Stanton, 2012) and salinity (Dekshenieks et al., 1996; Hidu & Haskin, 1978) elicit behavioural responses in bivalve larvae. For bivalves that inhabit estuarine environments, the behavioural response to salinity is important in ensuring that the larvae remain in areas of suitable habitat (Herbert et al., 2012; North et al., 2008; Wood & Hargis, 1971). The salinity in estuaries can fluctuate on a tidal basis, with reduced salinities at low tide due to freshwater input and higher salinities at high tide due to seawater input (McLellan, 1965). The potential range of salinities experienced by larvae on a tidal basis, highlights the importance of a tolerance to a wide range of salinities.

Manila clam larvae are able to tolerate a salinity range of between 12 to 33psu with low mortality (Numaguchi, 1998). The optimum salinity range for Manila clam larvae (discovered under experimental conditions) is 20-30psu (Numaguchi, 1998), this raises the question of whether the larval behaviour is modified to ensure that the larvae remain in this salinity. Numaguchi also found that at 2.5psu all exposed larvae died within 24 hours and that larvae actively swam at 15.5psu, but not at 12psu (Numaguchi, 1998).

Salinity can affect the settlement rate of Manila clam pediveliger larvae (Tezuka et al., 2013). Larvae settled at salinities ranging from 13psu to 30psu, with the highest number settling at 13psu (Tezuka et al., 2013). Once settled the larvae metamorphose in to juvenile or spat clams.

1.6 Predation on the life history stages of bivalves

All of the life history stages of bivalves, from the larval to the adult stage are subject to predation pressure. Larval bivalves are eaten by a wide range of predators including: adult bivalves (Troost et al. 2008), polychaete larvae (Johnson & Brink, 1998), fish and scyphozoans (Short et al., 2013). Once larvae metamorphose into the juvenile stage they are subject to a new suite of predators.

Predation can have an important impact on newly settled bivalves and result in high levels of mortality (Hiddink et al., 2002; McArthur, 1998). As a consequence many species of infaunal invertebrates have developed predator avoidance strategies such as the ability to burrow into the sediment to reduce the chances of being eaten (Griffiths & Richardson, 2006; Zwarts & Wanink, 1989). The depth an organism buries also influences survival rates due to the time required by predators to excavate it (Haddon, et al. 1987; Zwarts & Wanink, 1993).

The main limiting factor in bivalves as to how deep they can burrow is the length of their siphons (Zwarts & Wanink, 1989). The Manila clam is known to burrow to a depth of 10cm, the limit which is determined by the length of the siphons (Bourne, 1982). This is because food and oxygen availability is limited by access to the water column (Lardies, et al., 2001; Zwarts & Wanink, 1989).

Like other bivalves, the Manila clam uses burial as a mechanism to avoid predation (Mistri, 2004). Laboratory studies have found that burial results in a higher survivorship than clams remaining on the surface (Mistri, 2004). In this thesis an investigation was undertaken to determine whether the presence or absence of a predator influences the burial behaviour in the Manila clam. Other species of bivalve are known to modify their behaviour in response to predators. *Macoma baltica* have been reported to bury themselves deeper in the substrate in order to avoid predation (Griffiths & Richardson, 2006) whereas *Cerastoderma edule* were observed to bury at a shallower depth but present the strongest part of the shell towards the predator (Romano et al., 2011).

In its native distribution, the Manila clam is a prey item for a range of different organisms such as gastropods (Hasegawa & Sato, 2009), octopus (Ebisawa et al., 2011), starfish (S. Ponurovsky & Selin, 1988), crustaceans and many species of fish (CABI 2014).

Due to the fact that it has been introduced into many ecosystems around the world, the Manila clam has been exposed to new predators. In North America, these include: the Dungeness crab *Cancer magister* and the Hairy crab *Hemigrapus oregonensis* (Smith & Langdon, 1998) as well as crow species (O'Brien et al., 2005). The crab species prey upon juvenile Manila clams and can have serious impacts on clam aquaculture (Smith & Langdon, 1998). Crows only predate upon larger clams and exhibit a preference for larger heavier clams (O'Brien et al., 2005).

Little information has been reported on predators of the Manila clam in British waters. Caldow *et al.* (2007) focussed on the benefits to wading birds of the introduction of the Manila clam in to Poole Harbour. Caldow *et al.* (2007) estimated the impacts of the clams' introduction on bird mortality using a simulation model, and surmised that the overwintering mortality would decrease due to the clams' introduction. That study focussed on oystercatchers, *Haematopus ostralegus*, but there are other bird species such as gulls and crows that might predate upon Manila clams. Crows have been observed to predate upon Manila clams in North America (O'Brien et al., 2005) and gull species are also known to predate upon other bivalves (Ward, 1991).

There is limited literature detailing predation rates of European shore crabs on Manila clams, with the literature focussing on the effectiveness of protecting clam stocks with netting as opposed to the influence of environmental conditions on predation (Cigarria & Fernandez, 2000; Spencer et al., 1992). This study investigated the predation rates of *Carcinus maenas* on Manila clams under a range of environmental conditions including salinity and temperature in order to determine if these factors affect predation rates. A study investigating the effect of the presence or absence of a predator on Manila clam burial was also carried out because the Manila clam uses burial to avoid predation (Mistri, 2004). Crustaceans such as crabs have also been described as pest species affecting bivalve aquaculture with many methods designed to reduce their impact on the yield of bivalves (Cigarria & Fernandez, 2000; Spencer et al., 1992).

1.7 Aquaculture of the Manila clam

The methods used in bivalve aquaculture differ from species to species and location to location. Epifaunal bivalves such as mussels and oysters are cultured using different methods to infaunal bivalves such as clams. Mussels, due to their epibenthic habitat are often grown in rope cultures, where the mussels are allowed to attach to ropes which are then suspended in the water column (Camacho et al., 1991). The mussels are allowed to grow to marketable size and are harvested by pulling up the ropes and removing the mussels (Camacho et al., 1991). In other locations, notably in the Menai straight in north-west Wales, mussels are cultured directly on the seabed and are moved up and down the shoreline depending on the size of the mussel (http://www.menaioysters.co.uk/ accessed 2014).

Oyster culture methods depend upon the location. Floating oyster bags (suspended in the water column) and trestle tables are used in North America (Mallet et al.,2006) whereas in Poole Harbour, in the UK, adult oysters are laid directly upon the seabed (Gary Wordsworth, personal communication). Oysters, cultured on the trestle tables are harvested by hand, whereas the oysters laid on the seabed are harvested using mechanical dredges operated from boats.

Infaunal bivalves such as clams are cultured in the sediment, where spat clams are laid directly upon the substrate and allowed to burrow (Serdar et al., 2007; Spencer et al., 1991). To reduce predation, plastic netting is often laid upon the substrate to stop predators such as crabs from getting access to the bivalves (Cigarria & Fernandez, 2000; Spencer et al., 1992). The clams are harvested either using mechanical dredges attached to boats, by tractor or by hand at low tide (FAO 2013).

Since the early twentieth century the Manila clam has been introduced around the World both intentionally and accidently. Since an initial accidental introduction to the Unites States, the Manila clam has been intentionally introduced to many other countries for the purpose of aquaculture. These regions include: the Atlantic coast of France in 1972 (Dang et al., 2010),the North west of Ireland in 1982 (Drummond et al.,

2006), Venice Lagoon in Italy in 1983 (Pellizzato et al., 2011), Spain in the late 1980s (Flassch & Leborgne, 1992) and Poole Harbour in the UK in 1988 (Jensen et al., 2004).

The Manila clam is one of the World's most important aquaculture species and its production accounts for up to 20% of the Worlds shellfish market (Sladonja et al., 2011). The World's largest producer of Manila clams is China, which accounts for 97.4% of the global production (FAO 2013). In 1996, China produced 1.6 million metric tonnes of clams, the majority of which were Manila clams (Ximing et al., 1999). The three major producing countries in order are: China, Italy and the USA (Sladonja et al., 2011). In Europe the Manila clam is replacing the local palourde species *Ruditapes decussatus* in aquaculture due to the fact it is easier to culture, spat are easier to obtain and it is hardier and grows faster to marketable size (Sladonja et al., 2011).

An example of how important the Manila clam is to Chinese aquaculture is given by Jiaozhou Bay in Qingdao province. In 2003 over 10,000 hectares of seabed in Jiaozhou Bay were enhanced (by importing large numbers of spat clams and seeding them on the mudflats) for use in Manila clam production (Ren et al., 2008). This area alone yielded 320,000 tonnes of Manila clams (Ren et al., 2008). The enhanced area of seabed accounted for 45% of the whole Manila clam production for Jiaozhou Bay, with the remaining 55% coming from naturally occurring clams (Ren et al., 2008).The level of production for the enhanced area of Jiaozhou Bay alone is 8 times higher than the most productive site in Europe, Venice Lagoon, Italy (Pellizzato et al., 2011) and 320 times higher than the most productive area in France, Arcachon Bay (Dang et al., 2010).

Outside of China there are countries where Manila clam landings are falling, including Korea (Mitsuharu, 2004; Uddin et al., 2013) and Japan (Mitsuharu, 2004). An example is Tokyo Bay, Japan, which at its peak had yearly landings of 70,000 tonnes in the 1960s, reducing to under 20,000 tonnes in the 1970s (Mitsuharu, 2004). This was put down to land reclamation and reduced availability of wild spat clams (Mitsuharu, 2004). Korea is also experiencing a reduction in Manila clam production due to mass mortalities caused by Perkinsosis (FAO 2013).

Perkinsosis is a genus of protozoan parasites that infect molluscs including, oysters, clams, mussels and abalone (Villalba et al., 2004). Infection usually occurs due to the

ingestion of perkinsosis zoospores from the water column by uninfected bivalves. Initial infections often occur in the gills and siphons of the bivalve, but then spread to other tissue and organs in the host. Once infected, perkinsosis causes lesions to occur in the host tissue. This often results in reduced condition, slower growth rates and can result in organ failure and death (Villalba et al., 2004).

When these mass mortalities of Manila clams occur they can have devastating effects upon the local economy (Mitsuharu, 2004). Theories behind the causes of the mass mortalities in bivalves range from upwelling of oxygen poor water (Mitsuharu, 2004), diseases such as Perkinsosis (FAO 2013) and metabolic debt caused by increased metabolic demand and reduced food availability (Weiss et al., 2007). The causes of Manila clam mass mortalities in areas where they have been introduced for aquaculture are poorly understood.

The methods used to culture Manila clams often differ by location and farming intensity. Traditional methods rely upon the recruitment of spat clams from the local populations (Mitsuharu, 2004) or by transplanting wild seed clams from other locations to the culture site (Mitsuharu, 2004; M. Pellizzato et al., 2011; Ren et al., 2008). In countries where the Manila clam is not native, hatchery reared spat are introduced to the culture areas (Campos & Cachola, 2006; Jensen et al., 2004; Sladonja et al., 2011). Hatchery seed is produced by conditioning adult clams with high levels of food and inducing them to spawn by a temperature shock. The larvae are then collected and raised under optimum temperature and food conditions until they metamorphose and settle (Sladonja et al., 2011). The juvenile clams are then grown up to a size of 12-15mm before being ready to be sown on the culture site (Sladonja et al., 2011). In some culture sites the clams are covered with plastic netting to prevent predation, but not in others (Cigarria & Fernandez, 2000).

The clams are then allowed to grow up to marketable size, which depends upon the country. The minimum landing size of Manila clams in Poole Harbour is 35mm. In the rest of the European Union the minimum landing size is also 35mm (Dang et al., 2010). Once the clams have reached this minimum size they are harvested. Harvesting methods for Manila clams often depend on the location and the level of commercialisation. These methods range from collecting by hand using a rake (Sladonja et al., 2011), using a

specially designed dredge from the back of a boat (Sladonja et al., 2011), a specially designed pump scoop mechanism (Jensen et al., 2004) and a harvesting barge with a conveyor that brings up the catch from a pump scoop dredge (personal observation Poole Harbour). Once the clams have been harvested they are then depurated to remove any harmful pathogens and grit or sand and then sold either as live or frozen (Sladonja et al., 2011). The different methods both have advantages and disadvantages. Using a hand rake is not very invasive but can only be undertaken at low tide and is very labour intensive. The use of dredges and pump scoops are very efficient, however they can cause a reduction in the species diversity of the location and also re suspend harmful chemicals and metals from the sediment in to the water column (Parker & Pinn, 2005; Sladonja et al., 2011).

The efficiency of the dredge and pump scoop methods can also cause a problem due to the fact that they remove up to 75% of the targeted sized clams (Dang et al., 2010; J. Humphreys et al., 2007). This could lead to issues of sustainability and potentially cause the fisheries to collapse, especially in areas where the Manila clam is not native such as Poole Harbour.

1.8 Poole Harbour

Poole Harbour is a large intertidal estuary system located on the South coast of England, which at high tide has an area of water cover of approximately 3600ha (Humphreys, 2005). On spring tides it is estimated that 45% of the water in the Harbour leaves over the course of the tidal cycle, with 22% of the total water volume leaving on a neap tide (John Humphreys, 2005). The sediment in Poole Harbour is predominantly mud, with areas of muddy-sand, sand and gravel spread throughout the Harbour (Herbert et al., 2010).

Poole Harbour is classified as an estuary with a well-mixed water column for the majority of its area, however at the Wareham channel it is classed as partially mixed (John Humphreys, 2005). This is due to the main input of freshwater into the Harbour occurring in the Wareham channel from the River Frome and the River Piddle. As a

consequence of the main freshwater input occurring in the Wareham Channel, there is a salinity gradient across the Harbour with the maximum recorded salinities at the Harbour entrance and the minimum at the upper reaches of the Wareham channel at the western end of the Harbour (Humphreys, 2005). Salinity also varies with the state of the tide, with the lowest recorded salinities coinciding with low tide. Poole Harbour is unique in that it is both micro-tidal, with a range of 1.8m on a spring tide and 0.6m on a neap tide and that it has a double high water so that for 16 out of a 24 hour cycle the water level is above the mean tide level (Humphreys, 2005).

Poole Harbour is classed as a Special Protection Area (SPA) due to its importance to wildfowl for breeding, overwintering and feeding (Caldow et al., 2005). Poole Harbour is ecologically diverse, with over 61 species of macro-invertebrates found in the intertidal areas of the Harbour (Caldow et al., 2005), with subtidal areas containing 68 seaweed species, 159 invertebrate species and 32 fish species (Dyrynda, 2005). Of the 61 species that are found in intertidal habitats, 15 are mollusc species which means that molluscs account for nearly 25% of the species found in the intertidal regions (R. Caldow et al., 2005).

Although it is an area of ecological importance, Poole Harbour has been modified by human enterprise and supports a wide range of recreational and economic activities including recreational boating, fisheries and commercial shipping (see figure 1.7). The main navigation channels are regularly dredged to support the commercial shipping that uses the port facilities in the Harbour. There are a number of marinas for recreational boat users located around the Harbour as well and areas designated for jet skiing and windsurfing (Dyrynda, 2005).

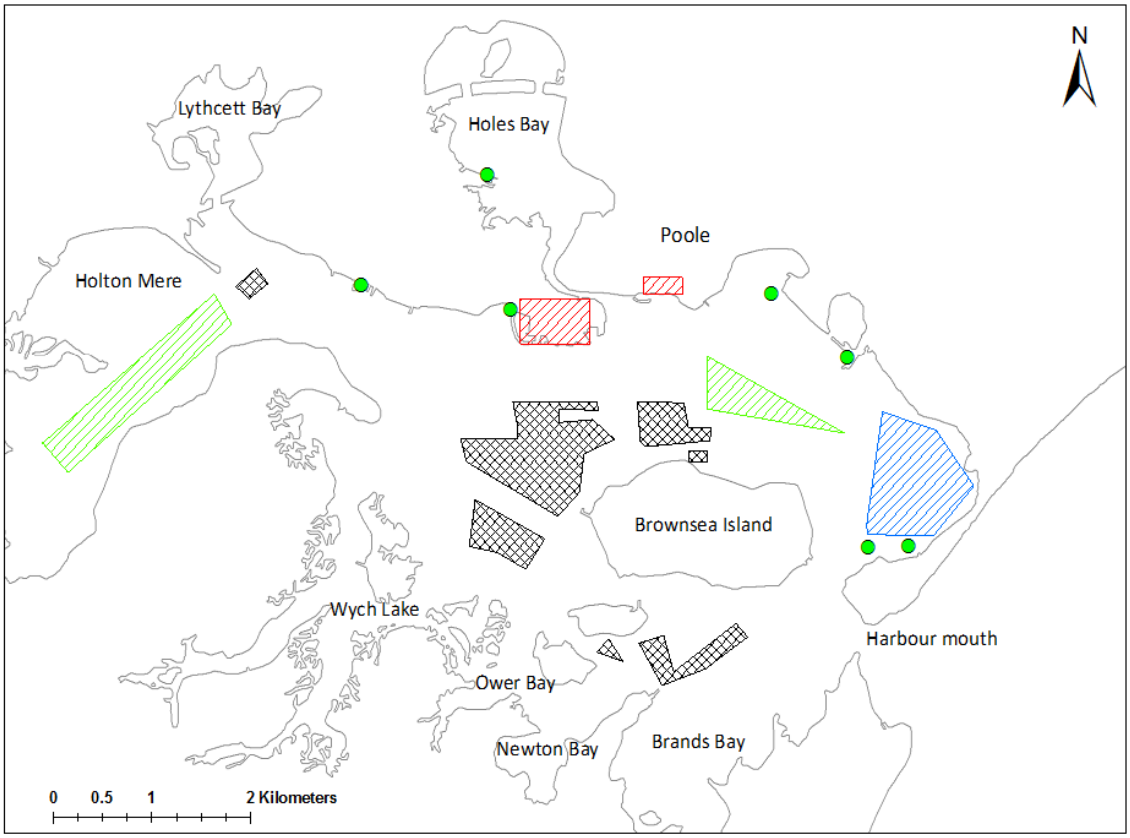

Figure 1.7 Map of selected human activities in Poole Harbour. Green circles indicate recreational marinas, green-hatched areas are an approximation of the designated areas for jet skiing, the blue-hatched area is an approximation of the designated area for windsurfing, red-hatched areas are an approximation of the commercial zones including Poole Quay (small box) and the Poole Ferry terminal and docks (large red box) and the black-hatched areas are an approximation of the shellfish lease beds.

Poole Harbour has a mix of both wild and aquaculture fisheries occurring in its designated area, with an estimated worth of £2 million per year (Jensen et al., 2005). The wild fisheries target cockles, clam species, shellfish including prawns and finfish including bass and grey mullet (Jensen et al., 2005), whereas the aquaculture fishery concentrates on the culturing of Oysters on the shellfish lease beds (Dyrynda, 2005). Manila clams were also originally cultured on the lease beds, but mass mortality events made the continuation of this fishery at an economic level unsustainable.

1.9 The naturalised population of Manila clams in Poole Harbour

The Manila clam population in Poole Harbour has become naturalised and is self-sustaining (Humphreys et al., 2007; Jensen et al., 2004). This is contrary to what MAFF thought would occur at the time of introduction (Laing & Child, 1996; Spencer et al., 1991). Due to its importance as the most northerly naturalised population of Manila clams in Europe, the Poole Harbour population has been the subject of several studies (Humphreys et al., 2007; Jensen et al., 2004).

The first study investigated the establishment of the Manila clam fishery in Poole and its spread across the harbour (Jensen et al., 2004). In Poole harbour, the clam had colonised large areas of the intertidal zone outside of its initial aquaculture site (Jensen et al., 2004). The species has also established populations in areas adjacent to the harbour mouth in the east, up to the Wareham channel in the west, and in many of the bays on both the north and south coasts of the harbour (Jensen et al., 2004).

The second study investigated the population dynamics of the Poole Harbour population between January 2003 and January 2004 (Humphreys et al., 2007). They found the population to be self-sustaining with 6 distinct age classes (Humphreys et al., 2007). The study also found that the local fishery was responsible for removing 75% of the clams above the legal minimum size of 40mm over the winter period (Humphreys et al., 2007). Since this study was completed the Minimum landing size (MLS) for Manila clams was reduced from 40mm to 35mm (Lambourn & Le Berre, 2007). This raises the question of whether this reduction in MLS will affect the population dynamics of the Poole Harbour population and the productivity of this population. The fact that the Manila clam became naturalised in Poole Harbour in the first place raises the question of whether it has managed to become naturalised elsewhere and whether it has begun to spread from the areas of initial introduction.

1.10 Distribution of the Manila clam in British waters and potential dispersal mechanisms

In the last 200 years the rate of reported marine invasions by non-indigenous species has greatly increased (Brandt et al., 2008; Ruiz, Fofonoff, Carlton et al., 2000). The most common invasive species are crustaceans and molluscs (Ruiz et al., 2000). In North America the vectors by which a non-indigenous species is introduced have previously been categorised as: shipping, fisheries, bio-control, ornamental escape, agricultural escape, research escape or multiple vector escapes (Ruiz et al., 2000). These vectors can also be used to categorise how a species might spread in other locations as well.

The northern most naturalised population of Manila clams in Europe is currently located in Poole Harbour in Dorset (Jensen et al., 2004). The term naturalisation refers to a non-native species that was introduced to a location by human agency but has subsequently adapted to the local conditions and is recruiting without further human influence (Hettinger, 2001).

Manila clams are found further north than Poole Harbour, in the Northwest of Ireland, but this population is currently not self-sustaining, so it is not classed as naturalised (Drummond et al., 2006). Because the larval stage of the Manila clam has a high dispersal potential (Kasuya et al., 2004) the Manila clam has the ability to spread out from the currently established populations and establish new populations. The fact that it is a commercially viable species also means that it could be spread by anthropogenic factors (Bourne, 1982) and could in fact be more widely distributed than currently reported.

1.11 The aims of this thesis

The introduction and subsequent naturalisation of the Manila clam in British coastal waters, has provided the opportunity to study the novel interactions between a non-native species and the environment and ecosystem into which it has been introduced. The distribution of the Manila clam throughout the South and South Eastern coastline of England is currently poorly understood due to a lack of scientific literature on its distribution and the absence of the Manila clam from many identification guides. This fact and a morphological similarity with the local species *Ruditapes decussatus* suggests that the reported distribution of the Manila clam could be under-reported. This study set out to determine the current distribution of the Manila clam along the South and South Eastern coastline of England and to determine possible reasons for this distribution (Chapter 2).

The first reported naturalised population of Manila clams in the UK was in Poole Harbour in Dorset. This population was last studied in 2004 over a period of 12 months and the population dynamics were reported. This thesis sets out to describe the population dynamics of the Holton Mere population over a longer timescale (between 2009 and 2012) and investigate the potential impacts of the reduction in minimum clam landing size. It also set out to determine whether recruitment occurred on an annual basis and how the population at Holton Mere compared with other native and introduced populations of Manila clams (Chapter 3).

Condition index in bivalves is known to vary with season and reproductive cycle. Condition index has also been found to influence levels of reproductive success. The importance of condition index to reproductive success highlights the importance of determining which environmental conditions influence condition index. This study set out to determine which environmental factors influenced the condition index of the Poole Harbour population (Chapter4).

The majority of natural dispersal undertaken by the Manila clam takes place during the larval stage. Larvae are able to influence their dispersal by modifying their swimming behaviour. As a result, the larval stage and its response to environmental stimuli are

important in determining the distribution and continued presence of a population. In order to clarify the response of larvae to salinities, this study set out to determine the tolerance of larvae to reduced salinities, the behavioural response to the presence of a halocline and the influence of reduced salinity on swimming velocity (Chapter 5).

Once settled, bivalve spat are vulnerable to predation, predators can greatly influence the success of a recruitment event. Bivalves have developed strategies such as burial behaviour in order to reduce levels of predation. This study set out to determine the burial response of Manila clams to the presence of a predator and a variety of salinities. This study also set out to determine the effect of environmental conditions such as salinity and temperature on the predation rates of the European shore crab on Manila clams and the size at which clams became safe from crabs. (Chapter 6).

Chapter 2: The distribution of the invasive Manila clam in the British Isles

2.1 Introduction

The Manila clam is non-native to British coastal waters and was introduced for the purpose of aquaculture in the 1980s (Humphreys, 2010; Humphreys et al., 2007; Jensen et al., 2004). Before licenses were granted to allow the Manila clam to be cultured, a series of experiments were carried out by the Ministry of Agriculture Fisheries and Food (MAFF) which determined that it would be unable to reproduce due to low sea water temperatures (Humphreys et al., 2007; Spencer et al., 1991; Utting et al., 1991). This proved incorrect and the Manila clam successfully reproduced and naturalised in Poole Harbour in Dorset within six years of its introduction (Jensen et al., 2004). The naturalisation in Poole Harbour raises the question of whether it has become naturalised elsewhere and what is its potential for further invasions?

Marine invasions and dispersal in bivalves

Invasions by marine organisms are predominantly recorded in bays and estuaries and consequently, tolerance to a range of salinities is essential for invasion success (Miller et al., 2007). Miller et al., (2007), cite Kennish (1990) that environmental tolerance is viewed as being the limiting factor in the distribution of organisms in their native ranges. Invasions are more likely to be successful if the environment that is being invaded is similar to that from which the organism originates (Miller et al., 2007). However similar environmental conditions are not the only factor that influences the success of an invasion. Disturbed and marginal environments are more likely to be successfully invaded than settled environments (Levinton, 2001), p430)

The species diversity of an environment can affect the success of an invasion, with environments with greater species diversity being more resistant to invasions than those with low species diversity (Stachowicz et al., 2002). Predation by native predators may influence the success of an invasion, as predation is known to influence bivalve recruitment success (Hiddink et al., 2002). In order for an invasion to be successful there needs to be a steady stream of recruits as without this the population will ultimately disappear (Levinton, 2001), p423).

The process of invasion requires many criteria to be met in order for it to be a success (Miller et al., 2007). These criteria include: a vector to move the organism to a new location, survival of the organism in transit, initial survival on release, establishment of a self-sustaining population and subsequent spread (Ruiz et al., 2000).

For bivalves the most important vector for introduction has been the deliberate transport by humans (Miller et al., 2007). Once in a new location the similarity of the environmental conditions to the source location plays an important role as to whether the species becomes established (Miller et al., 2007). This proposes that if a species is naturally distributed in estuarine environments, these areas will be the most likely to be colonised. Miller et al, (2007) state that bivalve tolerance to salinity is the most important aspect as to whether a species established or not. This implies that a species that is able to tolerate a wide range of conditions will have a greater potential for invasiveness and a greater range in which it can become established. The fact that the Manila clam is naturally found in estuarine environments (Cigarria & Fernandez, 2000) and is able to tolerate low salinities (Laing & Child, 1996) suggests that these locations are more likely to be colonised than other locations. The original distribution of the Manila clam in the UK is a result of human activity due to its introduction as an aquaculture species to create a series of fisheries (Humphreys, 2010; Humphreys et al., 2015).

Further dispersal by non-native species can be driven by either human introductions or larval dispersal (Bourne, 1982). In many bivalves the larval stage is the means of dispersal. The larvae are transported by tides and currents, although they cannot alter their horizontal position in the water column, they are able to influence their vertical distribution (Bayne, 1964; Raby et al., 1994). This ability to control vertical position in the water column, allows bivalves to influence their dispersal pattern by either avoiding or actively swimming into areas of the water column with higher levels of tidal flow (North et al., 2008). North (2008) found that larvae of both *Crassostrea virginica* and *Crassostrea ariakensis* had different distributions in Chesapeake Bay due to the behaviour of the larvae in relation to salinity (North et al., 2008). The ability to influence dispersal by vertical migration in the water column did not stop the larvae from being dispersed over a wide area, with larvae travelling between 7.1- 9.0km before settling

(see figure 2.1) (North et al., 2008). The Manila clam has also been reported to disperse widely during the larval stage (Kasuya et al., 2004). This suggests that during its pelagic larval phase it has the potential to colonise a wide geographical range.

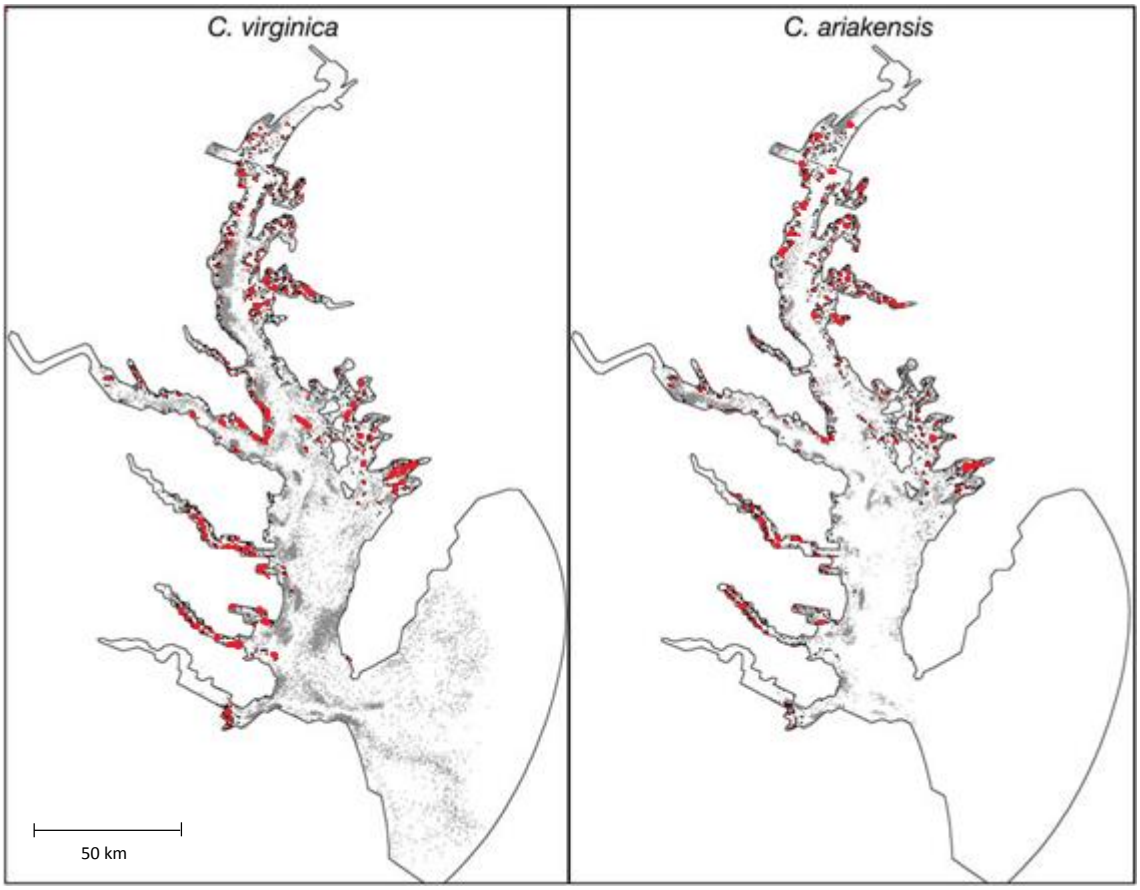

Figure 2.1: The predicted distribution of *Crassostrea virginica* and *Crassostrea ariakensis* in Chesapeake Bay (USA), taken from North et al. 2008. Still taken from a particle tracking model of Chesapeake Bay where hydrodynamic models and oyster larva behaviour were modelled to predict distribution. The red dots represent suitable habitats for oyster settlement and existing populations of adult oysters, the grey dots represent the dispersal of larvae as predicted by the model.

The Manila clam as an aquaculture species

Since the early twentieth century the Manila clam has been spread around the world, sometimes by accident (Bourne, 1982) and others times intentionally (Cigarria & Fernandez, 2000; Dang et al., 2010). The Manila clam was introduced into Europe in the 1980s for the purpose of aquaculture (Dang et al., 2010; Pellizzato et al., 2011; Pellizzato, et al., 2011) and into British coastal waters in the same decade by the Ministry of Agriculture Fisheries and Food (MAFF). This was to create a new fishery and to help diversify the UK shellfish industry (Humphreys, 2010). The Manila clam was not the only

non-native species of shellfish to be intentionally introduced into the UK. The Pacific Oyster, *Crassostrea gigas* had been introduced into British Coastal waters to create a fishery. The date for the initial introduction of this species is open to debate, with known deposits occurring in the 19th century, however the first disease free, UK-based-hatchery-cultured Pacific Oysters were introduced into the Blackwater Estuary in Essex in the 1960s (Herbert et al., 2012).

As with the Pacific Oyster, before licences were granted to culture Manila clams, a series of experimental trials were undertaken to investigate the suitability of Manila clams for aquaculture in the British Isles (Spencer et al., 1991). Manila clams were imported from the West coast of the United States to be used as brood stock and were quarantined to ensure they were disease free before the trials took place (Spencer et al., 1991). The aquaculture trials took place in: the Menai Strait Wales, Walton-on-the-Naze Essex, the River Beaulieu (Hampshire), the River Exe Devon and the River Helford Cornwall (Spencer et al., 1991). After the experiments had been undertaken, licences were then granted for aquaculture at several more locations including: Emsworth Yacht basin in Hampshire, The Blyth Estuary in Suffolk, West Mersea in Essex the River Teign in Devon, Poole Harbour in Dorset and Loch Creran in Scotland (Hansard 1985). Seed clams were provided to Seasalter Hatcheries in Kent in order to create a Manila clam hatchery in mainland UK (Spencer et al., 1991).

The locations where the initial trials and licenses were granted in the British Isles can be seen in Figure 2.2 with more detail in Table 2. The south and eastern coast of England had the greatest frequency of trial locations and licenses granted. Out of all of the 19 locations used in either trials or those that were granted licences for culture, 15 of these were along the south and eastern coast of England.

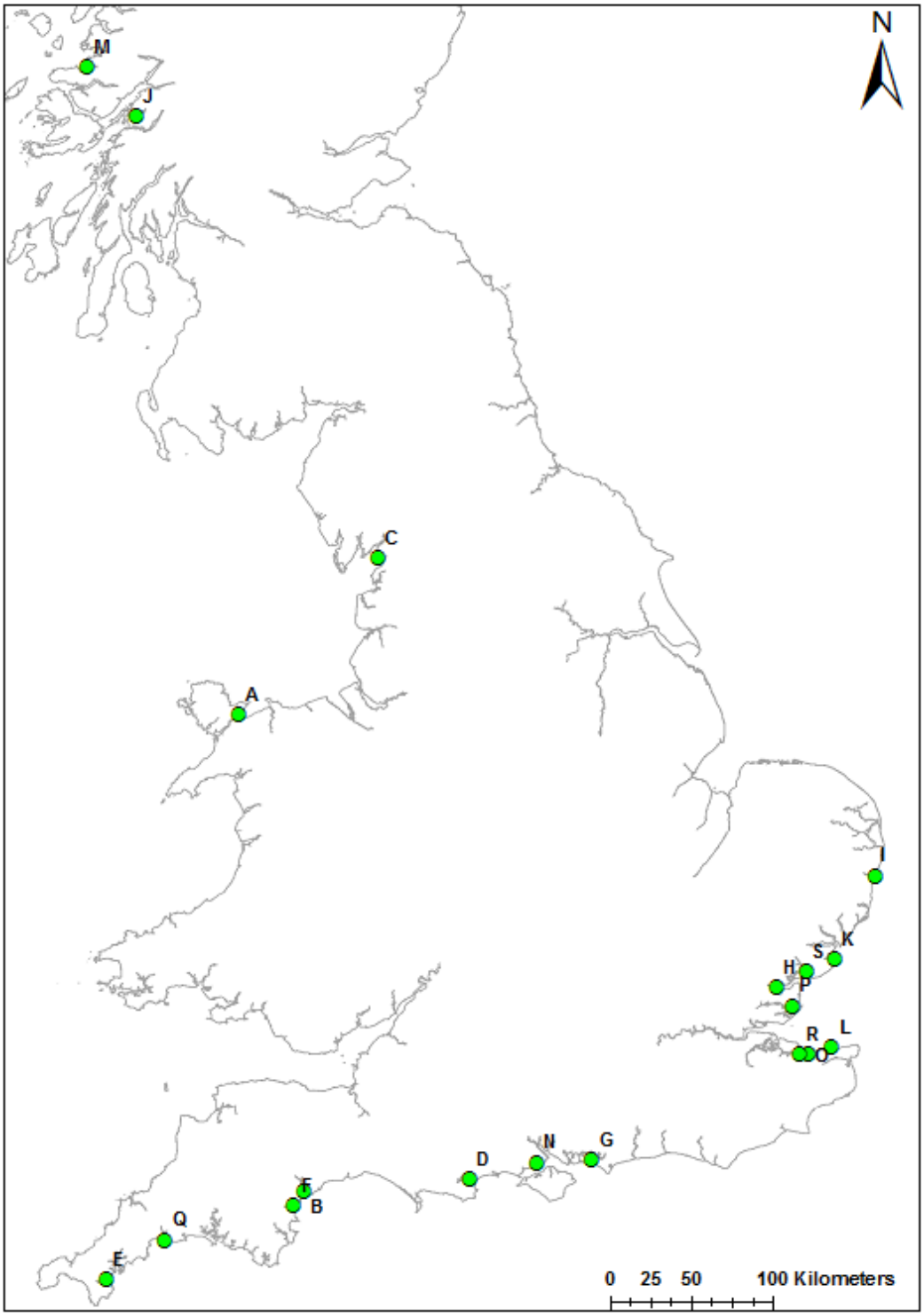

Figure 2.2: The location of the original trial plots and licensed introductions of the Manila clam in the British Isles. The locations are labelled A-S with more information on the locations detailed in table 2. The majority of the introductions were made along the South and Eastern coast of England, with one in Wales (A) and two in Scotland (M&J).

Table 2: The locations of the initial aquaculture sites from figure 2.2 and the year in which Manila clams were introduced.

Site	Location	Year introduced	Reference
A	Menai Strait, Wales	1982-1985	Spencer *et al*. 1991
B	Exe Estuary, Devon	1982-1985	Hansard 1985
C	Morecambe bay	1982-1985	Humphreys *et al*. 2015
D	Poole Harbour	1988	Humphreys 2010
E	Helford Estuary, Cornwall	1982-1985	Spencer *et al*. 1991
F	Teign Estuary, Devon	1982-1985	Hansard 1985
G	Chichester Harbour, Hampshire	1982-1985	Hansard 1985
H	Blackwater Estuary, Essex	1982-1985	Spencer *et al*. 1991
I	Blyth Estuary, Suffolk	1982-1985	Hansard 1985
J	Loch Creran, Argyll Scotland	1982-1985	Hansard 1985
K	Walton on the-Naze, Essex	1982-1985	Spencer *et al*. 1991
L	Reculver, Kent	1982-1985	Personal comm. J Bayes
M	Loch Miodart, Scotland	1988	N Lake 1992
N	Beaulieu River, Hampshire	1982-1985	Spencer *et al*. 1991
O	Seasalter, Kent	Late 1980s	Personal comm. J Bayes
P	Crouch-Roach Estuary	1982-1985	Hansard 1985
Q	Fowey Estuary, Cornwall	Pre 1996	Jack 2010
R	Sheppey, Kent	Late 1980s	Personal comm. J Bayes
S	Colne Estuary, Essex	1982-1985	Hansard 1985

One of the reasons that MAFF authorised the introduction of the Manila clam into British waters was because it would not pose a threat to local species or become invasive. This is because the scientific opinion at the time was that it could be grown to marketable size, but would be unable to reproduce in British waters (Spencer et al., 1991). This was due to the seawater temperature regime around the British Isles not being compatible with the reproductive requirements of this species (Spencer et al., 1991).

This was incorrect and the Manila clam became naturalised in Poole Harbour in Dorset (Jensen et al., 2004). Since its introduction, the Manila clam has spread throughout the harbour from the initial site of introduction on the clam aquaculture beds (Jensen et al., 2004). The distribution of the Manila clam in Poole Harbour in 2002/3 can be seen in

Figure 2.3 taken from Jensen et al (2004). The distribution is the result of larval dispersal throughout the harbour (Herbert et al., 2012).

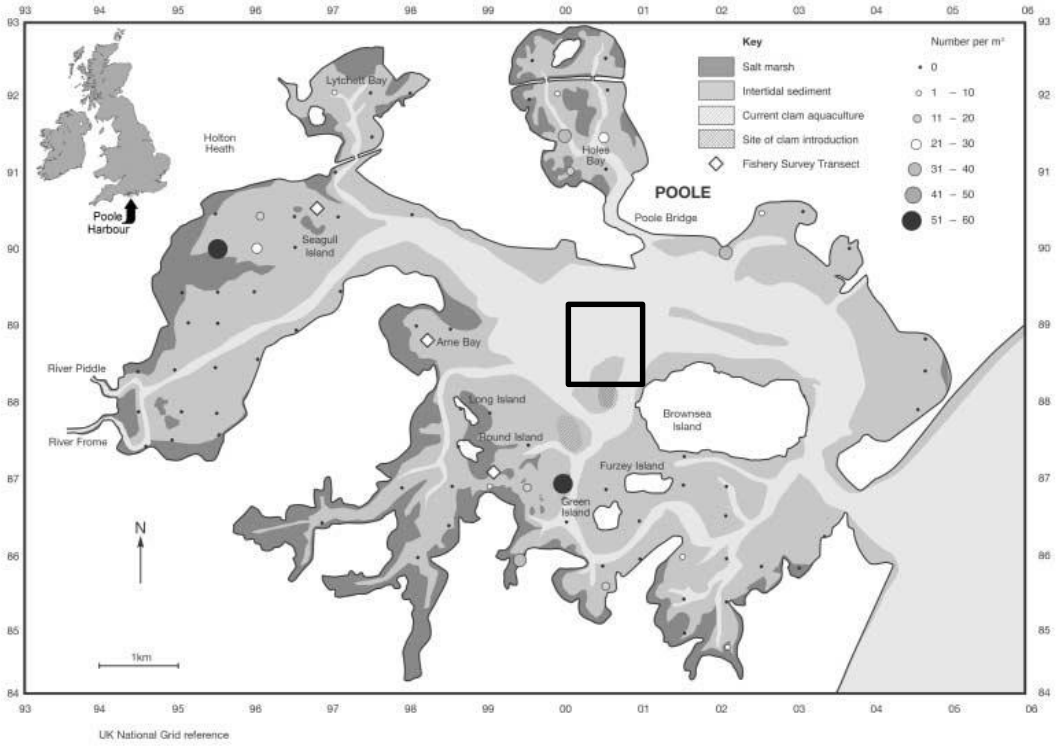

Figure 2.3 Distribution of the Manila clam in Poole Harbour in 2002/3 taken with permission from Jensen et al., 2004. The black square indicates the clam aquaculture beds, where the clams were laid on to the seabed to grow to marketable size. The graduated circles represent increasing densities of Manila clams.

The naturalisation and spread of the Manila clam throughout Poole Harbour raises the question of whether it has managed to successfully colonise new areas. If new populations have become successfully established is it due to dispersal as larvae or through human actions such as fishermen creating new fisheries?

The current reported distribution of naturalised populations of Manila clams in the British Isles is: Poole Harbour in Dorset (Humphreys et al., 2007), Southampton water in Hampshire (Tumnoi 2012), the Exe Estuary in Devon (personal communication Devon and Severn IFCA) and Seasalter Hatcheries in Kent. This chapter aimed to determine whether the Manila clam is in fact more widely distributed than currently reported and to use metadata analysis to attempt to identify underlying factors that may influence the distribution.

2.2 Materials and Methods

This study focused on the Harbours and Estuaries along the south and eastern coast of England, from the River Helford in Cornwall up to the Wash in Norfolk. This focus was selected due to the high number of Manila clam introductions along this stretch of coastline. The majority of the surveys were conducted between 2009 and 2011 with two surveys in Chichester Harbour occurring in 2013. Out of the 52 estuaries surveyed, the Author was actively involved in the sampling of 11, with John Humphreys providing data for the remaining 41.

Field surveys carried out by the Author

To determine where to begin the surveys, a literature search was undertaken to identify areas that had previously had licences to lay Manila clams granted, were one of the initial trial sites or had a reported presence of Manila clams. The sources included documentation from CEFAS for shellfish classification areas, Parliamentary papers and records, Journal papers and any mention of Manila clams in regards to aquaculture or in any other form of literature.

Locations that had previously had a presence of clams or that had clams reported were investigated first to determine whether there was still a presence of Manila clams. After all the locations that had reported sightings or a history of clam aquaculture had been sampled, the remaining estuaries and harbours located in the study area were surveyed. The field surveys consisted of an initial visual inspection of the strandline and the substrate surface for any dead shells. After the initial visual inspection, areas of substrate were sampled using either a rake (firm sediments such as sand) or sieve (muddy substrate) and any bivalves retained were identified on location if possible.

The presence or absence of Manila clams was noted and the GPS coordinates taken. All clams collected were taken back to the lab for positive identification as either Manila clam or local species. Reports of Manila clams by collaborators were also used to determine presence or absence of the Manila clam.

The methods used at each of the locations visited by the author are described in more detail for each individual location in the following section. GPS coordinates and maps where appropriate, are used to define the locations surveyed. As the aim of the field surveys was to determine the presence or absence of Manila clams, once they were found at a location, no further sampling effort was expended.

Southampton Water

Sampling in Southampton Water was undertaken on board the University of Southampton research vessel Callista on the 7-10-2011. A clam dredge with a width of 65cm was towed behind the vessel for approximately 100m and then the contents were sorted on deck using water pumps and sieves. A total of four dredge samples were collected giving a total area 260m^2. The presence or absence of Manila clams in each dredge was recorded. A total of four dredges were sampled, and the GPS coordinates of these dredges are recorded in table 3.

Table 3: The GPS coordinates of the dredge samples taken in Southampton Water.

Dredge	Start coordinates	End coordinates
1	N 50° 51.615, W 001°22.164	N 50° 51.527, W 001°21.816
2	N 50° 51.583, W 001°22.078	N 50° 51.437, W 001°21.678
3	N 50°51.500, W 001° 21.927	N 50° 51.385, W 001°21.549
4	N 50.51.685, W 001° 22.304	N 50° 51.564, W 001°21.877

The location of the dredge samples in Southampton Water is displayed in figure 2.4. The red hatched areas indicate the approximate area that was dredged.

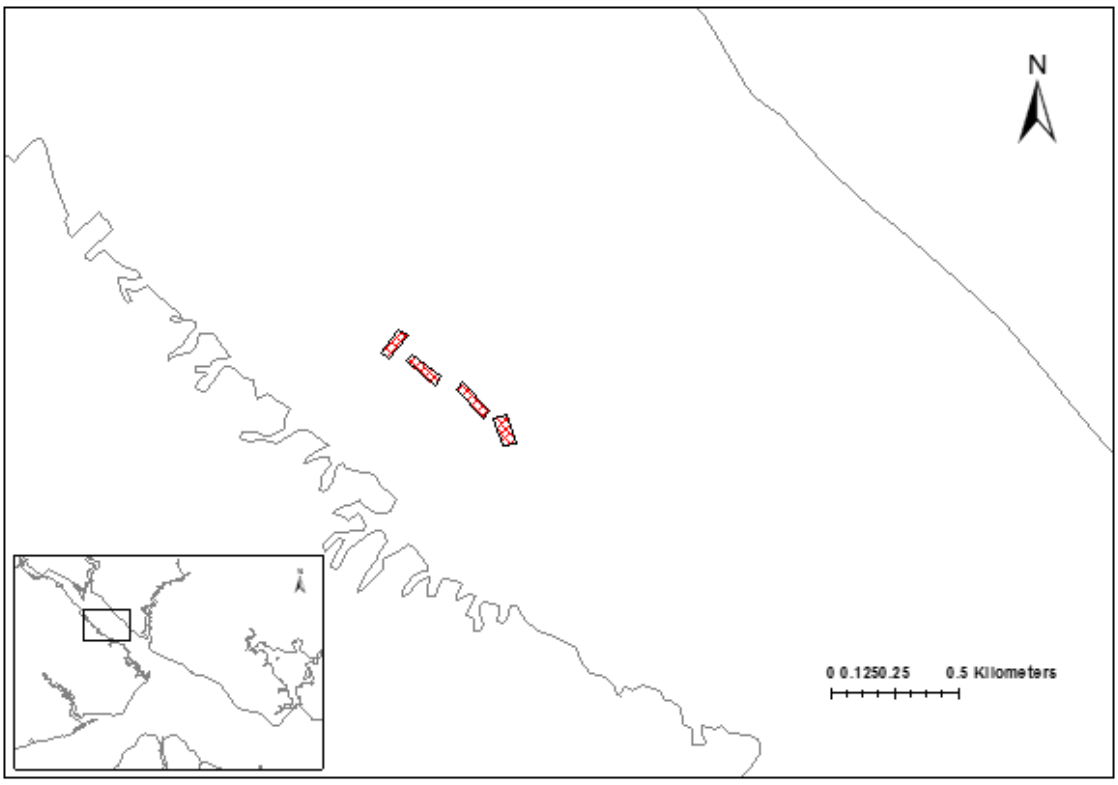

Figure 2.4: Map of the sampling locations in Southampton water. The red hatched areas represent the locations of the four dredge samples collected in Southampton water. Inset is the location of the sample area in relation to the rest of Southampton Water and the Solent.

Portsmouth Harbour

The boat survey in Portsmouth Harbour was conducted on the 28-5-2010 from the University of Southampton survey vessel Bill Conway. Trawl areas were designated by splitting the harbour into a grid system to ensure that no area of the harbour was missed, however the South and South East corners of the Harbour could not be sampled due to the proximity to the harbour mouth with its busy shipping channels and the Naval base with its restricted access. A clam dredge with a width of 65 cm was towed behind the vessel for approximately 100m and then sorted on deck using water pumps and the presence or absence of Manila clams was recorded. A total of ten dredges were sampled giving a total area of approximately 650m^2. The GPS coordinates were recorded at the beginning and end of each trawl to allow the area to be mapped (see table 4).

Table 4: The GPS coordinates of the dredge samples taken in Portsmouth Harbour.

Dredge	Start coordinates	End coordinates
1	N 50° 49.463, W 001°09.318	N 50° 49.462, W 001°093.421
2	N 50° 49.462, W 001°09.421	N 50° 49.458, W 001°09.426
3	N 50° 49.481, W 001°06.451	N 50° 49.520, W 001°06.430
4	N 50° 49.443, W 001°06.477	N 50° 49.517, W 001°06.443
5	N 50° 50.166, W 001°06.480	N 50° 50.072, W 001° 06.473
6	N 50° 49'59.4, W 001°06.159	N 50°49.537, W 001°06.098
7	N 50° 49.311, W 001°06.271	N 50° 49.373, W 001°06.375
8	N 50° 50.071, W001°06.495	N 50°50.025, W 001°06.453
9	N 50° 50.094, W001°07.447	N 50°50.139, W001°08.095
10	N 50°49.244, W 001°07.454	N 50°49.485, W 001°07.520

The location of the dredge samples in Portsmouth Harbour is displayed in figure 2.5. The red hatched areas indicate the approximate area sampled by the dredge. Dredge samples were sorted on deck for Manila clams.

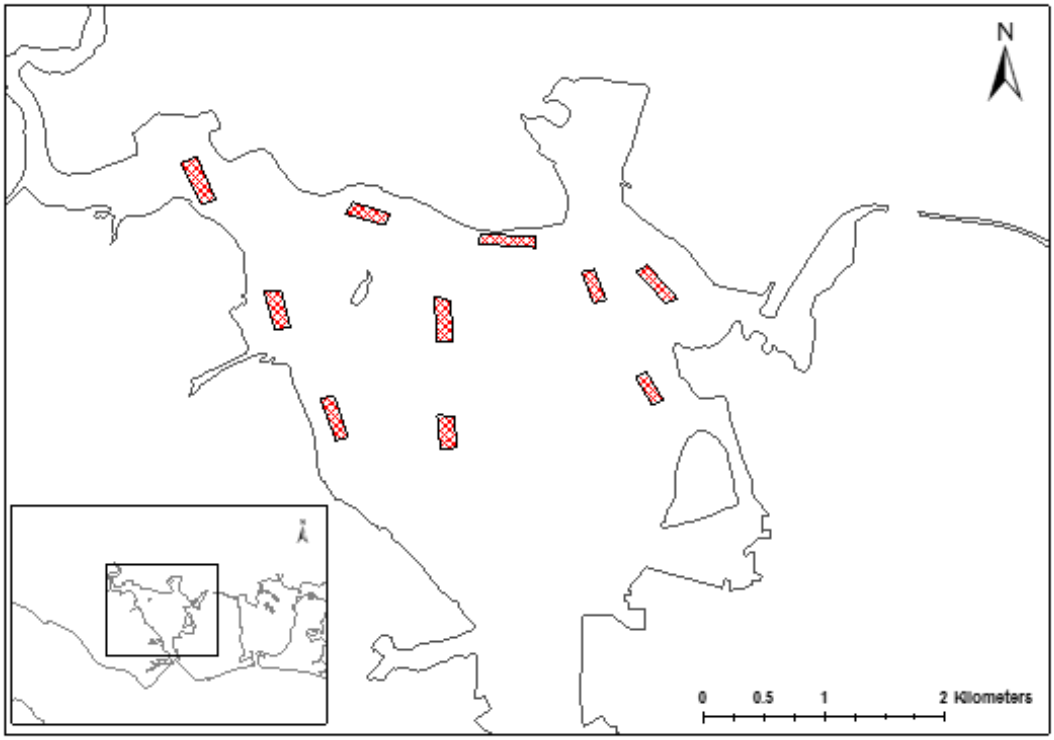

Figure 2.5: Map of the sampling locations in Portsmouth Harbour. The red hatched areas represent the location of the dredge samples. Inset is a map showing the sample area in relation to the rest of the Solent.

The Fleet Lagoon

The Fleet Lagoon was surveyed on the 30-9-2010 by a shore survey walking the strandline looking for dead shells for a distance of approximately 3km. The visual inspection covered an area of approximately 1m either side of the strandline, giving a total area of 6km^2. Two sites in the intertidal zone were then sampled. The two sites sampled in the Fleet were at the following GPS coordinates: N50°35.474 W002°29.139 and N50°35.318 W002°28.887.

Five random quadrats each with an area of 0.25m^2 were excavated to a depth of 5 cm using a hand trowel at each site (total area of 1.25m^2 at each site). Three adjacent areas of 5mx5m were also raked over to a depth of 5cm (giving an area of 75m^2 sampled at each site). The rake had a width of 29cm, with a gap of 1.6cm between tines which were 5cm long. The location of the shoreline survey and area sampled using quadrats and rakes can be seen in figure 2.6.

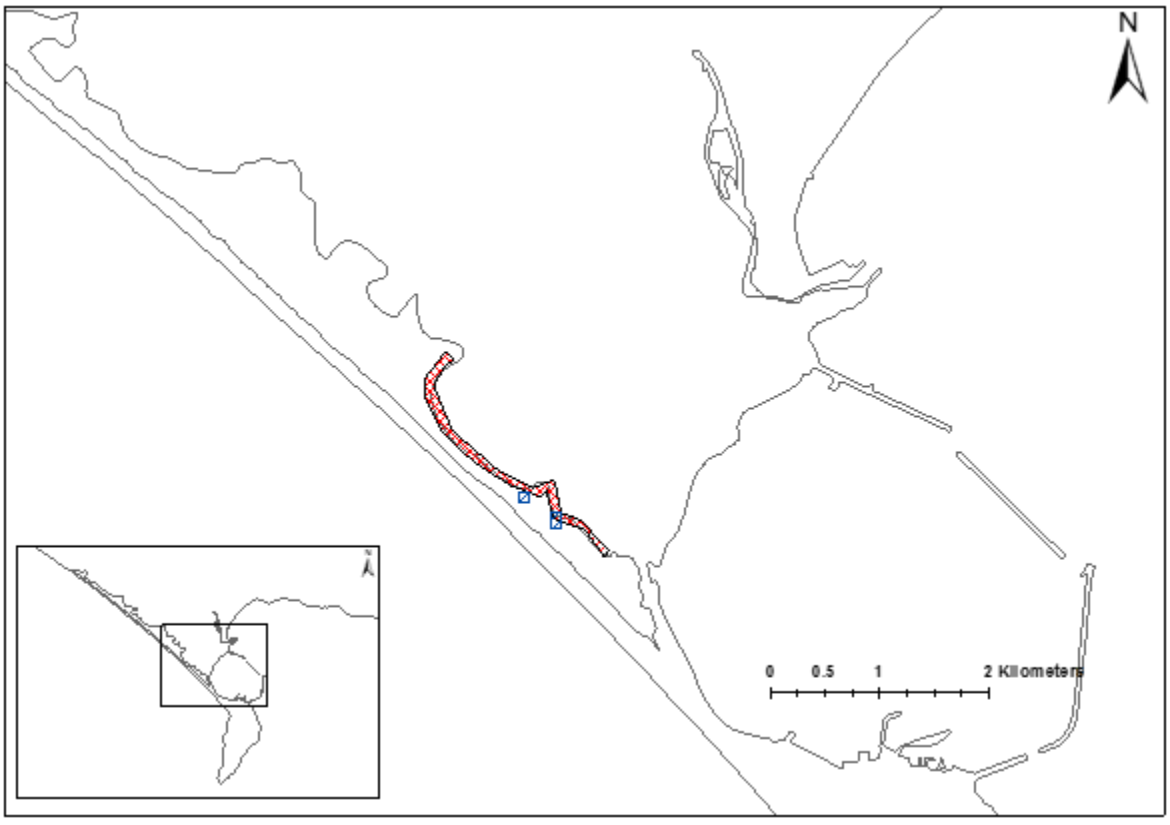

Figure 2.6: Map detailing the location of the areas sampled in the Fleet Lagoon. The red hatched areas denote the area covered by the shoreline survey, with the two blue hatched areas representing the two areas that were sampled using quadrats and were raked over.

Medina Estuary

The survey in the Medina Estuary was carried out on the 17-7-2009 by boat from the Odessa Boatyard at the southern end of the river down to Cavalier Quay at the river mouth. A sediment grab, with a width of 30cm, was used to sample the sub-tidal substrate and search for clams (see table 5 for the GPS coordinates of the grab samples). Twelve grab samples were collected giving a total area sampled of 3.6m^2. Bivalves collected from the grabs were identified and fixed. GPS coordinated were recorded at every grab site.

Table 5: The GPS coordinates of the grab samples taken in the Medina Estuary.

Grab no.	GPS Coordinates
1	N 50° 42.220, W 001°17.303
2	N 50° 42.246, W 001°17.294
3	N 50° 42.384, W 001°17.205
4	N 50° 42.414, W 001°17.187
5	N 50° 42.464, W 001°17.181
6	N 50° 42.495, W 001°17.173
7	N 50° 42.527, W 001°17.164
8	N 50° 42.432, W 001°16.528
9	N 50°43.519, W 001°16.492
10	N 50° 44.037, W 001°16.578
11	N 50° 44.147, W 001°17.080
12	N 50° 44.240, W 001°17.162

A survey of the intertidal zone was undertaken adjacent to the Medina Valley Centre (GPS coordinates: N 50°42.552 W 001°17.161) using a handheld dredge with a diameter of 30cm and a mesh bag with a 1mm diameter. Ten dredge samples were collected giving a total sampling area of 3m^2. Samples were then sieved through an 8mm sieve and all clams were collected. The locations of all of the grab samples and the dredge samples can be seen in figure 2.7.

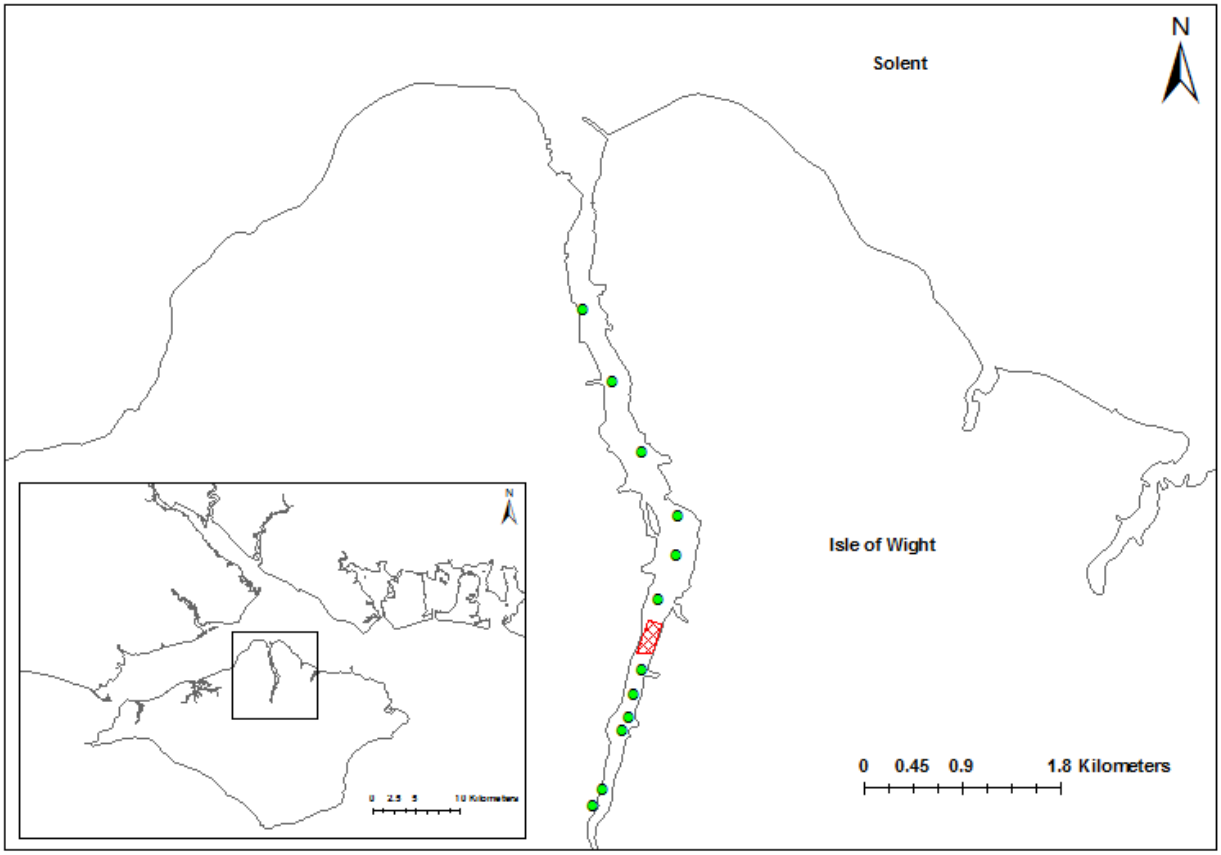

Figure 2.7: Map detailing the sampling methods used in the Medina Estuary. The green circles denote the sites of the grab samples, the red hatched box denotes the location where the dredge samples were collected. Inset is the location of the sample site in relation to the rest of the Solent.

Langstone Harbour

A shoreline survey was undertaken in Langstone Harbour on several occasions (1-8-2009, 14-8-2009 and 13-6-2010) initially searching for washed up shells 1m either side of the strandline (A total distance of 2.6km was surveyed across the three locations, giving an area of 5.2km^2) (see figure 2.8). One of the areas that had a presence of dead shells was then sampled using a rake in order to collect live specimens. Three 5m by 5m squares were raked to a depth of 5cm, giving a total area of 75m^2 with all live clams collected for further identification (See figure 2.8).

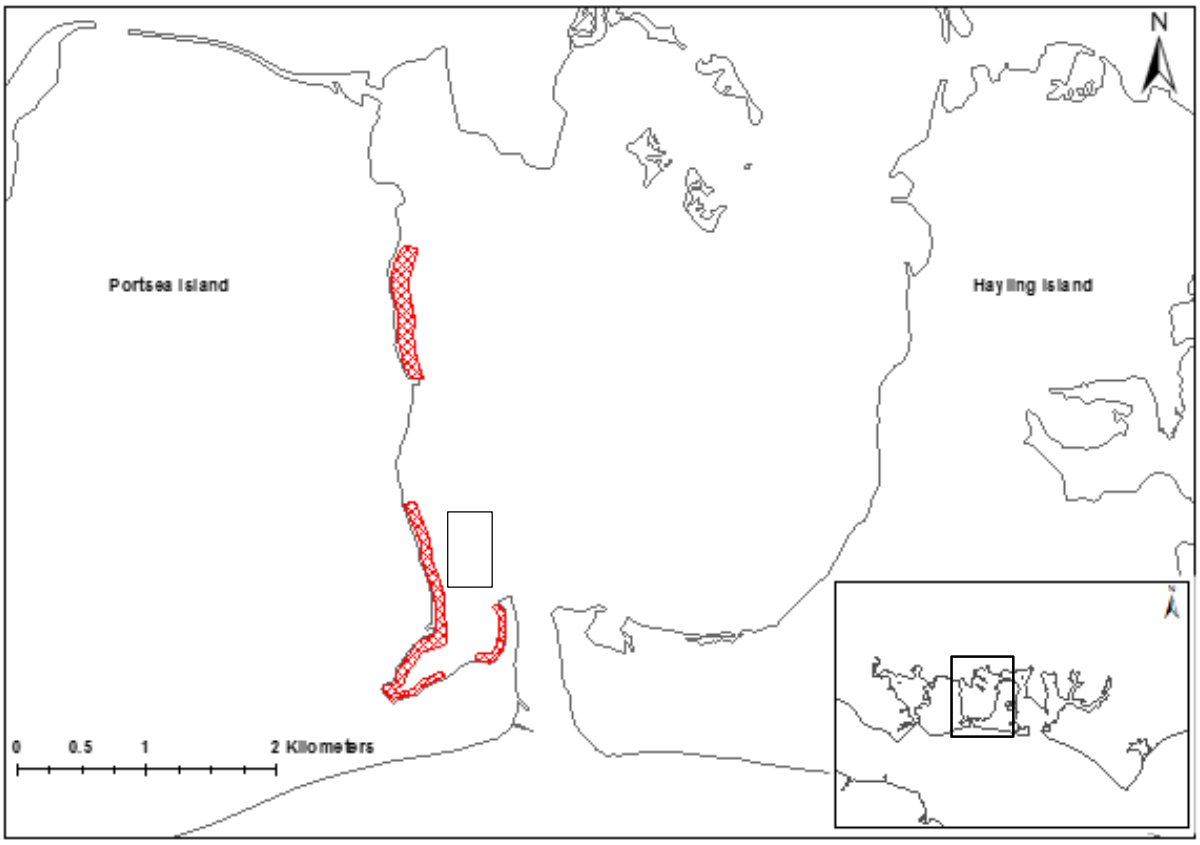

Figure 2.8: Map of the sampling locations in Langstone Harbour. The red hatched areas indicate the locations in Langstone Harbour in which the shore walking surveys were carried out to determine the presence or absence of Manila clams. The red hatched area located in the black box indicates the area that was sampled using rakes.

Chichester Harbour

A shoreline survey was undertaken in Chichester Harbour searching for washed up freshly dead shells along the strandline (see figure 2.9). The site of the initial Manila clam introduction, Emsworth Yacht Basin was chosen as a sample site as well as the area adjacent to the harbour mouth at the West Witterings and the area adjacent to Dell Quay, off the Fishbourne channel.

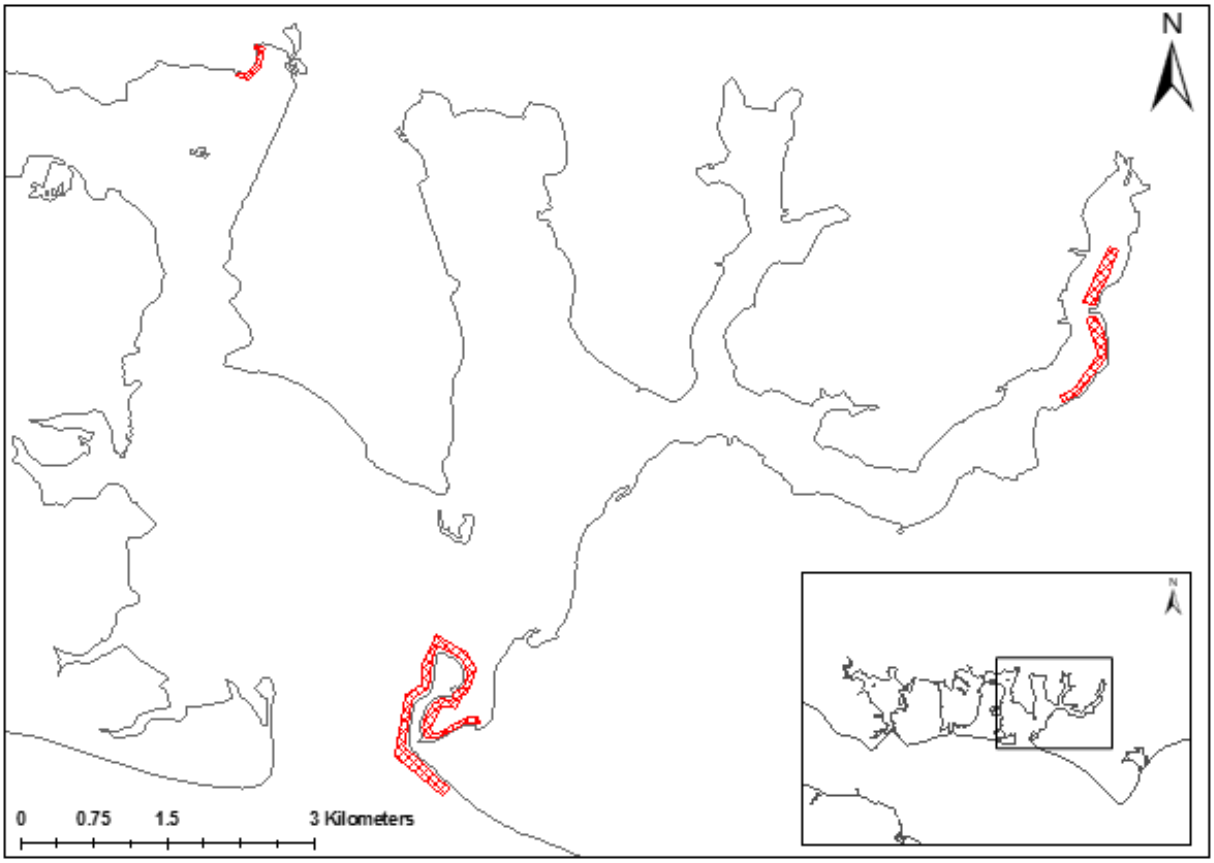

Figure 2.9: Map of the sampling locations in Chichester Harbour. The red hatched areas indicate the areas that were surveyed in order to determine the presence or absence of Manila clams. Emsworth Yacht Basin is located at the North West of the Harbour, Dell Quay is located at the North eastern corner of the Harbour and the Harbour mouth is located at the South of the Harbour.

The surveys at Emsworth Yacht Basin (22-5-2013) and at the mouth of the Harbour (7-3-2013) consisted of shoreline surveys, where the foreshore was walked and any freshly dead shells were collected. Freshly dead shells were those classed as those that were still articulated and connected by the ligament and with no sign of any growth on the internal and external portion of the shell and with a glossy white interior. The shells were then identified as either Palourdes or Manila clams using the shell morphology and the internal scarring of the valves. The total distance surveyed at Emsworth Yacht basin was ~335m. The area surveyed at the mouth of the Harbour was ~ 3.9km.

The survey at Dell Quay was undertaken on the 12-6-2009 (located at the eastern end of the harbour, alongside the Fishbourne Channel) and consisted of both a shore survey searching for dead shells and sampling of the intertidal zone. The intertidal sampling

consisted of using rakes and sieves to sample the mud and collect any live clams found. The total area raked over in Dell Quay consisted of five 5x5m squares, giving a total area sampled of 125m^2. An additional ten 0.3m^2 sieve samples were taking giving a total area of 3m^2.

Thames Estuary

Shoreline surveys were undertaken in the Thames Estuary along the North Kent coast at Whitstable and Seasalter (the location of the commercial beds on which Manila clams are cultured) and along the South Essex coast at Shoeburyness and Maplin sands looking for washed up shells. On the 27-7-2010 a shoreline survey searching for dead Manila clam shells was carried out at Shoeburyness in Essex. The survey consisted of a visual survey of the sand flats walking a square grid pattern of approximately 100m by 100m starting from the GPS coordinates N51°31.750 W000°47.982, any dead clams shells were collected for further identification. An area of approximately 5m by 5m was raked over to a depth of 5cm at the starting point of the survey area and random plots of approximately 5m by 5m were raked over inside the 100mx100m survey grid area. A total of five 5x5m areas were raked over giving a total area of 125m^2 sampled.

On the 8-8-2010 an area that had a reported presence of Manila clams in the intertidal zone was surveyed at Whitstable in Kent (GPS coordinates N51°22.019 W001°02.140). An area of 5m by 5m was sampled to a depth of 5cm by rake and all live clams were collected (Total areas 25m^2).

Crouch and Roach Estuary

Shoreline surveys searching initially for dead shells were undertaken at various locations in the Crouch and Roach Estuary on the 27-7-2010. At Pagelsham Boatyard on the River Roach (GPS Coordinates N51°35.380 E000°48.389) one meter either side of the strandline was surveyed for a distance of 50m either side of the slip way and any dead clam shells were collected for later identification.

At Hullbridge, on the River Crouch (GPS Coordinates of the public slipway N51°37.789 E000°36.788) a shoreline survey searching for dead shells along the intertidal zone and

one meter either side of the strandline was undertaken from the public slipway to a point 200m east of the start position.

At Burnham on Crouch a survey took place along the strandline from the Jetty located at the end of Remembrance Avenue to the eastern tip of the Burnham Yacht Harbour (GPS Coordinates N51°37.585 E000°48.412 to N51°37.339 E000°48.172). This was approximately 400m long. The total distance of shoreline surveyed along the crouch and roach estuary was 700m (100m+200m+400m) giving a total area of 1.4km^2 (See figure 2.10 for the locations of the surveys in the Crouch and Roach Estuary).

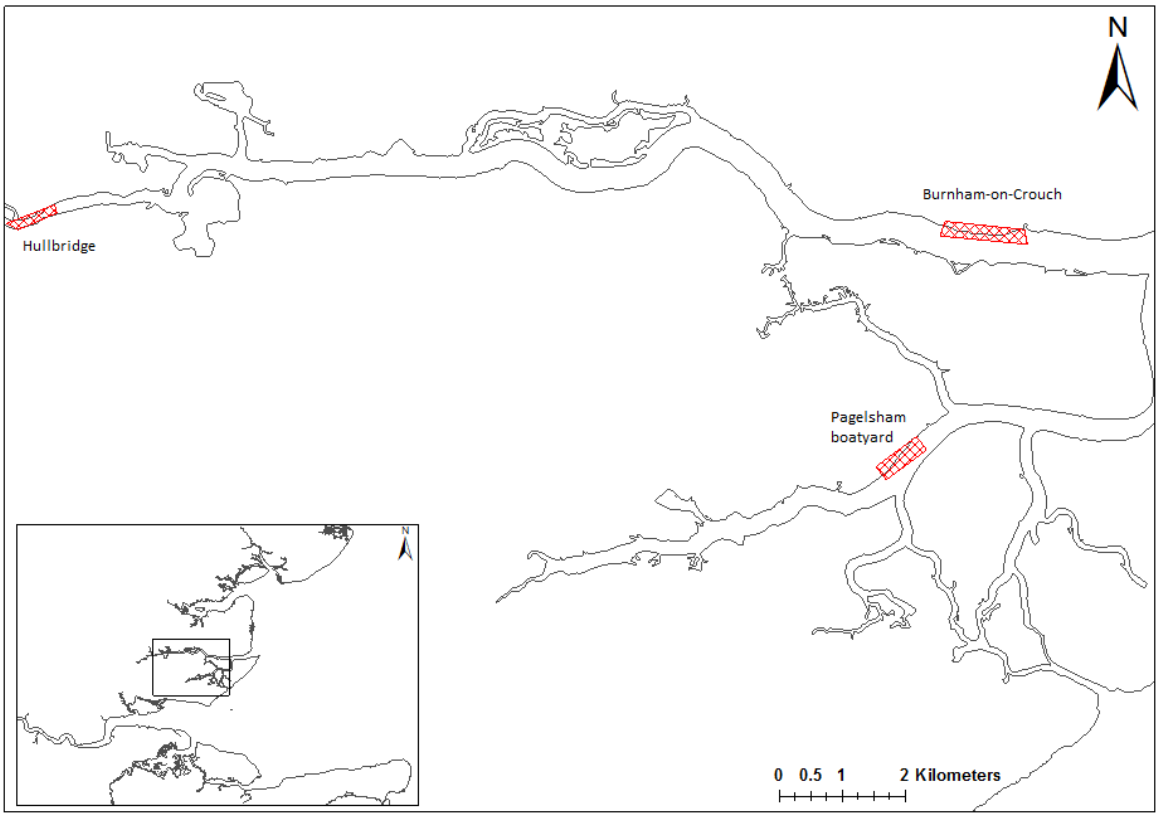

Figure 2.10: Map of the sampling locations in the Crouch and Roach Estuary. The red hatched areas indicate the approximate location of the shoreline surveys searching for evidence of Manila clams.

Hamford Water

A shoreline survey to identify the presence of Manila clams by the presence or absence of dead shells was undertaken on the 27-10-2010. One meter either side of the strandline was surveyed eastwards from Titchmarsh Marina (GPS Coordinates N51°51.841 E001°15.584) along the sea defence wall for a distance of approximately 200m, giving a total area of 400m^2 (GPS Coordinates N51°54.427 E001°15.493) and all clam shells were collected and identified (See figure 2.11).

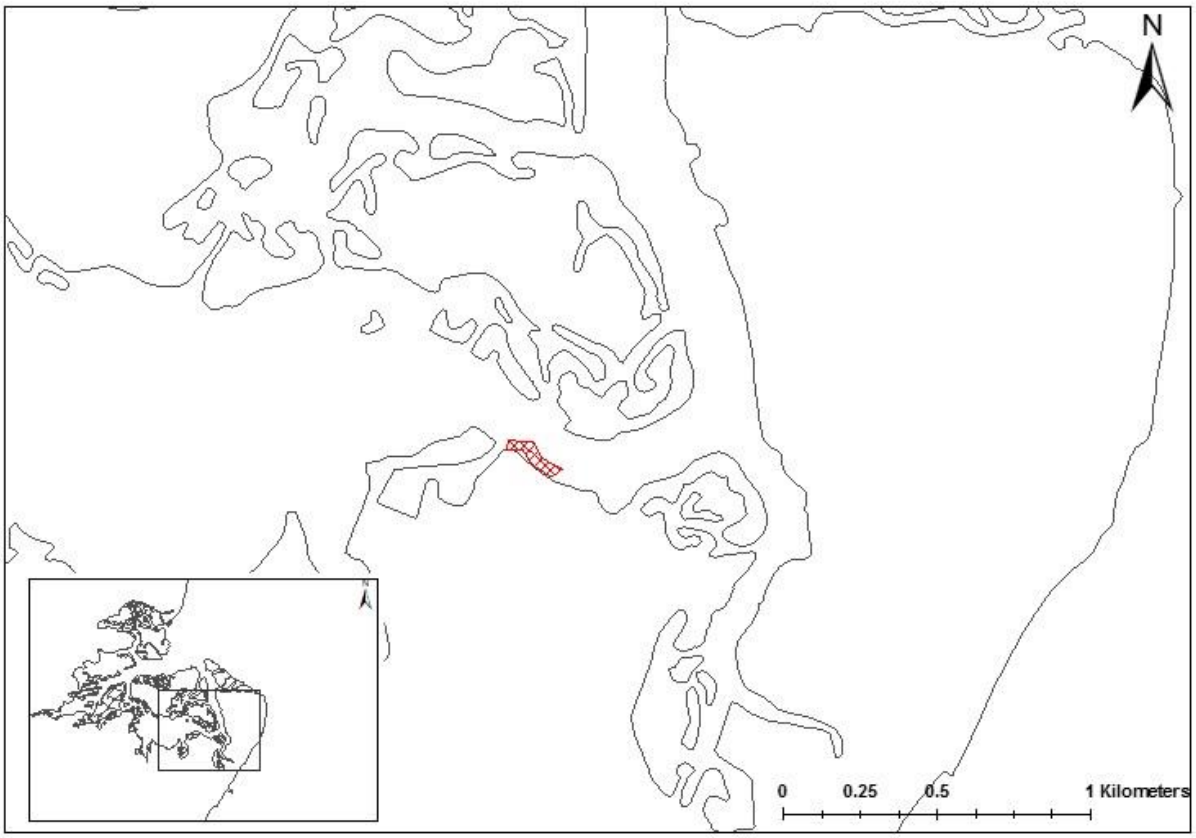

Figure 2.11: Map detailing the sampling location in Hamford Water. The red hatched area indicates the approximate location of the shoreline survey, looking for evidence of the presence of Manila clams.

Blackwater Estuary

Shoreline surveys were carried out in the Blackwater Estuary in Essex on the 27-7-2010. A shore walking survey searching for dead clam shells up to 1m either side of the strandline was carried out from Tollesbury Yacht harbour (GPS Coordinates N51°45.548 E000°51.022) for a distance of approximately 600m north eastwards along the flood

defences, giving a total area of 1.2km^2 (GPS Coordinates N51°45.444 E000°51.472)(see figure 2.12).

A search of the sediment using sieves with a diameter of 8mm was carried out at the Blackwater Nature reserve (GPS Coordinates N51°46.429 E000°50.413). Ten random samples of 30cm^2 were sorted through the sieves (giving a total area of 3m^2) and all bivalves present were collected (see figure 2.12).

At Salcott a shoreline survey along the flood defences at the high tide line was undertaken in order to locate evidence of Manila clam presence in the form of dead clam shells up to 1m either side of the strandline (The distance surveyed was approximately 610m, giving an area of 1.22km^2). The shore survey was carried out from GPS coordinates N51°47.161 E000°45.558 to N51°47.163 E000°50.123 and all dead clam shells were collected for identification (see figure 2.12).

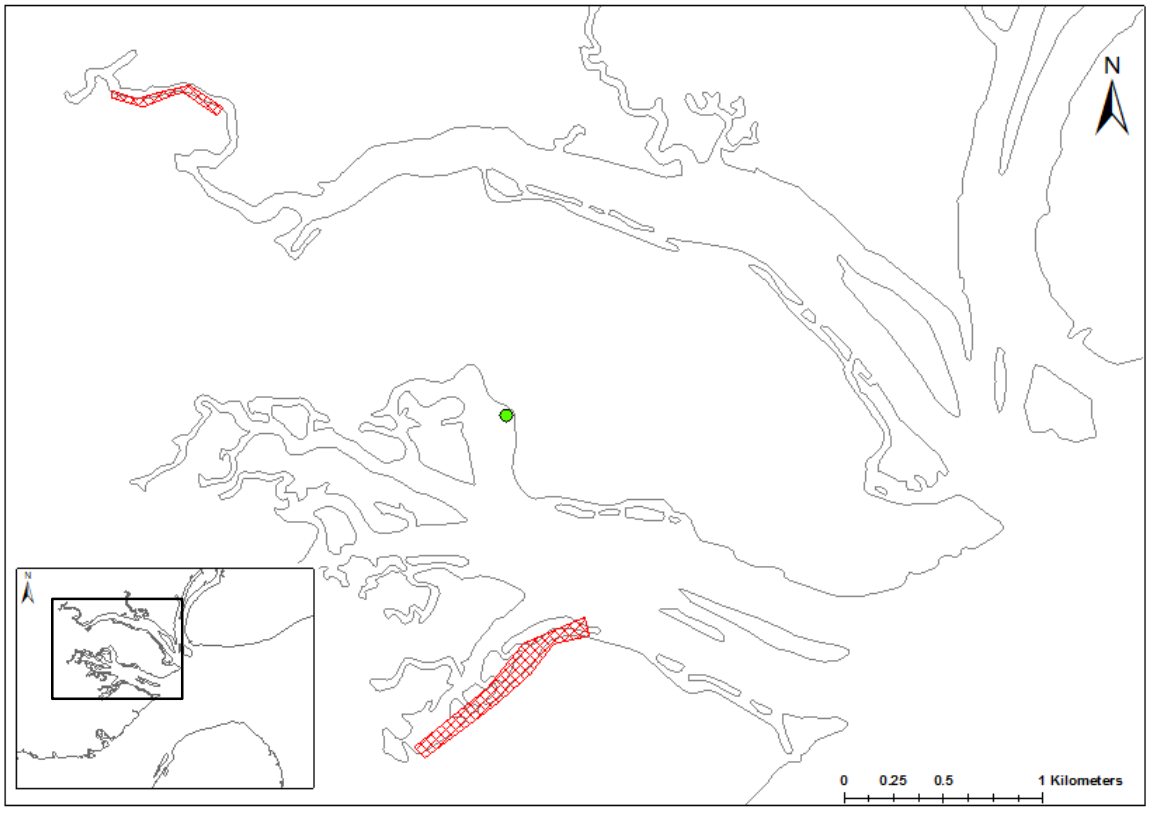

Figure 2.12: Map of the sampling locations in the Blackwater Estuary. The green circle denotes the location of the sieve sampling in the Blackwater Nature reserve, whilst the red hatched areas indicate the locations of the shore walking surveys (Salcott to the North West of the map and the Tollesbury Marina to the South of the Map)

Stour Estuary

A shoreline survey along approximately 350m of the strandline was undertaken at Mistley on the 27-10-2010. The visual inspection covered an area of approximately 1m either side of the strandline, giving a total area of 700m^2. All dead clam shells were collected and identified as Manila clams or local species. A survey of the intertidal zone was undertaken based on the presence of dead clam shells. The intertidal zone was sampled (GPS Coordinates N51°51.841 E001°15.584) by three 5m x 5m areas of the intertidal were raked to a depth of 5cm with a rake (Giving a total area of 75m^2) (See figure 2.13). All bivalves collected were identified on site and all clams were retained for positive identification.

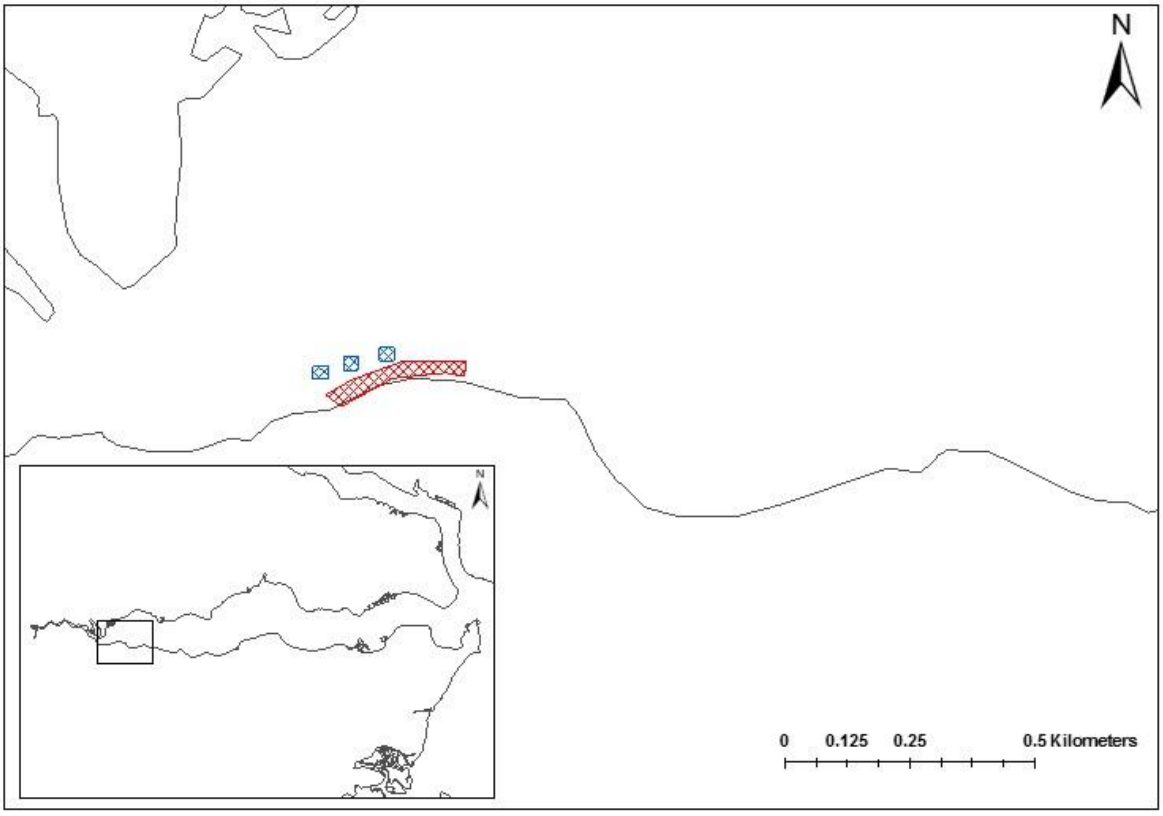

Figure 2.13: Map detailing the sampling locations in the Stour Estuary. The red hatched area denotes the location of the shore line survey, whilst the blue hatched areas denote the location of the 5x5m areas raked areas.

Summary of sampling methods and area sampled by the author

The total area sampled by the Author over the course of the surveys can be seen in table 6. The methods used at each site are included, for example boat deployed dredge and shore survey, the area sampled in included and the percentage of the whole estuary that these surveys represent.

Table 6: The sample effort and methods used at each of the locations surveyed by the Author. The total area sampled at each estuary in m^2 is included for each location, and expressed as a percentage of the total intertidal area of the estuary.

Estuary	Sampling method	Total area sampled	% of total intertidal area sampled
Southampton Water	Boat deployed dredge	260m^2	0.002
Portsmouth Harbour	Boat deployed dredge	650m^2	0.007
The Fleet Lagoon	Shore survey	6km^2	0.2
	Raking of sediment	150m^2	
	0.25m^2 Quadrats (x10)	2.5m^2	
Medina Estuary	Boat deployed sediment grab (0.3m^2x12)	3.6m^2	0.001
	Hand held dredge (0.3m^2)(x10)	3m^2	
Langstone Harbour	Shore survey	5.2km^2	0.03
	Raking of sediment	75m^2	
Chichester Harbour	Shore survey	8.5km^2	0.04
	Raking of sediment	125m^2	
	0.3m^2 Sieve samples (x10)	3m^2	
Thames Estuary	Shoreline Survey	100m^2	0.002
	Raking of sediment	150m^2	
Crouch and Roach Estuary	Shoreline survey	1.4km^2	0.01
Hamford Water	Shoreline survey	400m^2	0.003
Blackwater Estuary	Shoreline survey	2.4km^2	0.007
	0.3m^2 Sieve samples (x10)	3m^2	
Stour Estuary	Shoreline survey	700m^2	0.005
	Raking of sediment	75m^2	

Field surveys carried out by J Humphreys

Information regarding whether clams were found and not found in the remaining 41 estuaries in the study area was provided by J Humphreys. The methods used by J Humphreys to survey the remaining estuaries are the same as those carried out by the author and are covered in Humphreys *et al* 2015. The sampling method consisted of an initial literature search for each estuary looking for reports of Manila clams from a variety of sources. These included: scientific papers, technical reports, websites and any other records regarding the estuaries. Once the literature search had been completed, contact was made with organisations and individuals that had jurisdiction over or worked in the local area such as the Inshore Fisheries and Conservation Agencies (IFCA) or local fishermen. Once all the intelligence had been gathered from the literature search and local knowledge, each of the estuaries was visited in order to carry out land based surveys. These surveys were extensive, but not quantitative in nature and consisted of searching the strandline and intertidal area for the presence of washed up shells, followed by raking and sieving of mud at sites where it was accessible and safe to do so.

Once all of the data for the estuaries had been collected, the results were classified as either: Manila clams were found or not found by the surveys. The results were mapped onto an outline of the British Isles using the Geographical Information System software (GIS) ArcGIS 9.3.

Environmental data for all the estuaries located in the study area was collected from the literature (where available) and statistically analysed using metadata analysis to determine whether this may influence the presence or absence of Manila clams. The environmental data that was included in the analysis was: minimum and maximum seawater temperature, tidal range and sediment type. Sediment type was coded using the following scale to allow it to be used for statistical analysis: mud =1, sand=2, gravel=3, shingle= 4, rock =5. This data was used to help identify further scientific questions rather than to identify the reasons behind clam distribution.

Statistical analysis

Whether Manila clams were found or not found was analysed three times, the first analysis looked at all of the estuary locations to determine which environmental factors could influence clam distribution. The second analysis investigated estuaries that had been granted a licence to culture clams and attempted to determine which factors could have influenced successful establishment of a population. The final analysis investigated the estuaries that had not been granted licences to culture Manila clams to determine whether environmental conditions could identify the reasons behind the presence or absence of Manila clams.

The data was analysed using Primer 6.1 (PrimerE Ltd: Plymouth Routines in Multivariate Ecological research) software. Similarities of presence or absence of Manila clams between estuaries was analysed using PERMANOVA in Bray-Curtis similarity matrices. Once the PERMANOVA analysis had been completed, a distance-based linear model (DistLM) was used to try and verify whether there were any relationships between the locations where Manila clams were, and were not found and the environmental conditions found at these locations. The environmental conditions used in the PERMANOVA and distance-based linear models were: the minimum and maximum recorded seawater temperature and sediment type.

2.3 Results

The results from all of the field surveys have been plotted on to a GIS map of the South and Eastern coast of England and can be seen in figures 2.14. Further information regarding these sites, such as average temperature, tidal range and sediment type can be found in table 7.

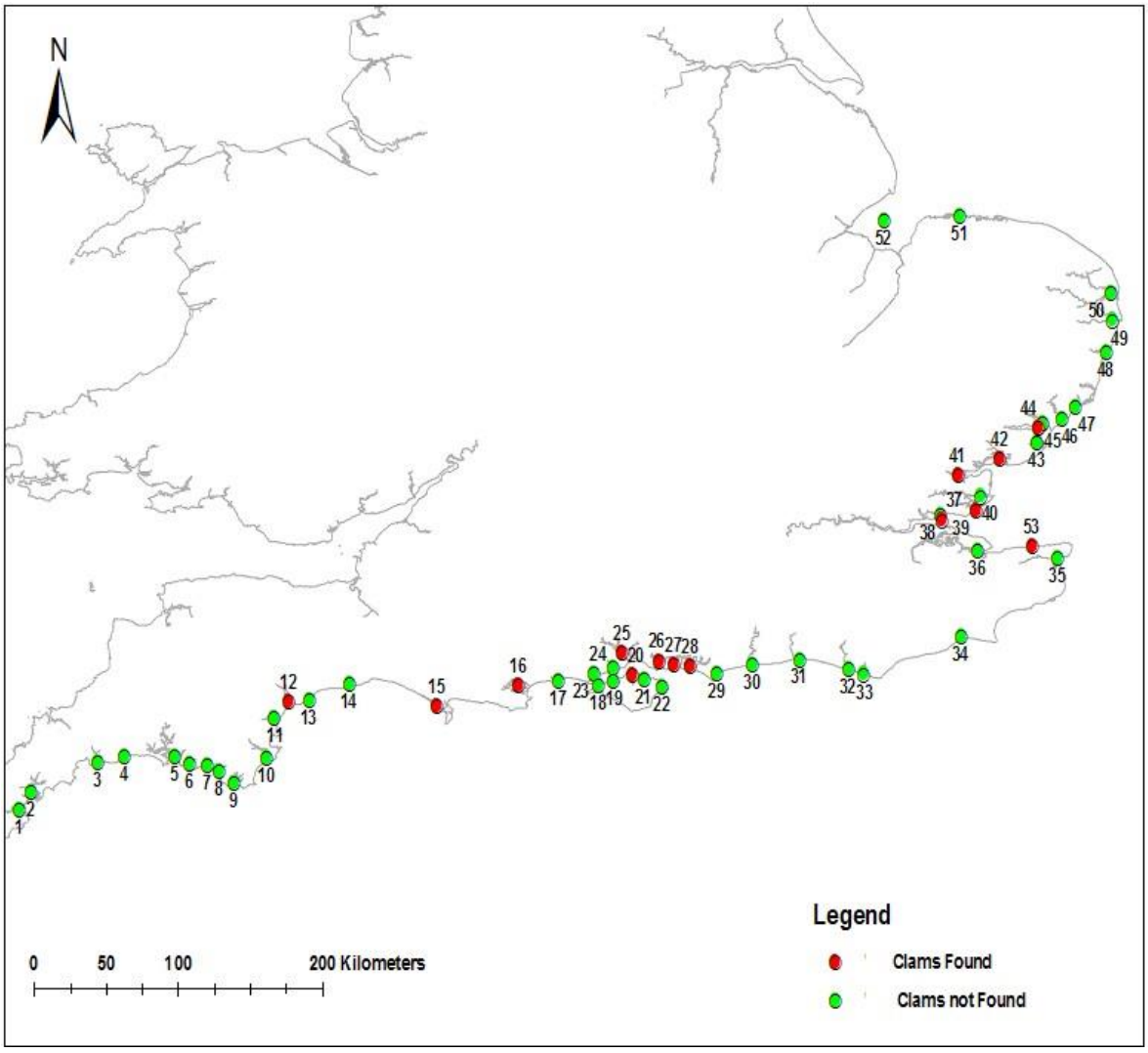

Figure 2.14: The distribution of Manila clam populations along the South and South eastern coastline of England up until 2011. The red circles denote estuaries in which Manila clams were found and the green circles denote estuaries in which Manila clams were not found by the surveys. The estuaries are all numbered and further information regarding these estuaries is found in table 7.

The results from the Boat surveys in Portsmouth Harbour and the Medina Estuary can be seen in Figures 2.15 and 2.16. In Portsmouth Harbour Manila clams were restricted to the northwest corner of the harbour, close to where the Wallington River joins the harbour.

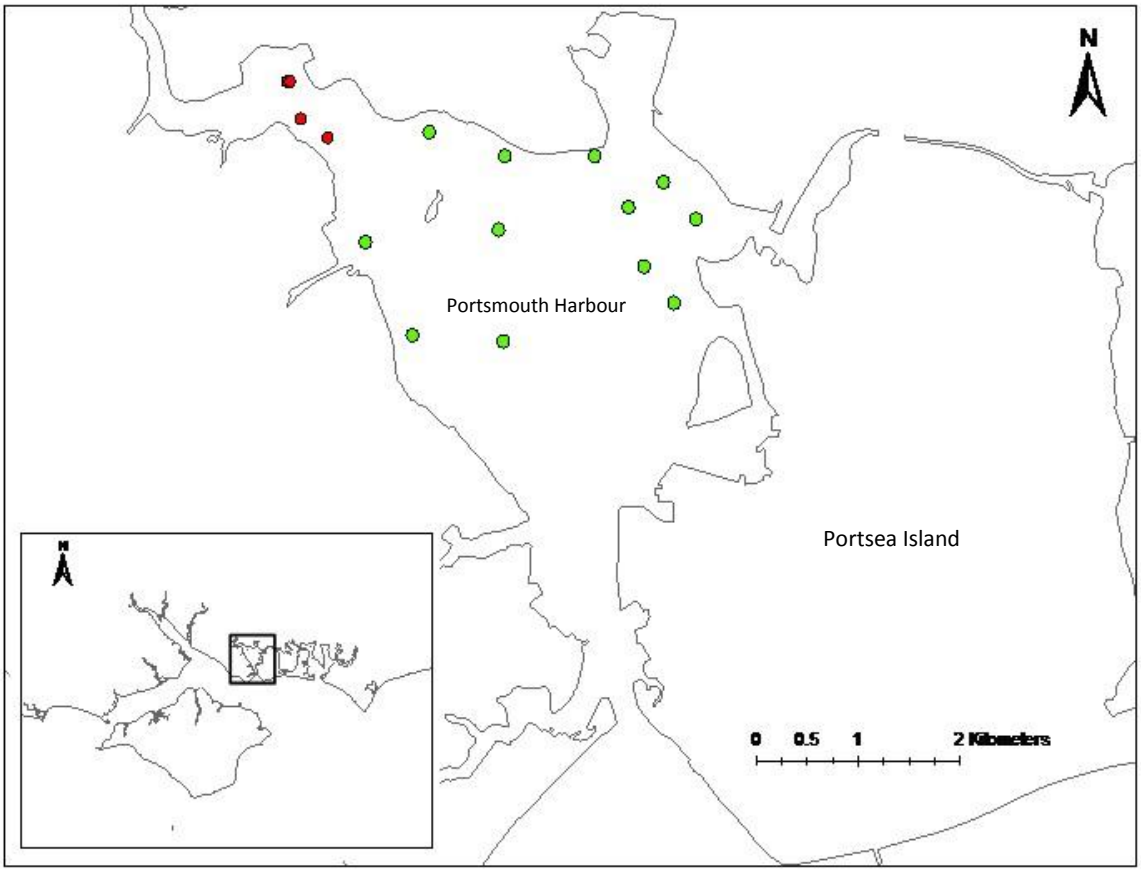

Figure 2.15: The distribution of Manila clams in Portsmouth Harbour. The presence of Manila clams is represented by the red circles; green circles indicate an absence of Manila clams.

In the Medina Estuary, Manila clams were only found in the mid-section of the river adjacent to the Medina Valley Centre (See figure 2.16). Areas of suitable habitat downstream from the Medina Valley Centre had no clams present.

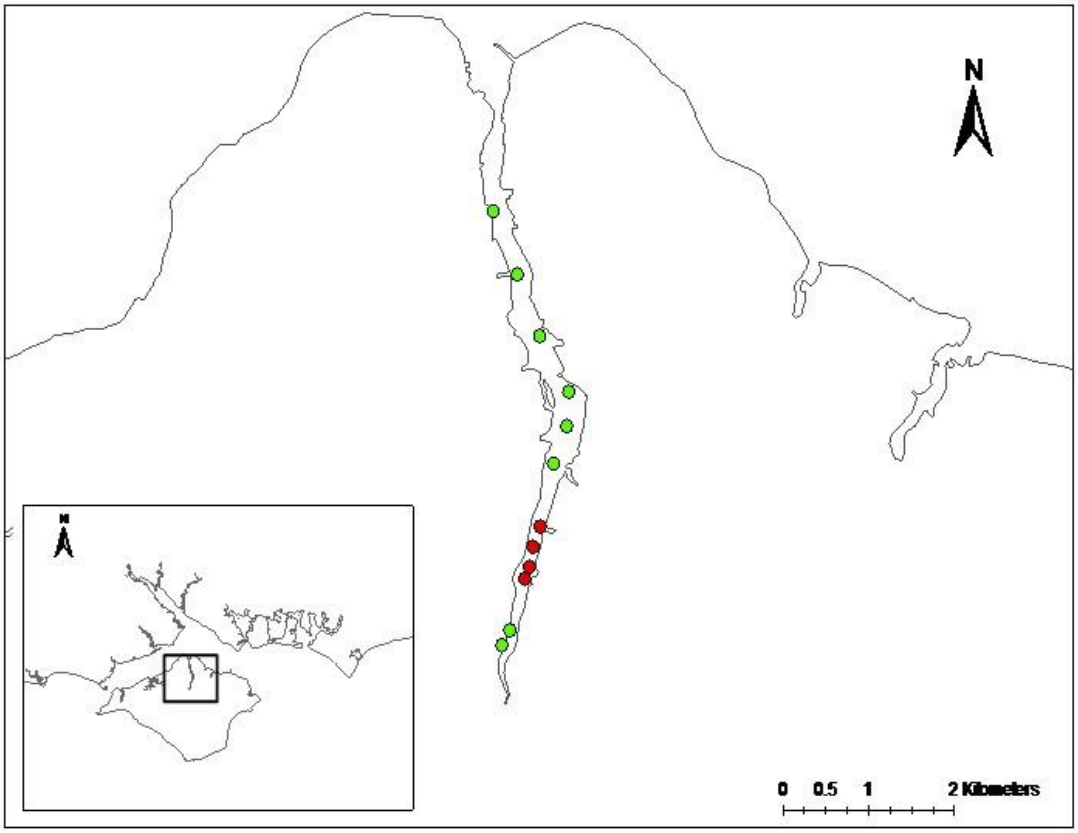

Figure 2.16: The distribution of Manila clams in the Medina Estuary Isle of Wight. Inset is the geographical location of the Medina Estuary in the Solent region of the UK. The Medina estuary is located on the northern coast of the Isle of Wight and can be seen in the centre of the map. The presence of Manila clams is indicated by the red circles, green circles indicate an absence.

Table 7: Information on the Estuaries along the South and Eastern coastline of England as depicted in figure 2.11. The table contains **w**hether Manila clams were found or not, whether licences were granted to culture them, the minimum and maximum average monthly temperatures, the tidal range and sediment type. Estuaries that had a presence of Manila clams found are in bold, n.d signifies no data available. ([1] denotes estuaries that were sampled by the methodology quoted in Humphreys *et al* 2015, whilst [2] denotes estuaries sampled by the author, details of which are in the methodology of this chapter).

Site	Location	Clams detected	Licensed	Temp range (°C)	Tidal range (m)	Sediment type	Reference
1	*Helford Estuary, Cornwall*	*No*[1]	*No*	*8-19*	4.7	*Mud, sand, shingle*	(Kershaw & Campos, 2008)
2	*Falmouth Estuary, Cornwall*	*No*[1]	*No*	*9.7-17*	5.3	*Mud*	Seatemperature.org, (Pratt & Aleem, 2010)
3	*Fowey Estuary, Cornwall*	*No*[1]	*Yes*	*9.7-17*	4.8	*Sand, mud*	Seatemperature.org, (Jack, 2010)
4	*Looe Estuary, Cornwall*	*No*[1]	*No*	*9.5-16.9*	4.8	*Sand, rock*	Seatemperature.org, (Davies, 1998)
5	*Plymouth Sound, Devon*	*No*[1]	*No*	*9.5-16.9*	4.7	*Rock, mud, sand*	Seatemperature.org, (Davies, 1998)
6	*Yealm Estuary, Devon*	*No*[1]	*No*	*n.d*	4.7	*Rock, sand, mud*	(Kershaw & Campos, 2010)
7	*Erme Estuary, Devon*	*No*[1]	*No*	*n.d*	4.7	*Sand, mud*	(Davies, 1998)
8	*Avon Estuary, Devon*	*No*[1]	*No*	*n.d*	4.7	*Sand, Rock*	(Davies, 1998)
9	*Salcombe and Kings bridge Estuary, Devon*	*No*[1]	*No*	*n.d*	4.6	*Mud, rock, sand,*	(Davies, 1998)
10	*Dart Estuary, Devon*	*No*[1]	*No*	*n.d*	4	*Mud, Rock, shingle*	(Davies, 1998)
11	*Teign Estuary, Devon*	*No*[1]	*Yes*	*9.1-16.6*	4.2	*Mud, sand*	Seatemperature.org, (Davies, 1998)
12	**Exe Estuary, Devon**	**Yes**[1]	**Yes**	**9.41-16.5**	**4.1**	**Mud, sand**	**Seatemperature.org, (Davies, 1998)**
13	*Otter Estuary, Devon*	*No*[1]	*No*	*n.d*	4.1	*Mud*	http://www.wildlifetrusts.org/
14	*Axe Estuary, Devon*	*No*[1]	*No*	*n.d*	3.7	*Mud, sand, shingle*	https://www.gov.uk/government/uploads
15	**The Fleet and Portland Harbour, Dorset**	**Yes**[2]	**No**	**8.8-17 (Weymouth)**	**1.9**	**Sand, mud, pebble**	**Seatemperature.org, (Davies, 1998)**
16	**Poole Harbour, Dorset**	**Yes**[2]	**Yes**	**5.6-18.2**	**1.4**	**Mud, sand, shingle**	**See chapter 4**
17	*Christchurch Harbour, Dorset*	*No*[1]	*No*	*8.7-17.2*	1.2	*Mud*	Seatemperature.org, (Colenutt, 2013)
18	*Yar Estuary, Isle of Wight*	*No*[1]	*No*	*8.15-19.57 (Bramble bank)*	2.5	*Sand, gravel*	http://www.coastalwight.gov.uk/, Bramblemet

19	*Newtown Estuary, Isle of Wight*	*No*[1]	*No*	*8.15-19.57 (Bramble bank)*	2.9	*Sand, gravel, mud*	http://www.coastalwight.gov.uk/, Bramblemet
20	**Medina Estuary, Isle of Wight**	**Yes**[2]	**No**	**7-20**	**4.2**	**Mud**	**Medinavalleycentre.org, (Kershaw & Acornley, 2008)**
21	*Wootton Creek and Ryde Sands, Isle of Wight*	*No*[1]	*No*	*8.6-17.3 (Ryde)*	3.8	*Mud, sand*	Seatemperature.org, http://www.coastalwight.gov.uk/
22	*Bembridge Harbour, Isle of Wight*	*No*[1]	*No*	*8.15-19.57 (Bramble bank)*	3.1	*Mud, sand*	http://www.bembridgeharbour.co.uk/, Bramblemet
23	*Lymington Estuary, Hampshire*	*No*[1]	*No*	*8.15-19.57 (Bramble bank)*	2.5	*Mud*	Bramblemet.co.uk, (Pinnion, Mackie, Somerfield, & Warwick, 2007)
24	*Beaulieu River, Hampshire*	*No*[1]	*Yes*	*8.15-19.57 (Bramble bank)*	3.2	*Mud*	(Carlos Campos, Morgan, Mitchard, & Udal, 2012a), Bramblemet
25	**Southampton Water, Hampshire**	**Yes**[2]	**No**	**8.6-17.3**	**4**	**Mud**	**Seatemperature.org, (Kershaw & Acornley, 2009)**
26	**Portsmouth Harbour, Hampshire**	**Yes**[2]	**No**	**8.6-17.3**	**4.1**	**Mud, sand**	**Seatemperature.org, (Carlos Campos, Morgan, Mitchard, & Udal, 2012b)**
27	**Langstone Harbour, Hampshire**	**Yes**[2]	**No**	**7.1-19.3**	**4.2**	**Mud, sand**	**IMS, (Kershaw & Campos, 2013)**
28	**Chichester Harbour, Hampshire**	**Yes**[2]	**Yes**	**5.4-18.6**	**4.2**	**Sand, mud, shingle**	**CHIMET, (Kershaw, 2013b)**
29	*Pagham Harbour, West Sussex*	*No*[1]	*No*	*n.d*	4.9	*Mud*	(Pinnion et al., 2007)
30	*Arun Estuary, West Sussex*	*No*[1]	*No*	*n.d*	5.3	*Mud*	(Pinnion et al., 2007)
31	*Adur Estuary, West Sussex*	*No*[1]	*No*	*5.8-18.4 (Shoreham)*	5.5	*Mud*	Cefas.Defra.gov.uk, (Pinnion et al., 2007)
32	*Ouse Estuary, East Sussex*	*No*[1]	*No*	*8.2-17.3 (Brighton)*	6.1	*Mud*	Seatemperature.org, (Pinnion et al., 2007)
33	*Chuckmere Estuary, East Sussex*	*No*[1]	*No*	*8.2-17.3 (Brighton)*	6.5	*Mud*	Seatemperature.org, (Pinnion et al., 2007)
34	*Rother Estuary, East Sussex*	*No*[1]	*No*	*n.d*	5.3	*Sand*	(Pinnion et al., 2007)
35	*Pegwell Bay, Kent*	*No*[1]	*No*	*7.5-17.7 (*Ramsgate)*	4.5	*Mud, sand*	Seatemperature.org, bto.org

36	**Swale Estuary, Kent**	**Yes[1]**	**Yes**	**7.8-17.4 (Gillingham)**	**4.9**	**Sand, shingle , mud**	**Seatemperature.org (Kershaw, 2011)**
37	**Thames Estuary**	**Yes[2]**	**Yes**	**8-17.3 (Grays)**	**6.5**	**Sand, shingle, mud**	**Seatemperature.org, (Kershaw, 2011)**
38	*Southend-on-Sea, Essex*	*No[2]*	*No*	*7.2-17.4*	5.2	*Sand*	Seatemperature.org, (Kershaw, 2011)
39	**Maplin Sands, Essex**	**Yes[2]**	**No**	**8-17.3 (Grays)**	**4.6**	**Sand**	Seatemperature.org, (Kershaw & Cook, 2012)
40	*Crouch-Roach Estuary, Essex*	*No[2]*	*Yes*	*n.d*	5	*Mud, sand*	(Kershaw & Campos, 2011a)
41	**Blackwater Estuary, Essex**	**Yes[2]**	**Yes**	**4.1-20.9 (Bradwell P.S)**	**4.6**	**Mud, sand**	Cefas.Defra.gov.uk, (Kershaw, 2013a)
42	**Colne Estuary, Essex**	**Yes[2]**	**Yes**	**1.5-24**	**4.6**	**Mud, sand, shingle**	(Dong, Thornton, Nedwell, & Underwood, 2000),(Kershaw, 2013c)
43	*Hamford Water, Essex*	*No[2]*	*Yes*	*1-21*	3.8	*Mud, sand*	(Kershaw & Campos, 2011b)
44	**Stour Estuary, Suffolk**	**Yes[2]**	**No**	**7.1-17.7**	**3.6**	**Mud, sand**	**Seatemperature.org, geo-east.org.uk**
45	*Orwell Estuary, Suffolk*	*No[1]*	*No*	*n.d*	3.6	*Mud*	(Cottle, Gibson, & Robinson, 1997)
46	*Deben Estuary, Suffolk*	*No[1]*	*No*	*n.d*	3.2	*Mud, sand, gravel*	(Kershaw & Cook, 2011)
47	*Ore/Alde/Butley Estuary, Suffolk*	*No[1]*	*No*	*n.d*	2.2	*Sand, gravel*	(Pye, 2005)
48	*Blyth Estuary, Suffolk*	*No[1]*	*Yes*	*n.d*	2.1	*Mud*	(Cottle et al., 1997)
49	*Oulton Broad, Suffolk*	*No[1]*	*No*	*6.8-17.2*	1.9	*Mud, sand*	Seatemperature.org, (Holve, Doarks, & Jane, 1996)
50	*Breydon Water, Norfolk*	*No[1]*	*No*	*n.d*	1.9	*Mud*	(Bines, 2001)
51	*North Norfolk Coast*	*No[1]*	*No*	*4.7-17.9 (Blakeney)*	2.6	*Mud, sand, gravel*	Cefas.Defra.gov.uk, (Bines, 2000)
52	*The Wash, Norfolk*	*No[1]*	*No*	*4.7-17.9 (Blakeney)*	6.5	*Mud, sand, gravel*	Cefas.Defra.gov.uk, (Bines, 2000)
53	**Reculver, Kent**	**Yes[2]**	**Yes**	**7.5-17.7**	**6.5**	**Sand, shingle, mud**	**Seatemperature.org**

The surveys found seven populations of Manila clams in areas that had previously been granted licences or were the initial trial locations. These locations from west to east included: The Exe Estuary in Devon, Poole Harbour in Dorset, the Beaulieu River in Hampshire, Emsworth Yacht basin in Chichester Harbour Hampshire, the Thames and Swale Estuaries, the Black Water estuary in Essex and the Colne Estuary in Essex.

There were seven recorded sites which had a presence of Manila clams but no aquaculture history. These locations were from west to east: The Fleet Lagoon in Dorset, Southampton Water in Hampshire, The Medina Estuary Isle of Wight, Portsmouth Harbour and Langstone Harbour in Hampshire, Maplin Sands in Essex and the Stour Estuary in Essex.

There were four sites where Manila clams were previously trialled or had licences granted that no longer have Manila clams present. These estuaries from west to east were: The Helford in Cornwall, The River Teign in Devon, The Crouch and Roach in Essex and Bulcamp Marshes in Suffolk.

The Manila clam is present in at least 14 of the 52 estuaries between the Helford Estuary in the south west of England and the Wash in the east of England. Ten of the 14 estuaries that contain Manila clams are clustered together, the first cluster is centred on the Solent on the south coast and contains six clam populations and the second cluster is centred on the Blackwater, Colne and Thames estuaries and contains four populations of clams. The other four estuaries containing Manila clams are outliers; these include the Exe estuary in Devon, The Fleet Lagoon in Dorset, Poole Harbour in Dorset and the Stour Estuary in Essex (The Stour estuary is classed as an outlier as it isolated from the nearest population by over 30km).

The presence or absence of Manila clams across all of the estuaries along the south and south east coast of England differ significantly between location (PERMANOVA, $F_{1,51}$ = 6.3, p = < 0.05). Seawater temperature influences the distribution of the Manila clam with the minimum seawater temperature accounting for 9.3% of the variance (DistLM marginal test Pseudo-$_F$ 5.2, P <0.05) with maximum seawater temperature accounting for 12.4% of the variance (DistLM marginal test Pseudo-$_F$ 7.2, P <0.05).

The presence or absence of Manila clams in estuaries that had previously been granted licenses to culture clams differ significantly between location (PERMANOVA, $F_{1,12}$ = 3.3, p = < 0.05). Sediment type influenced the presence or absence of Manila clams accounting for 61% of the variance (DistLM marginal test, Pseudo-$_F$ 18.9, P <0.05).

The presence or absence of Manila clams in estuaries that had not been granted licenses to culture clams differ significantly between locations (PERMANOVA, $F_{1,38}$ = 8.7, p = < 0.05). Seawater temperature influences the distribution of Manila clams in previously unlicensed estuaries with minimum seawater temperature accounting for 24.5% of the variance (DistLM marginal test, Pseudo-$_F$ 11.9, P <0.05) and maximum seawater temperature accounting for a further 20% of the variance (DistLM marginal test, Pseudo-$_F$ 18.9, P <0.05).

2.4 Discussion

The discussion has been divided into four sections: the current distribution of Manila clams, why did some of the introductions not result in established populations, the potential dispersal mechanisms for further spread and the general conclusions and the potential for further work generated by this study.

2.4.1 Current distribution of Manila clams

Manila clams were found during the surveys in 14 of the 52 estuaries (including the recently identified population in the Crouch and Roach Estuary) along the South and Eastern coastline of England. However this is likely to be an under-representation of the actual distribution, based on the limitations of the survey methods and the size of the estuaries involved (An example being the Wash, which has an intertidal area of 29770 hectares). It is possible that with a more intensive sampling effort undertaken at each Estuary, populations of Manila clams could be discovered that were missed by this survey. Unfortunately this study did not have the time or resources to carry out

intensive surveys at each location, so any conclusions drawn, should be based on the fact that only positive results (i.e. clams found) are 100% reliable.

When the presence or absence of Manila clams was analysed alongside the environmental data, sediment type and tidal range did not influence the overall distribution of Manila clams along the South and South-East coastline. This is unsurprising as the sediment types reported for the estuaries located in the study area, are in the majority, comparable to conditions in which the Manila clam is found in its natural distribution i.e. low wave energy sediments (Nakamura, 2001). The only variable that did influence distribution was seawater temperature. The maximum average seawater temperature accounted for 12.4% of the variance in distribution whilst the average minimum seawater temperature accounted for a further 9.3%.

The maximum average sea water temperature could be important in determining whether Manila clams will be able to successfully reproduce, because the reproductive cycle is subject to minimum temperature requirements (Mann, 1979). The average maximum seawater temperatures are above the minimum temperatures required for gametogenesis (8°C), gamete ripening (12°C) and spawning (14°C) (Mann, 1979) in the locations in which they were found. However reproductive success does not occur on a regular basis (See chapter 3 for the Poole Harbour population), Manila clams were observed to resorb gametes in Southampton Water when the water temperatures were not sufficient to elicit spawning (Tumnoi, 2012). This could suggest that these populations of clams are reliant on good years for recruitment to maintain the population. Intermittent recruitment has also been observed in populations of the Pacific Oyster in the North Wadden Sea, with populations dominated often by a single cohort (Diederich et al., 2005). An overreliance on intermittent recruitment events could put the populations at risk of collapse, if a series of years with unfavourable conditions run consecutively.

The locations where Manila clams were found, had on average, a lower minimum seawater temperature (6.9°C) than the locations in which they were not found (7.6°C). The minimum temperatures occurred in the winter months, with this period typically coinciding with the lowest levels of phytoplankton (Edwards et al., 2006). If the minimum temperature of 1°C from Hamford water is omitted, the average minimum

temperature for the estuaries without clams is 7.9°C. This is on threshold of the minimum temperature required to initiate gametogenesis in Manila clams (Mann, 1979). Manila clams are able to withstand short periods of time without food at low temperatures (three weeks at 3°C), however prolonged exposure (11 weeks at 9°C) results in the loss of condition and weight and results in the utilisation of energy reserves (Laing & Child, 1996). This could suggest that low levels of phytoplankton, coupled with temperatures above the threshold to initiate gametogenesis (8°C) could lead to a metabolic debt, this has been observed in the Southampton water population of Manila clams (Tumnoi, 2012). Mass mortalities caused by metabolic debt have been reported for populations of *Mercenaria mercenaria* in North America (Weiss et al., 2007). This could suggest that the lower winter temperatures in the estuaries where Manila clams were found could indirectly result in reduced overwinter mortalities, due to the reduced metabolism of the clams at a time when food availability is at its lowest.

It should be noted that seawater temperature data was not available for all of the estuaries in the study area, so these conclusions will need to be revised when further temperature data becomes more readily available. Seawater temperature is also unlikely be the only factor that is influencing the distribution of Manila clams, as only 21.7% of the total variance was accounted for by temperature, leaving a further 78.3% unaccounted for. The Manila clams status as a non-native, introduced species (Humphreys, 2010; Jensen et al., 2004), could be significant in understanding the cause of its current distribution. Of the 14 locations in which Manila clams were found during the survey, seven of these had previously been granted licenses to cultivate Manila clams. Of the remaining seven estuaries that had Manila clams found during the surveys, five were within close proximity of a licensed population. This could suggest that anthropogenic factors have had the biggest influence on Manila clam distribution up until the present-day, however distribution may also be influenced by natural dispersal mechanisms. A similar pattern of distribution was observed for the Pacific Oyster, with the initial distribution being limited to licensed areas, with subsequent further spread being limited to the vicinity of licensed populations (Diederich et al., 2005; Herbert et al., 2012; Troost, 2010).

If the Manila clam follows the same dispersal pattern as the Pacific Oyster, it suggests that the initial distribution will be the result of anthropogenic influences, followed by patchy recruitment and slow dispersal to areas adjacent to the aquaculture locations. The slow initial dispersal of the Pacific oyster in the Northern Wadden Sea was the result of irregular reproductive events, which slowed the dispersal rate (Diederich et al., 2005). The Manila clam also has irregular reproductive events, with gametes being resorbed by Manila clams in Southampton Water in some years (Tumnoi, 2012). This irregular supply of larvae coupled with high levels of predation upon newly settled clams, could potentially explain why Manila clams are not more widely distributed than they are.

2.4.2 Success/Failure of initial introductions

The results of the metadata analysis and subsequently the potential factors that could explain the success or failure of Manila clam introduction should be treated as a hypothesis that requires further investigation. This is due to the nature of the analysis and the fact that it uses data taken from the literature. This does not mean that the conclusion presented in the following section are not potentially correct, but that they should be used as the basis for further investigation.

The success or failure of the trial introductions of Manila clams could be due to the predominant sediment type. The results from the metadata analysis suggest that sediment type could account for approximately 61% of the variance in whether clams were found or not. The average sediment score for the estuaries where the Manila clam was not found by the surveys is 2.3, this equates to a mud or mud and sand substrate. The locations in which the Manila clam were found, have an average sediment score of 5.85, which equates to a sand and shingle substrate. Aquaculture trials carried out with Manila clams in the Eo Estuary in North-west Spain found that survivorship was higher in sand-gravel sediments compared to muddy sediments (Cigarria & Fernandez, 2000). This was because predation rates are lower in the coarser sediments due to reduced predator efficiency, caused by the larger grain size which required predators to expend more energy in foraging behaviour (Arnold, 1984). Coarser sediments are also more stable than finer sediments, which reduces mortality rates, by lowering the mobility of

the sediment and reducing the chance of infaunal organisms being smothered or having their feeding apparatus clogged up (Cigarria & Fernandez, 2000; Levinton & Bambach, 1970; Thompson, 1995). This could suggest that the presence of Manila clams in these Estuaries is the result of increased survival rates due to the coarser sediments present in these locations.

However the potential for increased survivorship of adult clams under these conditions alone does not explain why clams are still present in these estuaries up to twenty years after the trials finished. The coarser sediments may have initially given the clams an increased chance to survive in larger numbers, but eventually to sustain a population, recruitment would need to occur. To become successfully established in a new location, an invasive species needs to survive the transit to the new location, have a high initial survivorship on release and establish a self-sustaining population (Miller et al., 2007). Without these criteria being met, the species will not become established. When the Manila clams were initially trialled and aqua-cultured, seed clams were laid on to the culture areas and allowed to grow to marketable size (Spencer et al., 1991). Without seeding, the recruitment would have to be the result of natural spawning events. If the population did not become self-sustaining it would not become established.

The seven initial culture sites that still have a presence of Manila clams are: the Exe Estuary, Poole Harbour, Emsworth Yacht basin, the Thames Estuary (Reculver), The Swale Estuary, the Blackwater Estuary and the Colne Estuary. These locations still have a presence of Manila clams because the populations receive a steady supply of juvenile clams. This is from either natural reproduction or the laying of spat clams by fisherman. The reproductive cycle is dependent upon water temperature regime (Mann, 1979) and food availability (Uddin et al., 2012). If local conditions are not compatible with the requirements then spawning will not occur, due to the clams resorbing the gametes by the process of atresia to use as an energy source (Tumnoi, 2012). This could be used to explain why some populations have not become established, as there were not enough spawning events to maintain the population. A spawning event will not always result in a successful recruitment event, this could be because the water temperature is too cold for the larvae to survive (Drummond et al., 2006) or the larvae are not retained in the local area due to hydrodynamic action.

Natural recruitment occurs in Poole Harbour, with successful spat falls and spawning events (Humphreys et al., 2007; Jensen et al., 2004). The populations in the Blackwater Estuary (Kershaw & Campos, 2011a), the Colne Estuary (Kershaw, 2013c) and Emsworth Yacht basin (Kershaw & Campos, 2013) are classed as wild populations where no seeding is carried out, with the populations sustained by natural recruitment. Licences have been granted to lay Manila clams in the Beaulieu River (Campos et al., 2012a) the Thames Estuary (Personal communication John Bayes, Seasalter Hatcheries) and the Exe Estuary (Devon and Severn inshore fisheries personal communication).

The Poole Harbour population is self-sustaining and has successfully naturalised (Humphreys et al., 2007; Jensen et al., 2004). This population is potentially successful due to the geography and hydrodynamics of the harbour itself. Poole Harbour has a small tidal range and has many lagoon-like characteristics (Humphreys, 2005). This small tidal range means that the majority of larvae spawned in the harbour are retained in the harbour (Herbert et al., 2012). A study carried out by Herbert et al, (2012) mathematically modelled the transport of larvae in Poole Harbour based upon a hydrodynamic model of the Harbour and larval behaviour. The model concluded that a large proportion of the larvae would be retained in areas close to initial spawning locations; however some larvae would be ejected out of the Harbour into Poole Bay. The hydrodynamic regime in Poole Harbour coupled with its status as a shallow (Humphreys, 2005) eutrophic system (Wardlaw, 2005) make it an ideal location for larval development and retention.

Four of the initial eleven culture sites no longer have a presence of Manila clams. These sites are: The Helford Estuary in Cornwall, The Teign Estuary in Devon, The Crouch and Roach Estuaries in Essex and The Blyth Estuary in Suffolk. The Helford Estuary (133km) and The Blyth Estuary (50 km) are geographically isolated from other areas of initial introduction, whereas the Teign Estuary (13km) and Crouch and Roach Estuaries (7km) are located close to areas where Manila clams were found by the surveys.

The Helford estuary is the furthest west of all the sites where Manila clams were introduced but doesn't have a population of Manila clams present. The reason behind this could be due to the tidal regime of the Estuary. On a neap tide, the tidal range is 3m and then 5.4m on a spring tide. The mouth of the estuary is also open to the sea, which

could reduce the numbers of recently spawned larvae retained in the system, especially as the larval stage can last up to two weeks (Ruesink et al., 2013). The temperature regime based on the nearest monitoring station in Newlyn would be compatible with the reproductive cycle, with average monthly temperatures above 12°C for five months of the year from May to November and temperatures above 15°C for three months between July and September (CEFAS 2014).The temperature regime in the Helford Estuary also has a higher upper temperature than the Exe Estuary which does have a population of Manila clams. This suggests that the temperature regime in the Helford may not be a limiting factor to the establishment of Manila clams. The sediment found in the Helford Estuary, is also compatible with Estuaries that do have a population of Manila clams (See table 7) such as the Exe, so this could potentially be ruled out as a reason for Manila clams not becoming established.

The Teign Estuary is another estuary that no longer sustains a population of Manila clams. It is in close proximity to an established population in the Exe Estuary. Both Estuaries are semi-enclosed at the mouth and have a similar tidal range, with a 2.3m range on neap tides and a 4m range on a spring tide. The low tidal range and semi-enclosed mouth could prevent the loss of larvae and natural barriers have previously been proven to prevent the spread of larvae (Bourne, 1982). The seawater temperature regime could potentially be ruled out as a reason why the Manila clam has not become established, because a population became established in the neighbouring Exe estuary. Sediment type could potentially explain the absence of Manila clams in the Teign Estuary, as the sediment consists of mud and sand. This could have resulted in reduced survival rates of the introduced population and reduced its ability to self-recruit due to a small adult population size.

The Crouch and Roach Estuaries in Essex now have a reported presence of Manila clams (Kershaw & Cook, 2012), however no clams were found by the surveys carried out in this chapter. A CEFAS shellfish classification survey in 2012 reported that Manila clams are thought to be present throughout the estuary and come up in dredges targeting other species. This fishery for Manila clams is not currently classified so Manila clams are not currently able to be sold as by-catch, and currently there is no interest in getting it classified by local fisherman (Kershaw & Cook, 2012).

The Blyth Estuary in Suffolk is the last licensed area that no longer has a presence of Manila clams. This site is the furthest north out of all the trial locations and is isolated from the nearest other licensed site, the Colne Estuary by 70km, and by 50km from the nearest population of clams. The geographical isolation of this population and lack of continual introduction could be the reason why this population is no longer present. After the initial introduction there are no reports of licences being granted for continual seeding of Manila clams or their presence in the estuary. This could suggest that the population did not reach sufficient densities to become self-sustaining.

2.4.3 Potential dispersal mechanisms

The spread of the Manila clam from the initial trial locations could have been driven by natural dispersal as larvae, or as deliberate introductions by fishermen to create new fisheries (Bourne, 1982). The appearance of Manila clams in locations adjacent to culture and trial sites, could be the result of larval dispersal, as this was also reported for the Pacific oyster (Herbert et al., 2012). Surveys to map the spread of the Pacific oyster reported that oysters had begun to appear in locations adjacent to culture areas, however they did not appear in locations that were geographically isolated from existing populations of oysters (Herbert et al., 2012).

This raises the question of how did Manila clams get to the isolated locations for example the Stour Estuary in Suffolk and the Fleet Lagoon in Dorset? The economic activities practiced in these locations may provide an insight into how Manila clams were introduced. The Fleet Lagoon has a licensed Oyster fishery (Langston et al., 2003) and the Stour Estuary, had a licensed Manila clam fishery up until 2011 (http://www.eastern-ifca.gov.uk/). These economic activities could suggest that Manila clams were intentional introduced in order to create a fishery. The high sale price of Manila clams at between £6.50 (fishmarketportsmouth.co.uk) and £8.50 per kilo (freshfoodcornwall.co.uk 2014) could also add evidence to the theory that these isolated populations of Manila clams are the result of intentional introductions to create fisheries as opposed to the result of natural dispersal.

The region with the highest density of Manila clam populations is the Solent. In the Solent there are five locations in which Manila clams were found. Of these locations only Chichester Harbour was previously licensed to culture clams. There is anecdotal evidence to suggest that several of the Estuaries in the Solent (including Southampton Water and Langstone Harbour), were deliberately seeded with Manila clams in order to create new fisheries (personal communication, Peter Hill, SIFCA). However as this is anecdotal evidence and does not have any quantitative information to describe exactly when and how many clams were introduced, we cannot solely use this piece of evidence to determine the spread of the Manila clam around the Solent.

The current overall distribution of Manila clam populations in the Solent area is unlikely to be solely the result of natural larval dispersal at present, due to the "patchiness" of clam populations. An example of this is the distribution along the Northern coastline of the Isle of Wight, with Manila clams only being found in the middle of five estuaries, the Medina Estuary. Natural methods of dispersal would suggest that all of the estuaries along this coastline would have the same potential to receive larval recruits, however Manila clams were only found in the Medina Estuary. The failure of the Manila clam to establish populations in all of the Estuaries on the North coast of the Isle of Wight, maybe due to an insufficient or an irregular supply of larval recruits.

The irregular supply of larvae may be due to inconsistent spawning events caused by unfavourable conditions in the Solent. The population of Manila clams in Southampton Water (which is the closest population to the northern coastline of the Isle of Wight), has been observed to resorb gametes by the process of atresia when conditions are not compatible with the reproductive cycle (Tumnoi, 2012). In July 2009, 80% of the clams sampled from Southampton Water were observed to be in a state of atresia, compared to just 20% in July 2010 (Tumnoi, 2012). This suggests that the supply of viable larvae from the Southampton Water population is highly variable in nature and varies from year to year. A similar pattern was noted for the Pacific oyster in the Wadden Sea, with successful reproductive events, and subsequently recruitment not occurring on a regular basis (Diederich et al., 2005). This irregular supply of larvae may influence the ability of the Manila clam to establish new populations, because insufficient numbers of larvae are able to settle and avoid predation. The level of predation upon newly settled

bivalve larvae is high and the success of recruitment events is often determined by levels of predation (Hiddink et al., 2002; Hunt & Scheibling, 1997). This could suggest that low availability of larvae coupled with predation upon newly settled larvae may explain why populations of Manila clams have not become established along the whole of the Isle of Wight, however it raises the question of how did a population become established in the Medina Estuary?

A potential explanation as to why only the Medina Estuary was successfully colonised on the Isle of Wight, could be that the natural supply of larvae that arrived in the Estuary was augmented by the introduction of spat clams by fishermen in order to "seed" the Estuary. Evidence for this theory could be the distribution of Manila clams in the Estuary. The survey found that the Manila clams were located in the upper reaches of the estuary, adjacent to the Medina Valley centre. This could be a deliberate attempt by a fisherman to seed a new fishery, (these activities have been reported elsewhere (Bourne, 1982)). Some more evidence to support this, is the close proximity of the Medina Estuary to Portsmouth Harbour and the main wholesaler of Manila clams along the south coast, Viviers UK Ltd™ (an application to have the Manila clam fishery classified for harvesting was received by CEFAS in 2008 from Viviers UK ltd™ (Kershaw & Acornly, 2008)).

This could suggest that the potential spread of Manila clams throughout the Solent may be mostly due to anthropogenic influences rather than a result of larval transport. A similar phenomenon was noted in British Columbia where populations of Manila clams were found in locations that could only be the result of human interventions (Bourne, 1982). Naturally distributed populations of bivalves that are the result of larval dispersal are also more continuous in nature and do not exhibit high levels of "patchiness" (North et al., 2008). This does not mean that natural dispersal has not influenced the distribution of the Manila clam, but that it is not currently the main driver.

2.4.4 Main conclusions and potential for further work

The Manila clam is no longer confined to Poole Harbour, but has spread to other locations along the South coast including Southampton Water, Portsmouth, Chichester and Langstone Harbours and the Medina Estuary on the Isle of Wight. Population have also become established in the Thames Estuary as well the Blackwater, the Stour and the Colne Estuaries along the South Eastern coastline of England.

The current distribution (up until 2011) of the Manila clam along the South and Eastern coastline of England appears to be mainly influenced by human introductions, as opposed to the result of natural larval dispersal. This can be argued by the fact that based on the findings of this thesis; the Manila clam is not uniformly distributed throughout the Estuaries along the South and Eastern coast of England. There are areas, most notably the Stour Estuary in Suffolk, that have a presence of Manila clams, whilst the adjoining Orwell Estuary was not found to have a presence. With predicted increases in sea water temperatures the populations of Manila clams may begin to spread naturally, but until then the distribution is influenced by human intervention.

The aim of this chapter was to determine the current distribution of Manila clams along the South and Eastern coastline of England and then produce a map of these populations. Consequently the sampling methods utilised in this chapter were designed to determine whether Manila clams were present or absent in the Estuaries surveyed.

The large number (52) and size of the Estuaries located in the study area limited the amount of time available to survey each Estuary and made it impossible to survey each Estuary in its entirety. This lead to the decision to undertake an extensive set of surveys as opposed to intensive surveys at each estuary. As a consequence of the sampling methods the Estuaries were classified as either having Manila clams found or not found during the surveys, as opposed to having a presence or absence of Manila clams. This distinction is important as although Manila clams were not found in the large majority (38 out of 52) of the estuaries surveyed it does not mean that they are not present. The Crouch and Roach Estuary is a fine example of the limitations of the survey, as although no Manila clams were found during the surveys, they were reported to be present in the

Estuary by CEFAS (Kershaw & Cook, 2012). A good example of why a population of clams may not have been detected by the surveys is the distribution of clams in Portsmouth Harbour. If the North-west corner of the Harbour had not been surveyed, then Manila clams would have not been reported as being found in Portsmouth Harbour. This indicates that, as the surveys carried out did not cover every single part of the Estuaries, Manila clams may be more widely distributed than reported in this chapter. As a result of this, the conclusions and analysis drawn from this chapter need to be considered with the caveat that Manila clams are likely to have a wider distribution than reported in this chapter. This however does not mean that the results in this chapter are not useful, but they should be used as a guide for further studies as opposed to being the definitive distribution of Manila clams along the South and South eastern coastline of England. A more detailed sampling strategy at all of the locations in which Manila clams were not found would enable a more definitive map of the Manila clams distribution to be produced.

Once the definitive distribution of Manila clams along the South and Eastern coast has been produced it would then allow the causes of the distribution to be explored. The physical properties of the locations in which they have become successfully established such as: sediment type, water temperature, salinity and tidal range could be analysed alongside other parameters such whether they were introduced by man, the size of stock introduced and the proximity of other populations of Manila clams to develop a model to predict the future spread of this species. It would also allow for the determination of whether the main cause of dispersal of Manila clams along the English coastline is anthropogenic or natural in origin. This data could also be used to help predict the further spread of Manila clam larvae.

Once the dispersal of Manila clams can be predicted it would then allow the populations that were deemed to become established by natural means to be investigated and determine whether they would be able to become naturalised themselves and produce viable offspring or they would be reliant on parent populations to provide new recruits. The naturalisation of the Manila clam in Poole Harbour raises the question of whether the scientific findings by MAFF that Manila clams would be unable to reproduce were incorrect or whether climate change has played a part in the successful naturalisation.

It also raises the question of whether the Manila clam has truly naturalised in Poole Harbour or whether it is at the extreme of its range and is vulnerable to extinction from high mortality caused by suboptimal conditions and poor recruitment. This indicates that it is important to monitor the Poole Harbour population and determine whether the population has naturalised or is at the extreme of its tolerance.

Chapter 3: Population dynamics of the UK pioneer population of Manila clams

3.1 Introduction

Introductions of the Manila clam

Due to its high economic value, the Manila clam has been introduced around the world for the purpose of aquaculture. It has been introduced to: North America (Bourne, 1982), France (Dang et al., 2010), Spain (Juanes et al., 2012), Italy (Pellizzato et al., 2011), Ireland (Drummond et al., 2006) and the United Kingdom (Jensen et al., 2004). In Europe the Manila clam was able to successfully naturalise in France (Dang et al., 2010), Spain (Juanes et al., 2012) and Italy (Pellizzato et al., 2011). These naturalisations lead to the establishment of successful fisheries in the Bay of Biscay in Spain (Juanes et al., 2012) and Arcachon Bay in France (Dang et al., 2010) which are replenished by natural recruitment. Overexploitation of these populations had led to the removal of the majority of clams over the minimum size limit of 40mm (Dang et al., 2010; Juanes et al., 2012). A study was commissioned to investigate the sustainability of the Arcachon Bay population due to the pressure of overfishing (Dang et al., 2010). This intense fishing pressure and removal of a large proportion of the clams above the minimum landing size now requires these fisheries to be intensely managed to prevent the collapse of the stocks (Dang et al., 2010; Juanes et al., 2012).

The Manila clam was introduced into the United Kingdom in the 1980s as a species for aquaculture (Humphreys et al., 2007). The scientific opinion at the time was that Manila clam would be suitable for growing to marketable size but would be unable to become naturalised (Laing & Child, 1996; Spencer et al., 1991). This was because the seawater temperature regime in UK waters was believed to be incompatible with the requirements of the reproductive cycle (Laing & Child, 1996). This would make it an ideal species for on-growing, due to the fact that it was faster growing than the local species of clam, but could not spawn (Spencer et al., 1991).

Contrary to the scientific opinion at the time, the Manila clam did manage to become naturalised in the UK. The first reported naturalised population was in Poole Harbour in Dorset (Humphreys et al., 2007; Jensen et al., 2004). The Manila clam had been

introduced in to Poole Harbour in 1988 by Othniel fisheries and this population had successfully reproduced by 1994 (Jensen et al., 2004). The term "successfully reproduced" describes a spawning event that results in a spat fall or recruitment event of juvenile clams. Not all reproductive events are successful as some spawning events do not result in a spat fall, an example being the population of Manila clams in North West Ireland (Drummond et al., 2006). The Manila clam larvae that were spawned in North West Ireland, did not survive the larval stage due to an incompatible temperature regime (Drummond et al., 2006). The successful reproductive event in Poole Harbour was not a one off and the population has become self-sustaining and recruitment continues to occur (Humphreys et al., 2007). Irregular recruitment events had been reported in the Pacific oyster populations in the Wadden Sea (Diederich et al., 2005). It was suggested that the irregular recruitment was due to the Pacific oyster being at the northern-most limit of its range and that successful reproductive events were linked to warmer than average summers (Diederich et al., 2005). The Poole Harbour population of Manila clams status as the most northern naturalised population in Europe (Jensen et al., 2004) raises the question of whether recruitment occurs on a regular basis or whether it is intermittent, with recruitment occurring in some years and not in others.

Reproductive cycle of the Manila clam

The Manila clam is a broadcast-spawner and fertilisation takes place in the water column (Utting et al., 1991). This reproductive strategy is also known from other species of bivalves and many studies have described this strategy in more detail in bivalves such as oysters (Brandt et al., 2008; North et al., 2008; Utting et al., 1991), mussels, cockles (Bouma et al., 2001) and other species of clam (Bouma et al., 2001). The duration of the larval stage is influenced by temperature with low temperatures extending the duration of the larval stages by several weeks (Brandt et al., 2008; Ruesink et al., 2013). In commercial hatcheries, the larvae are maintained at elevated temperatures in order to shorten the duration of the larval stage (personal communication, John Bayes Seasalter Hatcheries). The influence of temperature on the duration of larval stage can also determine whether a reproductive event is successful or not (Drummond et al., 2006). The Manila clam larval stage typically lasts from 1.5-2 weeks (Ruesink et al., 2013) which is comparable to some other species of bivalves such as *Macoma balthica,*

Cerastoderma edule and *Crassostrea gigas* (Bouma, Duiker, et al., 2001; Brandt et al., 2008).

The reproductive cycle of the Manila clam is also influenced by food availability (Uddin et al., 2012). The relationship between environmental conditions such as temperature (Serdar & Lok, 2010) and food availability (Darriba et al., 2004) has been described in other species of bivalves. The minimum lower temperature limits reported by Mann (1979) for gametogenesis in the Manila clam was 8°C, with 12°C for gamete ripening and 14°C for spawning (Mann, 1979). Low seawater temperatures have been found to cause some populations of Manila clams to resorb gametes and integrate them back into somatic tissue rather than release them in spawning events (Drummond et al., 2006; Tumnoi, 2012). This response to unfavourable environmental conditions has resulted in populations of clams experiencing inconsistent recruitment events (Tumnoi, 2012). This process (atresia) does not just occur when seawater temperatures fail to reach the threshold to initiate spawning, atresia can also occur when food availability is a limiting factor (Tumnoi, 2012). Populations of clams that have low food availability have gametes that are of poorer quality than populations where food is not a limiting factor (Uddin et al., 2013). This indicates that food availability also has a large impact on reproductive success.

Temperature influences the number and duration of spawning events in bivalves, including the Manila clam. Some populations of Manila clams have been observed to have two spawning events a year (Dang et al., 2010; Drummond et al., 2006; Humphreys et al., 2007; Pellizzato et al., 2011; Uddin et al., 2012). This has also been recorded in other species of bivalves (Baron, 1992; Caddy, 1967) . Not all bivalve species have two spawning events, some only have one (Kautsky, 1982; Sahin et al., 2006) whereas some tropical species may have prolonged spawning seasons (Cantillanez et al., 2005; Rebelo, 2003).

Manila clam spawning events often consist of a small late spring/early summer event with a larger late summer spawning event (Dang et al., 2010; Ren et al., 2008; Uddin et al., 2012). In some countries where the Manila clam is at its temperature limit, such as Ireland, spawning events do not always lead to a successful recruitment of juvenile clams (Drummond et al., 2006). In the UK the Manila clam is at the northern extreme of

its range and the temperature required to trigger spawning is reached later in the year (Drummond et al., 2006) than in other countries such as France (Dang et al., 2010). Consequently larvae spawned in late summer often do not survive due to declining seawater temperatures post spawning (Drummond et al., 2006), because below 10°C larval survival is poor (Ruesink et al., 2013). At the end of the spawning season, any gametes that have not been released are resorbed by the clam and are incorporated into somatic tissue (Drummond et al., 2006; Uddin et al., 2012). The atresia of gametes is used by the clam to regain condition and food reserves after the reproductive season is over (Tumnoi, 2012; Uddin et al., 2012).

Condition index as a proxy for bivalve physiology and reproductive state

Condition index is the proportion of flesh weight to shell weight and gives a good indication of the meat content of an individual bivalve. The condition index of a bivalve is often used to determine the status of a population (Crosby & Gale, 1990) and the state of the reproductive cycle (Dang et al., 2010; Kang et al., 2007; Sahin et al., 2006). In bivalve molluscs, condition index is linked to the reproductive cycle, with the highest levels of condition index correlating with ripening of the gonads and sudden reductions in condition index corresponding with spawning events (Rebelo, 2003; Sahin et al., 2006; Serdar & Lök, 2009). The relationship between condition index and the reproductive cycle has been reported in many species including; *Anadara inaequivalvis* (Sahin et al., 2006), *Tapes decussatus* (Serdar & Lök, 2009*)*, *Crassostrea rhizophorae* (Rebelo et al., 2005), *Crassostrea gigas* (Dridi & Romdhane, 2007) and *Ruditapes philippinarum* (Dang et al., 2010; Drummond et al., 2006; Robert et al., 1993). In Northwest Ireland, the maximum reported values for condition index corresponded with the ripening of gametes and spawning corresponded with a drop in condition (Drummond et al., 2006).

Current understanding of the Manila clam in Poole Harbour

In Poole Harbour the Manila clam has successfully reproduced and spread to other areas around the Harbour from the site of initial introduction (Jensen et al., 2004). A large population of Manila clams became established at the Holton Mere Mudflat at the Western end of the Harbour in the Wareham Channel (see figure 3.1)

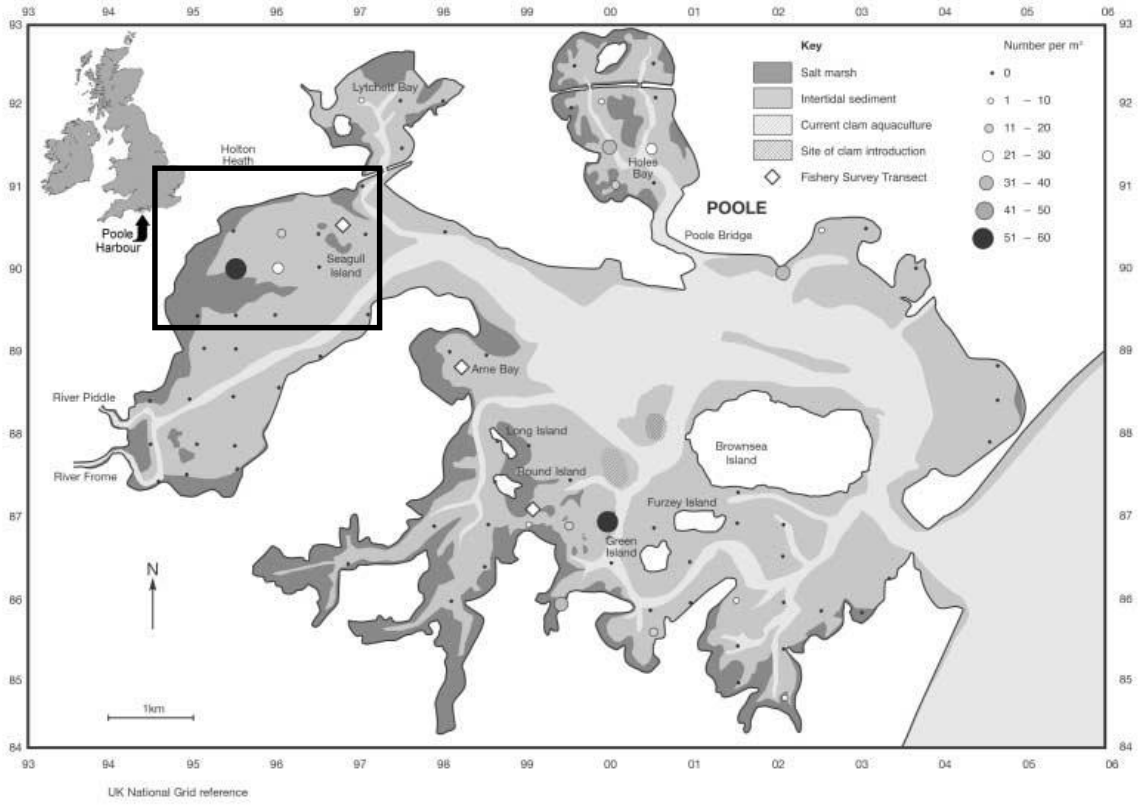

Figure 3.1: The distribution of Manila clams in Poole Harbour in Dorset (taken with permission from, Jensen *et al* 2004). The large black circles signify locations that have high densities of Manila clams. The Holton Mere population of clams is located north-west corner of the harbour. The study site is denoted by the black box at the western end of the harbour.

Holton Mere is a homogeneous intertidal mudflat, located towards the Western end of Poole Harbour in Dorset. The sediment is well sorted and consists of medium and fine sand (125 -355 microns grain size) as well as very fine sand and coarse silt (<63-125 microns grain size).

A study carried out in 2003 on the Holton Mere population found that there were two distinct spawning events a year, characterised by two spat falls, one in early summer and one in late summer (Humphreys et al., 2007; Jensen et al., 2004). This study

focussed on the area denoted by the black box in figure3.1. In 2003, the Holton Mere population had six distinct age cohorts, with seasonal density of clams influenced by recruitment events (Humphreys et al., 2007). The majority of the annual production of Manila clams was due to clams of 3, 2 and 1 years of age, at which time they would be between 25 to 40mm in size (Humphreys et al., 2007). The larger clams contributed more biomass to the population than smaller individuals.

The population was subject to pressure from a winter fishery which removed circa 75% of clams above the minimum landing size of 40mm (Humphreys et al., 2007). The clams were targeted using a specially designed pump scoop dredge which was reported as unique to Poole Harbour (see figure 3.2). The dredge is attached to a water pump which is used to liquefy the mud so that only bivalves are retained. That this method is particularly efficient is evident from the data reported later in this chapter. Clams are then sorted on deck and any undersized clams are thrown back.

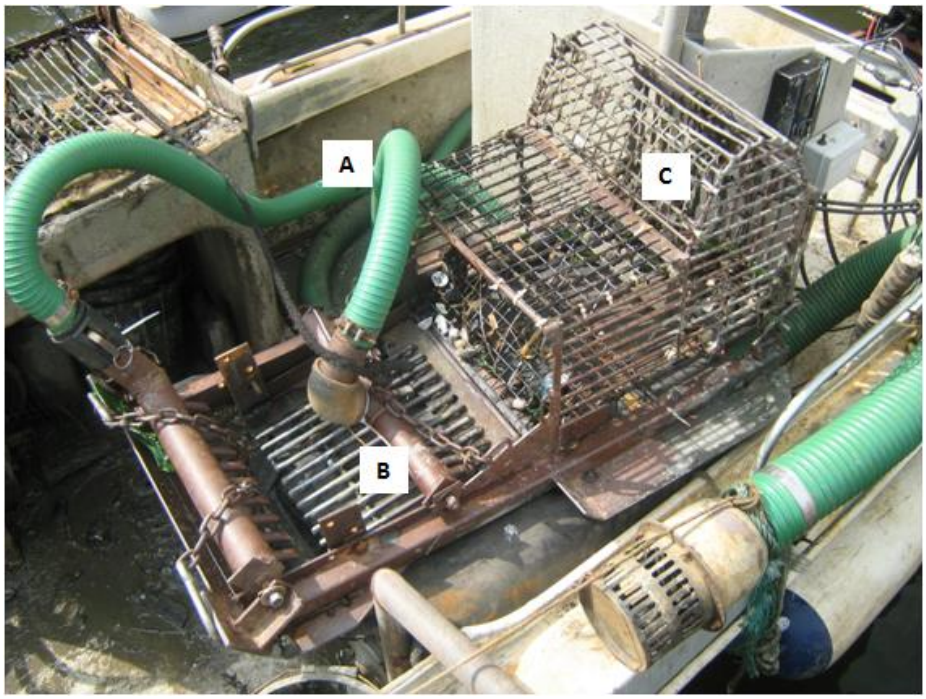

Figure 3.2: A pump scoop dredge used in Poole Harbour to harvest Manila clams. The dredge has two water jets (A), green hoses) which liquefy the mud. The dredge is pulled through the liquefied mud and with clams collected by the tines of the dredge (B) and moved to the basket (C) where they are collected.

Since the study in 2003, the minimum landing size (MLS) for Manila clams has been reduced from 40mm to 35mm by the European Union in 2008 (North Western waters Advisory Council).

The main aim of this study was to monitor the population dynamics of the Manila clam population in Poole Harbour over a long term timescale from 2009 to 2012, also building on the 2003 dataset. It also served to investigate the implications of fishing activity over a longer timescale, including the effect of the reduction in minimum landing size on both the clam population itself and the wider aspects of the fishery.

3.2 Materials and Methods

Quantitative sampling methods for the Holton Mere study site

The study site was unsuitable for approach from the shore due to a reed bed and therefore required boat work. The site was only accessible by boat two hours either side of a spring high tide due to the depth of the water. When accessible by boat there was a depth of at least 50cm of water over the seabed. The depth of the standing water prevented the use of quadrats and box cores to collect samples. The use of an underwater corer was an option; however the small diameter of the corer (15cm) was not suitable due to the densities of clams previously reported at the study site. Previous studies used wire framed nets with a small mesh size which were dragged through the sediment (Humphreys et al., 2007; Jensen et al., 2004). This study used a specially designed naturalist's dredge with a width of 30cm attached to an aluminium handle at a 45° degree angle to allow the dredge to be dragged through the sediment and collect any organisms in the attached 1mm mesh bag (see figure 3.3).

Figure 3.3: The hand held dredge designed to work in shallow water on the Holton Mere mudflat. The opening of the dredge is 30cm wide and the bag has a mesh size of 1mm. The dredge is pulled through the sediment for a distance of 1m in order to collect quantitative samples.

The dredge was pulled through the top layer of sediment for a distance of 1m. The sediment collected in the dredge bag and was then sorted through two vertically stacked sieves of 8mm and 2mm mesh size respectively. The sediment was washed through the sieves on the boats using a water jet from a pump and all live Manila clams were collected for analysis. The dredge was capable of collecting juvenile bivalves down to 1 mm in shell length as evidenced by the collection of *Mya arenaria* and *Cerastoderma edule* spat. All of these species were found on multiple occasions throughout the year.

Sampling the Holton Mere Manila clam population

Holton Mere was sampled from April 2009 to November 2010 and again from July 2011 to August 2012, with various intervals (see table 8). Unfortunately it was not possible to sample every month and subsequently there are some gaps in between sample months.

Table 8: The sampling regime over the duration of the study. Samples were collected from April 2009 until August 2012. The months in which samples were collected are indicated by an X.

Month	2009	2010	2011	2012
January				X
February		X		X
March		X		X
April	X	X		X
May				X
June	X	X		X
July	X	X	X	X
August	X	X	X	X
September	X	X	X	
October		X	X	
November	X	X	X	
December	X			

Random coordinates were used to determine the initial sampling locations before visiting the sampling sites; however the presence of illegal fishing vessels prevented these from being used. Issues with illegal fisherman meant that sampling had to take place in the areas in-between where clam boats were already fishing. An example of the issues encountered with illegal fisherman included threatening behaviour, which

included the threat of being run down with the fisherman's vessel whilst in the water sampling and on another occasion physical violence at Poole Quay. For each sample month two sampling locations were used. Only two sampling locations were chosen due to the time constraints of a sufficient depth of water on the study site and to prevent antagonising illegal fisherman further. It was also decided that sampling at two sites and having replication of sampling as opposed to several individual samples across the mudflat would give a better coverage and understanding of the clam population and would account for patchiness of clams.

At each sampling location four dredge samples were collected. The Global Positioning System (GPS) coordinates of each location were recorded and used to generate a map of the study area (see figure 3.4a). A depth profile for the study site can be seen in figure3.4b.

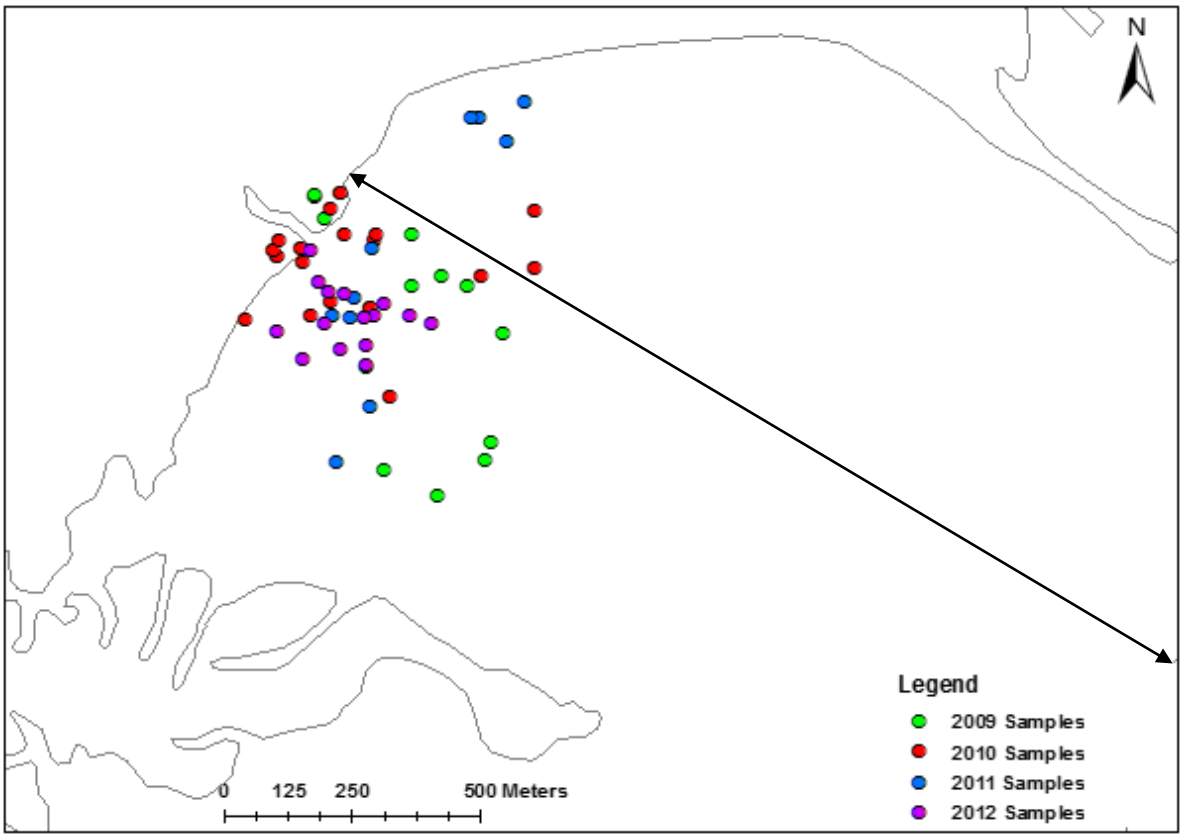

Figure 3.4a: The sampling locations on the Holton Mere mud flat between April 2009 and August 2012. Each of the circles indicates a sampling location; green circles indicate samples collected in 2009, red circles 2010, blue circles 2011 and purple samples 2012. Over the course of the study some of the sampling locations overlapped, hence why not all are visible for each year. The black line signifies the depth profile in figure 3.4b. The samples that appear to be on land are in fact at the edge of the reed bed in the intertidal area, this was caused by the accuracy of the GPS unit used and the map layer.

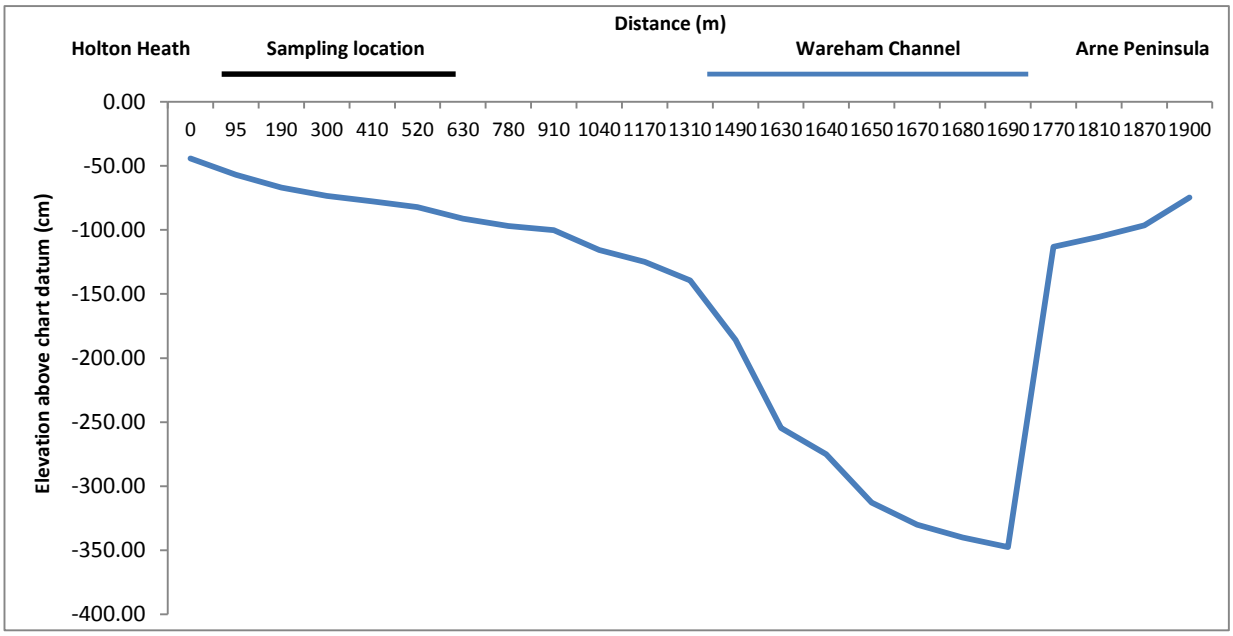

Figure 3.4b: The shore profile of the Holton Mere mud flat from Holton Heath in the North West, across the mudflat and the Wareham channel to the Arne Peninsula in the South East. The sampling location is indicated by black horizontal line, the elevation changed by a maximum of 30cm across the sampling location.

The sediment from each dredge sample was sieved and all Manila clams were retained. Clams were measured to determine the maximum shell length using digital callipers. The maximum length was determined as the length from the anterior to the posterior margin of the shell (see figure 3.5).

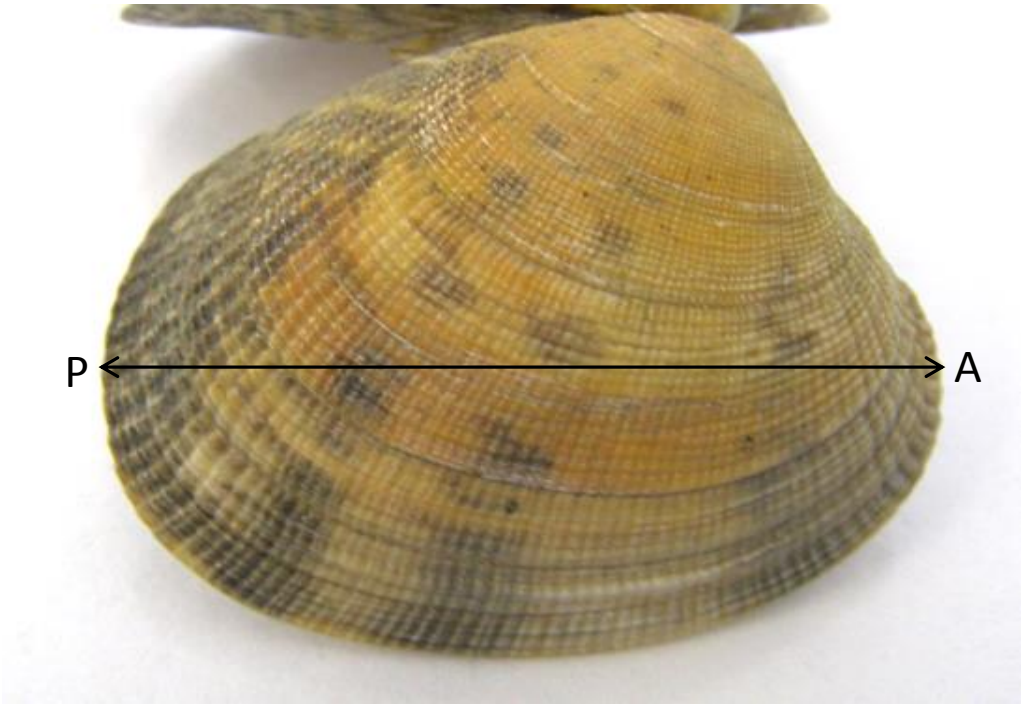

Figure 3.5: The maximum shell length measurements on a Manila clam. The clams were measured from the anterior margin (A) to the posterior margin (P) using digital callipers.

Each clam was measured in triplicate and the average length was used for statistical analysis. The number of winter growth rings was recorded for each clam to determine age. Growth rings were also used in the previous study on this population (J. Humphreys et al., 2007). If growth rings were not visible on the shell or if it was unclear or ambiguous how many there were the clam was not aged. Once the clams had been measured they were fixed in 10% formalin in seawater for 48 hours and then transferred into 70% ethanol for long-term storage due to restricted freezer space.

Population and stock assessment using cohort analysis

The shell lengths were used to assign clams to size cohorts and determine the population dynamics by using the freeware stock-assessment software FISAT II FAO-ICLARM Stock Assessment Tool. FISAT II is a descriptive piece of software and has been used by fishery managers and researchers to describe and categorise populations of shell and fin-fish (R. Cardoso & Veloso, 2003; Fiori & Morsán, 2004; Zeichen et al., 2002). It uses shell or body lengths in order to describe the population and calculate a number of parameters including population dynamics, mortality and growth rate.

To determine the age composition of the Holton Mere population, Modal progression analysis was used. Modal progression analysis (MPA) is used to infer growth rates, using the shift of the mean length of a cohort over a time series. MPA was used because the first stage of the process is to split the population into its component cohorts, each cohort with a mean length and population size. This data can then be used to determine the growth rate of each cohort using the change in mean length over time. To determine the number of cohorts in the population, Bhattacharyya's method was used (Fiori & Morsán, 2004).

The first stage in the process was to assign the shell length data for each month into arbitrary size classes. The size classes used were: 0-4.99, 5-9.99, 10-14.99, 15-19.99, 20-24.99, 25-29.99, 30-34.99, 35-39.99, 40-44.99 and 45mm+ shell length. Once the raw clam size data had been converted into size class frequency data it was then entered into the FISAT II software.

The FISAT software creates a series of histograms using the size frequency data which are then used to determine the number of cohorts in the sample (see figure 3.6a). The software requires the user to initially visually identify the cohorts in the sample (see figure 3.6a) before it uses this information to then calculate the upper and lower size limits for each cohort an then assign a proportion of the population into each cohort and calculate the mean length of the individuals in each cohort. The mean length of each cohort can then be used to calculate growth rate.

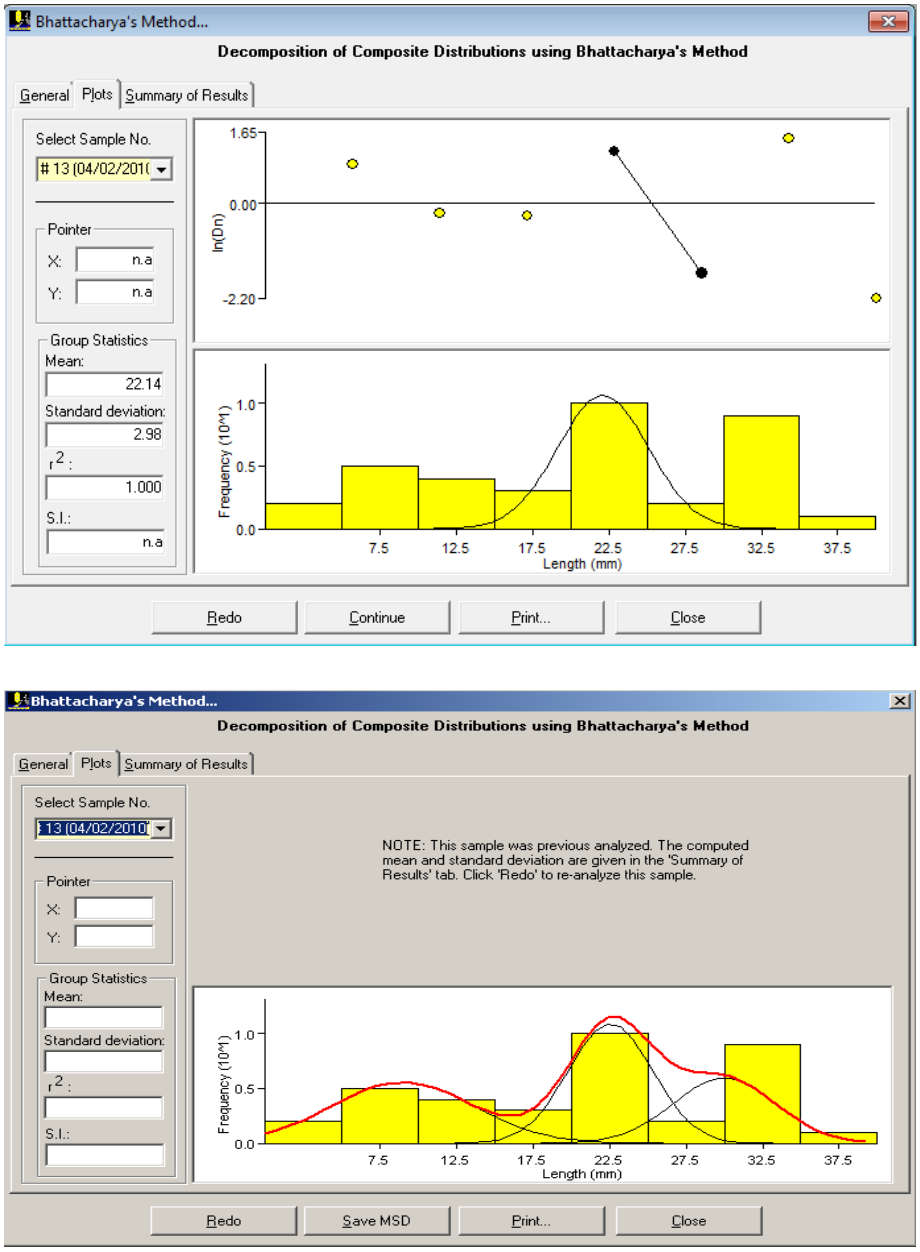

Figure 3.6a: The initial identification of the cohorts can be seen in the top image with the final cohort identification complete, bottom image. Connecting the dots identifies the initial cohort before the software then uses this information to identify any additional cohorts in the sample. For this sample month, three cohorts have been identified with a mean shell length of 7.5, 22.5 and 30mm.

Calculation of the growth rate of the Holton Mere population

The growth rate of the Holton Mere population was calculated using the mean lengths of each cohort generated by Bhattacharya's method. The mean lengths for each cohort were then linked over the time series (See figure 3.6b). This generated the growth rate of each cohort in mm's per day.

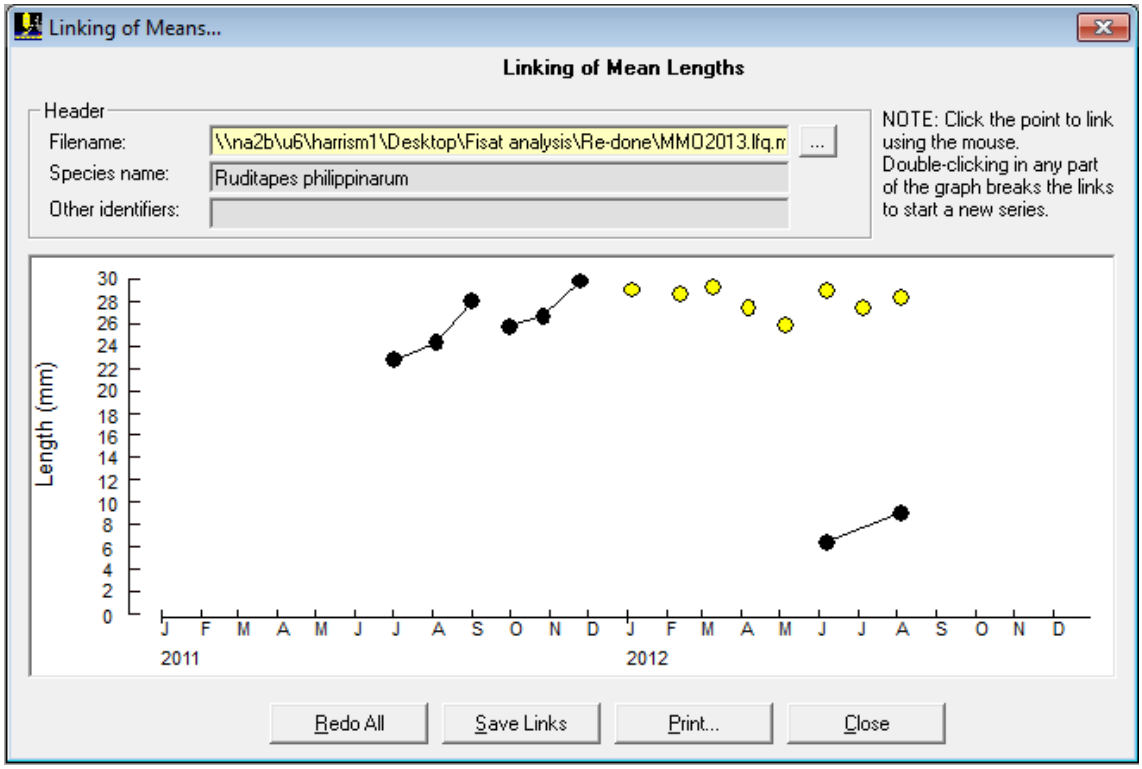

Figure 3.6b: Linking of the mean lengths of the cohorts identified using Bhattacharya's method. The linking of means is then used to generate a series of growth rates in mm per day. The dots linked by the black lines indicate the growth of that particular cohort.

Condition index

Condition index was used to monitor the physiology of the clams throughout the study period and to identify spawning events. Using condition index rather than histology also allowed the clams to be used for other analysis such as calculating secondary production. There are various methods in use for calculating the condition index of bivalves. These methods are diverse- some studies investigate the relationship between dry flesh weight and internal shell volume (Kripa et al., 2009; Rebelo, 2003) and others the relationship between dry flesh weight and dry shell weight (Dang et al., 2010; Kang et al., 2007; Sahin et al., 2006; Shriver et al., 2002). This study used the method utilising dry shell weight:

$$Condition\ Index = \frac{\text{Dry flesh weight (g)}}{\text{Shell dry weight (g)}} \text{x}100$$

For this study the method used by Sahin et al (2006) using the dry shell mass was used. This was because it was the same method used in other studies investigating condition in Manila clams (Dang et al., 2010; Kang et al., 2007; Matozzo et al., 2012) and allowed for comparisons between populations. This method was also used due to the difficulty in calculating the internal shell volume for Manila clams on a consistent basis and allowed for confident comparisons between months and populations.

Estimating the condition index of the Holton Mere population

All of the samples used to determine condition index from Holton Mere had been stored in 70% ethanol. This was to fix the samples and prevent changes in condition due to the length of time between sample collection and the condition index procedure being undertaken. The samples were fixed in ethanol rather than frozen, due to restricted freezer space at the Institute of Marine Sciences (A trial experiment had found that there was no difference in condition index between frozen and fixed clams).

For each sample date, when numbers made it possible, a minimum of 20 clams were sampled to determine condition index. There were two sample months where it was not possible to sample 20 clams, March 2010 (11 clams) and July 2011 (19 clams). A range of clam sizes was selected based on the cohort analysis when available.

The samples were flushed free of ethanol in running tap water for at least two hours before any procedure began. Once free of ethanol the maximum shell length was measured and the flesh carefully removed from the shell using forceps and a scalpel. The flesh was again washed in distilled water to remove any residual salt and then placed in a pre-weighed foil envelope on a glass Petri dish before being placed into a pre-heated drying oven. The empty shell was also cleaned using distilled water to remove any residual salt or sediment and placed onto a separate glass Petri dish to be dried at the same time as the flesh.

The samples were dried to constant weight at 105°C for 24 hours (Sahin et al., 2006). They were then re weighed separately on a precision balance to give the dry masses of the flesh and the shell. The flesh was then stored under moisture-free conditions for further analysis.

Secondary production estimates of the Holton Mere population

The secondary production was estimated by calculating the ash free dry mass (AFDM) of each clam. This gave the organic content of each individual. The dried flesh from the clams used in the condition index protocol was weighed, then combusted for four hours at 560°C (Cardoso et al., 2007). The flesh was then re-weighed to give the value of the inorganic matter which would allow for the calculation of the organic content of each clam.

The AFDM was calculated as follows:

$$\text{Ash free dry mass (mg)} = \text{Dry mass of tissue (mg)} - \text{residual post combustion mass of ash (mg)}$$

The AFDM per individual could then be used to determine the secondary production of each age cohort and the secondary production per m^2. This would be useful to determine whether there were any changes in production over time and could be compared to earlier studies.

The mass increment per individual was calculated by subtracting the previous monthly value for AFDM per individual from the current monthly AFDM per individual. The mass increments could be either positive or negative, a positive value would potentially indicate a growth in body mass and a negative one would potentially indicate a drop in body mass. The mean number during sampling period was calculated by adding the number of clams from the previous sample month to the number of clams in the current month and dividing by two to give an average density over the period

The production increment is the increase or decrease in AFDM per month of an age class. The monthly production increment for each cohort was calculated by subtracting the previous month's total AFDM from the current months AFDM (Eleftheriou, 2013). The production increment could be either positive or negative.

Statistical analysis

All of the data was first tested for normality and then the appropriate statistical tests were used. The only data that was transformed was the shell length which was log transformed to carry out a regression analysis when analysing the daily growth rate of the Holton Mere population. The clam density data was analysed using a two way ANOVA to determine whether there were any statistical differences between clam density across sample months and between age cohorts. A one way ANOVA was used to analyse the individual AFDM between months and cohorts over the duration of the study period. This was used as there were not equal numbers of AFDM data for each cohort in each month, this lead to an unbalanced design which would not allow a 2 way ANOVA to be run.

The average size of clams across years was analysed using a one way ANOVA using the statistical package Minitab. The size frequency distribution of clams across study years was analysed using the statistical package Primer 6.1 (PrimerE Ltd: Plymouth Routines in Multivariate Ecological research) software. To determine whether there were any differences between sample years a PERMANOVA using Bray-Curtis similarity matrices was undertaken. The data was then analysed using a Principal Component Ordination analysis, with hierarchical clustering using Bray-Curtis similarity matrices to explore similarities between sample years.

The condition indices were analysed using Kruskal-Wallis to determine whether there were any statistical differences between sample months. Transforming the condition indices did not normalise the data so a non-parametric test was used.

3.3 Results

Historical population dynamics of the Holton Mere Manila clam population

The Manila clam population at Holton Mere in Poole Harbour was sampled from January to December 2003 by Humphreys et al (See figure 3.7). The raw data of the clam sizes was provided by those authors and has been plotted as histograms in figure 3.7. At the time of the 2003 sampling, the MLS for Manila clams was 40mm (Jensen et al., 2004)

The fishery did not remove all clams of the legal minimum size of 40mm+ (See figure 3.7). These size clams were found in all of the samples collected. From September to December the majority of the clams in the samples were over 30mm in shell length. By December 60% of the clams were over 35mm in shell length with 25% of these being greater than 45mm. The majority of the population in 2003 was made up of larger individuals with a shell length of between 20-45mm. A small number of clam spat appeared in the February sample and again in June. The size class with the highest frequencies in the population is the 35-40mm sized clams. Clams over 45mm in shell length were found in 11 out of the 12 months sampled.

In 2009 the population was still dominated by individuals greater than 30mm but a larger proportion of the population was composed of smaller individuals under 30mm in shell length (See figure 3.8). There were individuals larger than 35mm present, but very few above 45mm in size.

The population in 2010 has very few individuals over 30mm with the majority of individuals under 25mm in length (See figure 3.9). In 2010 there were no individuals present over 45mm in size and the proportion above 35mm in size is very low.

By 2011 the population was composed mainly of individuals between 20-35mm in shell length, with few above this and a low proportion of the population under 20mm (See figure 3.10). There are no individuals present over 40mm in shell length. The proportion of clams above 35mm has also reduced.

The population by 2012 is composed mainly of individuals between 25-30mm and with a small proportion of individuals over 40mm in shell length (See Figure 3.11). A small proportion of clams <5mm also appear in the population.

Holton Mere Manila clam population January-December 2003

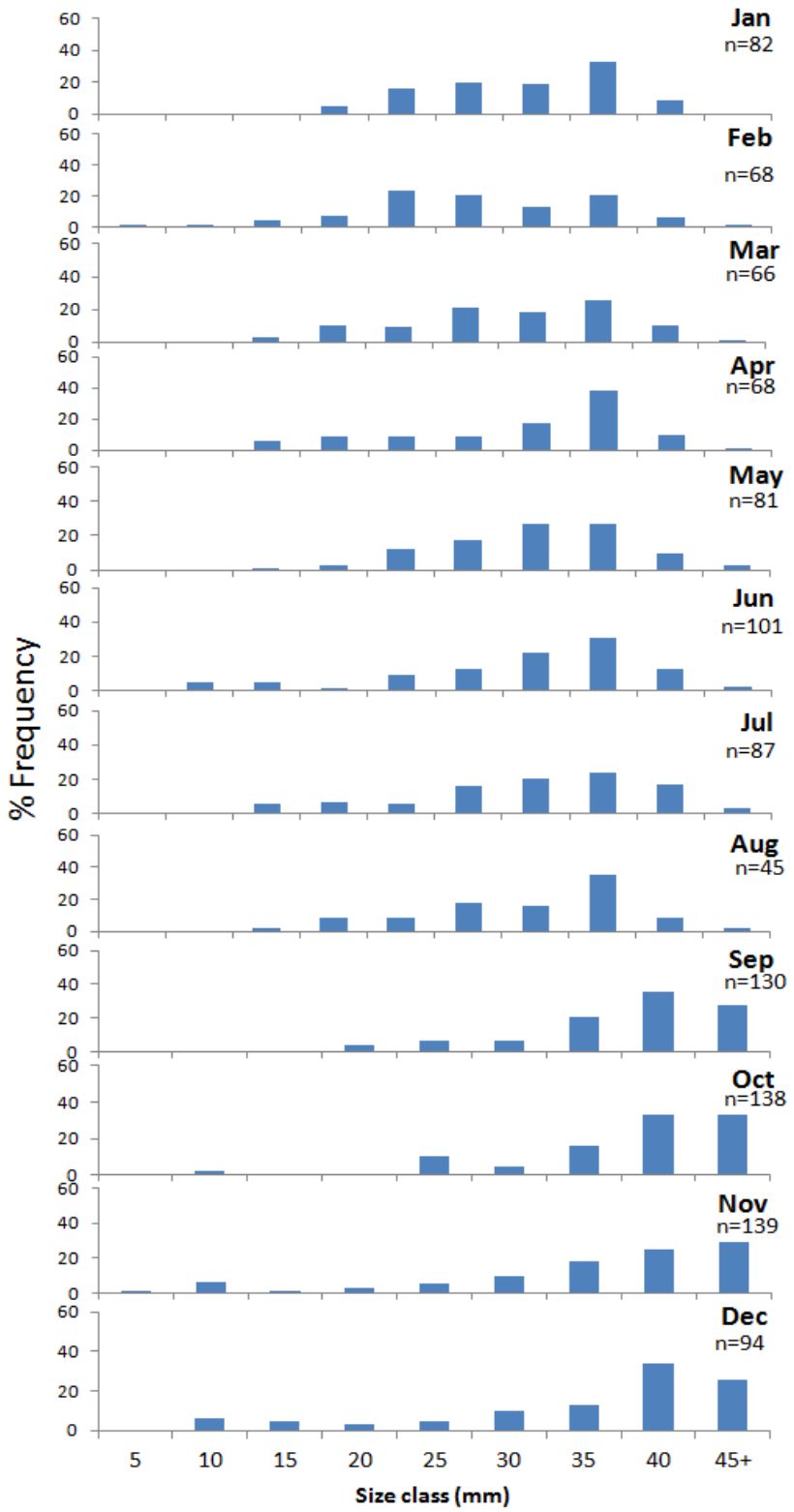

Figure 3.7: The size distribution of the Manila clam population at Holton Mere from January to December 2003. The percentage of the population made up of each size class is plotted sequentially by month, the total number of clams collected per month is also included.

Holton Mere population recruitment and life history from April 2009 - August 2012

At the time of sample collection, the minimum landing size for Manila clams in Poole Harbour had been reduced from 40mm to 35mm. Over the course of the sampling period, only one clam was found to be over 45mm in shell length and it was rare to find clams above 35mm.

Figures 3.10 and 3.11 indicate that there were very few clams of a legal size left at Holton Mere by this point (2011 and 2012 respectively). Legal sized clams are those with a maximum shell length of 35 mm or larger, these are notable absent by 2011 and 2012.

Spawning events can be identified in the histograms by an influx of juvenile or spat clams (clams <10mm maximum shell length). A good example of this is in June and July 2010 when the juvenile clams began to appear in the samples (see figure 3.9).

It is possible to follow the growth of the year classes of clams as they increase in size over the summer growth period. A good example of this is in 2010 (figure 3.9), where the Year 0 clams appear in June and July (at 5-10mm) and grow up until November (15-20mm) the end of the growing period in 2010.

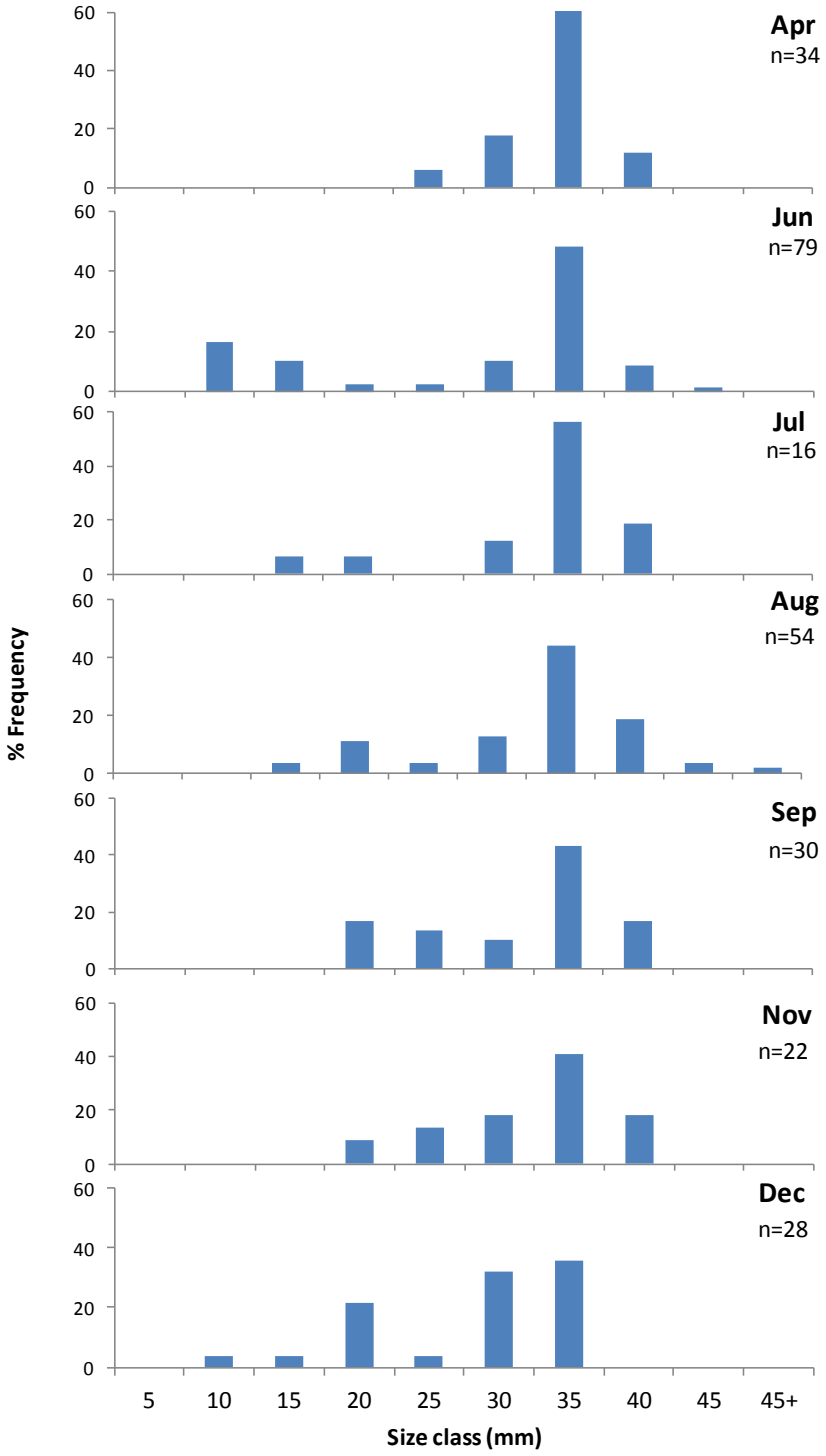

Figure 3.8: The size distribution of the Manila clam population at Holton Mere from April 2009 to December 2009. The percentage of the population made up of each size class is plotted sequentially by month, the total number of clams collected per month is also included**.**

Holton Mere Manila clam population February- November 2010

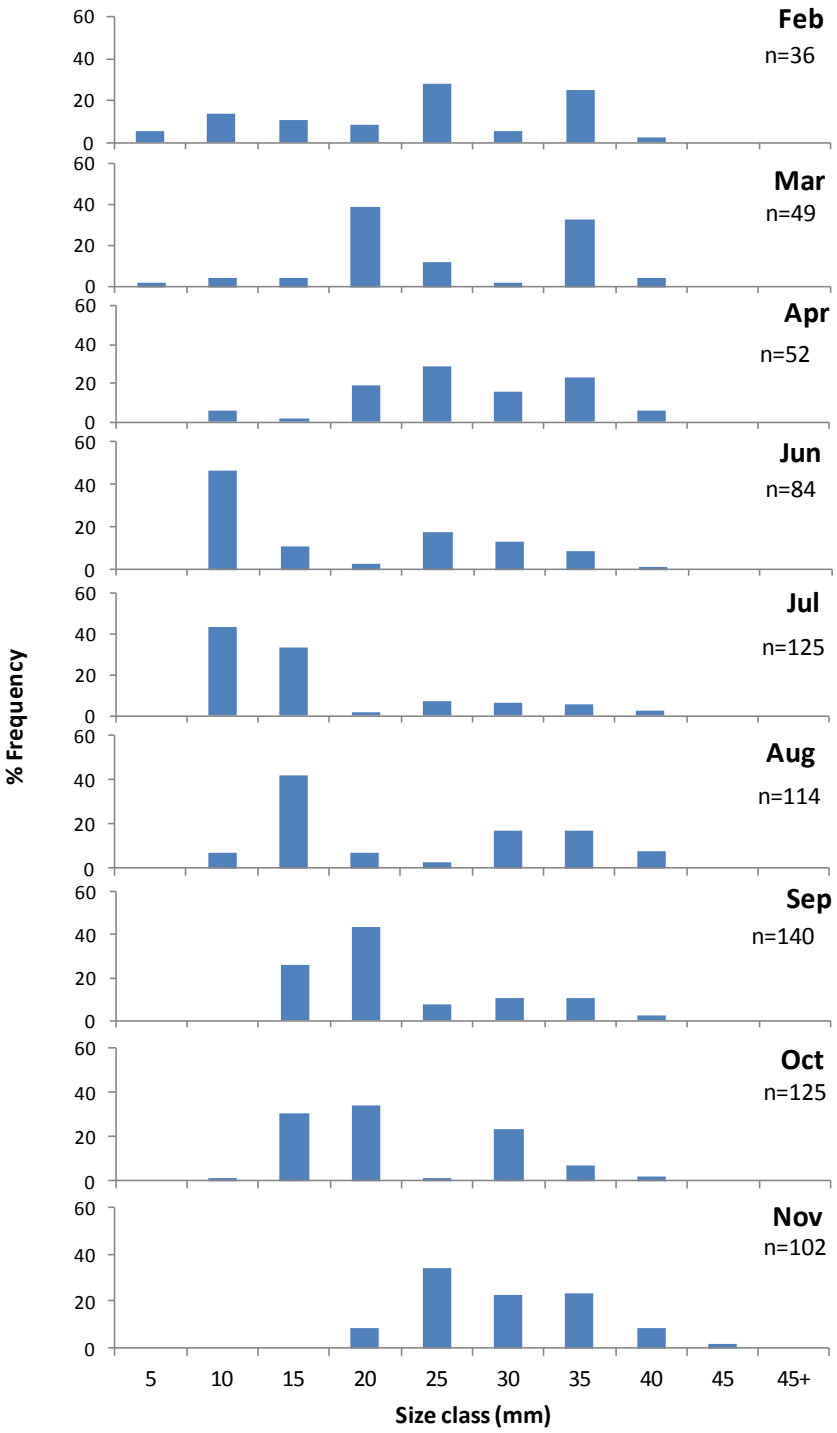

Figure 3.9: The size distribution of the Holton Mere Manila clam population from February 2010 to November 2010. The percentage of the population made up of each size class is plotted sequentially by month, the total number of clams collected per month is also included.

Holton Mere Manila clam population July-December 2011

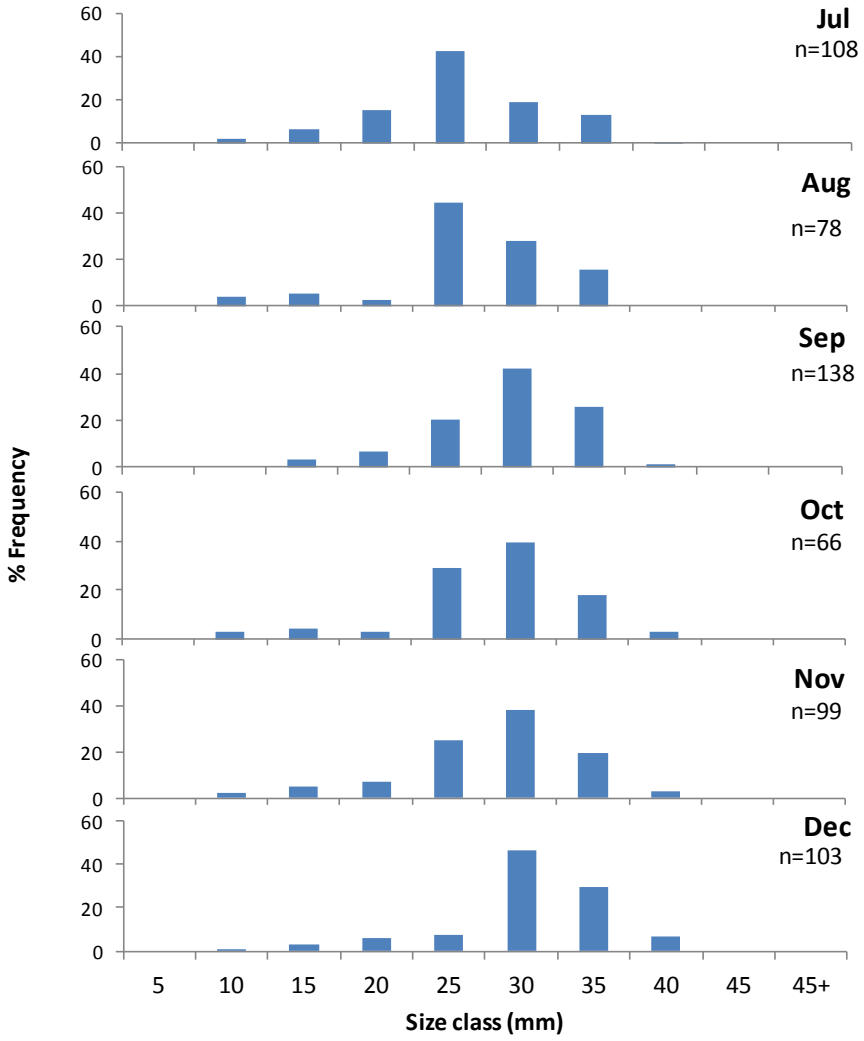

Figure 3.10: The size distribution of the Holton Mere Manila clam population between July 2011 and December 2011. The percentage of the population made up of each size class is plotted sequentially by month, the total number of clams collected per month is also included.

Holton Mere Manila clam population January- August 2012

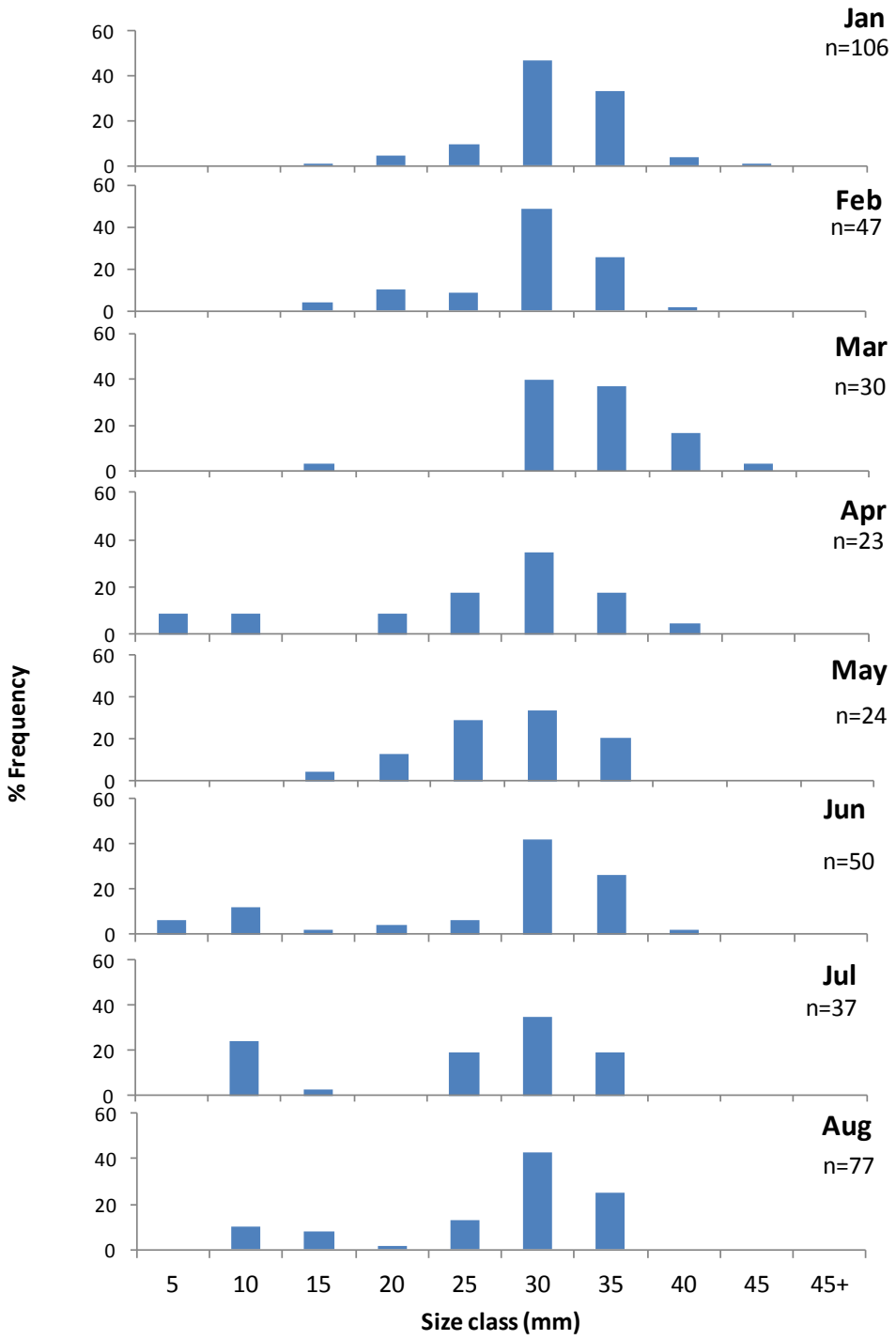

Figure 3.11: The size distribution of the Holton Mere Manila clam population from January to August 2012. The percentage of the population made up of each size class is plotted sequentially by month, the total number of clams collected per month is also included.

Analysis of the average clam size across the study period

There was a significant difference in mean clam size between the years in the study (One way ANOVA, size vs year, D.F: 4, F = 304.1, *p* = < 0.001). The largest average clam size was 32.9mm, which was recorded for 2003, the smallest average clam size was 20.1mm in 2010. The average clam size decreased from 2003 to 2010 before increasing in size again (See figure 3.12). There was no significant difference in clam size between 2011 and 2012.

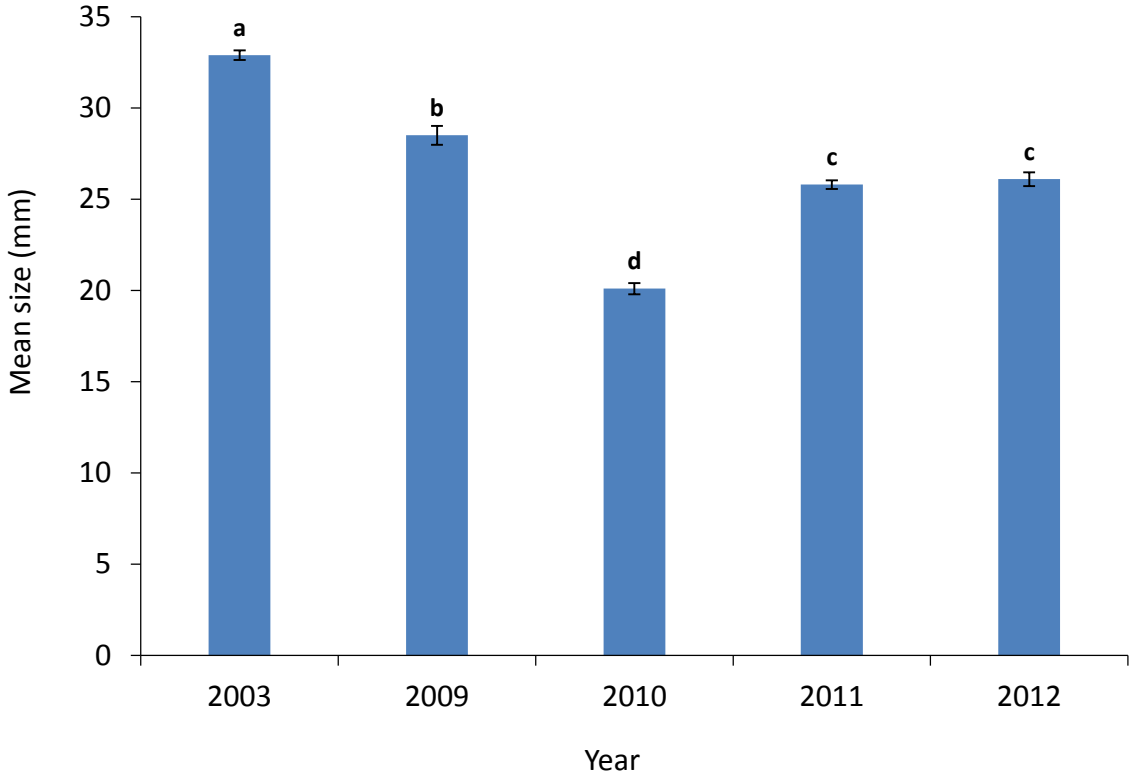

Figure 3.12: Average clam size from Holton Mere plotted against year (Mean, ±SE).

Analysis of size frequency distribution across the study period

There were significant differences in the size-frequency composition of the Manila clam population between the years in the study (PERMANOVA: $D.F{:}4_{,37} = 8.6$, $p = < 0.001$). Analysis by Principal component ordination analysis found that 55.3% of the data could be explained on two axis (See figure 3.13). PCO determined that the size frequencies of the population in 2003 were different to the rest of the years in the study period and that it was dominated by the larger size classes (35, 40, 45 & 50mm). The rest of the years in the study (2009, 2010, 2011 & 2012) were dominated by the smaller clam size classes (5, 10, 15, 20, 25 & 30mm).

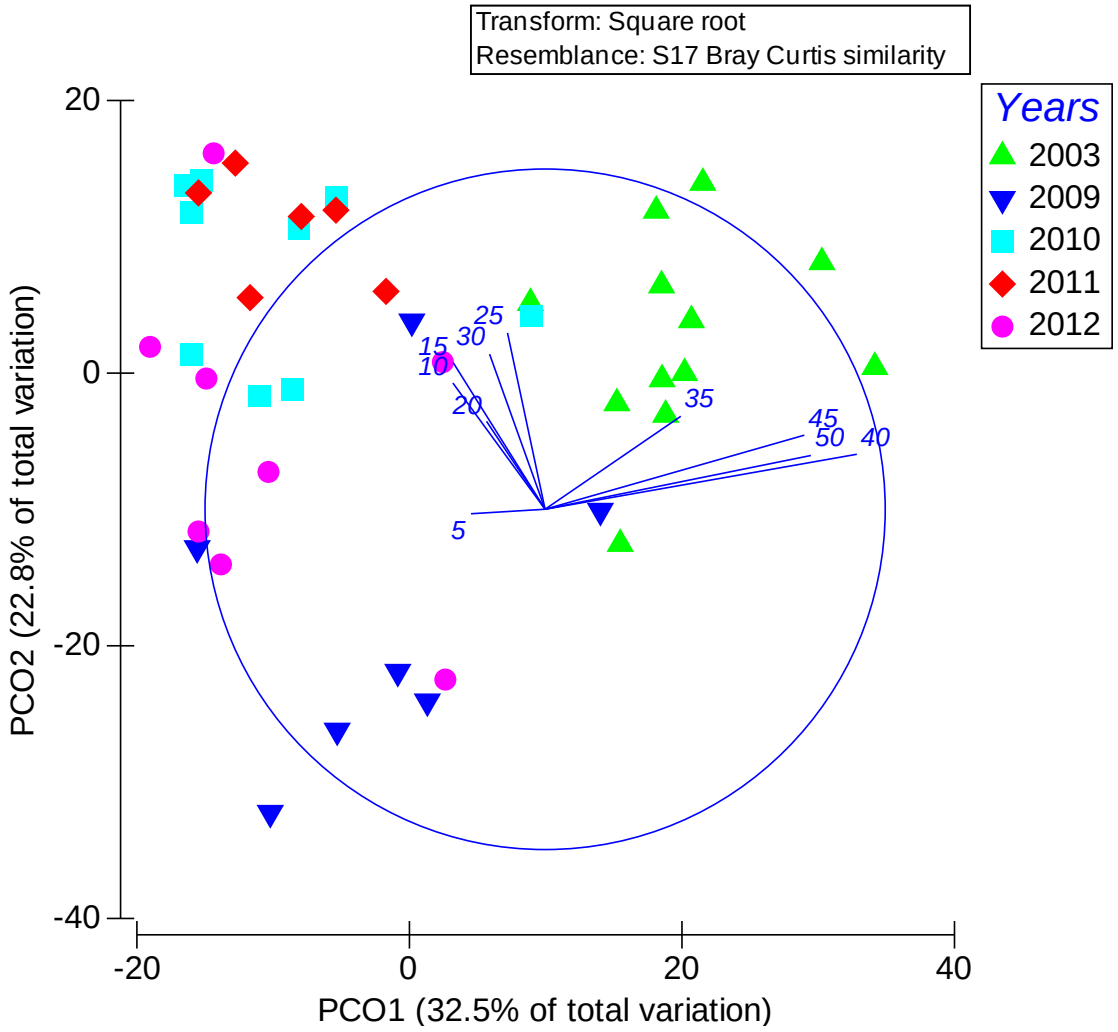

Figure 3.13: Principal component ordination analysis of the size frequency distribution of the Holton Mere population of clams between 2003 and 2009-2012.

Growth in the Holton Mere clam population

The Holton Mere population of Manila clams reached a length of approximately 24mm by the end of their first year and reached a shell length of approximately 35mm by the end of their second year (See figure 3.14a). The maximum predicted shell length for the Manila clam population at Holton Mere was 48.14mm, and would take individuals over three and half years to reach this shell length.

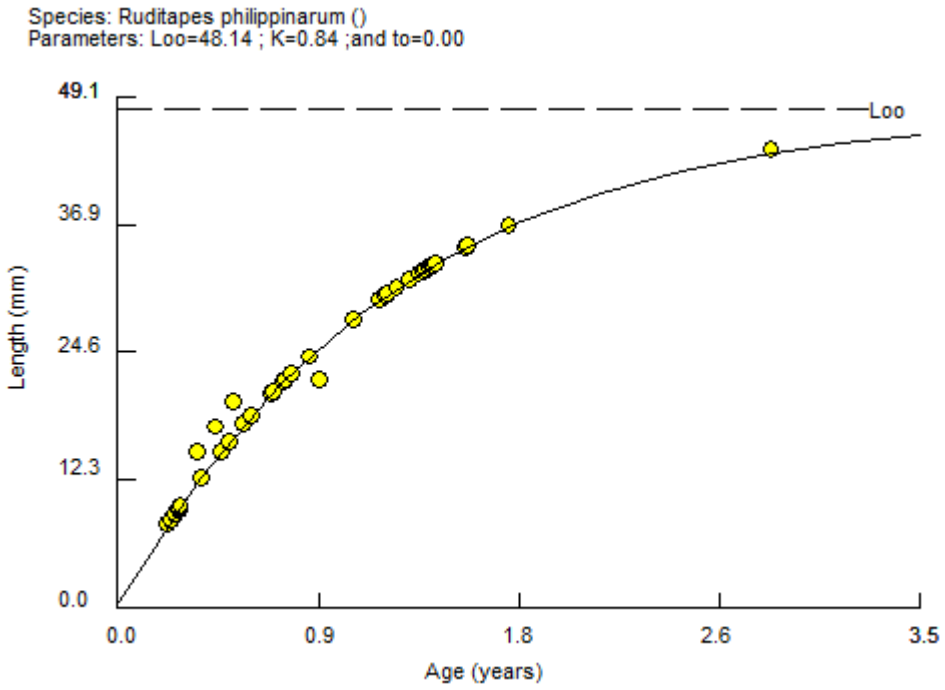

Figure 3.14a: The growth rate of the Holton Mere Manila clam population during the study period. Length in mm has been plotted against clam age in years. A Von Bertalanffy growth curve has been fitted to the data. The average maximum shell length predicted for the Holton Mere population is 48.14mm.

The growth rate of the Holton mere population of Manila clams was influenced by clam size (Regression analysis, F (1, 23) = 11.2, P value= <0.05) with an R^2 of 32.8 (see figure 3.14b), year and season (see figures 3.13a&b). As the clams increased in size the growth rate reduced. The fastest growth rate observed was 0.21 mm per day in clams of 9.29mm in shell length, whereas the slowest growth rate observed was 0.002 mm per day in clams with a shell length of 32.77mm. The mean daily growth rate for clams circa 10mm in shell length was: 0.10 mm per day.

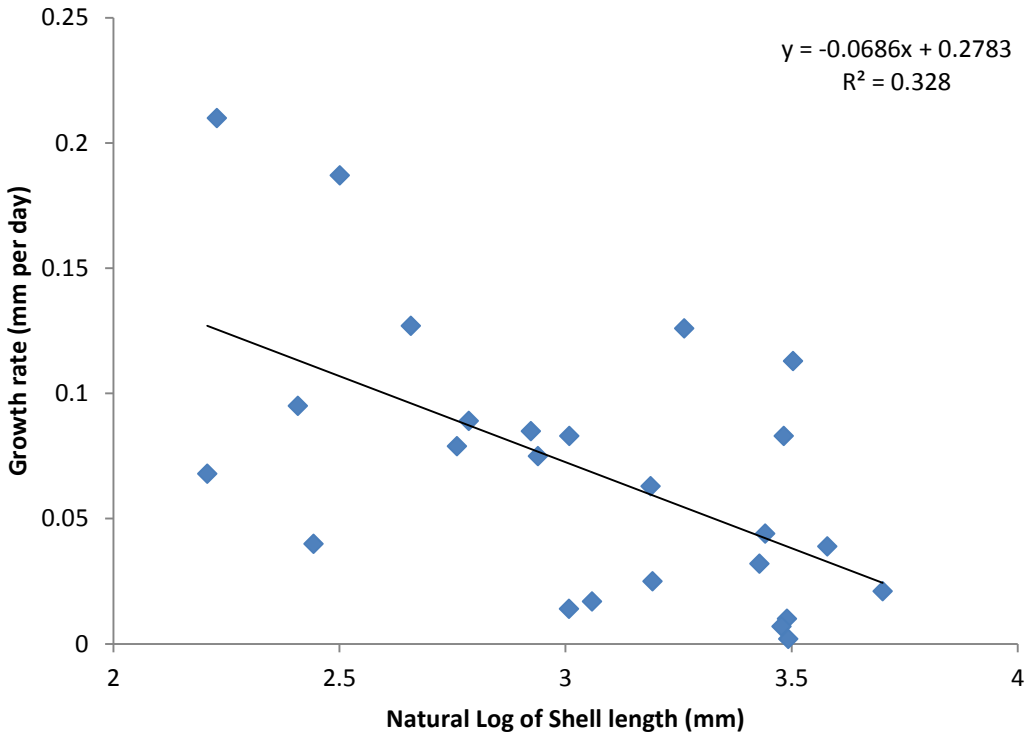

Figure 3.14b: Growth rate of the Holton Mere population of Manila clams plotted against the natural log of the shell length. The shell length was log transformed to aid in the analysis of the data.

The cohorts recruited in 2009 and 2010 have similar shell growth rates (see figures 3.15a & 3.15b). The 2009 cohort reach an average shell length of 30mm approximately 630 days after the 1st of January 2009. The 2010 cohort also reaches an average size of 30mm approximately 650 days after the 1st of January 2010. The population of clams display a seasonal growth rate, with rapid growth in the summer (180-270 days after the 1st of January) and then a decline in autumn (270-330 days after the first of January) and almost cessation of growth over the winter (330-420 days after the 1st of January).

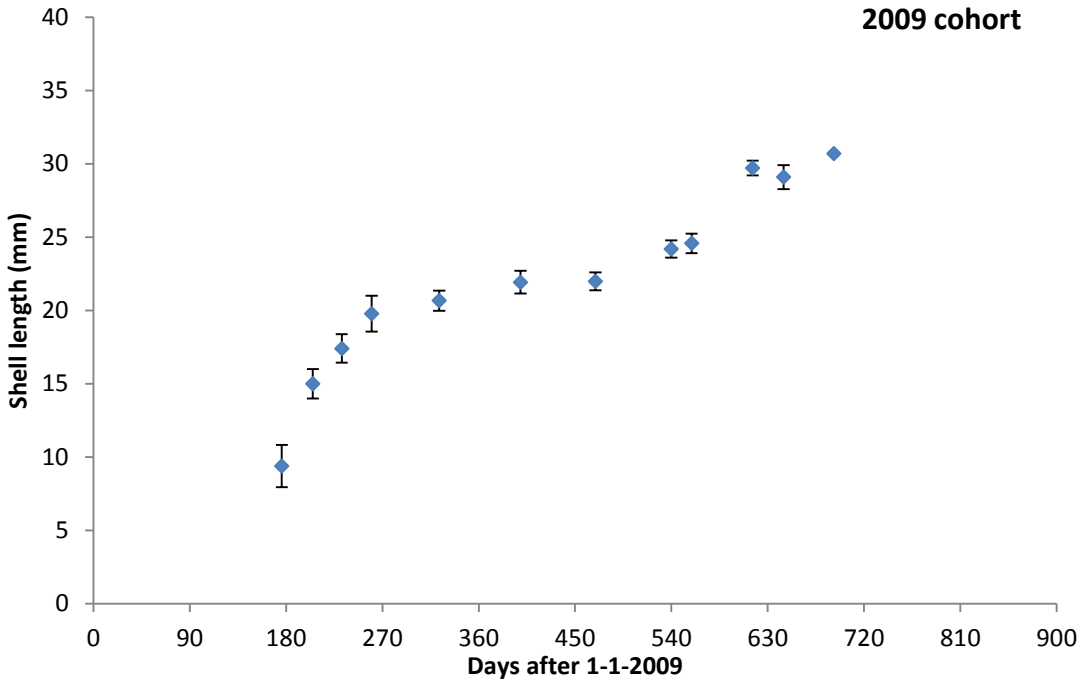

Figure 3.15a: The growth of the 2009 cohort of Manila clams from Holton Mere. The cohort has been tracked for two years.

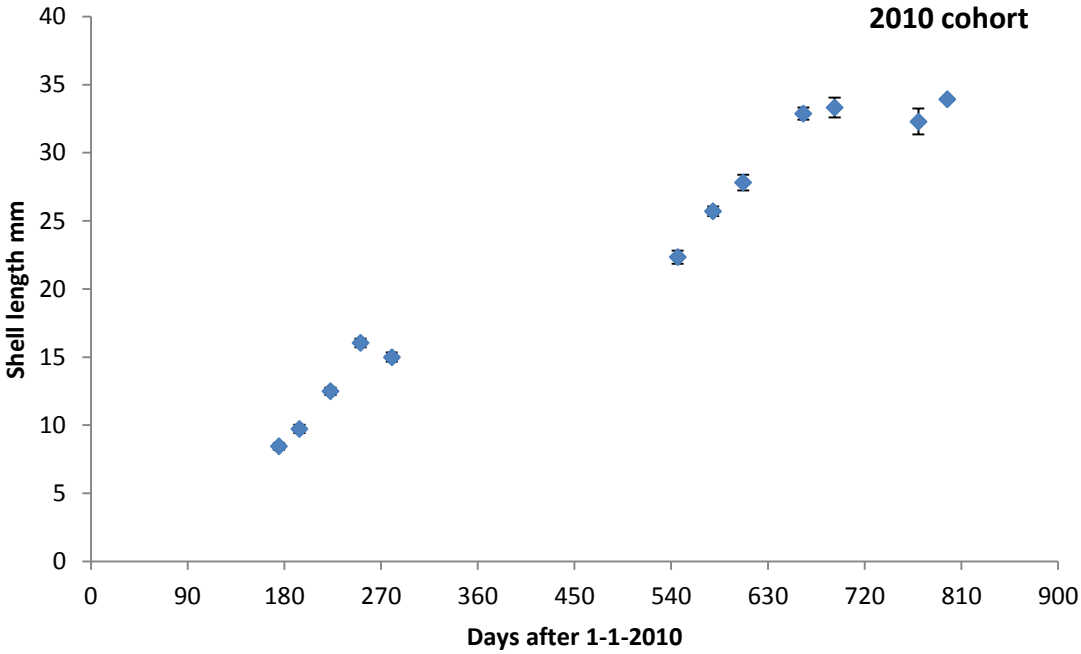

Figure 3.15b: The growth rate of the 2010 cohort of Manila clams from Holton Mere. The cohort has been tracked for two years.

Holton Mere clam production tables

Tables 9a-9c contain data sorted by the different age cohorts as detected by the FISAT II software, with each table containing the data for one year i.e. table 3a contains the data from 2010, table 8b contains the data from 2011. The figures in bold in brackets indicate the total annual production increment for that year class.

Table's 9a-c contain the production increment data for the Holton Mere clam population between February 2010 and August 2012. Tables 9a to 9c contain the density, mean ash free dry mass per individual clam, the mean number per individual and the production increment for each class on a monthly basis when samples were collected.

In 2010 there was a significant difference in overall clam density between the sample months (Two way ANOVA, d.f=8, F=7.05, P value = <0.001). There was also a significant difference between the densities of the cohorts identified in the sample (Two way ANOVA, d.f=3, F=75.08, P value= <0.001), and there was a significant interaction between sample month and clam cohort (Two way ANOVA, d.f=24, F=8.14, P value = <0.001). The highest density of clams recorded in 2010 was 42.92 clams per m^2 and these were in the year 0 cohort. The density of clams was negatively correlated with age, with the densities being lowest for the older age classes. In 2010 clams in cohort 3 were found in seven out of the nine months sampled.

In 2010 there was a significant difference in Ash Free dry mass per individual between sample months (ANOVA, d.f=8, F=7.43, P value = <0.001) and cohort (ANOVA, d.f=3, F=133.16, P value =<0.001). The highest AFDM per individual was 452.7mg for the year 3 cohort in November. The lowest AFDM per individual was 12mg in February for the year 0 cohort.

In 2011 there was no significant difference in overall clam density between the sample months (Two way ANOVA, d.f= 4, F= 2.22, P value= >0.05), but there was a significant difference between the densities of the individual cohorts across the months (Two way ANOVA, d.f= 3, F= 44.99, P value =<0.001). There was an interaction between sample month and cohort (Two way ANOVA, d.f= 12, F=4.60, P value= <0.001). The highest density of clams recorded in 2011 was 27.08 clams per m^2 and this was for the year 1 cohort. In 2011 clams in cohort 3 were found in three out of the five months sampled.

In 2011 there was a significant difference in AFDM per individual between sample months (ANOVA, d.f=4, F=3.53, P value =<0.05) and cohorts (ANOVA, d.f=3, F=118.96, P value =<0.001). The highest AFDM per individual was 216.3mg for the year 3 cohort in November. The lowest AFDM per individual was 14.78 mg for the year 0 cohort in October.

In 2012 there was a significant difference in overall clam density between sample months, (Two way ANOVA, d.f= 7, F= 7.81, P value =<0.001). There was a significant difference between the densities of the different cohorts (Two way ANOVA, d.f=3, F=3293, P value = <0.001), and an interaction between cohort and sample month (Two way ANOVA, d.f=21, F=2.78, P value =<0.001). The highest density of clams recorded in 2012 was 21.25 clams per m^2 and this was for the year 2 cohort. In 2012 clams in cohort 3 were only found in two out of the eight months sampled.

In 2012 there was no significant difference in AFDM per individual across the sample months (ANOVA, d.f=7, F=1.86, P value =>0.05), however there was a significant difference in AFDM per individual between cohorts (ANOVA, d.f=3, F=84.65, P value =<0.001). The highest AFDM per individual was 271.8mg for the year 3 cohort in June, whilst the lowest was the 2.7mg for the year 0 cohort in April.

The cohort of clams spawned in 2010 can be followed through table's 9a-9c. The 2010 cohort first appear as the year 0 cohort in table 9a and they are then represented in table 9b as the year 1 cohort and then the year 2 clams in table 9c. Throughout the study period the 2010 cohort form the majority of the Manila clam population at Holton Mere.

Table 9a: Calculated densities, ash free dry mass per individual, monthly increments of ash free dry mass, production increment and positive production increment for the different age classes of the Holton Mere clam population in 2010 from February to November. The figures in bold and brackets are the total production increment for that year class of clam.

Year	Month	Age Class	Clams per m^2 (Mean ± SE)	Mean no. during period (No. $m^{2)}$	AFDM per individual (mg) (Mean ± SE)	MI per Individual (mg)	Production Increment mg per m^2	Positive Production mg per m^2
	Feb	0	3.67 (±0.3)		12 (±0.3)			
	Mar	0	2.08 (±0.2)	2.87	12	0	0	
	Apr	0	1.11 (±0.3)	1.59	14	2	3.18	3.18
	May		No	samples	taken			
	Jun	0	20.0 (±1.7)	10.55	16.56 (±0.3)	2.56	27.01	27.01
	Jul	0	32.0 (±1.6)	26.00	28.1	11.54	300.04	300.04
	Aug	0	21.33 (±1.3)	26.67	13.5 (±0.2)	-14.6	-389.38	
	Sep	0	42.92 (±1.3)	32.13	22.92(±0.2)	9.42	302.66	302.66
	Oct	0	34.58 (±2.2)	38.75	19.52 (±0.2)	-3.4	-131.75	
	Nov	0	16.25 (±1.0)	25.42	62.15 (±1.0)	42.68	1084.93	1084.93
							(1196.69)	**(1717.82)**
	Feb	1	4.00 (±0.8)		67.1 (±1.1)			
	Mar	1	10.42 (±1.5)	7.21	66.5 (±3.6)	-0.6	-4.33	
	Apr	1	12.22 (±0.8)	11.32	61.0 (±0.7)	-5.5	-62.26	
	May		No	samples	taken			
	Jun	1	11.67 (±0.6)	11.95	83.0 (±1.3)	22	262.9	262.9
	Jul	1	3.67 (±0.4)	7.67	60.47 (±0.8)	-22.53	-172.81	
	Aug	1	13.67 (±1.4)	8.67	96.39 (±2.1)	35.92	311.43	311.43
	Sep	1	13.75 (±0.7)	13.71	116.88 (±0.9)	20.49	280.92	280.92
	Oct	1	10.0 (±1.3)	11.86	65.0	-51.88	-615.29	
	Nov	1	18.75 (±0.7)	14.38	182.77 (±1.8)	117.77	1693.53	1693.53
							(1694.09)	**(2548.78)**
	Feb	2	2.33 (±0.3)		201.7 (±2.5)			
	Mar	2	7.92 (±0.7)	5.13	183.49 (±1.4)	-18.21	-93.42	
	Apr	2	4.81 (±0.5)	6.37	189.3 (±1.7)	5.81	37.01	37.01
	May		No	samples	taken			
	Jun	2	2.92 (±0.6)	3.87	248.2 (±2.7)	58.9	227.94	227.94

2010

Jul	2	5.67 (±0.2)	4.29	119.04 (±1.2)	-129.16	-554.09	
Aug	2	3.0 (±0.8)	4.34	199.03 (±1.3)	79.99	347.16	347.16
Sept	2	1.25 (±0.5)	2.13	172.21 (±1.5)	-26.82	-57.13	
Oct	2	6.25 (±0.2)	3.75	109.0	-63.21	-237.04	
Nov	2	6.25 (±0.5)	6.25	270.15 (±2.0)	161.15	1007.19	1007.19
						(677.62)	**(1619.3)**
Feb	3	1.67 (±0.7)		231.4 (±0.7)			
Mar	3						
Apr	3	1.11 (±0.5)	1.39	297.9 (±3.3)	66.5	92.44	92.44
May		No	samples	taken			
Jun	3	0.42 (±0.2)	0.77	401.6	103.7	79.85	79.85
Jul	3	0.33 (±0.2)	0.36	230.6	-171	-61.56	
Aug	3						
Sep	3	0.42 (±0.2)	0.38	87.5	-143.1	-54.38	
Oct	3	1.25 (±0.2)	0.84	210.0	122.5	102.9	102.9
Nov	3	1.25 (±0.5)	1.25	452.7 (±10.2)	242.7	303.38	303.38
						(462.63)	**(578.57)**
						(4031.03)	**(6464.47)**

Table 9b: Calculated densities, ash free dry mass per individual, monthly increments of ash free dry mass and production increment for the different age classes of the Holton Mere clam population in 2011 from July to November. The figures in bold and brackets are the total production increment for that year class of clam.

Year	Month	Age Class	Clams per m^2 (Mean ±SE)	Mean no. During period (No. m^2)	Mean AFDM per individual (mg) (Mean ±SE)	MI per Individual (mg)	Production Increment mg per m^2	Positive production Mg per m^2
2011	Jul	0	9.58 (±0.9)		24.9			
	Aug	0	15.83 (±0.4)	12.71	24.6 (±0.5)	-0.3	-3.81	
	Sep	0	2.08 (±0.3)	8.96	18.96 (±0.3)	-5.64	-50.53	
	Oct	0	5.83 (±0.4)	3.96	14.78 (±0.2)	-4.18	-16.55	
	Nov	0	5.0 (±0.2)	5.41	25.95 (±0.31)	11.17	60.43	60.43
							(-10.46)	**(60.43)**
	Jul	1	21.67 (±0.6)		105.4 (±0.9)			
	Aug	1	25.00 (±1.6)	23.34	107.39 (±0.8)	1.99	46.45	46.45
	Sep	1	20.42 (±1.8)	22.71	76.26 (±0.6)	-31.13	-706.96	
	Oct	1	27.08 (±1.2)	23.75	71.24 (±0.5)	-5.02	-119.23	
	Nov	1	24.17 (±1.2)	25.63	84.35 (±1.1)	13.11	336.01	336.01
							(-443.73)	**(382.46)**
	Jul	2	12.92 (±0.7)		146.36 (±0.7)			
	Aug	2	15.42 (±0.8)	14.17	174.94 (±0.6)	28.58	404.98	404.98
	Sep	2	3.75 (±1.3)	9.59	139.69 (±1.0)	-35.25	-338.05	
	Oct	2	6.25 (±1.2)	5	215.0	75.31	376.55	376.55
	Nov	2	10.42 (±1.3)	8.34	130.7 (±1.3)	-84.3	-703.06	
							(-259.58)	**(781.53)**
	Jul	3	0.42 (±0.2)		205.4			
	Aug	3						
	Sep	3	0.83 (±0.2)	0.63	220.8	15.4	9.70	9.70
	Oct	3						
	Nov	3	2.92 (±0.9)	1.86	216.3 (±2.7)	-4.5	-8.37	
							(1.33)	**(9.70)**
							(-712.44)	**(1234.12)**

Table 9c Calculated densities, ash free dry mass per individual, monthly increments for ash free dry mass and production increment for the different age's classes of the Holton Mere population of Manila clams in 2012 from January to August. The figures in bold and in brackets are the total production increment for that year class of clam.

Year	Month	Age Class	Clams per m² (Mean ±SE)	Mean no. during period (No. m²)	Mean AFDM per individual (mg) (Mean ±SE)	MI per individual (mg)	Production Increment mg per m²	Positive production Mg per m²
	Jan	0	8.33 (±0.5)		22.55 (±0.9)			
	Feb	0	2.92 (±0.4)	5.63	26.94 (±0.7)	4.39	24.72	24.72
	Mar	0	0.42 (±0.1)	1.67	5	-21.94	-36.64	
	Apr	0	1.67 (±0.3)	1.05	2.7	-2.3	-2.42	
	May	0	1.25 (±0.2)	1.46	27.43 (±0.4)	24.73	36.11	36.11
	Jun	0	5.00 (±0.3)	3.13	28.45 (±0.5)	1.02	3.19	3.19
	Jul	0	4.17 (±0.5)	4.59	9.85 (±0.6)	-18.6	-85.37	
	Aug	0	6.25 (±0.6)	5.21	8.12 (±0.1)	-1.73	-9.01	
							(-69.42)	**(64.02)**
2012	Jan	1	14.17 (±0.9)		83.61 (±0.7)			
	Feb	1	11.25 (±0.9)	12.71	103.89 (±0.7)	20.28	257.76	257.76
	Mar	1	7.50 (±0.6)	38.54	100.21 (±0.6)	-3.68	-141.83	
	Apr	1	6.67 (±0.6)	36.25	95.3 (±1.2)	-4.91	-177.99	
	May	1	6.25 (±0.5)	6.46	74.35 (±0.7)	-20.95	-135.34	
	Jun	1	10.00 (±0.8)	8.13	82.39 (±0.8)	8.04	65.37	65.37
	Jul	1	7.5 (±1.0)	8.75	93.1 (±1.1)	10.71	93.71	93.71
	Aug	1	16.25 (±1.7)	11.88	94.09 (±0.8)	0.99	11.76	11.76
							(-26.56)	**(428.6)**
	Jan	2	21.25 (±1.3)		148.18 (±0.8)			
	Feb	2	5.00 (±0.4)	13.13	149.63 (±1.1)	1.45	19.04	19.04

Mar	2	3.33 (±0.3)	4.17	120.73 (±1.0)	-28.9	-120.51	
Apr	2	1.25 (±0.3)	2.29	196.33 (±1.7)	75.6	173.12	173.12
May	2	2.50 (±0.3)	1.86	127.05 (±1.1)	-69.28	-128.86	
Jun	2	5.00 (±0.9)	3.75	172.52 (±2.6)	45.47	170.51	170.51
Jul	2	3.33 (±0.5)	4.17	169.17 (±1.9)	-3.35	-13.97	
Aug	2	8.75 (±0.7)	6.04	176.66 (±1.6)	7.49	45.24	45.24
						(144.57)	**(215.75)**
Jan	3						
Feb	3						
Mar	3	2.50 (±0.4)		222.36 (±2.8)			
Apr	3						
May	3						
Jun	3	0.42 (±0.1)	1.46	271.8	49.44	72.18	72.18
Jul	3						
Aug	3						
						(72.18)	**(72.18)**
						(120.77)	**(780.55)**

Holton Mere clam density

The densities of the age cohorts identified in tables' 9a-c have been plotted between February 2010 and August 2012 (See figure 3.16). The clam density varied between cohorts and also between sample months. The highest recorded density was 42.92 clams per m^2 in September 2010 for the 2010 cohort. The lowest recorded density was 0.3 clams per m^2 also in 2010 for the 2007 cohort. Cohorts were identifiable for up to three years, after this time they were no longer detected in the samples. The 2010 cohort had the highest recorded density of clams from June 2010 until January 2012. From February 2012 the cohort recruited in 2011 had the highest reported density at Holton Mere.

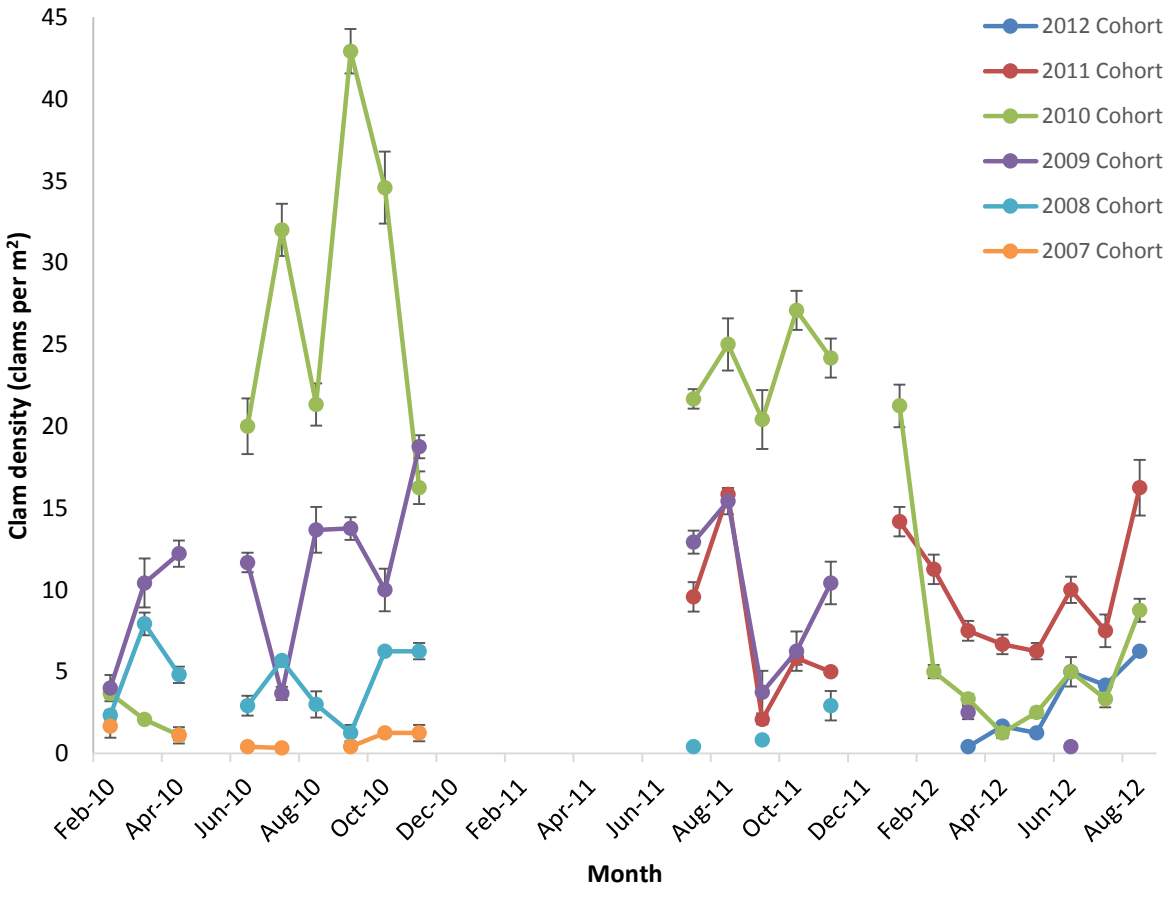

Figure 3.16: Fluctuations in population density by age cohort in the Holton Mere population of Manila clams between February 2009 and August 2012 (Mean, ±SE). Six distinct age cohorts were detected during sampling. Breaks between lines indicate either an absence of that size cohort of clams or a sampling interlude (Samples were not taken in May 2010 and December 2011 and was suspended between November 2010 and June 2011).

Holton Mere condition index

The condition indices for the Holton mere population did not have a normal distribution and transformations were unable to normalise the data, so non-parametric analysis was carried out on the data. The analysis used was the Kruskal-Wallis test.

The condition index of the Holton Mere population from April 2009-Augst 2012 shows seasonal variation, with the highest condition indices recorded in spring/summer and the lowest in winter (see figure 3.17). The highest condition index recorded was 5.03 in April 2010 and the lowest condition index was in 2.9 in March 2012. Spawning events are followed by a drop in condition (Dang et al., 2010; Uddin et al., 2012) and can be seen on 3 occasions over the study period. The drop in condition between June and July 2010 also coincided with the large spat fall seen in figure 3.9. The less successful spawning events coincided with lower condition 2011 and 2012.

In 2010 there was a significant difference in condition index across the sample months (Kruskal-Wallis, d.f=8, H=97.29 p value= <0.001). The highest condition index was recorded in June, followed by a sharp drop in condition index in July. The lowest condition index was recorded in October.

In 2011 there was a significant difference in condition index across sample months (Kruskal-Wallis, d.f=4, H=25.46, p value= <0.001). The highest condition index was recorded in August, with the lowest condition recorded in September.

In 2012 there was a significant difference in condition index across the sample months (Kruskal-Wallis, d.f=7, H=58.51, p value= <0.001). The highest condition index was recorded in August with the lowest in March.

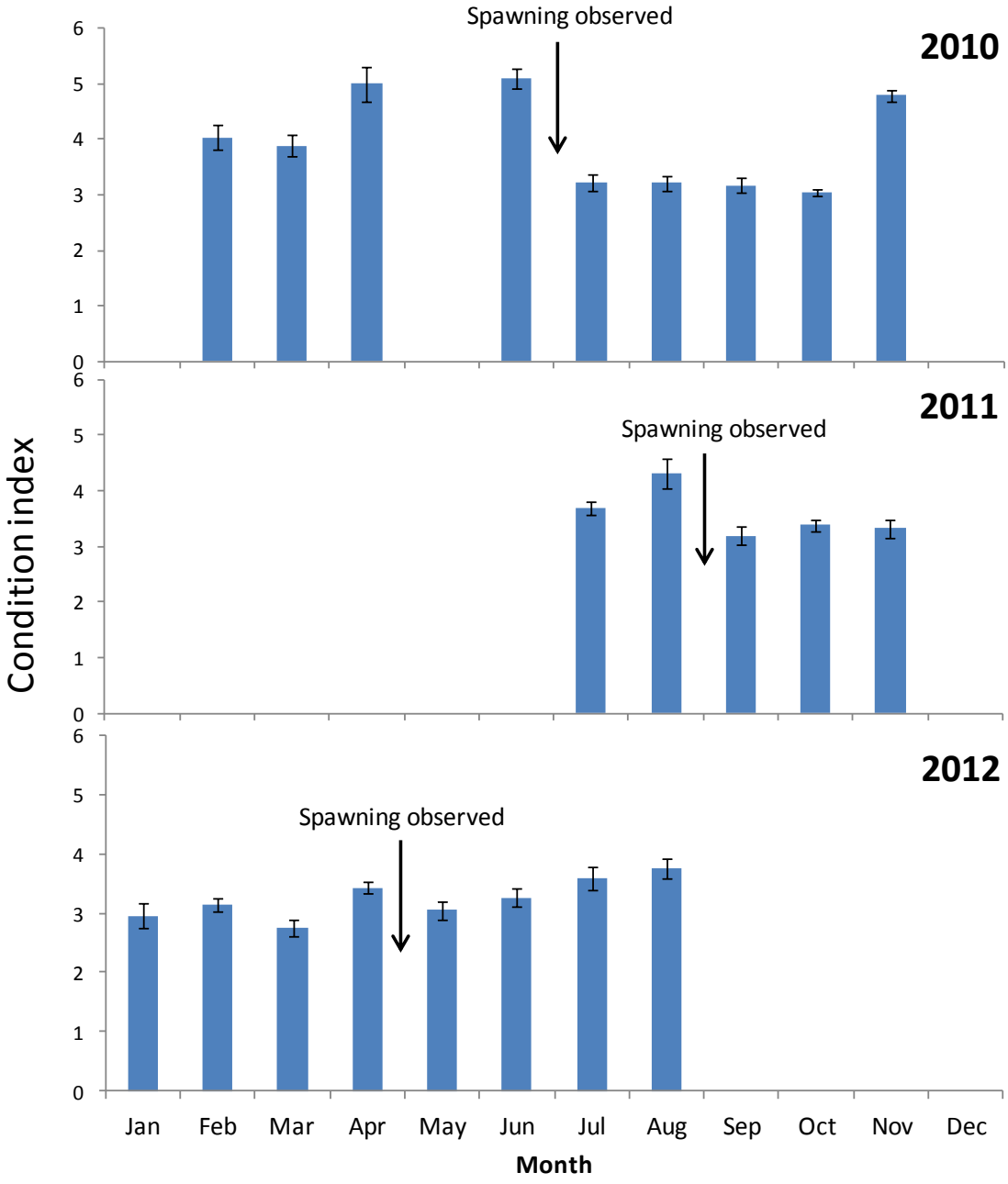

Figure 3.17: Condition indices of the Holton Mere Manila clam population from 2010 to 2012. For each month in the survey between 15 and 30 clams were processed for condition index.

Year classes as a percentage of the population

The Holton Mere population of clams did not have as many ages classes present in 2009-2012 (4 age classes) as it did in 2003 (6 age classes). Form 2009-2012 the population composed mainly of individuals between 0-2 years old with a small proportion of 3 year old clams (See figure 3.18)

The population in 2010 has four age classes present, Year 0, 1, 2 and 3. The year 0 clams are those that had been spawned that year and are the predominant class of clam found in the population from June onwards. Very few clams of age 2 and 3 were left in the population. The clams spawned in 2009 also make up a large proportion of the population.

In 2011 the population was still dominated by the clams spawned in 2009 and 2010. Unlike 2010 there was no successful spawning event (see figures 3.8-3.11). The 2009 and 2010 age classes account for between 70 and 80% of the clam population in 2011. The year 3 clams make up less than 5% of the remaining population.

In 2012, the 2010 cohort were still the dominant cohort in the population. The 2010 cohort are now 2 years of age and make up approximately 50% of the population in January. From February the 2010 cohort begin to make up less of the population and are replaced by clams spawned in 2011. There is a small spawning event and spat fall begins to occur from April onwards. By July there are only 3 age classes left in the samples.

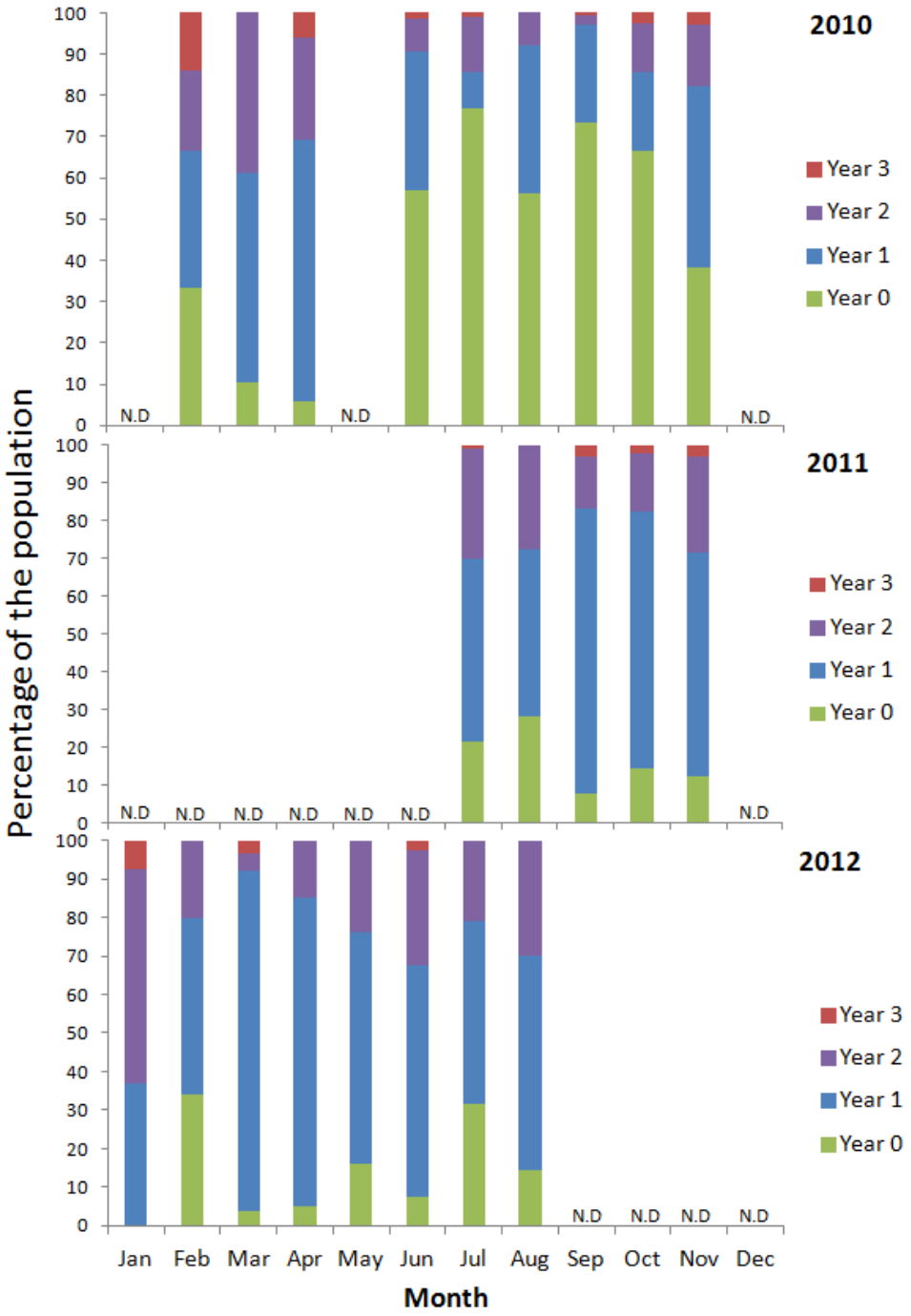

Figure 3.18: Age classes of the Holton Mere Manila clam population expressed as a percentage of the total population over time. N.D signifies no data.

3.4 Discussion

This is the first study to monitor the population dynamics of the Holton Mere Manila clam population over a period longer than 12 months. This allowed for comparisons within the years of the study and with the study carried out in 2003. The minimum landing size for Manila clams was reduced from 40mm to 35mm in 2008 by the European Union (SIFCA website). This presented the opportunity to investigate the impacts of a reduction of MLS on a population of introduced bivalves.

The presence of illegal fisherman at the study site necessitated a change in sampling method, from using ten random sample locations over the whole mudflat, to two sampling locations in-between where illegal fishing was taking place. Instead of taking one sample at each of the ten random sampling locations, four samples were taken at each location to produce an average and try to reduce the impact of the samples no-longer being random. The fact that the sites were no longer completely random may have influenced the results, however the fact that four replicates were taken at each location should increase the accuracy at each location. Sampling in-between where illegal fishing was taking place may have actually provided a benefit to the study as it would reduce the chance of sampling a location in which all of the clams had been removed by fishing activity.

3.4.1 Comparing Holton Mere with other populations of Manila clams

The growth rates of the Holton Mere population of Manila clams are comparable to those of the Manila clam population in Arcachon Bay in France, Fanny Bay in British Columbia and the Bisan Seto Channel in Japan. The spring cohort of year 0 Holton Mere clams reached an average shell length of between 15-20mm by their first winter, which has also been observed in Japanese populations of Manila clams (Ohba, 1959). The period of time for 10mm sized clams taken to reach a size of 30mm (14 months) was comparable to the population of Manila clams in Arcachon Bay in France (Flye-Sainte-Marie et al., 2007; Robert et al., 1993) and Fanny Bay in British Columbia (Bourne et al.,1998).

The daily growth rate for juvenile clams (~10mm shell length) at Holton Mere of 0.1mm per day is comparable to growth rates reported in China. In China juvenile clams have been reported to grow 0.02mm per day at 2.8°C and 0.29mm per day at 12°C (Zhang & Yan, 2006). The growth rate at Holton Mere is between these figures and is the average growth rate over the year, so would include growth at both cold and warm periods. This could suggest that growth at Holton Mere is not limited by temperature, as growth rates do not seem to be reduced and growth it is in fact faster than that reported in some natural populations.

There is a reduction in shell growth rate with increased clam size, which has also been reported in Japanese and French populations of Manila clams (Ohba, 1959; Robert et al., 1993). Temperature also influences growth rate, with populations of clams that experience colder temperatures growing slower and living longer than those in warmer waters (Ponurovsky & Selin, 1988). The growth rate of the Holton Mere population appear to suggest that the Manila clam experiences better conditions for growth in Poole Harbour than it does in Vostock Bay, as the growth rates are faster than that of the Vostock Bay population (Ponurovskii, 2000) and comparable to the Tokyo bay Population (Ohba, 1959).

However if the conditions in Poole Harbour were optimal for all biological processes, high levels of recruitment would occur every year, but over the course of the study there was only one year (2010) with a high level of recruitment. Increased food availability as a result of reduced intra-specific competition due to lower densities of clams at Holton Mere may also influence the growth rates. This is because increased densities of clams have been reported to reduce growth rates (Ohba, 1959). This means densities may influence the growth rate of the Holton Mere population. This raises the question of what influences the density of the Holton Mere Manila clam population. The maximum recorded density of Manila clams at Holton Mere was significantly lower than those recorded for both native and introduced populations of Manila clams (see table 10).

Table 10: The maximum reported densities and ages of both native and introduced populations of Manila clams. Native populations are those found in the natural geographic range.

Location	Status	Maximum density (individuals per m^2)	Maximum age (Years)	Reference
Mukasa shoal (Japan)	Native	2500	3	Ohba 1959
Starfish Bay (Hong Kong)	Native	258	n/d	Lee 1996
South Primor'e (Pacific Russia)	Native	896	13	Ponurovskii and Selin, 1988 Ponurovskii 2000
Holton Mere (UK)	**Introduced**	**58.3**	**4**	**This study**
Arcachon Bay (France)	Introduced	120.9	4	Dang et al. 2010
Bay of Santander (Spain)	Introduced	22.3	3	Juanes et al. 2012
Venice Lagoon (Italy)	Introduced	2000	n/d	Pellizzato et al. 2011
Willapa Bay (USA)	Introduced	500	n/d	Ruesink et al. 2013

Under optimal conditions, Manila clams can reach densities of between 2000-2500 clams per m^2 (Ohba, 1959; Pellizzato et al., 2011), over 40 times higher than the maximum recorded in Poole Harbour. This raises the question of why are the densities of clams lower for the Holton Mere population? The density of clams is not low because the Holton Mere population is introduced, because high densities of clams have been recorded in both native, 2500 individuals per m^2 (Ohba, 1959) and introduced populations, 2000 individuals per m^2 (Pellizzato et al., 2011). A potential explanation for the low densities at Holton Mere could be that the Manila clam is at the northern-most limit of its geographical range (Jensen et al., 2004). If this was the case, then slower growing older clams would be present at Holton Mere, as this species grows slower and lives longer in the northern extreme of its natural distribution (Ponurovsky & Selin, 1988). In Vostock Bay in Russia, Manila clams are known to live for up to 13 years, and can take 4 years to reach 30mm in shell length (Ponurovsky & Selin, 1988). The densities reported at Holton Mere, are unlikely to be due to the clams being at the northern extreme of their range, as clams routinely reach a size of 30mm within the first 2 years

of life. This growth rate is twice as fast as that in Vostock Bay. The lower densities of clams could in fact be the result of fishing pressure at Holton mere.

The majority of clams at Holton Mere were under 3 years of age, this is unlikely to be due to environmental conditions, as clams regularly lived for over 6 years on the lease beds in the Harbour. The low maximum recorded age could be due to the influence of fishing activity and the removal of the older larger individuals. Fishing activity has been reported to have modified the population dynamics of Manila clams in other locations such as Arcachon Bay in France (Dang et al., 2010) and the Bay of Santander (Juanes et al., 2012). Fishing activity has also affected other species as well such as the wedge clam *Donax hanleyanus* (Defeo & De Alava, 1995), cockles *Cerastoderma edule* (Piersma et al., 2001) and the razor clam *Ensis arcuatus* (Robinson & Richardson, 1998). These affects range from removal of larger individuals (Dang et al., 2010; Defeo & De Alava, 1995; Juanes et al., 2012) to a reduction in future recruitment (Piersma et al., 2001) and increased mortality of discarded individuals (Robinson & Richardson, 1998). This implies that fishing activity could have a wider range of effects upon the Holton Mere population rather than just reducing density and retarding maximum age.

3.4.2 Impact of fisheries activity on the Holton Mere Manila clam population, comparisons between the 2003 population and later years

The Holton Mere population has been affected by increased fishing pressure as a result of the reduction in the MLS of clams from 40-35mm. The MLS reduction has resulted in the mean size of clams reducing from 32.9mm in 2003 to of 20.1mm in 2010. The population dynamics of Holton Mere population have also been altered between 2003 and 2010. In 2003 the population was made up mainly of clams over 30mm in shell length, with the population being dominated by clams under 30mm from 2010 to 2012. The removal of the larger “legal sized clams” has subsequently led to a reduction in the Manila clam landings in Poole Harbour (see table 11).

Table 11: The reported landings of Manila clams in tonnes from Poole Harbour between 2004 and 2011, from the Southern Inshore Fisheries and Conservation Agency. The total number of licensed fishing boats and reported value of the catch is also displayed.

Financial year ending	Licensed clam boats	Total reported catch (Tonnes)	Reported value (£)
2004	31	500	1,500,000
2005	30	400	1,000,000
2006	25	350	900,000
2007	21	23.9	60,000
2008	19	33.8	84,575
2009	19	48.1	120,000
2010	18	39.8	99,500
2011	15	69.1	172,000

The landings were at their minimum in 2007, when only 23.9 tonnes were landed, but landings began to increase again from 2008. The high levels of exploitation from 2004 to 2006 were unsustainable and lead to a crash in the clam population. A reason behind the crash could be the fact that clams in Poole Harbour take at least 2.5 to 3 years to reach the minimum landing size, so the legal sized clams were not being replaced as quickly as they were being fished out. Irregular reproductive and recruitment events would also reduce the sustainability of the fishery due to the domination of the population by single age cohorts (see figure 3.15 for the dominance of the 2010 cohort). This imbalance in population dynamics could lead to a population crash once the dominant cohort reaches the MLS.

A result of the reduction of clam numbers was a drop in the number of licensed clam boats from 31 in 2004 to 15 in 2011. The reduction in licensed boats has not lead to a reduction in fishing pressure on the remaining clams. The remaining boats are still exerting a high level of fishing pressure which can be seen in figure 3.19.

Figure 3.19: Aerial photograph of the effects of pump scoop dredging in Poole Harbour, image courtesy of Dorset Wildlife Trust. The round circles are the scars left from pump scoop dredging, the area of fishing is 90m by 60m, this highlights the intensity of the fishing effort.

The reduction in larger clams has been caused by overexploitation by fisherman, as opposed to natural mortality events. Natural mortality could potentially be ruled out due to the lack of freshly dead shells collected in the sampling. The absence of clams just above the minimum size limit also suggests that it is fishing pressure that has removed the larger individuals. This phenomenon has also been reported in Arcachon bay in France, where circa 75% of legal sized clams were removed by fishing activity (Dang et al., 2010). More evidence for fishing being the cause of the reduction in larger individuals, is the presence of large numbers of clam boats on spring tides over Holton Mere throughout 2010 (see table 12).

Table 12: The number of clam fishing boats present on a spring tide over Holton Mere in 2010. The table also displays the proportions of the clam population that are over and just under the legal minimum size limit.

Date	No. of clam boats present	% of clam population between 30-35mm	% of clam population above 35mm (MLS)
4-2-2010	9	25	2.7
4-3-2010	12	32.3	4.08
15-4-2010	10	23.1	5.8
25-6-2010	9	8.3	1.2
14-7-2010	2	5.6	2.4
11-8-2010	5	16.7	7.9
12-8-2010	2	16.7	7.9
9-09-2010	2	10.7	2.14
8-10-2010	0	7.2	2.4

Many of the boats operating on Holton Mere are unlicensed and are fishing illegally for clams. The illegal fishing vessels are known to remove sub legal sized clams. This may explain the high numbers of boats fishing in March 2010. There were 12 boats fishing, even though only 4.08% of the clam population at this time were above the minimum landing size. There was however a large proportion of clams just under the MLS, which may have been what the fisherman were targeting. Fishermen have also been found to be taking undersized clams by the fisheries patrol in Poole Harbour (Simon Pengelly, personal communication).

The illegal fishery does appear to be self-regulating, however, as from July 2010 the number of boats operating over Holton Mere began to steadily decline. In August the number of vessels had reduced to 5 from a maximum of 12 in March, this reduction coincided with a reduction in the proportion of legal and sublegal sized clams. In October 2010 the number of fishing vessels had reduced to 0, which coincided with low levels of legal, and sub legal clams. The absence of fishing vessels when the proportion of legal sized clams are low, implies that the fishery is self-regulating and that periods of high fishing activity are followed by periods of low activity. The levels of fishing activity are determined by its profitability, as when large clams are present, there are large numbers of boats and when large clams are absent there are low numbers of boats.

The reduction in MLS and increased fishing pressure (John Humphreys, personal communication) has had an effect upon the Holton Mere clam population. The increased fishing activity has led to a decrease in productivity between 2003 and 2010 (see table 13).

Table 13: Mean density and average production of the Holton Mere Manila clam population in 2003 and 2010. The densities and average production have been displayed for the different age cohorts present in the population (2003 data taken from Humphreys et al. 2007).

Cohort	Mean density of clams per m^2 in 2003	Average production g per m^2 2003	Mean density of clams per m^2 in 2010	Average production g per m^2 2010
0	4.36	0.66	19.33	1.72
1	13.23	4.29	10.91	2.55
2	9.6	3.49	4.49	1.62
3	8.97	3.43	0.72	0.58
4	3.27	1.70	------	------
Total	**39.43**	**13.57**	**35.45**	**6.47**

The reduction in productivity has been caused by the removal of the clams from the year 4 cohort and a reduction in the density of the years 2 and 3 cohorts. In 2003 the majority of the production was by the year 1, 2, 3 and 4 year old clams (Humphreys et al., 2007). In 2010 the majority of the production came from the 0, 1 and 2 year old clams. The reduction can be explained by the removal of the larger individuals by fishing. This implies that the Holton Mere population is being over exploited by fisherman.

The current fishing practice of the legal and illegal boats on Holton Mere will not totally remove the Manila clam from the mudflats as the population will recover due to recruits from the un-fished populations. The current practices however, will not give an optimal yield for fisherman, and fishing will be controlled by a presence or absence of clams as opposed to a policy by the fisherman.

Between 2003 and 2010 the densities for the older age cohorts have dramatically declined, with very few clams of 3 years of age and above found in the 2010 samples. The population in 2003 was composed of relatively few year 0 clams as opposed to 2010. This raises the question of how does the population in 2010 have larger numbers of year 0 clams compared to 2003 when there has been a reduction in the number of clams of

reproductive size? Is a depleted Holton Mere population still able to produce large numbers of viable larvae or are the larvae coming in from a population elsewhere?

The argument that the Holton Mere population, although depleted, is still able to successfully recruit is based upon the fact that Manila clams are known to produce gametes after their first year (Ponurovsky & Yakovlev, 1992) and that Manila clams are known to have a high level of fecundity (well-conditioned individuals can produce 5-8 million eggs) (Utting et al., 1991). However gametes produced by smaller individuals are often of a smaller size and of poorer quality than those produced by larger individuals (Ponurovsky & Yakovlev, 1992; Tumnoi, 2012; Utting et al., 1991). The Manila clams from Holton Mere also do not have a high level of condition, which implies that the levels of fecundity would be lower than those reported under optimum conditions. This makes it unlikely that the higher numbers of year 0 clams found in 2010 compared to 2003 are due to recruitment from larvae spawned by the Holton Mere population alone.

This raises the question of where do the new recruits to the Holton Mere population originate from? Do they originate from another population of Manila clams within the Harbour or from a population outside of the Harbour? The origin of larvae from a population of Manila clams from outside the Harbour can be ruled out based on the results of a study that modelled the dispersal of larvae throughout the Harbour (Herbert, et al., 2012). The model used a hydrodynamic model of the harbour and the larval swimming behaviour in response to salinity (see figure 3.20). When larvae targeted salinities of 23 psu, larvae were transported out of the Harbour and were localised in the south of the Harbour. When targeting salinities of 17psu, larvae would be transported from Holes bay (location 3) to Holton Mere and also from Holton Mere to the leased beds of Othniel Shellfish Ltd (Herbert et al., 2012). This implies that if larvae targeted lower salinities, they could be transported to Holton Mere from other locations around the Harbour including Holes Bay and also the shell fish lease beds.

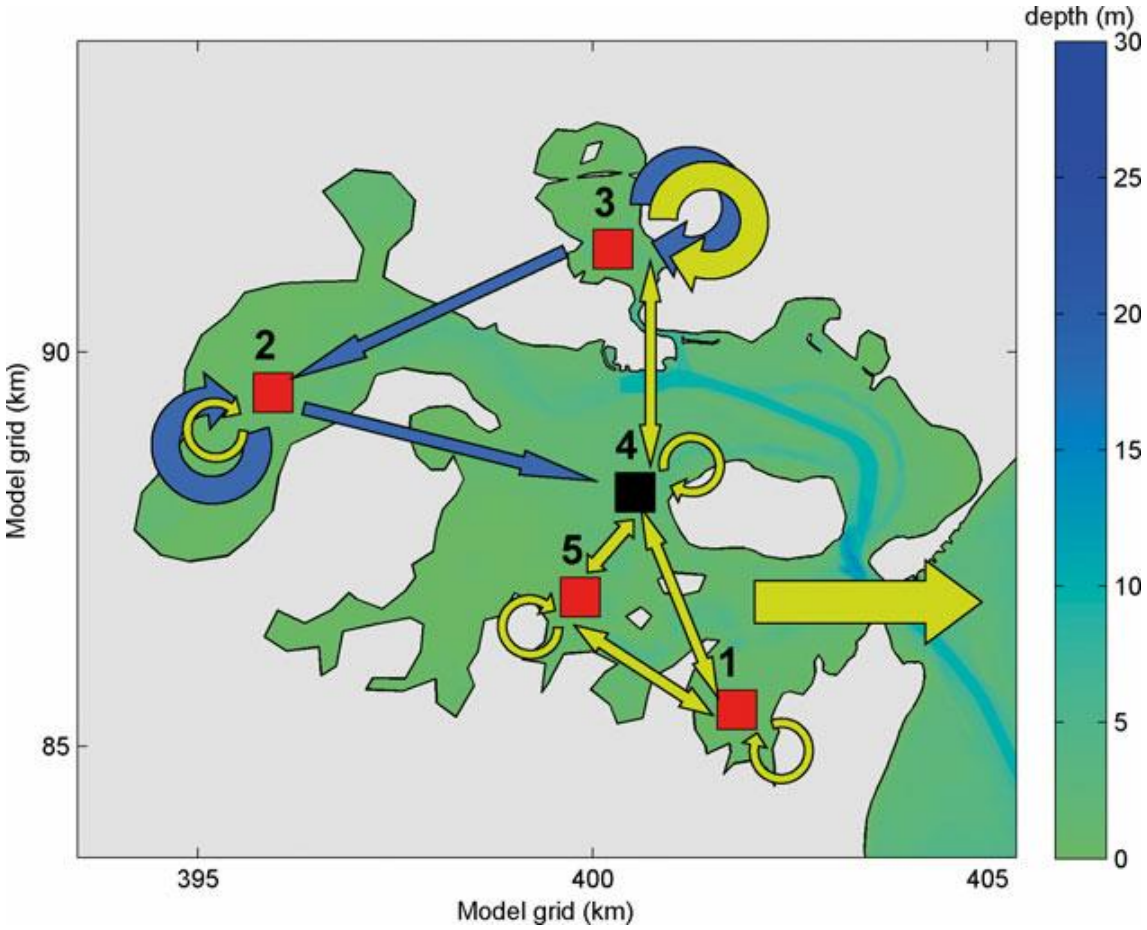

Figure 3.20: Patterns of Manila clam larval dispersal in Poole Harbour, Dorset based on salinity preference, taken from Herbert et al. 2012. The numbers 1-5 designate known populations of Manila clams, location 2 is the Holton Mere population, location 3 is Holes bay and location 4 are shellfish lease beds managed by Othniel shellfisheries. The blue arrows signify larval transport when targeting salinities of 17psu, whereas the yellow arrows signify larval movement when targeting salinities of 23psu.

The clams on the shellfish leases beds (location 4 on figure 3.18) are not regularly fished by Othniel Shellfish Ltd, due to the fact that they concentrate on oyster culture (Gary Wordsworth, personal communication). When dredging for oysters on the lease beds, clams are often discarded back into the water (John Humphreys, personal communication). As a consequence, the clams on the lease beds regularly attain shell lengths above that recorded from Holton Mere and clams have been recorded to attain a shell length of 59.88mm (unpublished data from chapter 4). The condition index of the clams from the lease beds are also on average higher than that of the Holton Mere population.

The population of Manila clams in Holes bay (Location 3 on figure 3.17) are also not fished on a regular basis by legal or illegal fisherman. This could be due to the inaccessibility of the location and also its proximity to Poole Quay and the fisheries patrol. This means that the Holes Bay population of Manila clams, like the lease bed population may act as a brood stock when conditions are optimal for reproductive success. This could imply that the clam fishery on Holton Mere does not rely upon self-recruitment in order to maintain the population. However this does not mean that it is not vulnerable to collapse if there is a bad year for recruitment.

3.4.3 Influence of condition index on recruitment success

The sharp reductions observed for condition index of the Holton Mere clams coincide with recruitment events; an example of this is the drop in condition index between August and September 2011. Spat clams appear in the October and November samples. The larval stage of the Manila clam lasts between 9-10 days at temperatures between 17-19°C (Zhang & Yan, 2006). The average seawater temperature in August 2011 was 18.2°C (see figure 4.3.1 in chapter 4) so theoretically the larval stage would last up to 10 days. The larvae metamorphose into spat clams at a size of approximately 0.23mm (Utting et al., 1991) and settle onto the substrate. The growth rate is variable until the spat clams reach a size of 0.7mm, at which point the growth rate increases to 0.25mm per day (Zhang & Yan, 2006). This means it would take spat clams of 0.7mm shell length 30 days to reach a size of 8.2mm. This could suggest that it would take approximately 50 days from the spawning event for a juvenile clam to reach a shell length of 8.2mm. Using this information, we can predict that if a drop in condition index was the result of a spawning event between August and September 2011, then spat clams should appear in the samples between October/November 2011. This is exactly when the spat clams appear, so it suggests that the drop in condition index in August is the result of a spawning event. An alternative explanation could be that the drop in condition is due to poor environmental conditions experienced by the clams, however this is unlikely due to the appearance of spat clams and the fact that condition increases again after the drop.

The appearance of spat clams in the samples does not always coincide with a reduction in condition index. The appearance of spat clams in the April samples in 2012 (see figure 3.11) does not correspond with any drops in condition index in the proceeding months. There is a small reduction in condition between February and March, however this reduction in condition is minimal and unlikely to be the result of a spawning event due to the average seawater temperatures in February (5.7°C) and March (9.4°C) not reaching the minimum threshold (14°C) required for spawning (Mann, 1979). This could suggest that the spat clams that appear in April are not the result of a spawning event in February/March 2012, but are in fact the progeny of a late spawning event from 2011 and overwintered as juvenile clams and may have come from one of the other populations of clams within the Harbour as the spawning event detected from the reduction in condition indices for the Holton Mere population in 2011 was between August/September.

Although the Holton Mere population may not rely totally on self-recruitment, the highest level of recruitment coincided with the year in which the Holton Mere clams had the highest condition index (see table 14). This suggests that in 2010, the clam population in Holton Mere also contributed to a successful recruitment event.

Table 14: Condition index and percentage of the Holton Mere population comprised of year 0 spat clams. The reduction in condition index is measured in condition index units.

Year	Condition index prior to spat fall	Reduction in Condition index	% of population composed of spat (n=total number of clams of all ages)
2010	5.6	2.68	46.4 (n=84)
2011	4.43	1.26	3.9 (n=78)
2012	4.41	1.18	16.4 (n=23)

In 2011 and 2012 the recruitment events were not as successful as in 2010 but still occurred. Both of these years had a similar condition index prior to spawning: 4.43 in 2011 and 4.41 in 2012. The numbers of spat clams that were found in the samples were comparable with three spat clams found in 2011 and four found in 2012. This implies that recruitment takes place on annual basis but that the level of recruitment is dependent upon the level of condition of the adult population. The link between condition index and reproductive success raises the question of what environmental factors drive condition of the Manila clam population in Poole Harbour?

Chapter 4: Correlating environmental variables with Manila clam health and condition index in the Poole Harbour population

4.1 Introduction

Influences of environmental conditions on bivalve condition index and the reproductive cycle

The condition index in bivalve molluscs has a seasonal pattern that is influenced by environmental conditions and the state of the reproductive cycle (J. Cardoso et al., 2007; Dang et al., 2010; Narváez et al., 2008; Norkko & Thrush, 2006; Orban et al., 2002; Ramesha & Thippeswamy, 2009; Sahin et al., 2006; Saxby & Australia, 2002). Condition index has been used to monitor the reproductive cycle in bivalves, with high levels of condition associated with the period prior to spawning (Cano, et al., 1997; Dang et al., 2010; Dridi & Romdhane, 2007), with spawning events causing a rapid reduction in condition index (Dang et al., 2010; Narváez et al., 2008) due to the release of gametes. Following a spawning event, bivalves enter a resting stage in which they replenish spent energy reserves and regain condition. Condition is often highest in the summer coinciding with high levels of food availability and lowest in the winter, (Dang et al., 2010; Dridi & Romdhane, 2007; Narváez et al., 2008; Weiss et al., 2007).

Condition index has been positively correlated with food availability in many species of bivalve, including: *Austrovenus stutchburyi* (Norkko & Thrush, 2006), *Mytilus galloprovincialis* (Orban et al., 2002), *Mercenaria mercenaria* (Weiss et al., 2007), *Anadara inaequivalvis* (Sahin et al., 2006), *Mytilus edulis* (Lesser, et al., 2010) and *Perna perna* (Narváez et al., 2008). In the past, phytoplankton, and subsequently the abundance of food available for bivalves has been measured by collecting water samples at the time of sampling. The water samples were then processed back at the laboratory to estimate species composition and abundance. This is a valid method, however it only provides a snapshot of the levels of phytoplankton at the time of sampling. In order to draw a more confident conclusion of the impact of food availability on a natural population of bivalves, a continuous monitoring of phytoplankton abundance would be

required. This study set out to correlate long term continuous monitoring of food abundance with bivalve condition index.

High levels of food availability in the form of phytoplankton coincide with increased seawater temperatures (Eppley, 1972; Norkko & Thrush, 2006). Seawater temperature has also been correlated with condition index in bivalves including: *Crassostrea gigas* (Dridi & Romdhane, 2007), Mytilus galloprovincialis (Orban et al., 2002), *Panopea generosa* (Marshall et al., 2012), *Perna perna* (Narváez et al., 2008) and *Spisula subtruncata* (J. Cardoso et al., 2007). Seawater temperature is also known to influence the reproductive cycle in bivalves (Cardoso et al., 2007; Honkoop & vanderMeer, 1997; Roger Mann, 1979).

Temperature and food availability are not the only environmental conditions that affect bivalves. Salinity has been reported to influence growth (Serdar et al., 2007), condition (Matthews & Fairweather, 2004; Norkko & Thrush, 2006; Peddicord, 1977) and survival (Rupp & Parsons, 2004) in bivalves. The Influence of salinity on bivalves is often the result of low salinity events such as flooding (Elston et al., 2003; Matthews & Constable, 2004). Low salinities can lead to mass mortalities in bivalves (Elston et al., 2003; Matthews & Constable, 2004). Prolonged exposure to suboptimal salinities can also influence the long term condition of bivalves, with juvenile *Austrovenus stutchburyi* having higher levels of condition than adults due to the cumulative effects of suboptimal conditions (Norkko & Thrush, 2006).

Influence of environmental conditions on the Manila clam condition index and reproductive success

Condition index in the Manila clam, like in other species of bivalves is influenced by environmental conditions and the state of the reproductive cycle (Dang et al., 2010; Drummond et al., 2006; Uddin et al., 2012). Manila clam condition index has been positively correlated with food availability (Kang et al., 2007; Kasai et al., 2004). The Manila clam is able to successfully filter out both small (cyanobacteria) and large (eukaryotic algae) particles with the same efficiency, which gives it a large potential source of nutrition (Yasuo Nakamura, 2001). Manila clam populations from estuarine environments, are also able to exploit terrestrial particulate organic matter (Kasai et al.,

2004), which suggest that Manila clams are able to utilise a large range of potential food items. The Manila clam's ability to assimilate a wide range of particle sizes and types raises the question of what food source would have the greatest influence on condition index in Poole Harbour? This study set out to determine using environmental monitoring, whether phytoplankton, blue green algae or particulate organic matter was the most important food source in driving Manila clam condition index.

Feeding rates in the Manila clam are influenced by seawater temperature, with increasing clearance rates linked to higher temperatures (Nakamura, 2004). The reproductive cycle of the Manila clam is driven by seawater temperature (Mann, 1979), with minimum seawater temperatures reported for gametogenesis at 8°C, gamete ripening at 12°C and spawning at 14°C (Mann, 1979). Condition index in the Manila clam is at its highest prior to a spawning event (Dang et al., 2010; Drummond et al., 2006). Following spawning the Manila clam enters a resting stage and any remaining gametes are broken down by the clams and resorbed back into somatic tissue (Drummond et al., 2006; Tumnoi, 2012). Under stressful or suboptimal conditions spawning does not occur and gametes are resorbed by the adult clams by the process of atresia (Drummond et al., 2006; Tumnoi, 2012). Low reproductive effort triggered by suboptimal conditions has also been observed in other bivalves including: *Ensis americanus* (Cardoso et al., 2009) and *Macoma balthica* (Honkoop & Van der Meer, 1998). Gametes were resorbed and used for metabolic activity due to suboptimal conditions.

There is evidence that populations of Manila clams outside of their native distribution have begun to adapt to local conditions and are reproducing under conditions previously considered suboptimal (Tumnoi, 2012). However, this has not occurred in all of the locations in which Manila clams have been introduced. An example of this is the population of Manila clams in North West Ireland, which has so far been unable to successfully reproduce (Drummond et al., 2006).

Temperature and food availability have been reported to be the most important factors in determining condition index in bivalves, in particular the Manila clam. However it is not known whether environmental variables, such as dissolved oxygen and pH also influence condition index in a wild population of clams. This study set out to determine

the influence of dissolved oxygen, salinity and pH on Manila clam condition index in Poole Harbour.

4.2 Materials and Methods

4.2.1 Environmental monitoring by the sonde

A multi-parameter sonde (an electronic monitoring device with a number of probes) was deployed from the Othniel Shellfish Platform in Poole Harbour. The Shellfish platform was in close proximity to a population of Manila clams on the Lease Beds operated by Othniel Shell fish Ltd. The water passing through the monitoring station was the same as that passing across the Lease Beds population of clams (see figure 4.1).

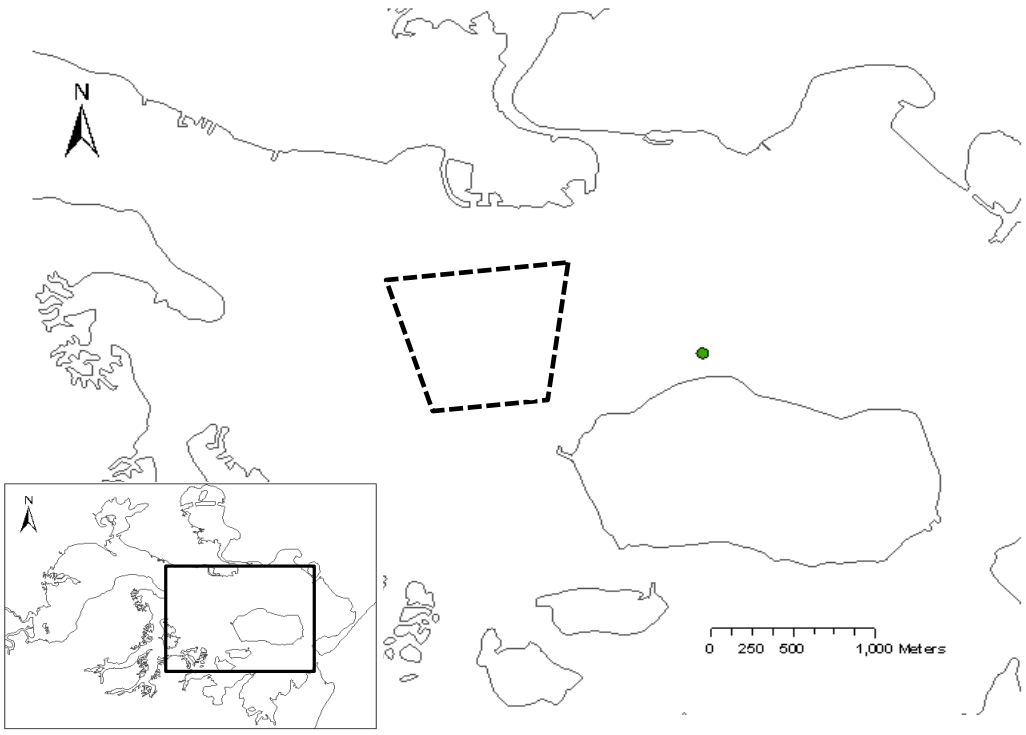

Figure 4.1: GIS map of the study area including the position of the clam population at the lease beds and the Othniel Shellfish Platform from which the sonde was deployed. The shellfish platform is marked by the green circle and the Manila clam population was sampled from the lease beds marked by the dashed line. The mouth of the harbour is located at the eastern end of the harbour. Inset is the location of the map in relation to the rest of the harbour.

To monitor the seawater conditions for the duration of the study, a multi-parameter sonde (YSI 6600v2-4) was immersed at a depth of 1m from the Othniel Shellfish Platform in Poole Harbour Dorset (see figure 4.2). This site was chosen because the platform is moored in a depth of water of between 3-5m, and the probe would always be immersed. The Platform is located in a position so that it experiences a strong tidal flow, from both the mouth of the harbour and from the shellfish lease beds from which Manila clams would be sampled. The Platform also provided a safe and secure environment for the sonde to be attached too, and it allowed for easy data collection and sonde maintenance.

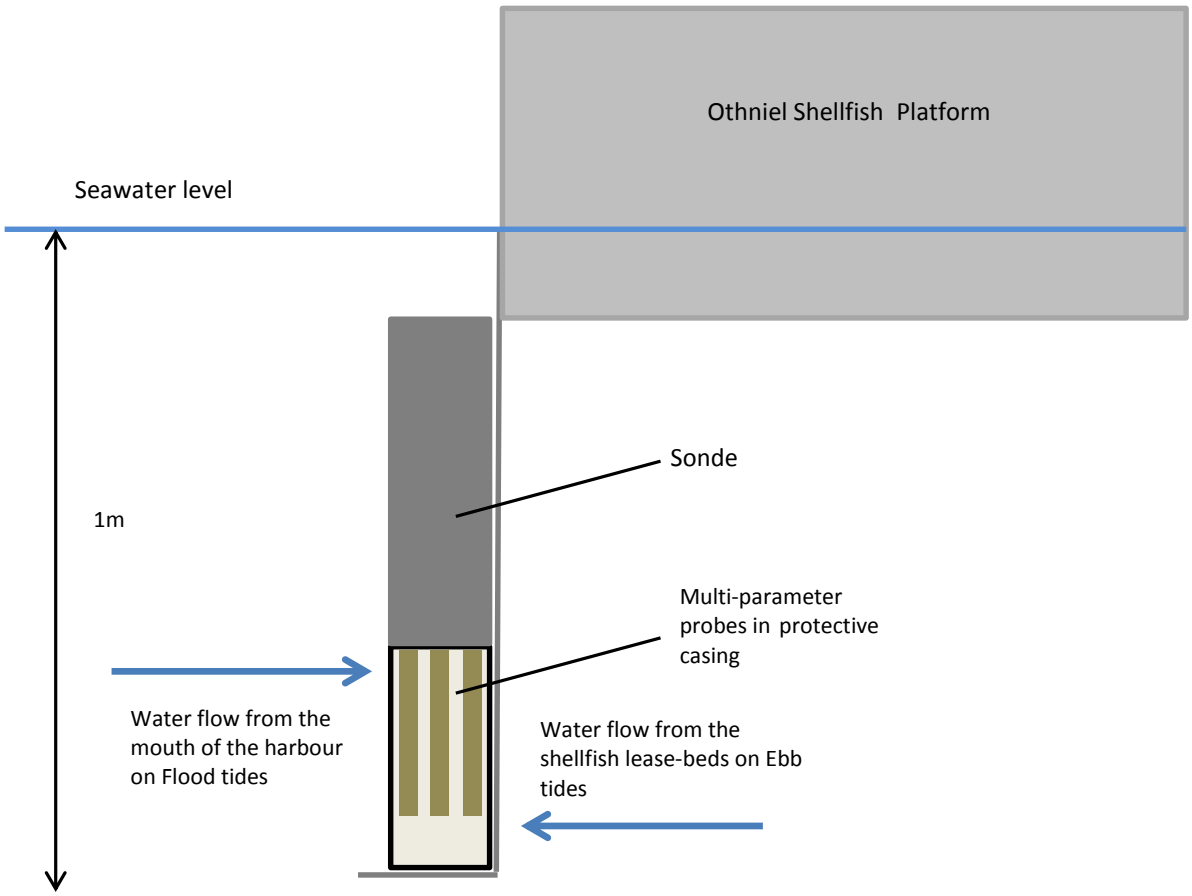

Figure 4.2: The sonde suspended to a depth of 1m from the Othniel Shellfish Platform, showing the directions of water flow across the probes.

The sonde was initially deployed from the platform at the start of the study on the 14/06/2011 and was permanently removed on the 03/08/2012. During the time in which the sonde was deployed, it was periodically removed from the water for up to a maximum of two hours to allow the data to be downloaded, the batteries to be changed

and for the probes to be cleaned of fouling organisms. The sonde was removed from the water and cleaned every three to four weeks in the summer to prevent the build-up of fouling organisms and to maintain the accuracy of the probes.

During the course of the study period, the sonde was removed for two extended periods of time due to a recalibration at the suppliers workshop: 27/11/2011 to 05/12/2011 and a failure of the data cable, which meant that it had to be taken back to the laboratory to download the data: 05/04/2012 to 12/04/2012. Each occasion in which the sonde was removed from the water for an extended period time, lasted seven days so that the data set was not unduly interrupted.

The sonde had seven probes to measure the following environmental parameters: salinity (psu), temperature (°C), pH, turbidity (nephelometric turbidity units), dissolved oxygen (mg/l), phycoerythrin fluorescence (Relative Fluorescence Units) and chlorophyll fluorescence (RFU). These parameters were measured every 12 minutes and the data stored before being downloaded on a monthly basis.

Turbidity measurements were used as a measure of the amount of particulate matter in the water column (both organic and inorganic). The chlorophyll and phycoerythrin relative fluorescence units were used as proxies to estimate the abundance of phytoplankton in the water column, this is a standard method that has been used by many studies (Aiken, 1981; Falkowski & Kiefer, 1985; Kolber & Falkowski, 1993). It was not possible to record phytoplankton densities or species composition using the sonde due to the complex assemblages of photosynthetic organisms in the plankton. However the amount of pigment present in the water column was used as a proxy for the food available for filter feeding organisms.

The sonde was set to measure the environmental parameters at 12 minutes intervals to increase the accuracy of the data generated. The sonde had a mechanical self-cleaning mechanism to prevent the build-up of fouling organisms on the probes. This worked by mechanically scouring the surface of the probe to remove any fouling organisms and to ensure that the accuracy of the probes was not compromised. The sonde was cleaned of any fouling organisms not removed by the self-cleaning mechanism when it was removed from the water to download the data. The average monthly values for the

environmental parameters were calculated for one month before and one month after the dates in which the clams were sampled.

4.2.2 Calculating the condition index of the lease bed Manila clam population

The clams used to estimate the condition index for the lease beds in Poole Harbour, were provided by Gary Wordsworth, the owner of Othniel Fisheries. The lease beds were in close proximity to the sonde monitoring station and the water passing over the sonde would also pass over the lease beds. The clams were sampled on a monthly basis from July 2011 until July 2012 and each month between 18 and 30 clams were sampled.

All of the clams used to determine the condition index of the population were of the same age cohort and the shell lengths ranged from 47-54mm maximum shell length. The clams were dredged up from the lease beds and provided to us on the same day as capture. The clams were immediately frozen at the laboratory to prevent a reduction in condition.

The clams were slowly thawed out at room temperature and then rinsed in tap water to remove any excess salt. The flesh was removed from the shell using a scalpel and then placed in a pre-weighed foil packet. The shell was then cleaned of any residual mud from the valves and then placed on a glass Petri dish along with the foil parcel containing the flesh. The flesh and the valves were placed in a drying oven set at 105°c for 24 hours.

After 24 hours the flesh and the valves were weighed on a precision balance and the following equation (as used by (Sahin et al., 2006) was used to calculate clam condition index:

$$Condition\ index = \frac{\text{Flesh dry weight (g)}}{\text{Shell dry weight (g)}} \text{ x } 100$$

Statistical analysis

The condition index for the Lease bed population was analysed using a one way ANOVA to determine whether there was a significant difference in condition index over the duration of the study period. The condition index data was not normally distributed, so was square root transformed to allow for parametric analysis. Regression analysis was used to determine whether there were any relationships between environmental variables and the transformed condition indexes. The environmental data for the month preceding the date the clam samples were taken was used for analysis. This was due to lag time for environmental conditions to influence clam condition.

To determine which of the environmental conditions had the largest influence on clam condition index, a stepwise regression analysis was performed including all of the environmental variables. This approach used a number of iterations to determine which factors could be removed from a model generated to explain the results.

The condition index of the Holton Mere and the Lease Bed populations of clams were analysed using an ANOVA general linear model, using site and month as fixed factors. All statistical analysis was undertaken using Minitab 16.

4.3 Results

The results of this chapter have been divided into three sections. The first reports the environmental data recorded by the sonde over the duration of the study period. The second section reports the condition index of the Lease Bed population of clams over the duration of the study. The final section analyses the condition index data in the context of the environmental conditions recorded by the sonde and compares condition index between the Lease bed and the Holton mere population of clams.

4.3.1 Environmental data recorded by the sonde

This section reports the environmental data recorded by the sonde over the duration of its deployment period. The sonde was deployed for a period of 15 months and all of the data collected has been represented in this section to give a clear picture of the seasonal variation. The sonde was deployed for a month before the start of the clam sampling regime and was recovered a month after it had finished. The mean monthly values for seawater temperature, salinity, dissolved oxygen, chlorophyll relative fluorescence units, phycoerytherin relative fluorescence units, seawater turbidity and seawater pH are all displayed individually in figures 4.3.1 to 4.3.7.

Seawater temperature

The average monthly seawater temperatures (± Standard deviation) in Poole Harbour between June 2011 and August 2012 are reported in figure 4.3.1. The highest temperature recorded was 18.2°C in August 2011, whilst the lowest recorded temperature was 5.6°C in February 2012. There is a clear annual cycle with low temperatures in the winter, with increasing temperatures through the spring until summer in which the highest temperatures were recorded. There were only two months of the year (January and February) where the seawater temperature was below the minimum reported threshold (8°C) for gametogenesis.

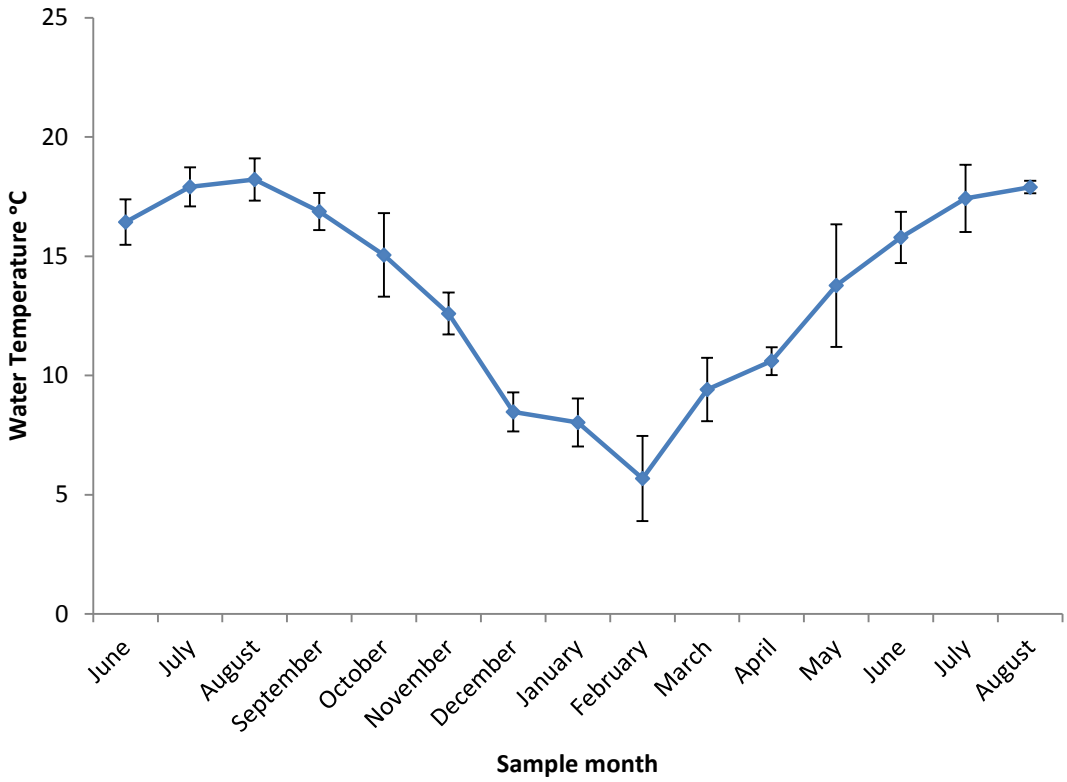

Figure 4.3.1a: Variation in seawater temperatures in Poole Harbour recorded by the sonde from June 2011 to August 2012 (monthly mean, ±SD, n=3400).

Mean daily seawater temperatures

Seawater temperature in Poole Harbour also fluctuated on a daily as well as seasonal basis in Poole Harbour between June 2011 and August 2012 (See figure 4.3.1b). The highest recorded temperature was 20.7°C on the 27-7-2012 whilst the lowest recorded temperature was 3°C on the 3-2-2012. When the average daily seawater temperatures are plotted there is still a seasonal trend with the lowest seawater temperatures occurring in winter and the highest in summer, however the short term variation becomes visible with large fluctuations occurring over short periods of time. In the winter the water temperature dropped by 5.5°C between the 26-1-2012 (8.5°C) and the 3-2-12 (3°C) a period of 8 days. This was a reduction of 0.69°C per day on average. Seawater temperatures also increased quickly, with a rise of 6.2°C recorded over 11 days between the 18-5-2012 (12.1°C) and the 29-5-2012 (18.3°C).

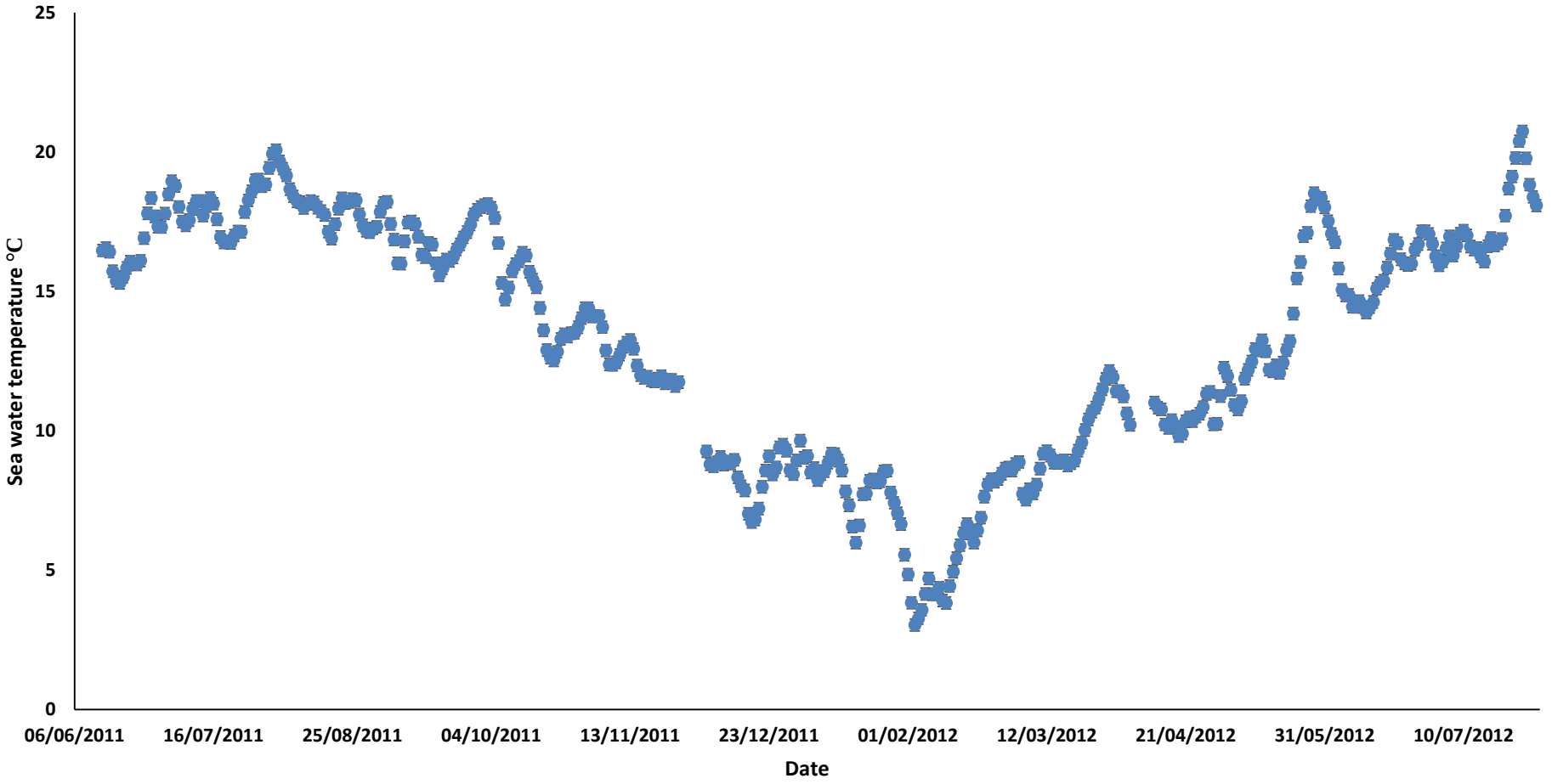

Figure 4.3.1b: Variation in daily seawater temperature in Poole Harbour recorded by the sonde between June 2011 and August 2012 (Daily Mean ± Monthly SD, n=1200).

Salinity

The average monthly salinities (± Standard deviation) in Poole Harbour recorded by the sonde between June 2011 and August 2012 are reported in figure 4.3.2a. The highest monthly salinity was 33.8psu in July 2011 and the lowest was 27.9psu in July 2012.There was no seasonal variation in salinity.

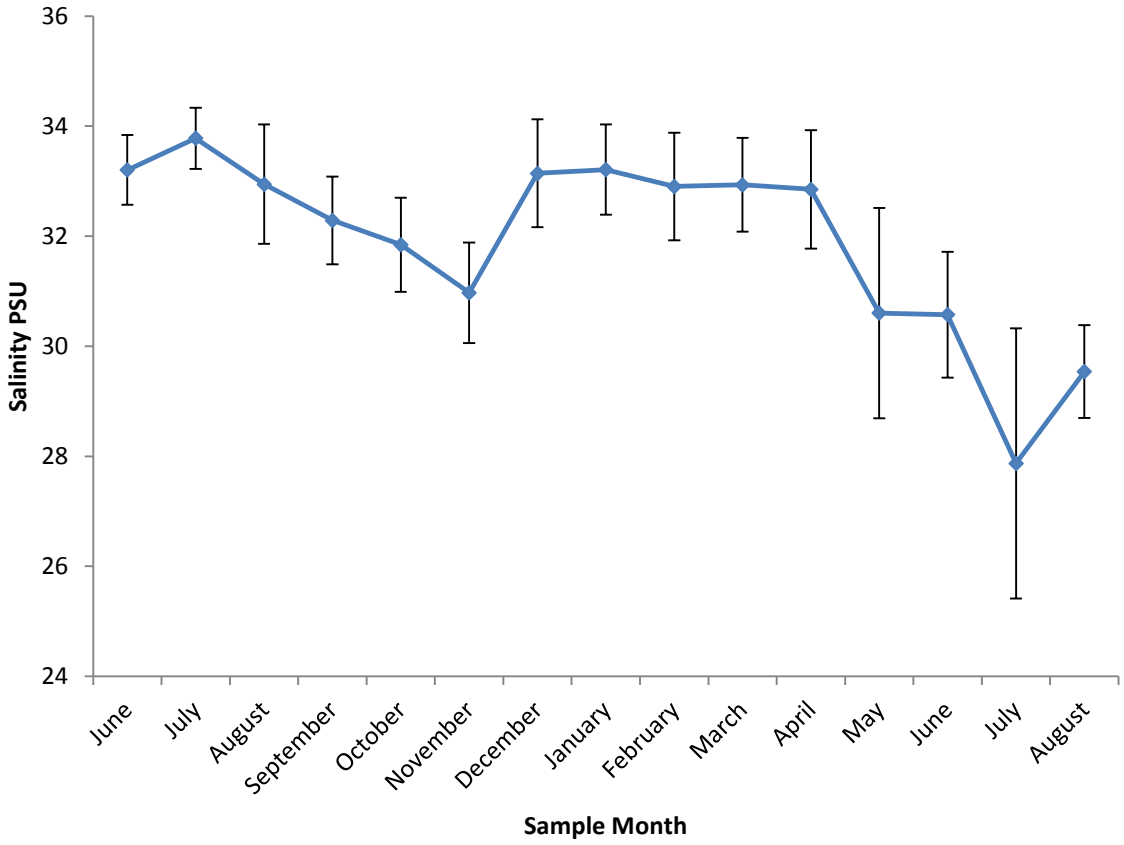

Figure 4.3.2a: Variation in salinity in Poole Harbour recorded by the sonde from June 2011 to August 2012 (monthly mean, ±SD, n=3400).

The salinity at the sonde monitoring station fluctuated on a tidal basis, with the lowest salinities coinciding with low tide (see figure 4.3.2b). This is due to the influences of the freshwater inputs from the western end of the harbour on the ebb tide and the seawater influx from the mouth of the Harbour on the flood tide. The salinity often fluctuated by up to 2psu over the course of the tidal cycle. Poole Harbour is also unique in that it has a second smaller higher water (see figure 4.3.2b).

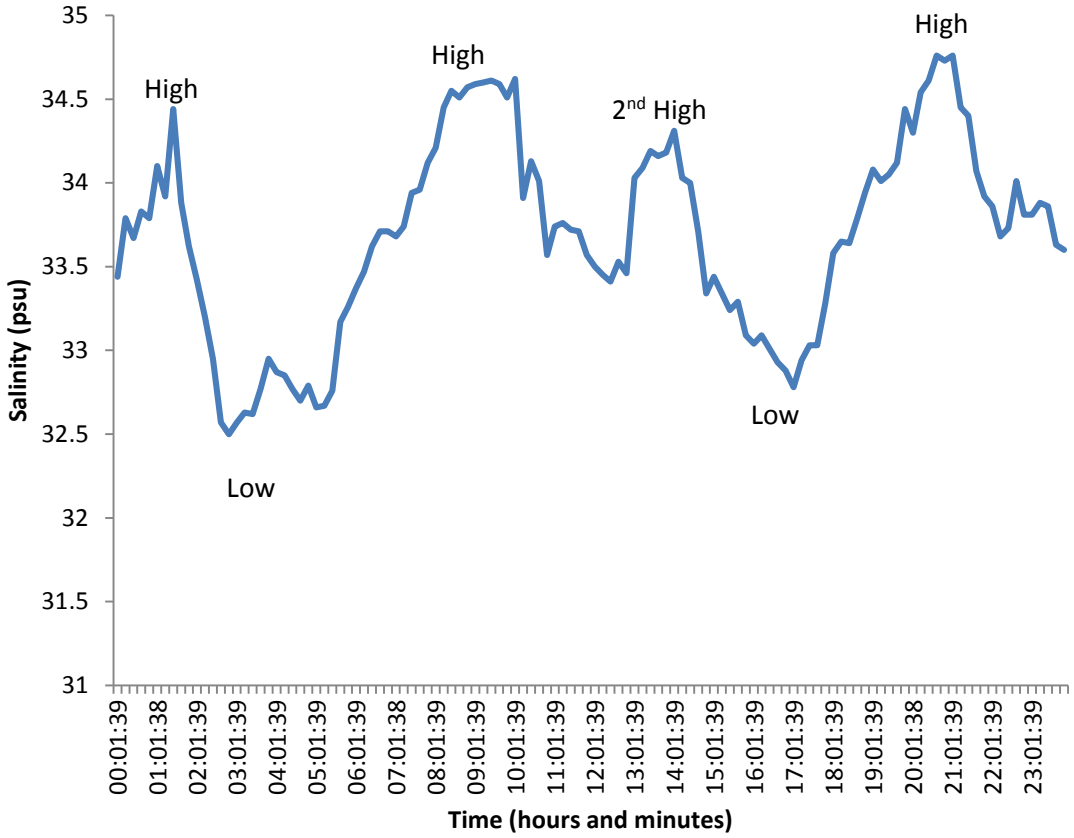

Figure 4.3.2b: Variation in salinity over the course of the tidal cycle in Poole Harbour recorded by the sonde over a period of 24 hours.

Dissolved oxygen

The average monthly dissolved oxygen (± Standard deviation) recorded by the sonde in Poole Harbour between June 2011 and July 2012 is reported in figure 4.3.3. The lowest recorded value for dissolved oxygen was 7.9mg/l in September 2011, the highest recorded value was 10.78 in March 2012. There was a slight seasonal trend in the levels of dissolved oxygen with the lowest levels recorded in summer and autumn with the highest levels in winter and spring. The higher levels of dissolved oxygen coincide with the time of year with the highest frequency of stormy weather. Throughout the monitoring period the seawater was saturated with dissolved oxygen.

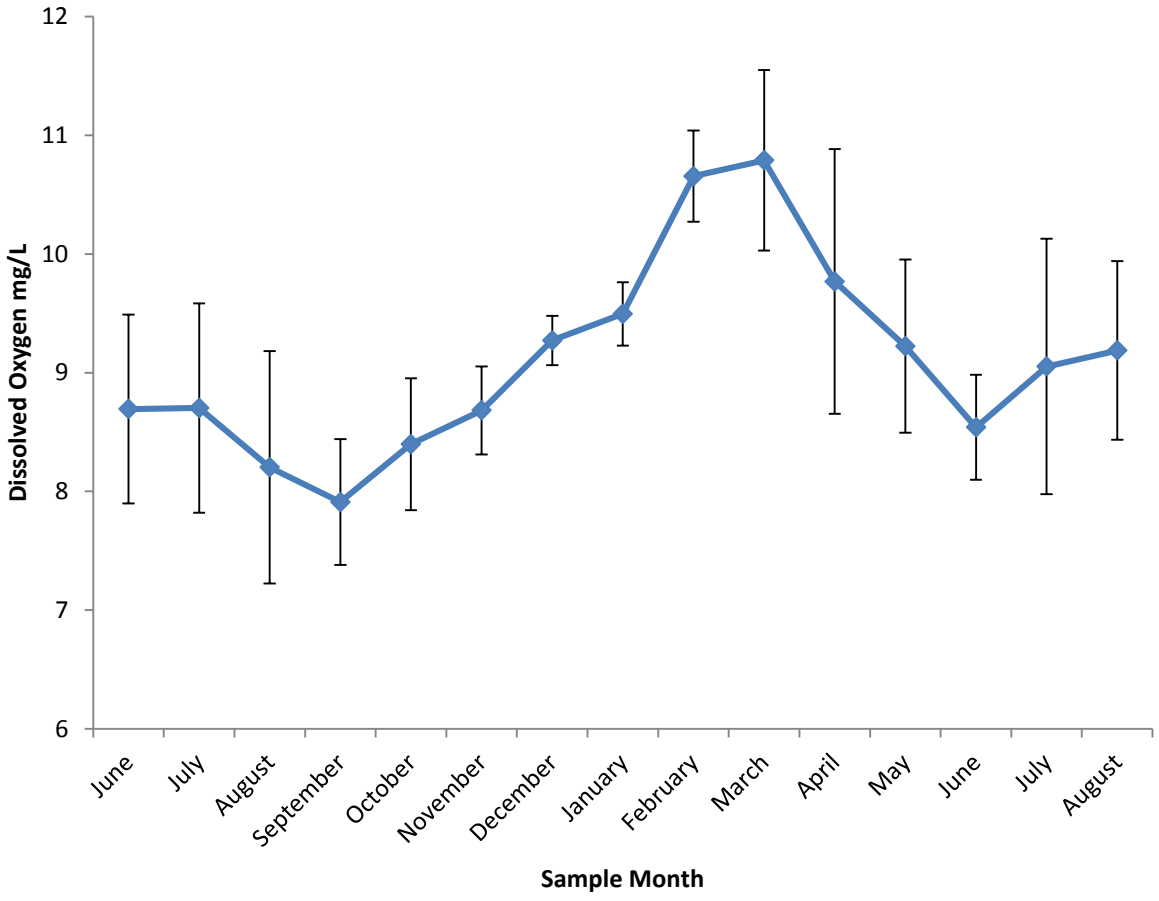

Figure 4.3.3: Variation in dissolved Oxygen in Poole Harbour recorded by the sonde from June 2011 to August 2012 (monthly mean, ±SD, n=3400).

Chlorophyll RFU

The average monthly chlorophyll abundance (+ Standard deviation) measured by the sonde in Poole Harbour between June 2011 and August 2012 in chlorophyll relative fluorescence units is reported in figure 4.3.4. Only positive values for standard deviation are included to aid clarity for interpretation of the seasonal trend. The highest recorded value was 4.89RFU in July 2011 whilst the lowest value was in December 2011. The levels of chlorophyll recorded by the sonde showed a seasonal pattern with the highest levels of chlorophyll recorded in the summer and the lowest levels recorded in the winter.

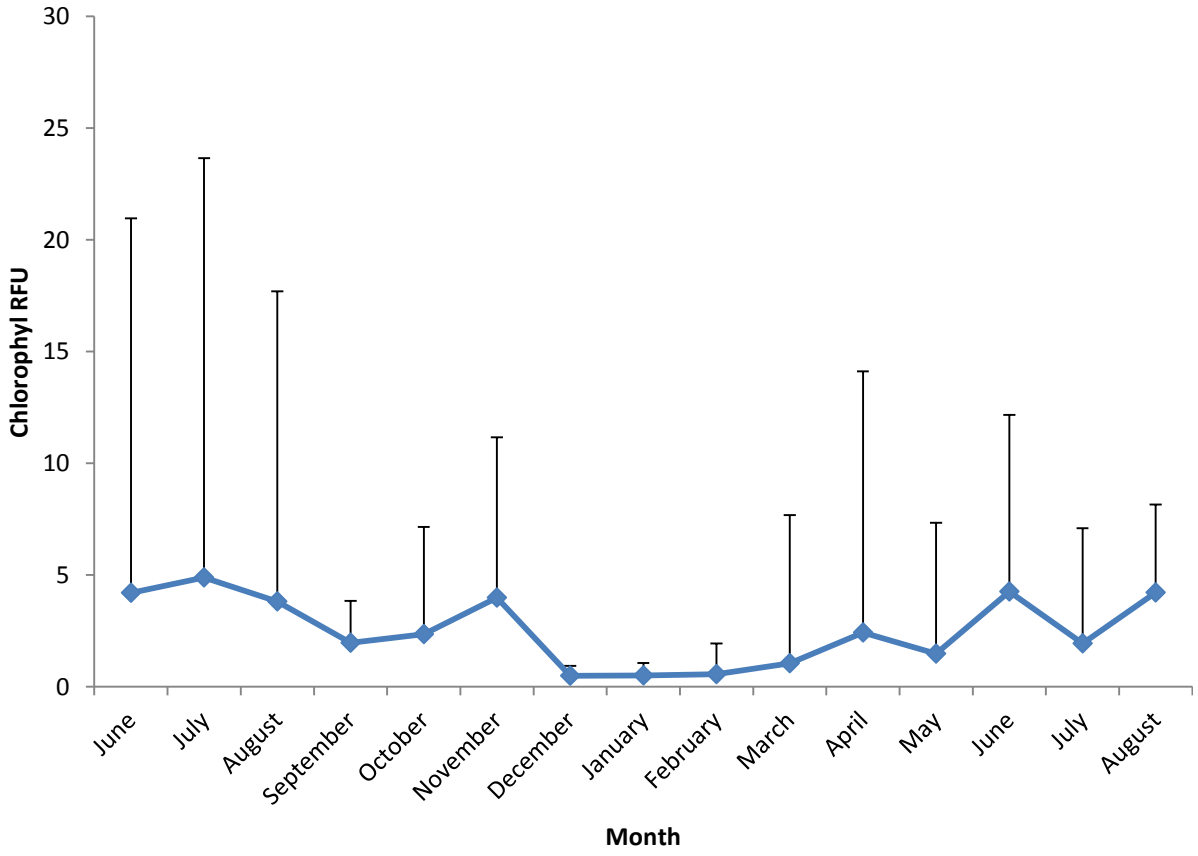

Figure 4.3.4: Fluctuation in chlorophyll relative fluorescence units recorded by the sonde in Poole Harbour from June 2011 to August 2012 (monthly mean, +SD, n=3400). Only positive values for standard deviation were included to aid in clarity of interpretation

Phycoerythrin RFU

The average monthly phycoerythrin abundance (+Standard deviation) recorded by the sonde in Poole Harbour between June 2011 and August 2012 is reported in figure 4.3.5. Only positive values for standard deviation are included to aid clarity for interpretation of the seasonal trend. There was a clear seasonal pattern, with a maximum abundance of 15.29RFU in August 2011 and a minimum of 0.39RFU in November 2012. The general trend was higher levels of phycoerythrin in the summer months and lower levels in spring and winter.

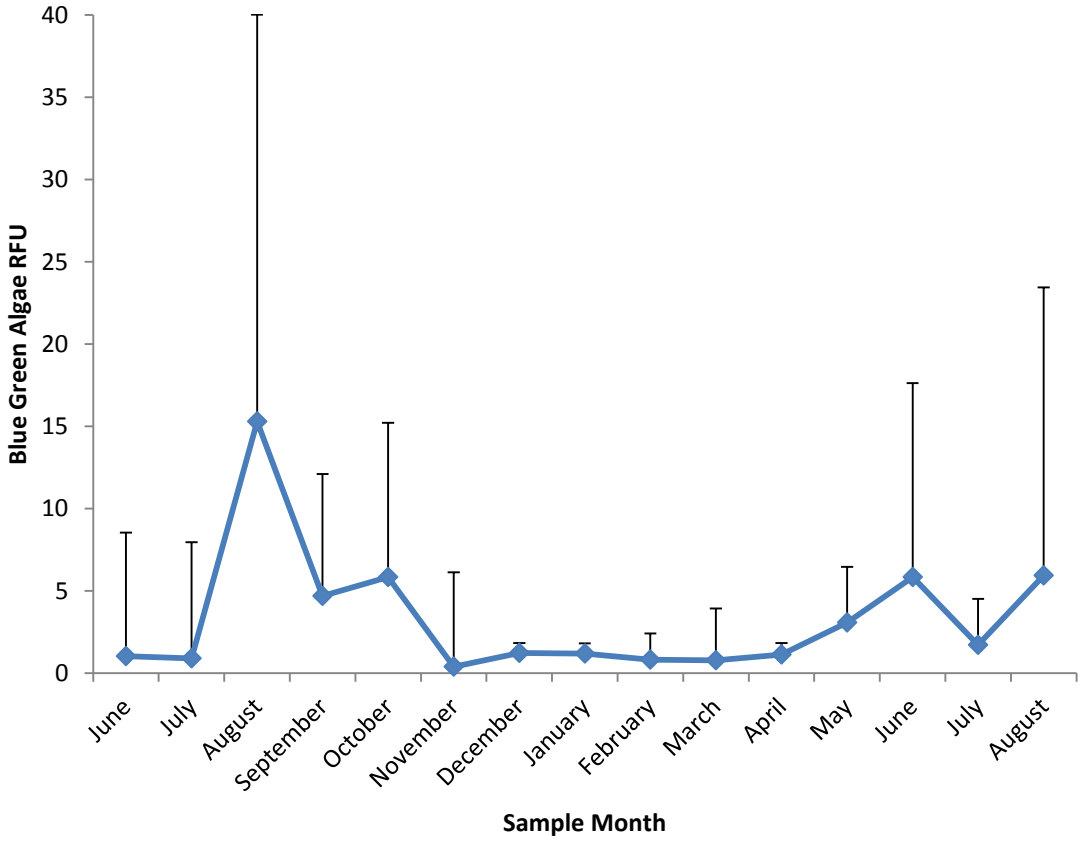

Figure 4.3.5: Fluctuations in phycoerythrin relative fluorescence units measured by the sonde in Poole Harbour from June 2011 to August 2012 (monthly mean, +SD, n=3400). Only positive values for standard deviation were included to aid in clarity of interpretation.

Turbidity of the seawater

The average monthly turbidity of the seawater (+ Standard deviation) recorded by the sonde in Poole Harbour between June 2011 and August 2012 is reported in figures 4.3.6a and 4.3.6b. Only positive values for standard deviation are included to aid clarity for interpretation of the seasonal trend. .The increase in turbidity between August and September was due to the commercial activities undertaken on the barge. It was found that the high levels of turbidity in August and September coincided with the pressure washing of the oyster bags to remove the Epifaunal growth on them. The runoff from this activity unfortunately was directed into the vicinity of the sonde so it recorded very high levels of turbidity for the periods during which the Oyster bags were washed. After it was noticed to effect the sonde the washing of the Oyster bags was undertaken at the other end of the barge. The lower values for turbidity can be seen by the values from November 2011 onwards (4.6.3b). Due to the issues with accuracy caused by human activity, turbidity was not used for analysis with clam condition.

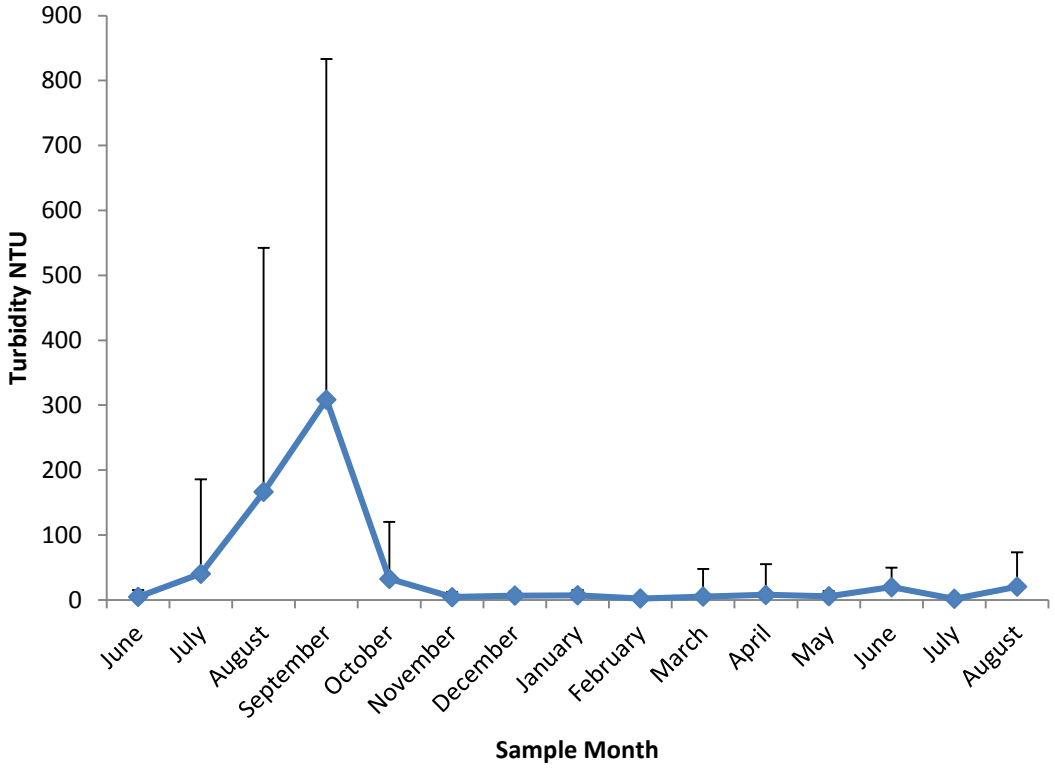

Figure 4.3.6a: Variation in the turbidity of the seawater in Poole Harbour measured in nephelometric turbidity units (monthly mean, + SD, n=3400) from June 2011 to August 2012. Only positive values for standard deviation were included to aid in clarity of interpretation.

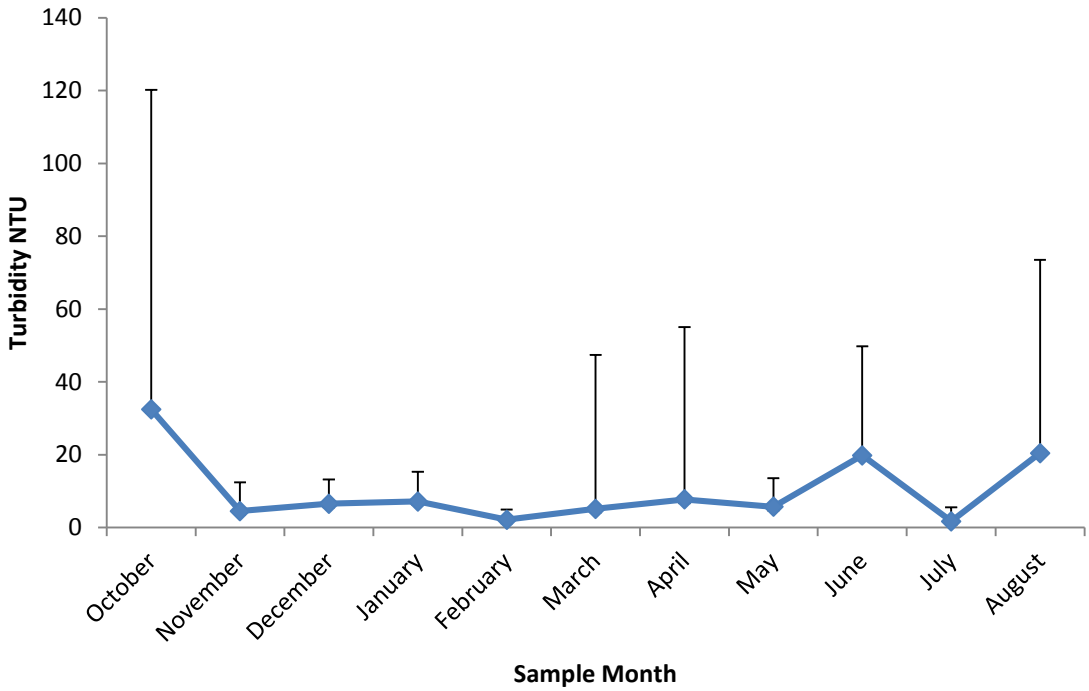

Figure 4.3.6b: Variation in turbidity of the seawater recorded by the sonde in Poole Harbour from October 2011 to August 2012 (monthly mean, + SD, n=3400). Only positive values for standard deviation were included to aid in clarity of interpretation.

Seawater pH

The average monthly seawater pH (± Standard deviation) recorded by the sonde in Poole Harbour between June 2011 and August 2012 is reported in figure 4.3.7. The maximum and minimum recorded values for pH are in 2012, with the minimum of 7.97 in January with the maximum of 8.33 in August. The range between the maximum and minimum recorded pH over the course of the study was 0.36 pH units, the pH in Poole Harbour is relatively stable.

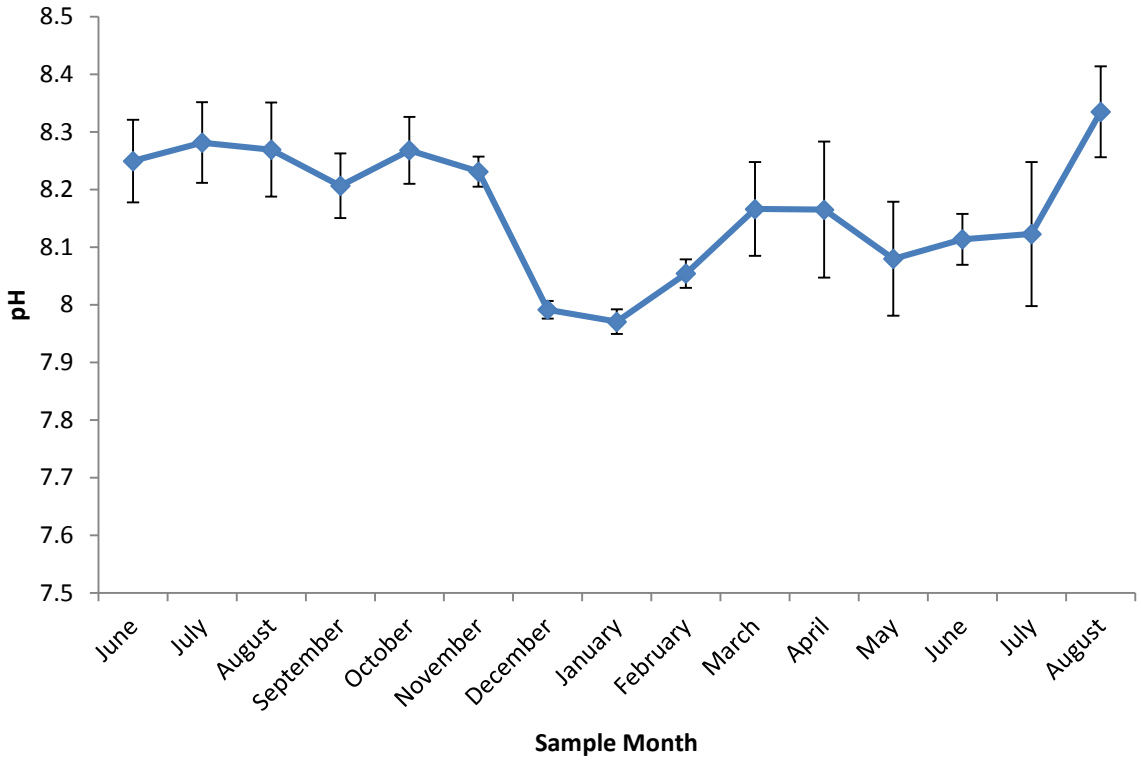

Figure 4.3.7: Variation in seawater pH recorded by the sonde in Poole Harbour from June 2011 to August 2012 (monthly mean, ±SD, n=3400).

4.3.2 Condition index of the lease bed population of Manila clams

The data was first tested for normality and found to be non-normally distributed (Kolmogorov Smirnoff test, DF 311, KS: 0.0506, P value <0.05), so the data was square root transformed to normalise it. There was a significant difference in the monthly clam condition indices between July 2011 and July 2012 (ANOVA, d.f. =11; F=7.99 p value= <0.001) (see figure 4.4). The highest condition index was recorded in August 2011 at 7.8 and the lowest condition index was 4.95 in February 2012. There was a seasonal trend with the highest condition indices recorded in the summer and the lowest condition indices recorded in the winter. There was a reduction in condition index between August (7.8) and September (6.35) 2011, this may have been due to a spawning event.

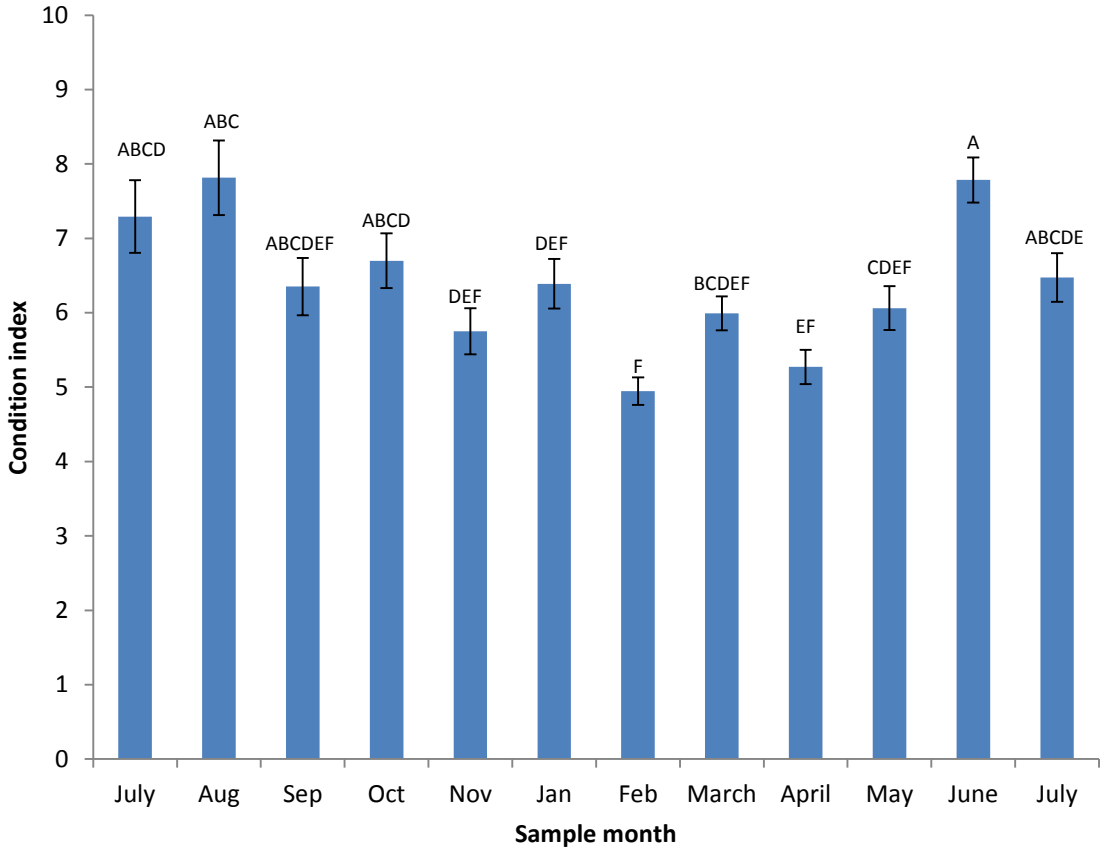

Figure 4.4: Condition index of the lease bed population of Manila clams from Poole Harbour from July 2011 to July 2012 (mean ±SE, n= 12-30).

4.3.3 Clam condition in relation to environmental variables

This section of the results analyses Manila clam condition index against the environmental conditions recorded by the sonde in the month prior to the clams being sampled. The sonde was deployed for a period of 15 months, from June 2011 until August 2012, with clams sampled in 12 of these months (from July 2011 until July 2012). The data is presented in Figures 4.5.1 to 4.5.6. The turbidity data has not been included due to problems with human activity affecting the readings as previously reported.

Clam condition index plotted against seawater temperature

There was a positive relationship between seawater temperature and the Square root of the condition index of the lease beds clams in Poole Harbour (Regression analysis, $F(1, 322) = 38.94$, P value= <0.001) with an R^2 of 10.8**.** As the average monthly temperature increases, so did the condition index of the Manila clams (see figure 4.5.1). The lowest clam condition index recorded was 2.21 at 8°C and the highest condition index was: 2.77 at 13.7°C.

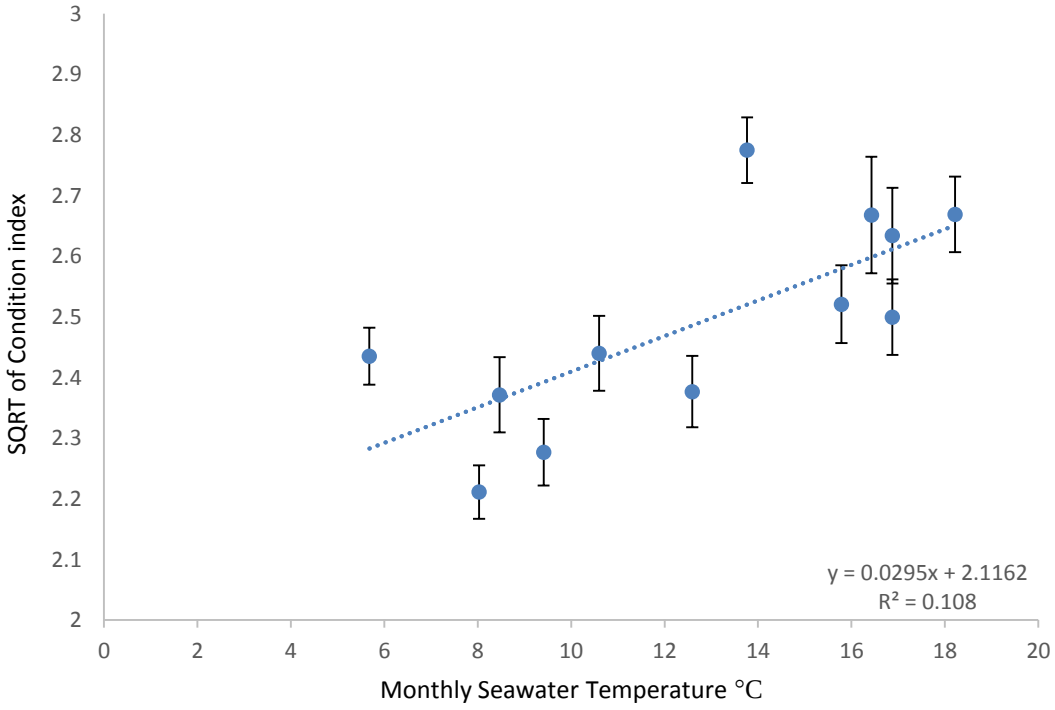

Figure 4.5.1: Variation of the square root of the condition index of the lease bed clams in Poole Harbour with seawater temperature (mean ± SE, n=20).

Clam condition index plotted against salinity

There was a negative association between salinity and the square root of the condition index in the clams from the lease beds in Poole Harbour (Regression analysis, F(1,322)= 12.04, P value=<0.001) with an R^2 of 3.6 (see figure 4.5.2). The lowest recorded Manila clam condition index was 2.21 at a salinity of 33.21 psu and the highest recorded condition index was 2.77 at a salinity of 30.6psu.

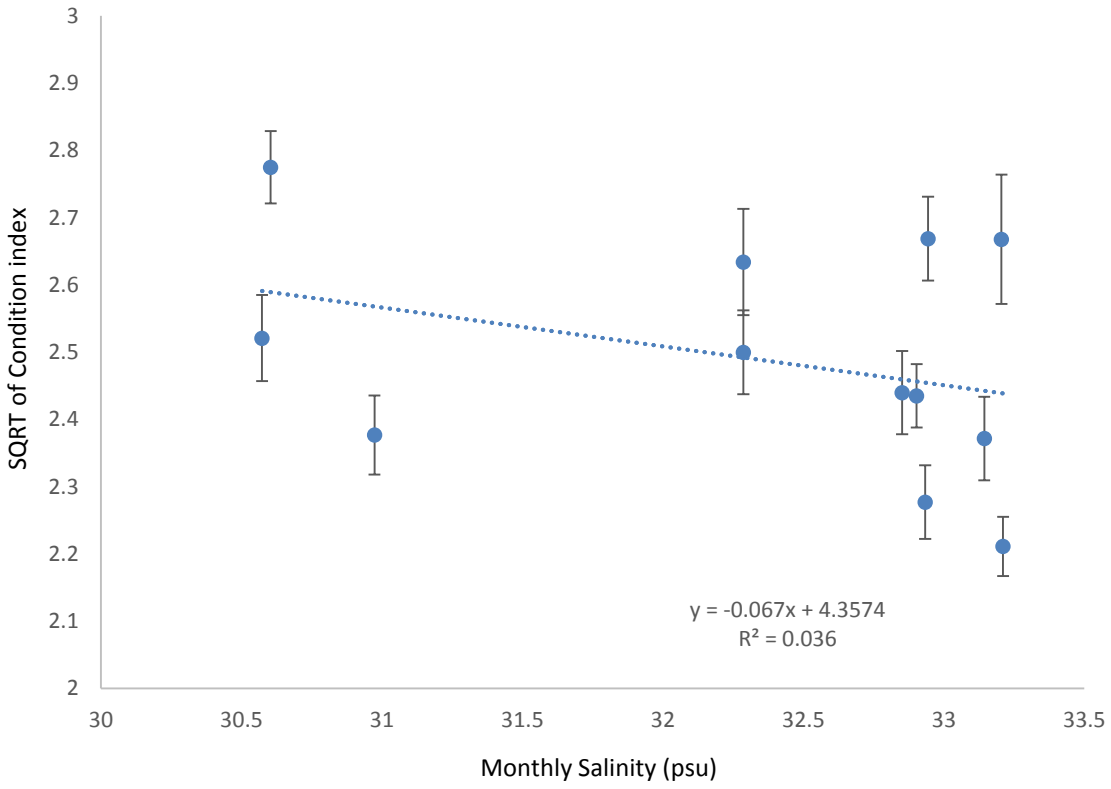

Figure 4.5.2: Variation of the square root of the Manila clam condition index in Poole Harbour with salinity (mean ± SE, n=20).

Clam condition index plotted against dissolved oxygen

The condition index of the clams from the lease beds in Poole Harbour was negatively correlated with dissolved oxygen (Regression analysis, F (1,322)=19.15, P value=<0.05) with an R^2 of 5.6 (see figure 4.5.3). The lowest Manila clam condition index was 2.21 at 9.5 mg of dissolved oxygen per litre of seawater, whilst the highest condition index was 2.77 at 9.2 mg of dissolved oxygen per litre of seawater.

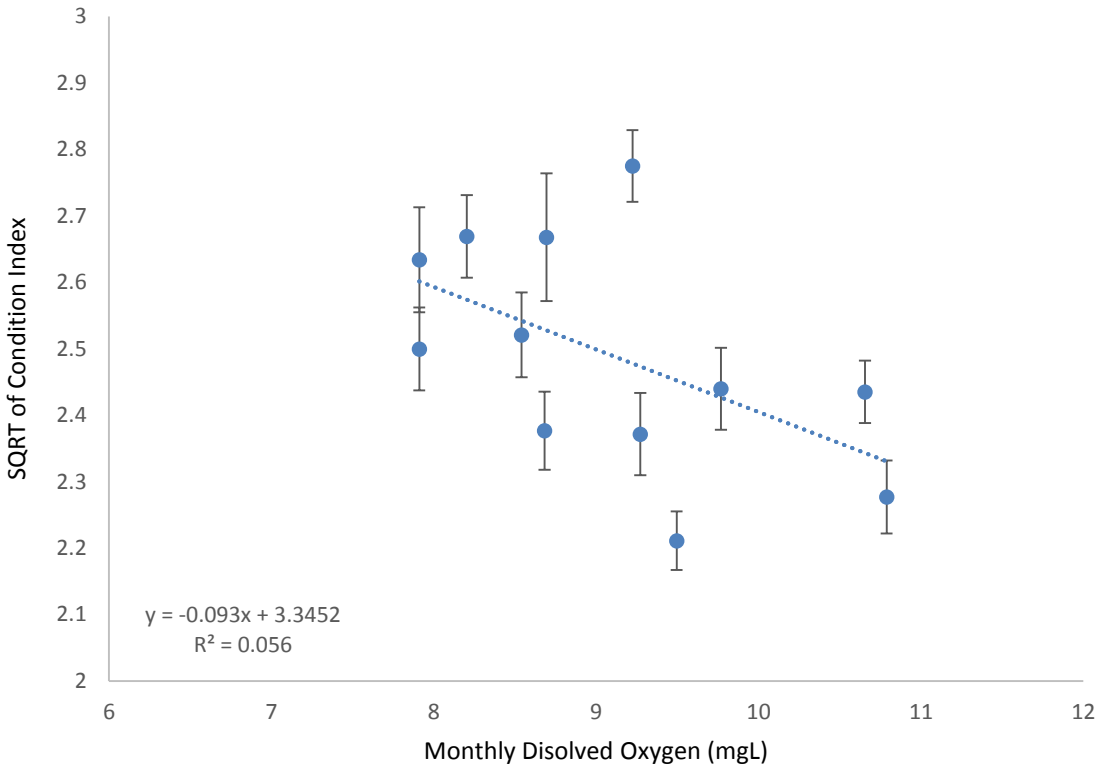

Figure 4.5.3: Variation of the square root of the Manila clam condition index in Poole Harbour with levels of dissolved oxygen (mean ± SE, n=20).

Clam condition index plotted against chlorophyll RFU

The square root of the condition index of clams from the lease beds in Poole Harbour was positively correlated with chlorophyll relative fluorescence units (Regression analysis, F(1,322)=13.00, P value=<0.001) with an R^2 of 3.9 (see figure 4.5.4). The lowest recorded clam condition index of 2.21 corresponded to a chlorophyll RFU value of 0.51 and the highest clam condition of 2.77 corresponded to a chlorophyll RFU value of 1.5.

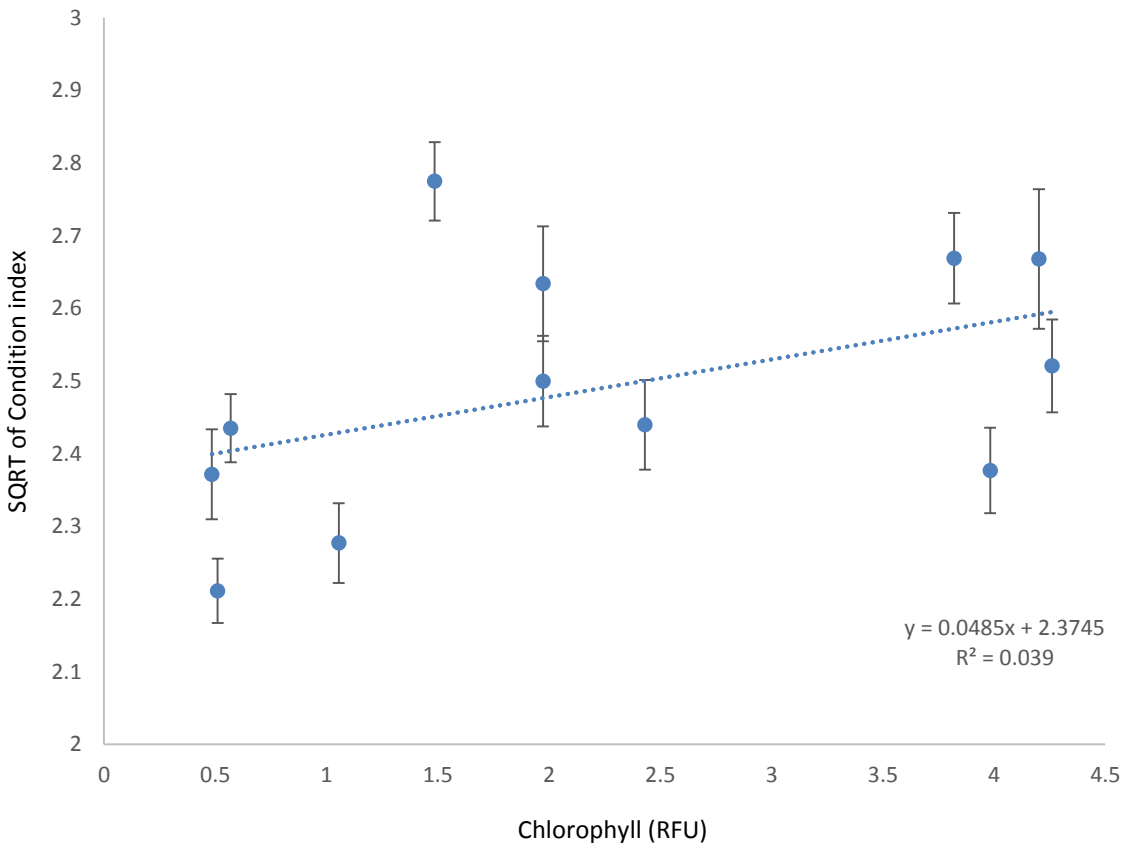

Figure 4.5.4: Variation in the square root of the Manila clam condition index in Poole Harbour with levels of chlorophyll relative fluorescence units (mean ± SE, n=20).

Clam condition index plotted against phycoerytherin RFU

There was a positive association between the square root of the condition index of clams from the lease beds in Poole Harbour and the phycoerytherin relative fluorescence units recorded by the sonde (Regression analysis, F(1,322)=19.19, P value= <0.001) with an R^2 of 5.6 (see figure 4.5.5). The lowest condition index of 2.21 corresponded to a phycoerytherin RFU value of 1.19 highest Manila clam condition index of 2.77, and the highest condition index corresponded to a phycoerytherin RFU value of 3.07.

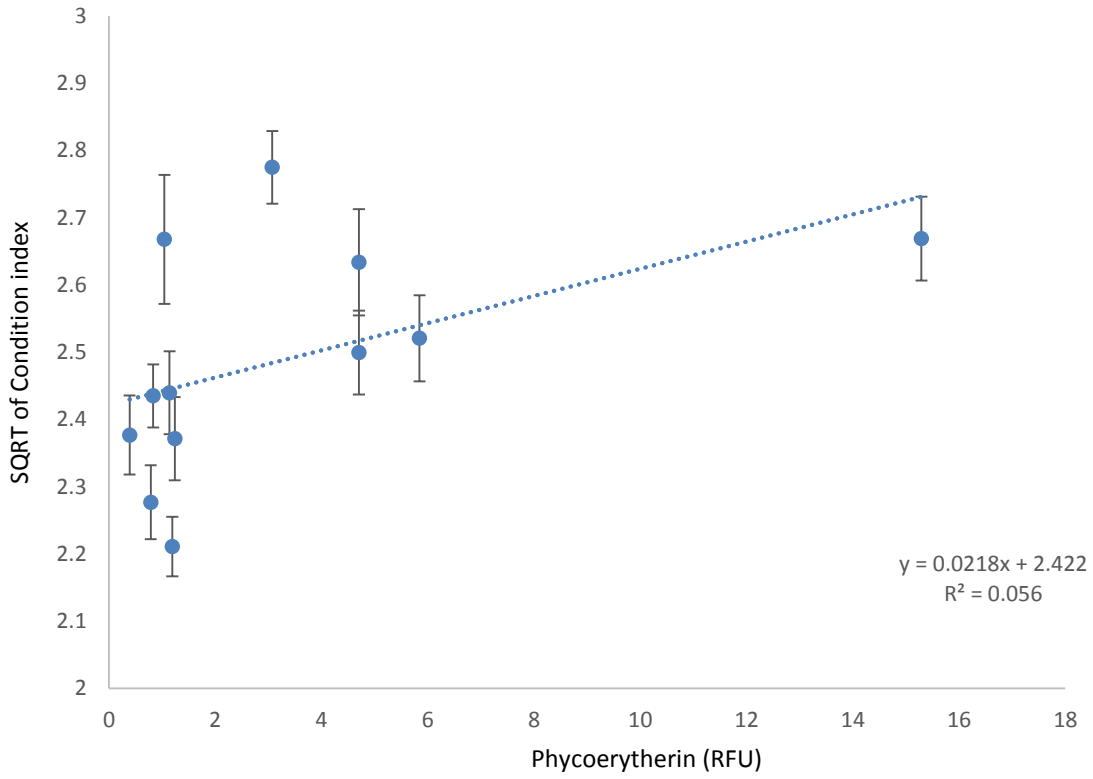

Figure 4.5.5: Variation in the square root of Manila clam condition index in Poole Harbour with increasing phycoerytherin abundance (mean ± SE, n=20).

Clam condition index plotted against seawater pH

There was a positive association between pH and the square root of the condition index of the clams from the lease beds in Poole Harbour (Regression analysis, F(1,322) =12.72 , P value=<0.001) with an R^2 of 3.8 (See figure 4.5.6). The lowest clam condition index of 2.21 was recorded at pH 7.97, with the highest condition index of 2.77 recorded at a pH of 8.07.

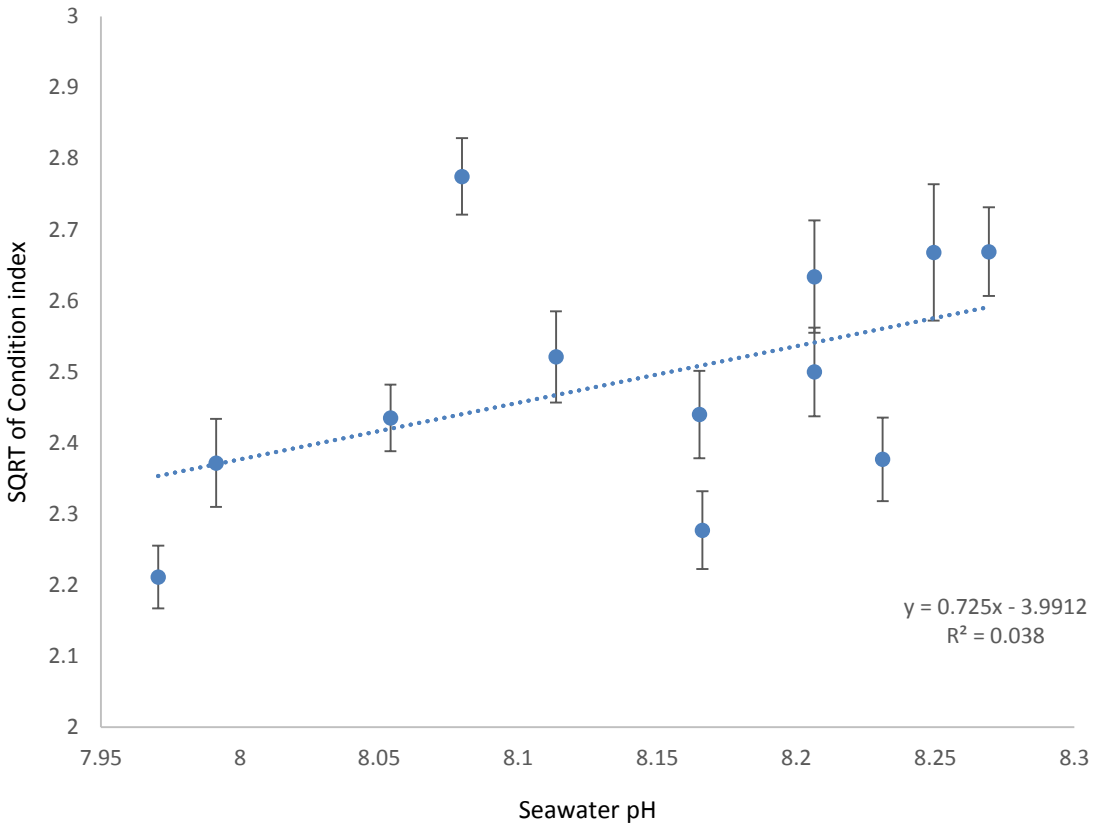

Figure 4.5.6: Variation in the square root of the Manila clam condition index in Poole Harbour with seawater pH (mean ± SE, n=20).

Analysis of clam condition index with all environmental variables

When analysed individually all of the environmental variables displayed a small but significant relationship with condition index. However when all of the environmental variables were analysed together using stepwise linear regression to create a model, only seawater temperature was found to have a significant relationship with Manila clam condition index. The results from the stepwise regression analysis are displayed below:

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations		
		B	Std. Error	Beta			Zero-order	Partial	Part
1	(Constant)	2.115	.061		34.770	.000			
	TEMP	.029	.005	.328	6.240	.000	.328	.328	.328

a. Dependent Variable: SQRT_CI

Excluded Variables[a]

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics		
						Tolerance	VIF	Minimum Tolerance
1	SALINITY	-.070[b]	-1.217	.225	-.068	.841	1.190	.841
	PHYCOERYTHRIN	.031[b]	.437	.663	.024	.551	1.814	.551
	CHLOROPHYLL	-.104[b]	-1.329	.185	-.074	.449	2.226	.449
	DO	.153[b]	1.536	.126	.085	.277	3.614	.277
	pH	-.077[b]	-1.031	.303	-.057	.496	2.015	.496

a. Dependent Variable: SQRT_CI

b. Predictors in the Model: (Constant), TEMP

The stepwise regression analysis did not detect any significant levels of multicollinearity between environmental variables. This means that there were no significant interactions between seawater temperature and the other variables recorded by the sonde. This was indicated by the calculated tolerance values for the environmental variables excluded by the model being above 0.1 and the VIF (Variation inflation factor) values being below 10. An example of this is salinity, with a VIF score of 1.190, this

indicates no interaction at all between temperature and salinity because the VIF score is just above 1.0. Out of all of the variables examined in the model, the variable that is most likely to have an interaction with temperature is dissolved oxygen, this is because it has a VIF score of 3.614, however this value is still below the value of 10 which would indicate that there wasn't a significant interaction between temperature and dissolved oxygen.

Comparison of the condition indices from the Holton mere and the Lease bed populations of Manila clams

There is a significant difference in the condition indices between the Holton Mere and the Lease bed populations of clams and there was also a significant difference in condition between months (ANOVA GLM, Site; F=649.26; d.f. =1; p value =<0.001, Month; F=10.54; d.f. =10; p value= <0.001)(see figure 4.6). The population of clams from the lease beds has a higher level of condition index than the Holton Mere population throughout the duration of the study. Both populations of clams show a seasonal pattern, however the lowest recorded condition index from the Lease bed clams is still higher than the maximum recorded condition index of the Holton Mere population.

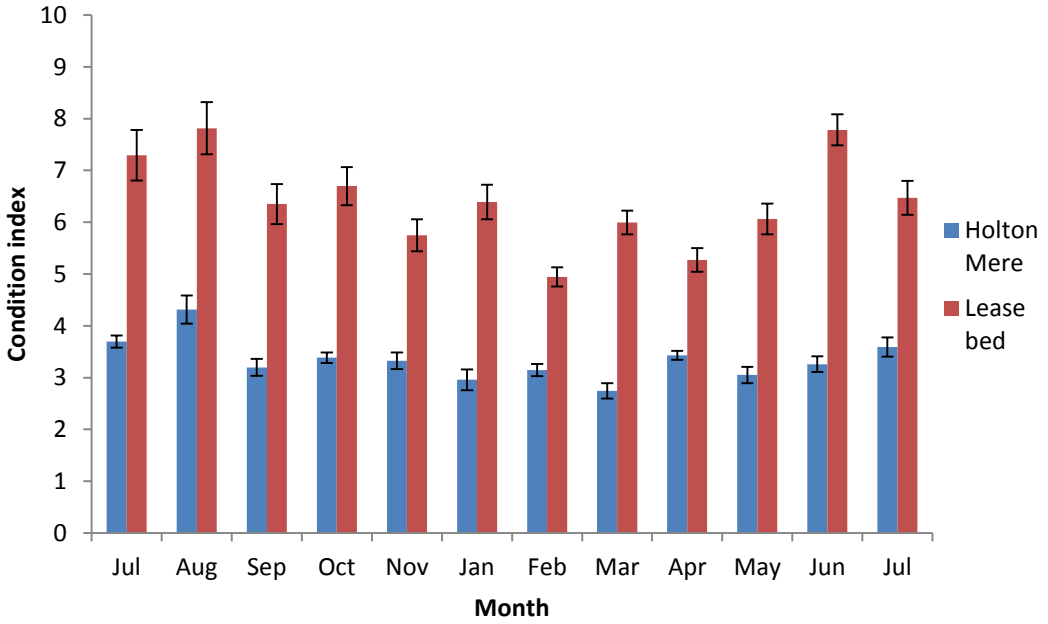

Figure 4.6: Condition indices of the Holton Mere and Leases bed population of Manila clams from Poole Harbour in Dorset from July 2011 to July 2012 (Mean ±SE, n=12-30).

4.4 Discussion

This study aimed to determine whether there were any relationships between environmental conditions and Manila clam condition index. During the course of the study period, there were no mass mortalities of clams in Poole Harbour, which suggests that the conditions experienced by the clams over the 13 month study period were not sufficiently detrimental to clam health to result in a mass mortality event. The influence of the environmental parameters measured by the sonde on clam condition index are discussed in the context of the stepwise regression analysis and then individually. A comparison of the condition index of the Holton Mere and the lease bed clam populations is also discussed.

The regular cleaning of the sonde (both when recovering data and by the automatic mechanical cleaning) and the recording of parameters at 12 minute intervals should have reduced the impact of fouling upon the accuracy of the sonde readings. Typically there was very little growth if any at all on the probes when they were removed for maintenance so loss of accuracy should be at a minimum. However there would still have been some minor loss of accuracy towards the end of the deployment period due to biofouling, which needs to be considered when drawing conclusions, however all steps were taken to reduce the loss in accuracy.

The sonde also recorded large fluctuations in monthly values for turbidity, chlorophyll RFU and phycoerytherin RFU during the course of the deployment. For turbidity the extreme values corresponded with periods of cleaning of the oyster bags on the barge, however this was not always the case with the chlorophyll and phycoerytherin. The period in which the chlorophyll and phycoerytherin showed the largest variation was during the spring and summer months. This is often the period in which phytoplankton blooms occur, this could suggest that phytoplankton blooms may explain the large variations in RFU recorded during the deployment.

Stepwise regression analysis of condition index with all measured environmental variables

The results of the stepwise regression analysis suggest that out of all of the environmental parameters measured during the sonde deployment, seawater temperature is the most likely variable to influence Manila clam condition index. Seawater temperature was also the variable that had the highest R^2 value (10.8%) when analysed individually against condition index. The stepwise regression analysis did not detect any significant levels of collinearity between the environmental variables measured during the study, as all of the calculated VIF values were below the threshold value of 10. This means that there was no significant interactions between the environmental variables examined, i.e. temperature did not significantly influence the level of phycoerytherin RFU recorded by the sonde.

Although there were no significant interactions between environmental variables, there may be a small interaction, between temperature and dissolved oxygen (VIF score: 3.614). Out of all of the variables examined, dissolved oxygen had the highest VIF score (3.614), the other variables had lower scores: salinity: 1.190, phycoerytherin: 1.814, chlorophyll: 2.226 and pH: 2.015, this suggests that if interactions did occur, dissolved oxygen is the most likely variable to have an interaction with temperature, however the model did not detect any significant levels of interaction.

Sea water temperature

The stepwise regression analysis suggests that seawater temperature was the environmental variable that had the greatest association with Manila clam condition index. Seawater temperature also had the highest R^2 value (10.8) when the environmental variables were individually analysed using regression analysis against condition index. This could suggest that seawater temperature is the most important factor in determining Manila clam condition in Poole Harbour.

The lowest recorded monthly temperature (5.6°C) was within the experimentally determined tolerance range of the Manila clam. Laboratory experiments have found that Manila clams can survive exposure for up to 11 weeks at this temperature both with and without food and not experience any mortality (Laing & Child, 1996). This suggests

that the winter seawater temperatures lowered the clam metabolic rate so that the low food availability did not have an effect upon then.

The relationship between higher temperatures and increased Manila clam condition index has also been described in other bivalve species such as the king scallop *Pecten maximus* (Ian Laing, 2000), the blood cockle *Anadara inaequivalvis* (Sahin et al., 2006) and the bay scallop *Argopecten irradians* (Shriver et al., 2002). A similar relationship between temperature and condition index has been described for populations of Manila clams in France (Dang et al., 2010) and South Korea (Uddin et al., 2012), which suggests that this is a typical physiological response to increased seawater temperature.

A possible mechanism by which increased temperatures leads to the increase in the condition index could be the effect of temperature on feeding rates and food availability in the form of phytoplankton. However we found no significant relationship between temperature and phytoplankton levels. Laboratory experiments investigating the effect of temperature of clearance rates by the bivalve *Modiolus barbatus* found that the highest clearance rates were observed between 20-28°C and the lowest were at 9°C (Ezgeta-Balić et al., 2011). The highest condition indexes in this study corresponded with seawater temperatures of between 16-19°C, whereas the lowest condition corresponded with a temperature of 4.9°C. This suggests that temperature does have an important influence on Manila clam condition. However if high water temperatures coincided with low food availability it could result in a drop in condition and potentially to mortality due to an increased metabolism and reduced food supply (Tumnoi, 2012). Temperature also influences the reproductive cycle in bivalves, with temperature driving the production, development, maturation and spawning of gametes (Honkoop & vanderMeer, 1997; Mann, 1979; Serdar & Lök, 2009). The reproductive cycle also affects the condition index of bivalves, and spawning events can reduce the condition index of an individual due to the release of gametes (Dang et al., 2010; Rebelo, 2003; Sahin et al., 2006).

In their natural geographical distribution, the reproductive cycle of the Manila clam starts from temperatures as low as 8°C where the gonads begin to develop oocytes, gonad maturation takes place at temperatures above 12°C and spawning often occurs at temperatures above 15°C (Mann, 1979; Ponurovsky, 1992). In 2011 the Poole

Harbour population of Manila clams had a potential spawning period of between June and October, because the seawater temperatures were above the threshold of the minimum temperature required for spawning. A reduction in condition index was due to a spawning event, but if spawning did not occur then the gametes could have been resorbed by the clam (Drummond et al., 2006).

Throughout the whole monitoring period, only one month, February 2012, had a temperature that was too low (below 8°C) for gamete production. During the rest of the study period only five out of the fifteen months monitored, had a temperature below that required for gamete maturation (12°C). This fact indicates that the temperature regime in Poole Harbour is compatible with the reproductive cycle of the Manila clam, however if the temperature does not reach at least 15°C for a prolonged period of time, spawning and recruitment may not occur.

Increasing average summer seawater temperatures around the UK could lead to increased reproductive success and could potentially push the distribution of the Manila clam northwards. Increasing average winter seawater temperatures could in fact prove to be detrimental to Manila clam health, as they would stimulate the metabolic processes of the clams whilst the food availability is low. This would lead to an energy budget deficit (Tumnoi, 2012) and potentially mass mortality events (Weiss et al., 2007). In the Dutch Wadden sea mild winter seawater temperatures have been linked to reductions in condition of the bivalves (P. Honkoop & Beukema, 1997).

Salinity

The negative relationship between condition index and salinity is surprising and probably not a true indication of the influence of salinity on the lease bed population of Manila clams. The variation in condition index that can be attributed to salinity is also low (only 3.6% of the variation in condition index can be attributed to salinity) which could suggest that salinity does not play a major role in determining the condition index in the lease bed population of Manila clams. Salinity was also not retained in the model created by the stepwise regression analysis, which suggests salinity does not influence condition index in the lease bed population of clams.

This could be because the range of salinities experienced by the clams during the study (30.6- 33.2 psu), is within the reported optimum range for this species (24-34) (Spencer et al., 1991; Utting et al., 1991) and above the minimum salinity (10 psu) that Manila clams can tolerate with low levels of mortality (Elston et al., 2003). The difference in average monthly salinities across the study period (a difference of 2.6 psu between the highest and lowest salinity) is also similar to what clams are exposed to over the course of the tidal cycle (a difference of 2.1 psu per tide) so is unlikely to influence clam condition index. The stepwise regression analysis also excluded salinity as a variable that influenced condition index so this could suggest that salinity does not influence the condition index of the lease bed population of Manila clams.

Dissolved oxygen

The negative relationship between dissolved oxygen and Manila clam condition index is probably not a true reflection of the influence of dissolved oxygen on clam condition index. This is because DO only accounted for 5.6% of the variation when analysed individually and was not retained by the stepwise regression model when analysed along with all of the other variables. Dissolved oxygen also had the highest VIF score (3.614) of all the environmental variables from the stepwise regression model. This suggests that there could be some small interaction between temperature and dissolved oxygen. This could suggest that the relationship between condition and dissolved oxygen is in fact an echo of the temperature-condition interaction rather than a relationship between dissolved oxygen and condition.

Chlorophyll RFU

Condition index displayed a positive relationship with chlorophyll a RFU when analysed separately using regression analysis. However it was not retained in the model when analysed using stepwise regression, this could suggest that there is no relationship between condition and chlorophyll a RFU and that it is purely an echo of the relationship with temperature. Also when individually analysed against condition index on its own, chlorophyll RFU only accounted for 3.9% of the variation in condition. This is a very low amount and could mean that chlorophyll does not play a large role in clam condition index in Poole Harbour.

This is noteworthy as there are documented cases of condition index in of bivalves being linked with food availability such as *Mercenaria mercenaria* (Weiss et al., 2007), *Paphies australis* (Norkko et al., 2005) and *Perna perna* (Narváez et al., 2008). This could suggest that although it was not retained in the model, Chlorophyll abundance may in fact influence condition index in some way, although it is not the main driver and only accounts for a small percentage of the variation.

It should be noted that chlorophyll a and levels of phycoerythrin RFU and by inference of cyanobacteria abundance is used as a proxy for algae biomass, as opposed to an accurate measure of concentration in the water column. The equipment is calibrated using known concentrations of algae in the lab (Aiken, 1981), however conditions in the field may interact and cause loss of accuracy. The measurement of the algal density and species diversity using water samples collected on a regular basis would give a more accurate concentration of phytoplankton density and community composition. Even though it does not give a fully accurate concentration of algal cells per ml of seawater, it is still useful for analysis as an increase in biomass will correlate with an increase in the food available to filter feeding organisms.

Phycoerythrin RFU

There was a positive relationship between phycoerythrin and Manila clam condition index. However it should be noted that the stepwise regression analysis did not retain phycoerytherin in the model and that it only accounted for 5.6% of the variation when analysed individually against condition index. This could suggest that the relationship is an echo of the relationship between temperature and condition index. This does not mean that phycoerytherin abundance does not influence condition index, but that it is not the main driver of condition for the sampled population.

In fact higher levels of phycoerythrin and subsequently cyanobacteria abundance could equate to higher levels of food for the clams to exploit. The phycoerythrin levels were consistently higher than the levels of chlorophyll throughout the course of the monitoring period (The sonde recorded higher RFU values for phycoerythrin than chlorophyll over the course of the deployment). This could be because cyanobacteria, (which include blue green algae) are able to grow more successfully under low light

intensity than chlorophytes (Flöder et al., 2002). This implies that cyanobacteria have the potential to have a longer growing season that chlorophytes, which would explain the higher levels of abundance of cyanobacteria than chlorophyll recorded by the sonde.

Laboratory studies have found that Manila clams can in fact successfully filter out particles from the water column down to ~1 micron in size (Nakamura, 2001), which means that they could successfully filter out blue green algae. This suggests that in Poole Harbour, blue green algae could constitute a more important part of the diet of Manila clams than other larger species of phytoplankton.

The spike in phycoerytherin RFU in August 2011 could be due to a late summer bloom, as the highest water temperatures were recorded in August 2011. Another reason for the spike could be due to the increased turbidity caused by the washing of the oyster bags, however this is unlikely as the highest turbidity value (September 2011) coincides with a reduction in phycoerytherin RFU, which suggests that the increases in phycoerytherin is due to a phytoplankton bloom.

Seawater pH

The regression analysis determined that only 3.8% of the variation in condition index was due to seawater pH, which could suggest that the pH of the seawater is not having much of an impact upon clam condition index in Poole Harbour. The fact that pH was not retained in the stepwise regression model also suggests that pH does not influence condition in this population.

When we consider that the pH values recorded during the sonde deployment ranged from 7.97 to 8.26, it raises the question of whether the pH of the seawater was low enough to have any impact upon clam condition index. Prolonged exposure to seawater with a pH below 7.5 is harmful to shelled molluscs and reduces the metabolism and growth rates in adult mussels (Michaelidis et al., 2005) with increased levels of CO_2 (and the subsequent reduction in pH) reducing shell calcification in mussels and oysters (Gazeau et al., 2007). The lowest pH value of 7.97 is above the value of 7.5 which is reported to be harmful to shelled molluscs. The variation between the lowest and highest pH values recorded was 0.29 pH units, which could suggest that another factor is responsible for the variation in condition index. More evidence for this theory is that

there was a positive association between seawater temperature and pH. The stepwise regression analysis also eliminated all other factors apart from temperature in determining clam condition index. This could suggest that the relationship between pH and condition is actually an echo of the interaction between temperature and condition rather than being a true reflection of the influence of pH on the Poole Harbour clams.

Comparison of condition index of the Holton Mere and the Lease Bed clam populations

The difference in condition index between the Holton Mere and Lease bed populations of Manila clams may be due to the difference in environmental conditions at both locations. Holton Mere can be considered a marginal habitat due to the intertidal nature of the mudflat coupled with the reduced levels of salinity caused by the influx of fresh water from the rivers Piddle and Frome. The Lease bed in Poole Harbour is sub tidal, with the clams only being exposed at extreme low tides and with little freshwater influence.

Immersion time influences the growth rate and condition index of cockles (De Montaudouin, 1996), which suggests that immersion time may be one of the factors that causes a reduction in condition index of the Holton Mere population. Reduced salinity may also influence the condition index of the Holton Mere population, as prolonged exposure to reduced salinities can causes a reduction in condition and death in bivalves (Elston et al., 2003; Matthews & Fairweather, 2004; Sarà et al., 2008).

The sub-optimal environmental conditions at Holton Mere that have allowed the Manila clam to become established, are also putting the clam population under physiological stress. This is evident from comparing the condition index of the Lease bed population with the Holton Mere population. The establishment of the Holton Mere population is due to the high tolerance range of Manila clams to environmental conditions such as salinity and immersion time. The establishment of the Holton Mere population also provides valuable insight into the potential spread of this species and indicates that future surveys should sample marginal habitats in order to determine the spread of this species.

4.4.1 Conclusions

The results from this chapter suggest that sea water temperature is the most important factor in determining clam condition index. This is because temperature controls metabolic processes in the Manila clam (Mann, 1979). Manila clams are able to survive periods of low food availability as long as metabolic activity is at a low level, this has also been reported for other species of bivalve (Honkoop & Beukema, 1997). The low seawater temperature could be the key factor in preventing a mass mortality event. This is because the lower temperature limits for gamete production is 8°C and gamete maturation is 12°C (Mann, 1979). A temperature above 8°C could trigger gamete production and deplete internal energy reserves of the clams because they are not able to filter out enough phytoplankton to replace the energy used up in the reproductive effort. The seawater temperature did not rise above 8°C until March by which time the levels of chlorophyll levels had begun to increase. The increased metabolic demand of the clams would be met by the increase in the available food resources.

This suggests that the ideal conditions in order for Manila clams to increase their distribution around the British coastline are winters with cold average seawater temperatures below 6°C in order to allow the clams a reproductive resting phase with summers with an average seawater temperature above 18°C for both the reproductive cycle and for larval growth.

During the course of the sonde deployment, fluctuations in temperature were recorded over short time periods (days) as well as the seasonal changes (months) already described. These short-term fluctuations included a reduction in temperature of 5.5°C over a period of eight days in the winter (from 8.5°C to 3.5°C) and an increase of 6.2°C over 11 days in the spring (12.1°C to 18.3°C). These short-term fluctuations may also influence Manila clam condition and physiology as the clams have to adapt to these rapidly changing conditions. Manila clams adapt to rapidly warming conditions by closing the shell and suspending feeding and pumping behaviour (Anacleto et al., 2014). This is in contrast to the local species *Ruditapes decussatus* which uses upregulated expensive metabolic responses to cope with temperature stress (Anacleto et al., 2014). This may give Manila clams a short term advantage over periods of rapid temperature

increase, by not using energy reserves. This could result in reduced mortalities for Manila clams compared to local species when un-seasonally warm temperatures coincide with periods of reduced food availability, for example high water temperatures in early spring.

Although the stepwise regression analysis suggests that seawater temperature is the most important factor in determining condition index in the lease bed population of Manila clams, interactions between other factors such as salinity cannot be completely ruled out. It could be argued that the relationships detected by the individual statistical analysis could be echoes of the interaction between condition and temperature, however relationships between environmental variables such as salinity have been reported for the population of Manila clams in North West Ireland (Drummond et al., 2006). This could suggest that the other environmental variables do in fact influence condition, but do not have as much of an influence as temperature.

The lack of a mass mortality event during the course of this study makes the environmental data recorded very valuable. This data set can be used as a baseline for future studies as a year in which conditions are favourable for clam survival. Comparing the environmental conditions of a year in which mass mortality does occur will allow for the rapid determination of the factors that influence this event. Future work could also include screening clams for parasites and infections such as perkinsosis using molecular methods. This screening process would also be useful in comparing the Holton Mere and Lease bed populations, as the Holton Mere population may be more susceptible to infection by pathogens due to environmental stressors.

Chlorophyll and phycoerythrin abundance are often positively correlated with seawater temperature due to the season and day length (Flöder et al., 2002). The relationship between temperature and phytoplankton abundance has been previously reported in the literature (Norkko & Thrush, 2006). Food abundance does influence clam condition, as increased temperatures with low levels of food availability reduce the condition of bivalves due to an energy budget deficit (Tumnoi, 2012; Weiss et al., 2007). Dissolved oxygen is also often negatively correlated with seawater temperature due to the increased metabolic demands of organisms. Salinity, pH and dissolved organic matter

are not correlated with temperature so these can be considered independent from temperature.

The influence of environmental conditions on the condition of the Manila clam populations, make it important for the larvae to locate and settle in suitable habitats. The differences in condition index of the Holton Mere and Lease bed populations of clams indicate that marginal habitats, although they allow the Manila clams to establish a population, also reduce the fitness of that population as well due to physiological stress. This highlights the importance of settlement by larvae in areas of suitable habitat.

Chapter 5: Manila clam larval response to reduced salinities

5.1 Introduction

Environmental conditions such as water temperature and food availability are important factors in determining adult Manila clam condition and ultimately reproductive success. Thus effective habitat selection plays an important role in ensuring the future success of a population. Bivalve larvae are able to influence, but not control dispersal and habitat selection by modifying their swimming behaviour. Dispersal and habitat selection takes place during the larval stage of the Manila clam (Herbert et al., 2012; Kasuya et al., 2004; Tezuka et al., 2013). This suggests that the ability of the larvae to detect and modify behaviour in order to maintain position in suitable conditions is vital to the future survival of the individual. This highlights the importance of understanding the behavioural response of larvae to a range of environmental conditions in particular salinity.

Bivalve reproductive strategies

The reproductive cycle of most marine benthic bivalves can be categorised into three main phases; gametogenesis and gamete production, spawning and external fertilisation and larval development and growth (Newell et al., 1982). The stages of the reproductive cycle are influenced by environmental conditions. Gametogenesis is influenced by environmental conditions such as temperature (Fabioux et al., 2005; Honkoop & vanderMeer, 1997; Mann, 1979) and food availability (Soudant et al., 1996; Uddin et al., 2012; Utting et al., 1991). Reduced levels of food availability can influence the quantity and quality of gametes (Hendriks et al., 2003; Soudant et al., 1996; Uddin et al., 2012) and populations of bivalves are known to break down gametes by atresia when food availability is low (Drummond et al., 2006; Soudant et al., 1996; Tumnoi, 2012). Low temperatures have been reported to slow down the production of gametes in some bivalves, notably oysters and clam species (Fabioux et al., 2005; Mann, 1979). Increasing temperatures are a cue that induces spawning in many species of bivalve, including oysters (Honkoop & Van der Meer, 1998), mussels (Chipperfield, 1953) and clams (Philippart et al., 2003; Utting et al., 1991).

Once released, gamete fertilisation and larval growth occurs in the water column (Bayne et al., 1983; Ponurovsky & Yakovlev, 1992; Utting et al., 1991). The larval stages of many species of bivalves, including oysters and clams are planktotrophic and feed upon micro algae and dissolved organic matter present in the water column (Baldwin & Newell, 1991; Hendriks et al., 2003; Inoue et al., 2007; Utting et al., 1991). The duration of the larval stage is determined by seawater temperature. Reduced seawater temperatures can increase the duration of the larval stage due to reduced growth rates (Calabrese, 1969; Herbert et al., 2012; Ruesink et al., 2013). The length of the larval stage can also influence the distance over which the larvae disperses (O'Connor et al., 2007)

Swimming behaviour and dispersal in bivalve larvae

It was previously thought that bivalve larvae were passive particles drifting with tides and currents with no way to influence dispersal. This has since been disproved and larval swimming behaviour is known to influence dispersal (Mann et al., 1991; North et al., 2008; Wood & Hargis, 1971). Due to their relatively small size and weak swimming ability, larvae are unable to control their horizontal movement , but can influence it by modifying their distribution within the water column (North et al., 2008; Wood & Hargis, 1971). This modification of position in the water column allows the larva to influence, its dispersal by tides and currents (Herbert et al., 2012; North et al., 2008; Wood & Hargis, 1971). Oyster species have been observed to maintain a position near the bottom of the water column in estuaries to ensure that they are transported inland on the flood tide. This behaviour allowed the larvae to be retained in the estuary system (Wood & Hargis, 1971).

Bivalve larvae have different swimming behavioural mechanisms that allow them to control their position in the water column. These behaviours consist of active swimming, controlled sinking and passive sinking (Cragg, 1980; Finelli & Wethey, 2003; Hidu & Haskin, 1978; Mann et al., 1991; Stanton, 2012). The active swimming in many species of bivalve larvae is facilitated by the velum, a specially developed organ that allows the larvae to swim (Cragg, 1980, 1989; Stanton, 2012). By beating the cilia of the velum, larvae are able to swim actively, often in a helical motion, controlling velocity and direction (Cragg, 1980; Hidu & Haskin, 1978; Mann et al., 1991; Stanton, 2012). The behaviour of active swimming has been associated with maintaining position in an area

of suitable habitat (Hidu & Haskin, 1978; Mann et al., 1991; North et al., 2008). Active swimming in larvae is indirectly associated with low swimming velocities typically 0.25-1.5 mms^{-1} (Cragg, 1980; Mann et al., 1991; North et al., 2008). Larvae can exceed these speeds by closing the shell and retracting the velum and passively sinking (Hidu & Haskin, 1978; Stanton, 2012). Passive sinking behaviour often occurs when larvae encounter suboptimal conditions (Hidu & Haskin, 1978) or turbulence in the nearby water (Cragg, 1980). Passive sinking could be a response to ensure that the larvae remain in areas of suitable conditions. This suggests that there may be many interactions between environmental variables and swimming behaviour.

Influences of environmental variables on swimming behaviour and dispersal in bivalve larvae

Understanding the response of bivalve larvae to environmental conditions allows the potential spread of a species to be predicted using hydrodynamic modelling (Dekshenieks et al., 1996; Herbert et al., 2012; North et al., 2008). These environmental conditions include light (Bayne, 1964; Garland et al., 2002), gravity (Bayne, 1964; Cragg, 1980), temperature (Hidu &Haskin, 1978; Stanton, 2012) and salinity (Davis, 1958; Dekshenieks et al., 1996; North et al., 2008; Wood & Hargis, 1971) which are all known to influence swimming behaviour in bivalve larvae. These variables are known to influence the swimming velocity (Cragg, 1980; Hidu & Haskin, 1978; Stanton, 2012) and the position of larvae in the water column (Davis, 1958; Garland et al., 2002; North et al., 2008). These behavioural responses are adapted to allow the larvae to ensure that they remain in areas of suitable habitat (Dobretsov & Miron, 2001; Hidu & Haskin, 1978; Knights et al.,2006; North et al., 2008; Sameoto & Metaxas, 2008; Wood & Hargis, 1971).

It has been reported that salinity influences the swimming velocity of clam and oyster larvae (Davis, 1958; Hidu & Haskin, 1978) and the position of larvae in the water column (Davis, 1958; Dekshenieks et al., 1996; Wood & Hargis, 1971). In estuaries, oyster larvae have been observed to sink to the bottom of the water column on the ebb tide and swim up into the water column during the flood tide (Wood & Hargis, 1971). In some estuaries, haloclines occur where the denser seawater and the lighter fresh water do not mix. In these haloclines the lighter fresh water sits on top of the denser seawater in layers, that have very little mixing (McLellan, 1965), p144). These haloclines elicit different

behavioural responses from different species. Some species, such as *M. mercenaria* and *C. virginica* actively swim through haloclines into low salinity water (Davis, 1958; North et al., 2008) whereas others such as *R. philippinarum* and copepod species congregate below the depth at which reduced salinities occur (Kasuya et al., 2004; Lougee et al., 2002).

The larvae of the clam species *Mercenaria mercenaria* have been observed to actively swim through haloclines until the larvae encounter seawater with a salinity of 15psu (Davis, 1958). Once the larvae encountered seawater with a salinity of 15psu, they no longer continued swimming upwards but remained in the boundary layer of salinity change and reduced their swimming velocity (Davis, 1958). This suggests that the response to suboptimal conditions was to remain below the level at which the suboptimal conditions occur (Sameoto & Metaxas, 2008).

A similar response to the presence of a halocline was recorded for Manila clam larvae in Tokyo Bay. When surface water salinities were reduced by heavy rain, Manila clam larvae were observed to congregate in the layers of seawater just below the fresh water. The region of water in which the larvae congregated was recorded to have a salinity of 30psu (Kasuya et al., 2004). This behaviour has also been observed in the laboratory. Experiments investigating the response of the different larval stages to a series of haloclines found that the different larval stages had different salinity preferences. The trochophore larvae actively swam into seawater with a salinity of 18-24psu, whilst early D larvae swam into salinities of 29psu with the late stages of the D larvae selecting water with a salinity of 21-23psu (Ishida et al., 2005). This suggests that Manila clam larvae have different tolerances to salinity at each development stage and will actively avoid conditions that may be detrimental to survival rates. This raises the question of what is the lowest salinity that Manila clam larvae will actively swim in to?

Ishida et al. (2005) also reported that the maximum swimming velocity for larval Manila clams was 3.3mm s^{-1}, but there have been no investigations to determine how salinity may affect the swimming velocity in this species. This study set out to investigate whether salinity influences the swimming velocity of Manila clam larvae.

5.2 Materials and Methods

5.2.1 Manila clam larval tolerances to salinities

The larvae used in this experiment were nine day old pediveliger larvae that had been reared under hatchery conditions (27°C with a salinity of 34 psu). The experiments were undertaken using pediveliger larvae as these were the stages available from the commercial hatchery. The experiment was undertaken at room temperature (21°C ±1), with actively swimming larvae. Only active larvae were used to ensure that any observed responses were due to the effects of the salinity rather than a lack of larval vigour prior to the start of the experiment.

The salinities examined were: 30, 26, 22, 20, 18, 16, 14, 12, 10, 5 and 0 psu. The salinities were created by mixing filtered seawater with distilled water to obtain the required value. The vessels used in this experiment were tissue culture plates, with each culture plate containing 12 wells. Every well on the culture plate contained one of the 12 experimental salinities, giving a total of 12 different salinities present on each culture plate. A total of eight plates were used, giving a total of eight replicates for each salinity

Actively swimming larvae were removed from the holding vessel using a Pasteur pipette and introduced to a well containing one of the 12 salinities. To minimise the effect of altering the salinity in the well by adding the larvae, the pipette containing the larvae was firmly tapped on the side to ensure that all the larvae dropped to the bottom of the pipette. This ensured that only one drop containing the larvae needed to be added to each well. The number of larvae in each well were counted at the end of the experiment and the numbers ranged from 6-77 larvae per well.

The wells were observed after one, three, five and twenty-four hours and the number of active and inactive larvae were counted. Active larvae were categorised as those that were actively swimming or had beating cilia. Non-active or dead larvae were those that had a closed shell and no observable movement of cilia.

5.2.2 Larval behaviour in response to a halocline

The experiment to determine larval response to the presence of a halocline was undertaken at room temperature (21°C ± 1) with a constant diffuse light source to prevent light conditions affecting the behaviour of the larvae. The experiment was carried out in 4ml plastic cuvettes in which a halocline was created. Three cuvettes were used for each halocline experiment, given a total of three replicates per treatment. The haloclines were created by adding 2ml of full strength seawater (34 psu) to the bottom of the cuvette and then carefully pipetting 2ml of the experimental salinity above it. A drop of seawater was taken either side of the halocline and the salinity measured using a refractometer to ensure that no mixing occurred, if there was mixing the cuvette was discarded. The experimental salinities were created by mixing filtered seawater with distilled water.

The salinities tested were: 10, 14, 15, 16, 18, 22 and 26psu. A control to determine distribution of larvae without a halocline was created by adding 4ml of 34 psu seawater to a cuvette. Each halocline and the control were replicated three times giving a total of 24 cuvettes. The larvae used in this experiment were from the same batch as those used in the salinity tolerance experiment. The larvae were collected from the water column in the holding vessel using a fine Pasteur pipette and then carefully added below the halocline. This was to ensure that there was limited mixing and that the larvae started the experiment in 100% seawater (see figure 5.1).

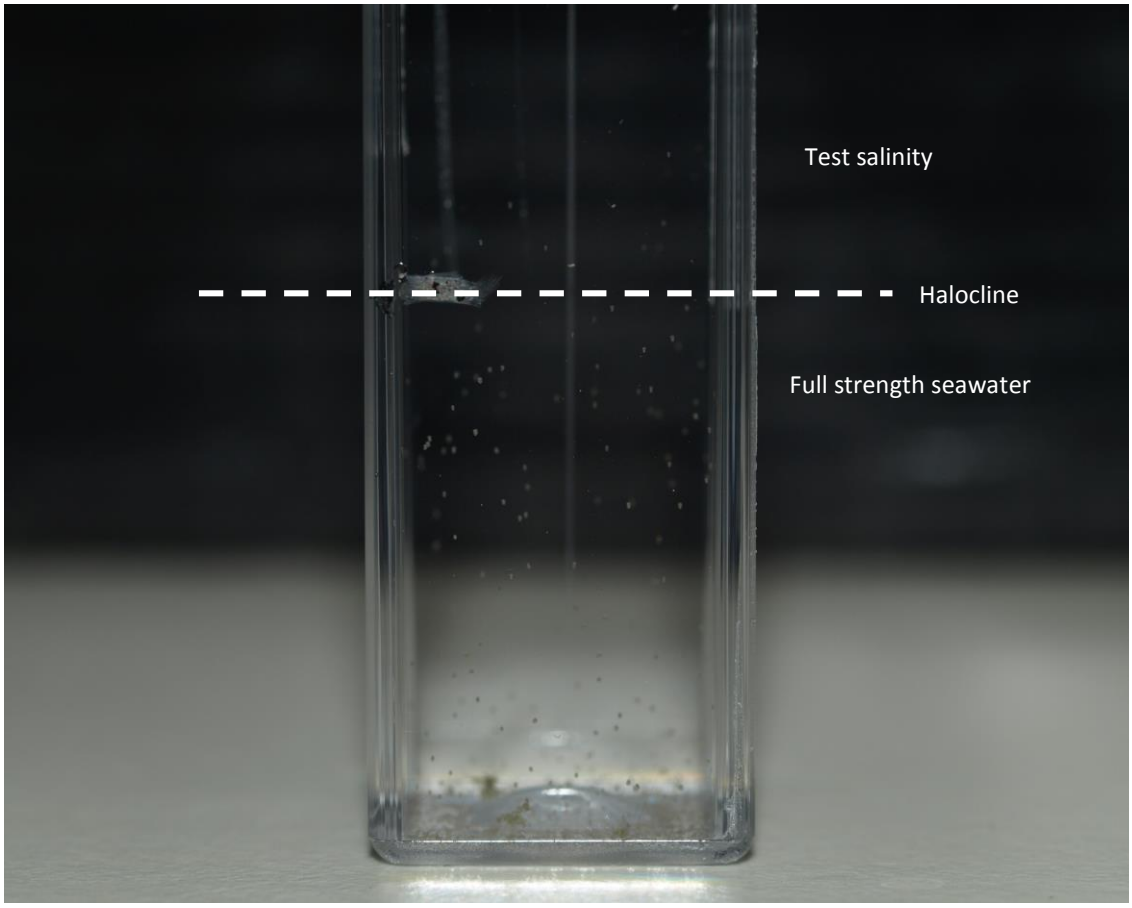

Figure 5.1: **Halocline experimental design, showing the approximate location of the halocline as a dotted line**. The salinity below the halocline was always full strength seawater and this is where the larvae were added. The small dots just visible in the photograph are Manila clam larvae. Photographs were taken using a digital SLR camera.

Once added to the cuvette, the larvae were allowed to acclimate for one hour, and then each cuvette was photographed using a digital camera with a macro lens. A drop of water from above and below the level of the halocline was sampled at the end of the experiment to ensure that no mixing of seawater had occurred and where mixing had occurred the cuvettes were discarded. The images obtained were then analysed for the numbers of larvae above and below the halocline.

5.2.3 Larval swimming velocity in response to salinity

The experiment to determine larval swimming velocity in response to salinity was carried out using a specially designed filming chamber shown in figure 5.2. The experiment was undertaken at Seasalter Hatcheries in Kent to maximise the health and vigour of the larvae tested. Larvae were sampled directly from the hatchery bins, which were maintained under optimised conditions for larval growth. All of the larvae used in this experiment were seven day old pediveliger larvae.

Larvae were removed from the culture bins at the Hatchery and concentrated in a 250ml glass beaker containing full strength seawater (34 psu). At the Hatchery the larvae were cultured at 27°C in full strength seawater (34psu) with a constant supply of food. The larvae were maintained at 27°C throughout the filming process to ensure that only the salinity was varied. The salinities that the larvae were exposed to in this experiment were: 5, 10, 14, 16, 18, 20, 22, 24, 26, 28, 30 and 34 psu. These salinities represented those which Manila clam larvae would be exposed to in the natural environment.

Actively swimming larvae were removed from the larval concentrate using a Pasteur pipette and added to a 4ml plastic cuvette fitted into the filming chamber. A total of three cuvettes were used for each salinity given a total of three replicates per salinity. To ensure that the experimental salinity was not changed by adding the larvae, the side of the pipette was struck to ensure the larvae sank to the bottom of the cuvette and were concentrated in a small volume of water. Each of the experimental salinities was replicated three times giving a total of 36 batches of larvae exposed to the 12 salinities.

Once the larvae had been added to the cuvettes in the filming chamber they were allowed to acclimate for 15 minutes before the filming started. A longer acclimation time would have been preferable however time constraints imposed by the conditions at the hatchery and the availability of larvae (all salinity exposures had to be carried out within 24 hours) meant that the acclimation period had to be restricted to 15 minutes. The acclimation time of 15 minutes had also been successfully used by Stanton (2012) using the same equipment and methodology.

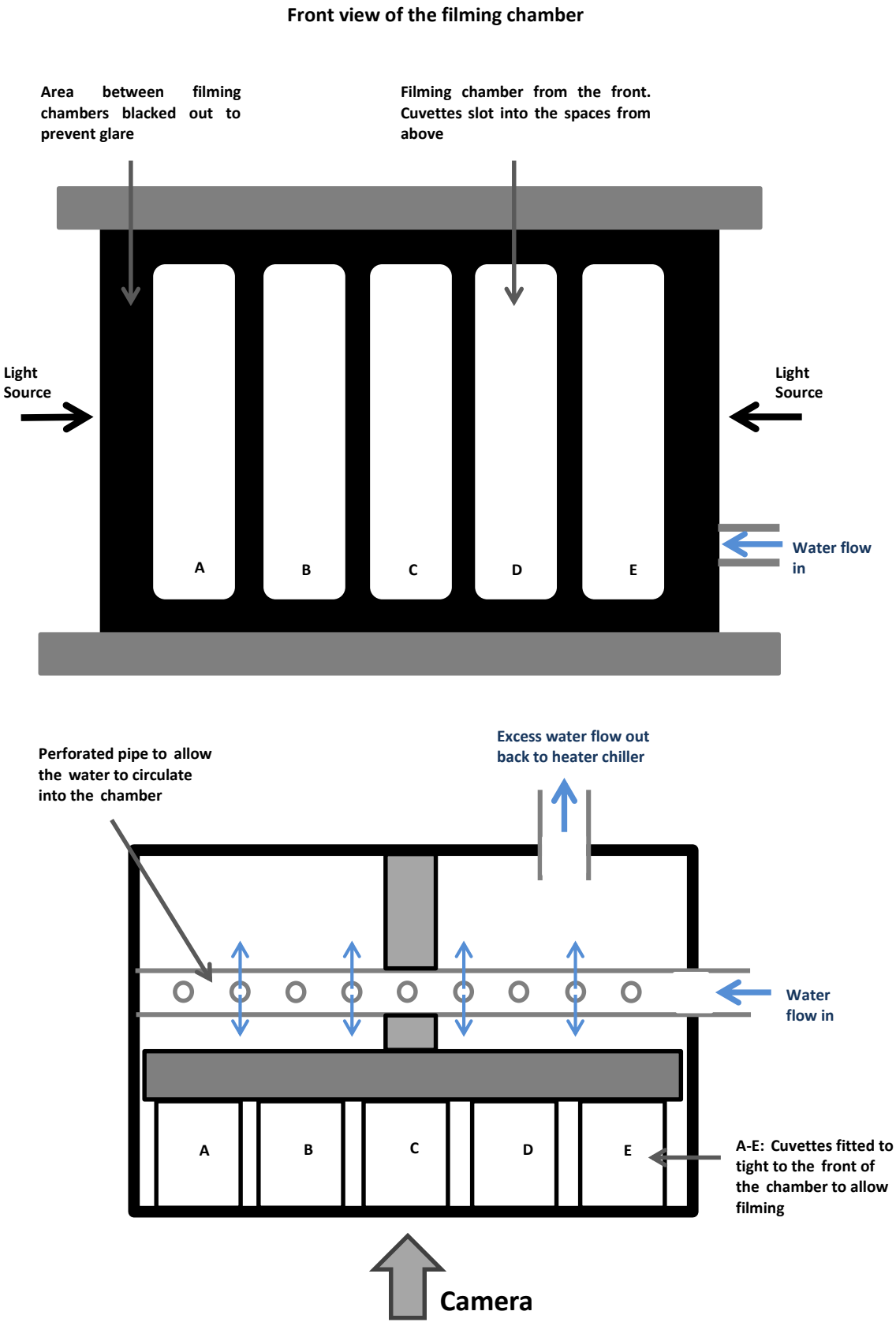

Figure 5.2: The experimental filming chamber used to film the larvae from above and from the front. The cuvettes were held flush against the front of the chamber by a holding bar, to prevent image distortion. The temperature was maintained by a constant flow of water in to the chamber, which insulated the cuvettes but did not mix with the water inside them.

The filming chamber was constructed from clear Perspex with five filming windows behind which 4ml plastic cuvettes containing the larvae could be slotted. The temperature in the cuvettes was kept constant throughout the experiment by a constant flow of water that was pumped from a heater-chiller unit in to the chamber around the cuvettes. A digital video camera was focussed in sequence on to each of the cuvettes separately.

The cuvettes were each filmed for a total of 60 seconds using a colour video camera with a macro lens connected to a Dazzle™ video creator. The Dazzle video creator was connected to a laptop which converted the images into a digital video. To prevent light direction being a factor affecting the larval vertical distribution, the chamber was illuminated from the sides with a cold light source. This was because some species of bivalves have larvae that react to light direction (Bayne, 1964).

The first stage in image analysis of the 60 second segments was to convert them from videos into individual frames, using Virtualdub™ a freeware program readily available on the internet. Each 60 second film segment was converted into 1499 frames with each frame being 0.04 seconds long.

To calculate the larval velocities, the freeware programme ImageJ™ with a particle tracking plug-in (also available as freeware) was used. The plug-in was a program that had been developed to track multiple particles from a digital image sequence. Due to the high processing power required to analyse the images, the films were analysed in 450 frame segments (the first and last 450 segments were used). The images were imported into ImageJ™ as image sequences and vertically stacked by frame number. The images were then turned into 8-bit greyscale images to reduce the memory required to process them. Once the images were converted to 8 bit greyscale, the threshold and colour balances of the frames were adjusted so that the larvae appeared as bright white dots. This was to aid the detection of the particles by the particle tracking software. Once the images had been converted, they were cropped so that only the cuvette was visible. This was then measured in pixels using the Analyze and Measure function in ImageJ. The width of the cuvette in each frame was 160 pixels. This measurement would later be used to calibrate the distance and velocity travelled by each larva.

Once the frames were prepared for analysis, the next step was to use the particle tracker plug-in. The particle tracker used three main parameters to detect and track particles and their trajectories, these were: the radius of the particle, the cut-off size of the particle and the percentile. The radius was the size of the particle detected, the higher the setting of the radius, the larger the particles the software would detect. The cut-off was used to exclude particles that are not in the size range required. The "percentile setting" adjusted the brightness of the particles that were detected, the higher the percentile the brighter the particle that would be selected.

After trial and error, the settings selected to detect the larvae were: Radius: 5, Cut-off: 0 and the percentile used was 0.500%. Once the particle tracking software had been run, the larval trajectories detected by the software were displayed as a series of coloured lines. These lines, when selected, gave the coordinates of the larvae at each frame in which the software was able to detect it. These coordinates were used for analysis. The software was able to discern between larval trajectories by taking the frame number into account and ensuring that only trajectories that appeared in adjacent frames were linked together. An example of the graphical representation of the trajectories can be seen in figure 5.3.

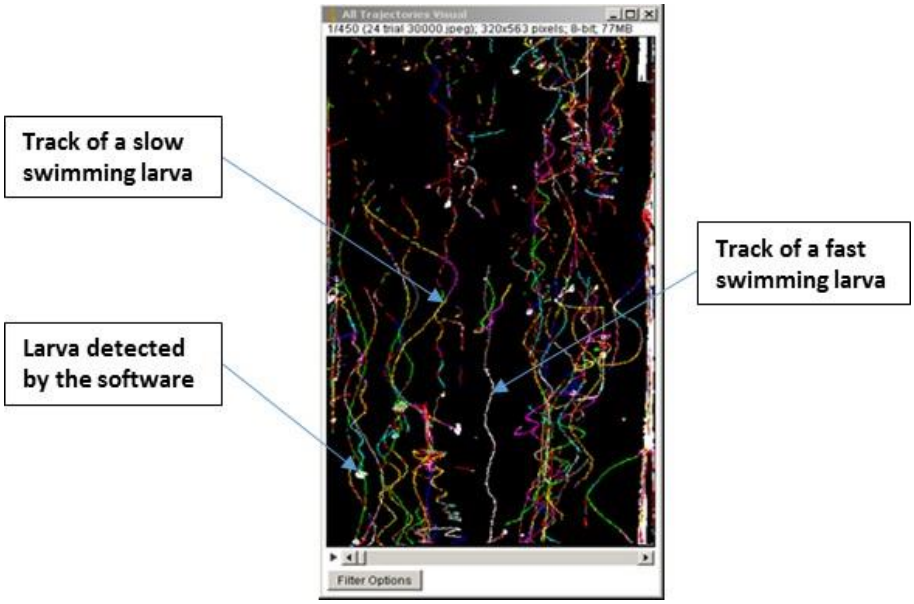

Figure 5.3: Particle tracking plug-in from ImageJ. The larvae appear as large white shapes, due to the process of converting the images to 8-bit greyscale. The different colour lines indicate individual trajectories of larvae that the software has detected. Slower swimming speeds were characterised by wide helical trajectories and faster speeds by tighter trajectories. Individual lines were selected and the coordinate information in each frame was extracted and used to determine the movement of the larvae between frames, which allowed for the calculation of larval velocity.

The coordinates of the tracked larvae in each frame were used to calculate the displacement of larvae between frames and hence the distance in pixels travelled. The software was only able to track the larvae in two dimensions (X and Y) due to the position of the camera and the limitations of the software. The fact that the larvae are only able to be tracked in two out of three dimensions will lead to a more conservative estimate of the larval velocity. The camera angle and setup used for the experiment was the optimum obtainable and had been used in previous experiments tracking bivalve larvae (Stanton, 2012). The displacement was calculated using the following equation (where X_1 is the x coordinate in the first frame and X_2 is the x coordinate in the second frame and Y_1 is the y coordinate in the first frame and Y_2 is the y coordinate in the second frame):

$$\sqrt{((X1 - X2)\text{^}2 + (Y1 - Y2)\text{^}2)}$$

The equation was squared and then square rooted to ensure that there was always a positive value for displacement. This was to prevent negative values for movement effecting the calculations for larval velocity. This was because downward movement could result in a negative value for movement.

The initial calculated values for displacement were in pixels, which then had to be converted into mm to determine velocity. The displacement was converted from pixels to mm by calibrating the images. The images were calibrated by using the width of the cuvette, this was because the width of the cuvette was known to be 9mm. Therefore the width of the cuvette in pixels equalled 9mm. Therefore if the cuvette was 160 pixels wide then the distance of each pixel in mm was calculated as follows: 9/160=0.05625mm. The total distance travelled by the larvae was determined by summing the frame to frame displacement values across the full sequence of images, summed across the period it was tracked. This summed distance was divided by the length of the image sequence in seconds in order to determine velocity.

Only larvae that were actively swimming in a helical motion were analysed for swimming velocity. Larvae that had closed the valves and were falling were not analysed for velocity.

Statistical analysis of results

All analysis was carried out using the statistical packages Minitab 16 and SPSS. Both packages were used, due to the different statistical tests both packages supported.

The data generated by the experiment investigating larval tolerances to salinity did not fit any of the models commonly used for statistical analysis. Consequently the response to salinity over time has been described in the text and the EC_{50} values at each time point, were estimated using the graphical method (EPA, 2002). Even though the model did not fit, the EC_{50} values generated by probit analysis were generated for comparison with the graphical method.

The experiment investigating the larval response to a halocline was statistically analysed using Logistic regression, with the presence of larvae above the halocline coded with a 1, and larvae below the halocline coded with a 0. The aggregation of larvae just below the halocline was also analysed using logistic regression, with larvae aggregating just below the halocline coded as 1 and all other larvae in the cuvette coded as 0. Binary logistic regression was unable to account for any variability between replicate cuvettes.

The larval swimming velocity response to salinity data was tested for normality using the Kolmogorov-Smirnoff test. The data was found to be non-normally distributed so was then $\log_{10}$ transformed. Only the velocities of actively swimming larvae were used for analysis. This was because at salinities at which larvae were actively swimming it was impossible to determine which proportion of the larvae were inactive, which meant that an average velocity for all of the larvae in the cuvette was impossible to calculate. Consequently only the velocities for actively swimming larvae were analysed using regression analysis.

5.3 Results

The results from the three experiments investigating the response of Manila clam larvae to a rage of salinities are presented in this section. The results are reported under the following headings: Larval tolerances to salinities, larval behaviour in response to a halocline and Manila clam larval swimming velocity in response to a range of salinities.

The larval swimming velocity experiment measured short term effects of reduced salinity upon Manila clam larvae, whilst the experiment investigating tolerances investigated longer term affects. These two experiments investigate both the behavioural and physiological response to reduced salinity exposure of Manila clam larvae.

5.3.1 Manila clam larval tolerances to salinity

Due to the fact that the data did not meet the assumptions required for statistical analysis the graphical method was used to determine the EC_{50} values at each of the time points during the course of the experiment. These EC_{50} values are plotted onto the graphs as green dots (See figure 5.4). For comparison, the EC_{50} values calculated by probit analysis are also plotted on the graph as red dots.

After one hour the salinity at which 50% of the larvae were inactive was 15.7psu, the value calculated by the graphical method and probit analysis is the same. After three hours the salinity that elicits an EC_{50} response reduces to 8psu, this value remains consistent through to 24 hours. The EC_{50} values calculated by the two methods (probit and graphical) generate similar results at one hour and then five and 24 hours, with a greater gap between the values at three hours exposure.

The general trend observed over the duration of the experiment was; an initial shock after one hour where levels of activity were low, followed by an acclimation period and then increased level of larval activity over all salinities. After five hours exposure the larval activity at salinities below 10psu began to decline, with activity at 5psu declining to low levels (below 10% activity) after 24 hours exposure.

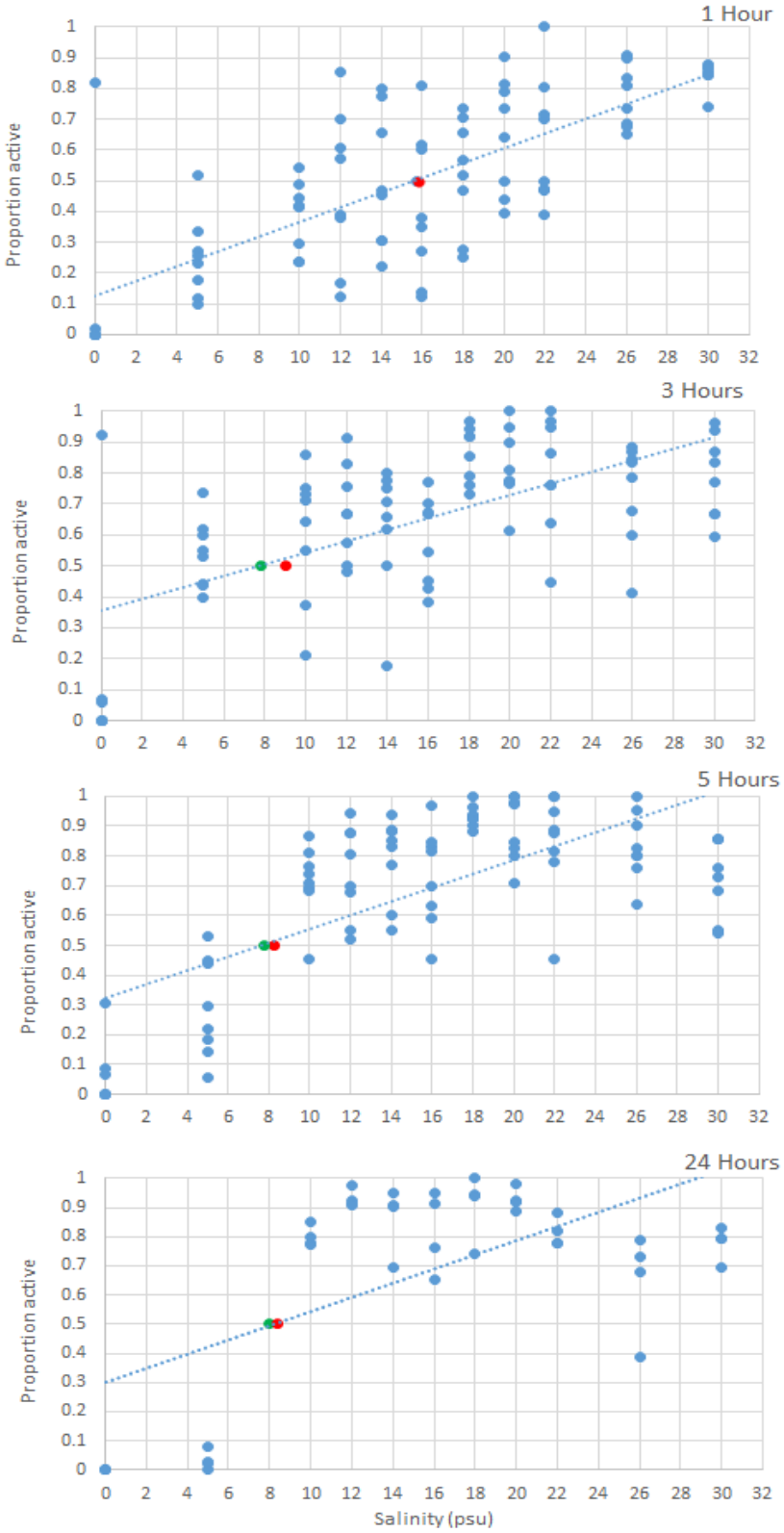

Figure 5.4: The effect of salinity upon the activity of Manila clam pediveliger larvae over time. (From one to five hours n=8, at 24 hours n=4) The green dots signify the EC_{50} value calculated by the graphical method and the red that calculated by probit analysis**.** The dotted line signifies the line of best fit.

The larval adaption to reduced salinities (0, 5 and 10psu) after the initial stress can be seen in figure 5.5. The larvae when initially exposed to salinities of 10psu and below did not show any signs of activity, but after one hour the larvae began to become more active across all of the salinities. Larvae exposed to salinities of 0 psu had the lowest proportion of active larvae, whilst those exposed to 10psu water had the highest activity. At salinities of 5psu and 0 psu, the number of active larvae was highest after three hours but began to decline after this time. By the end of the experiment all of the larvae were inactive or dead at 0 psu and 95% of the larvae were dead at 5psu. The larvae exposed to 10 psu seawater displayed an increase in activity, with the highest proportion of larvae active after 24 hours.

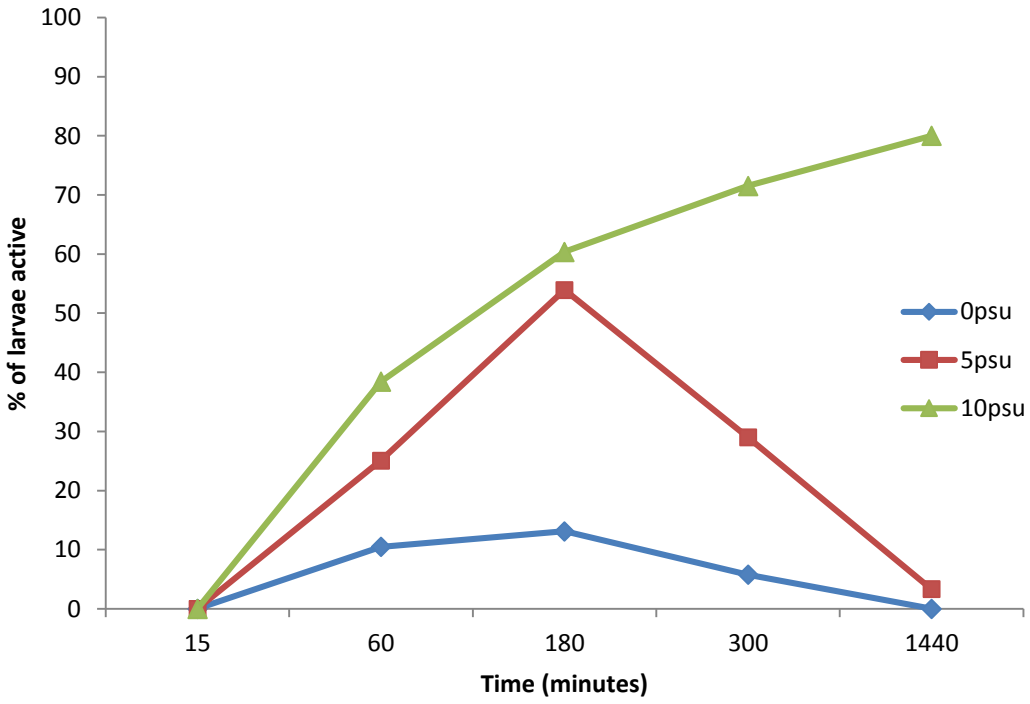

Figure 5.5: The response of larval Manila clams to three salinities: 0, 5 and 10psu over a period of 24 hours (mean, n=8). The proportion of the active at each of the three salinities over the duration of the experiment is displayed.

5.3.2 Manila clam larval response to a halocline

The salinity above the halocline had a significant effect upon the distribution of Manila clam in the cuvette when analysed using logistic regression analysis (See figure 5.6). The logistic regression model was statistically significant, (χ^2 (7) =487.4, p <0.05). The model explained 15.2% (Nagelkerke R^2) of the variance in larval position above the halocline and correctly classified 65% of cases. Larvae were 15.8 times more likely to be above the halocline when the salinity was 26 psu compared to when the value was 10 psu. As the salinity above the halocline increased from 10psu, the likelihood of larvae being above the halocline increased (1.74 times higher at 14psu, 3.6times at 15 psu, 7.7 times at 16 psu, 8.6 times at 18 psu, 10 times at 22 psu and 15.9 times at 26 psu). In the control (34psu throughout the cuvette) the larvae were evenly distributed throughout the whole cuvette, with 50% above and 50% below the point at which the halocline was created in the other cuvettes. The salinity into which the fewest larvae swam was 10psu, with 8% of the larvae actively swimming through the halocline. The salinity in to which the highest proportion of larvae swam was 26psu, with 56% of the larvae swimming through the halocline into this salinity. Binary logistic regression did not account for the variability in the behaviour of larvae between cuvettes with salinities between 14-22psu.

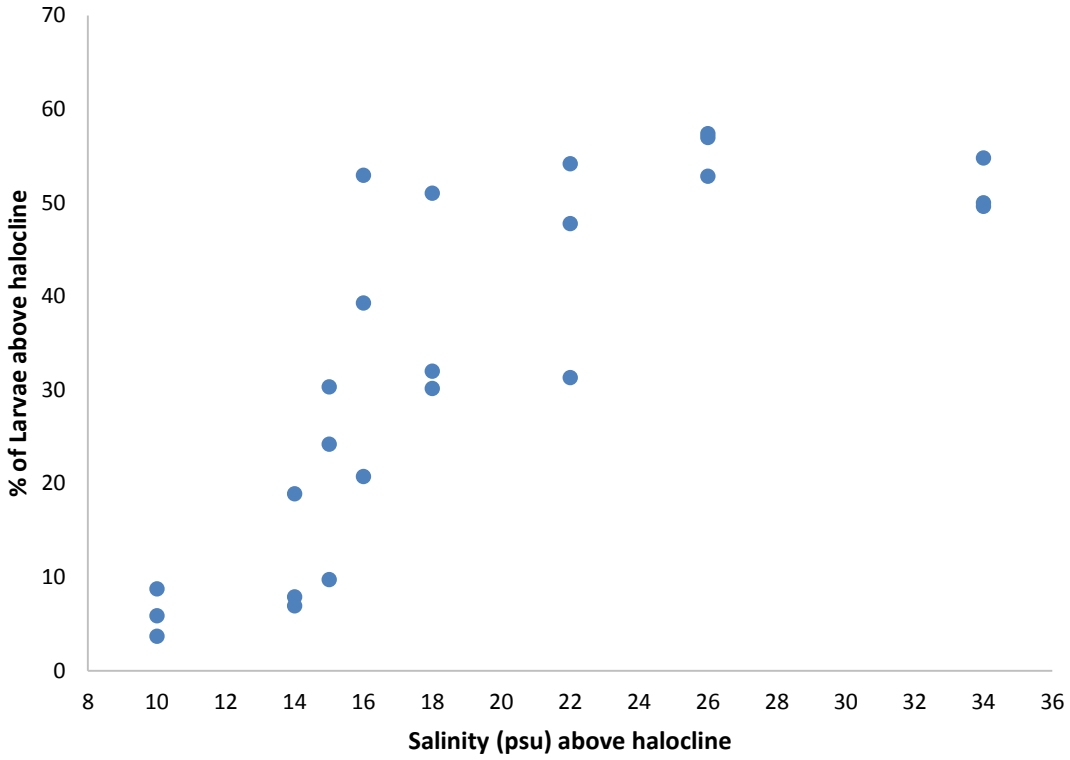

Figure 5.6: The dispersal of Manila clam larvae when presented with a halocline. The salinity above the halocline is displayed upon the x axis with the percentage of larvae that swam through the halocline on the Y axis.

As the differential between the salinities above and below the halocline decreased more of the larvae swam through the halocline into the lower salinity water. When the differential was large, a significant percentage of the larvae were observed to aggregate just below the level of the halocline (within 2mm of the halocline) (A logistic regression model was statistically significant, χ^2 (6) =219.843, p <0.05). The model explained 11.5% (Nagelkerke R^2) of the variance in larval position at the halocline and correctly classified 85.4% of cases:

Salinity above halocline (psu)	10	14	15	16	18	22	26
Percentage of larvae aggregating at Halocline	8.7	22.6	12.9	12.9	10.8	9.1	5.7

Once the difference between the two salinities began to decrease the likelihood of larvae aggregating at the halocline decreased. Larvae were 4.6 times more likely to aggregate below the halocline at 14 psu compared to at 10psu and half as likely to aggregate at the halocline at 26 psu, when compared to 10 psu.

5.3.3 Larval swimming velocity in response to salinity

The maximum average swimming velocity observed was 0.96mm per second at 34psu (with six larvae observed to be swimming). The lowest recorded swimming velocity was 0.58mm per second at 18psu. Below 18psu no larvae were observed to actively swim for the duration of the experiment (See figure 5.7a). The highest swimming velocity recorded during the experiment was 3.76mm per second at 28 psu (n=1) and the lowest was 0.08mm per second at 19spu (n=1).

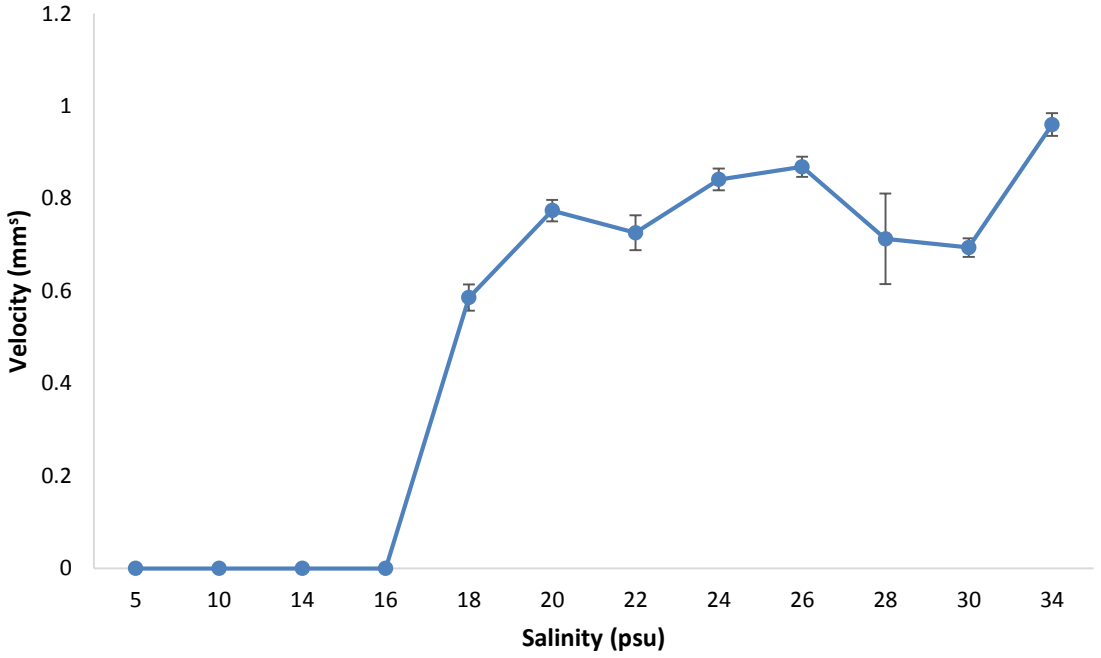

Figure 5.7a: The larval swimming velocity in response to a range of salinities (mean ± SE, n=3). The larvae were exposed to a range of salinities from 5-34 psu. The average swimming velocity after 15 minutes is displayed for each salinity.

Salinities at which no larvae actively swam were discarded from the statistical analysis. This was because only the velocities of actively swimming larvae were recorded, the number of larvae that did not swim were not recorded which made it impractical to compare salinities with active swimming larvae and those without active swimming larvae.

The velocity data for actively swimming larvae was first tested for normality and was found not to be normally distributed (Kolmogorov-Smirnoff test, DF (1,385), KS: 0.084, P value <0.05). The velocity data was first log transformed to normalise it and then analysed using regression analysis. The regression analysis detected no significant relationship between salinity and swimming velocity in actively swimming larvae (Regression analysis, DF (1, 385) F= 1.11, R^2=0.03, P value=0.293) (See figure 5.7b).

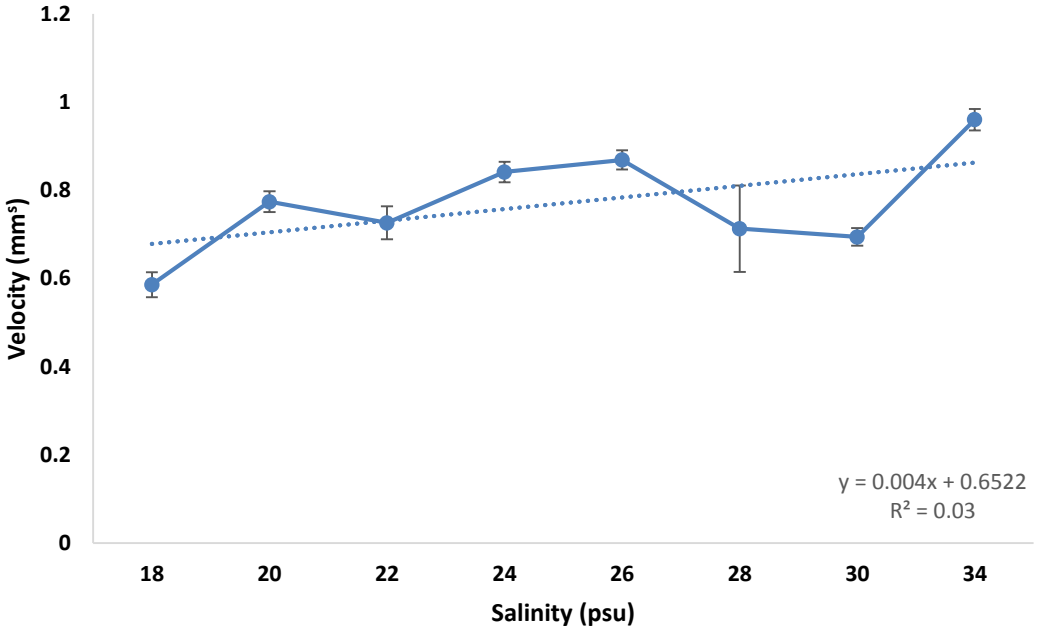

Figure 5.7b: The velocities of active swimming larvae only (Mean ± SE, n=3). No larvae were actively swimming below 18psu.

5.4 Discussion

This study aimed to determine both the short term (larval behavioural) and long term effects (physiological) of exposure of Manila clam larvae to reduced salinities through a series of experiments. The short term effects were the swimming velocities of the larvae, whereas the long term effects were the tolerances of larvae to reduced salinity over 24 hours. These short term and long term responses have been discussed separately in this chapter. This chapter also aimed to determine the behavioural response of Manila clam larvae, to the presence of a halocline. This is because larvae would be exposed to haloclines in the natural environment due to fact that Manila clams are often found in estuarine environments.

5.4.1 Manila clam larval tolerances to salinities

The data generated by the experiment investigating larval tolerance to salinity did not fit any of the statistical models commonly used to calculate EC_{50}s or LC_{50}s. As a consequence of this, the EC_{50} values were calculated using the graphical method. The EC_{50} values calculated using the graphical method were very close to those produced using probit analysis and those found in the literature. This study found that the salinity required for 50% of the larvae to become inactive within 24 hours was 8psu. This figure is comparable to that found by Tezuka *et al*. (2013), at 6.8 psu but higher than the figure of 2.5 psu suggested by Numaguchi (1998). The experimental conditions at which the larvae were kept prior to the trials would not influence the results as they were comparable with those used in the studies by Tezuka and Numaguchi. The fact that the EC_{50} value calculated by this study is higher than previously stated could potentially be down to the choice of salinities used in the experiment. A greater range of salinities investigated between 0 and 10psu may have resulted in a different EC_{50} value and the data fitting the models used for both probit and logistic regression. Unfortunately as only 0, 5 and 10psu were experimentally tested, conclusions must be drawn from the available data. It should be noted though, that there is variation between the EC_{50} values reported by both Tezuka and Numaguchi.

A potential reason behind the variation in the lower tolerance level could be the due to the larval stages used, or a variation in the tolerances of the parent populations of clams. The study by Tezuka (2013) used pediveliger larvae (as did this study) whereas Numaguchi (1998) used D larvae, which could explain why the results of this study matched that of Tezuka. There is evidence however, that Manila clams exhibit different tolerances to reduced salinities across clam populations (Liu et al., 2011). This suggests that within this species there are some populations that have become adapted to different conditions, or some selection pressures have led to different tolerance levels to salinity. This phenomenon of different populations of the same species having different tolerances to environmental variables has also been found in mussels as well. The population of *Mytilus edulis* on the Pacific coast of North America had a higher temperature tolerance range than the Atlantic population (Sorte et al., 2011).

It also raises the question of the origin of the Manila clams that were imported to the UK. Were they imported from a population of Manila clams that had adapted to an environment with a narrower salinity range? The larvae used in this experiment came from Seasalter Hatcheries in Kent, which are the main producers of Manila clam spat for the whole of the UK (John Bayes personal communication). The Hatchery received the brood stock to start producing Manila clam spat from MAFF, who originally imported Manila clams in to the UK from North America (Spencer et al., 1991). This could suggest that the tolerance of the larvae from the hatchery may be representative of the UK Manila clam population as a whole. This could also suggest that the UK populations of Manila clams may be descended from the same population that Tezuka et al. (2013) worked on. This theory could be tested by a genetic analysis of the populations of Manila clams across the UK to determine how closely related they are to each other and the Japanese population. Unfortunately this study did not have the scope to investigate this line of questioning, but it could prove important evidence to the origin of the Manila clam in the UK.

The lowest salinity at which there were low levels of larval mortality was 10psu (at this salinity 80% of the larvae were still active after 24 hours), the tolerance limit of adult Manila clams from North America is also 10psu (Elston et al., 2003). The fact that the lower tolerance limit for the larvae in the UK and adults in North America is the same is

unsurprising because the Manila clams imported by MAFF to create brood stock in the 1980s were imported from North America (Spencer et al., 1991).

The activity of the larvae when first exposed to reduced salinities appears to indicate a salinity shock that causes the larvae to suspend all activity. The larval activity remains low for the first hour, then the larvae begin to recover and the activity increases until after 24 hours at least 70% of the larvae are active at salinities above 10psu. At salinities of 5psu and below there are some noteworthy results. The initial activity at these salinities is very low, with a peak of activity after three hours which then reduces until all the larvae were dead after 24 hours. This suggests that there may be an interaction salinity and exposure time in influencing larval behaviour. This interaction was captured by the statistical analysis. This response appears to indicate that Manila clam larvae can adapt to reduced salinities for short periods of time before mortality occurs. This response of an initial shock (where all larval activity is suspended), followed by an increase in activity before mortality occurs has not been previously reported for the Manila clam or any other species of bivalve.

This behaviour suggests that the larval response to sub optimal conditions is to remain closed for a short period of time and then begin to become active again if the conditions do not improve. This response to reduced salinity by Manila clam larvae raises the question of whether similar behaviour is exhibited in other species of bivalve as well or of it is unique to the Manila clam.

Further experimentation with larvae from other species of bivalves is necessary in order to determine whether this behaviour is universal among bivalves or not. Further work could also be undertaken to determine the EC_{50} values for the different larval stages of the Manila clam. Experiments should be undertaken using all the larval stages, from the D larvae to the pediveliger stage as only pediveliger larvae were studied and these as previously mentioned have different salinity tolerances to D larvae. By using all of the larval stages, a more accurate picture can be drawn regarding tolerances. Future experiments should also investigate the effect of a greater range of salinities between 0 and 10psu. This may allow a more accurate assessment of the salinity required to cause 50% of the larvae to become inactive.

The calculation of the lower tolerance limits are also important in allowing for the prediction of future colonisations of sites by this species. By understanding the lower tolerance limits for larvae areas that do not meet these minimum requirements can be assumed as being unsuitable for colonisation. This allows for a more focussed approach for selecting survey locations in order to determine the spread of this species.

5.4.2 Larval behaviour in response to a halocline

It should be noted that as pediveliger larvae were used in this study, any conclusions drawn should only be applied to the pediveliger stage, as the different larval stages have different responses to salinity. The statistical analysis did not account for any variation between cuvettes. This was because when treating each cuvette as a replicate (with the number of larvae above the halocline treated as an event, and the total number of larvae as the number of trials), the data did not fit the model. When the response of each individual larvae was analysed, rather than the response in each cuvette, the model fitted well, so this method of analysis was used. It should also be noted that the number of larvae in each cuvette may not have an effect upon the larval response. This is because when the raw data was scrutinised, the cuvette with the highest number of larvae in it (Cuvette 1 at 16psu) was not an outlier and 39% of the larvae swam into the lower salinity water. If crowding had an effect upon larval behaviour it could be argued that the cuvettes with the most larvae in them would result in lower numbers swimming through the halocline. This is because high densities of larvae could result in disturbances to the water column, which could elicit a fright response where the larvae close the valves and sink rapidly (Stanton, 2012). As this did not occur it could suggest that the density of larvae in each cuvette did not affect larval behaviour.

The larvae used in this study exhibited a response to the presence of a halocline with a strong salinity differential by aggregating just below the halocline. When there was a weak salinity differential or no differential at all the larvae became evenly distributed across the whole of the cuvette. The same behaviour has also been observed in other species of bivalve, notably oysters (Dekshenieks et al., 1996), Mussels (Sameoto &

Metaxas, 2008) and *M. mercenaria* (Davis, 1958). When presented with salinities outside of the optimum range for that species, the larvae remained in water that was within the optimum range. This could suggest that larvae are able to detect salinities and will avoid those that are detrimental to survival.

By aggregating below the level of the halocline, larvae are ensuring they have the greatest chance of dispersal by being as high in the water column as tolerable (Dekshenieks et al., 1996), but are below salinities that will place them under physiological stress. Manila clam larvae can tolerate salinities as low as 12 psu (Numaguchi, 1998), which could suggest that the reason the larvae did not swim through the haloclines at the lower salinities was due to detecting sub-optimum conditions for survival. This has also been observed for other species such as *M. edulis* (Sameoto & Metaxas, 2008).

When the salinity above the halocline was 22psu, approximately 45% of the larvae actively swam into the lower salinity water. This is noteworthy as in Tokyo Bay in Japan, larvae were observed to aggregate in seawater with a salinity of 30psu, below a halocline created by a flood event (Kasuya et al., 2004). This could suggest that Manila clams will actively swim through a halocline into lower salinities than previously reported. Manila clams are not the only species of bivalves that have been observed to swim through a halocline into an area of reduced salinity, *Crassostrea virginica* (North et al., 2008) and *M. mercenaria* (Davis, 1958) have also been observed to swim through a halocline into reduced salinity seawater. It has been theorised that swimming upwards into these lower salinities may aid in dispersal due to the fact that these lower salinities are higher up in the water column (Dekshenieks et al., 1996; North et al., 2008). The velocity of water increases with height within the water column, which means that by positioning themselves higher in the water column the larvae could be transported further (North et al., 2008). This could suggest that Manila clams are swimming in to these salinities in order to maximise their dispersal potential.

Another factor could be that the optimum range for larval growth in Manila clams is 20-30psu (Numaguchi, 1998), which means that seawater within this range could elicit a response from the larvae in order to maximise growth and survival. Reduced salinities are a settlement cue for Manila clam larvae, with increasing settlement rates until

seawater reaches 13psu (Tezuka et al., 2013). This could indicate that the larvae were actively swimming into the reduced salinities as a response to suitable environmental conditions for growth and survival.

5.4.3 Larval swimming velocity in response to salinity

The fact that the software was only able to track the larvae in two dimensions rather than three, may have had an influence on the results, however this was unavoidable due to the constraints of the experimental set up. A set up with two cameras, one facing the side of the cuvette and another directly above the cuvette may allow for the larvae to be filmed in all three dimensions. This would pose additional problems in that larvae would have to be entered into the cuvettes individually, both cameras would have to be controlled simultaneously and new software would have to be written in order to combine the two sets of film and analyse it accurately. The method of recording the larval displacement across two dimensions used in this thesis was the most practical method available and has been used in other studies (Ishida et al., 2005; Stanton, 2012).

The maximum swimming velocity of 3.76mm per second is similar to that reported by Ishida et al., (2005) at 3.3mm per second and comparable for other species of bivalve including *Pecten maximus* , 2.2mm per second (Cragg, 1980) and *Crassostrea virginica*, 3mm per second, (North et al., 2008). The study by Ishida (2005) calculated the swimming velocities for larvae between 8 and 16 days old, and found that velocity increased with larval age. Ishida (2005) did not investigate the influence of salinity on swimming velocity however, which makes the results of this investigation important. The fact that swimming velocity increases with larval age, suggests that the experiments carried out in this thesis should be repeated with different larval stages. It also means that the conclusions drawn in this chapter should only be applied to pediveliger larvae, different larval stages may respond differently to salinity.

The maximum velocity recorded (3.76mm per second) would be fast enough to allow Manila clam pediveliger larvae to take advantage of tidal transport systems by adjusting their position in the water column (Hidu & Haskin, 1978; Wood & Hargis, 1971). More

evidence for this theory comes from a study by North *et al*. (2008), who modelled the dispersal of *Crassostrea virginica* in Chesapeake Bay using hydrodynamic modelling and larval swimming speeds of between 0 and 3mm per second. North (2008) found that these swimming velocities had significant influences on dispersal, so it can be argued that the velocities calculated in this study were sufficient for larvae to influence dispersal. The fact that the larvae were swimming at velocities well below the maximum they were capable of, could be because the larvae were trying to maintain their position in the water column. An alternative explanation could be that these salinities had a physiological effect upon the swimming behaviour of the larvae, however this is unlikely as the reduced velocities all occurred at salinities that have been classed as optimal for this species (18-34)(Numaguchi, 1998). Reduced swimming velocities in oyster species have been observed to coincide with maintaining a position in suitable conditions (Hidu & Haskin, 1978).

The reason that no larvae were observed to swim below 18psu, could be because it is outside of the optimum range for this species of 18-32 psu (Numaguchi, 1998).The larvae were not dead, as the tolerance experiment indicated that larvae can survive for up to 24 hours at 10psu with low mortality. The acclimation period of 15 minutes could have influenced the larval response at the lower salinities and a longer acclimation period may have yielded a different response, however experimental constraints dictated that the acclimation period be a maximum of 15 minutes. The fact that larvae were actively swimming at salinities of 18 psu and above however, could suggest that the acclimation period was sufficient and that the larvae were not swimming because they were responding to the sub-optimal conditions rather than being physiologically shocked. The response after 15 minutes at the lower salinities could also indicate that the response of Manila clam larvae to sudden reductions in salinity is to suspend swimming altogether.

A similar behaviour of not swimming when presented with suboptimum conditions has also been reported in oyster larvae. When oyster larvae were exposed to traces of formalin, they closed their valves and sank (Hidu & Haskin, 1978). This could suggest that the closing of the shell by bivalve larvae is a response to suboptimal conditions. By closing the shell the larvae are ensuring that they are not exposed to the suboptimal

conditions. There is more evidence to suggest that this is a behavioural response, rather than a physiological one, if the lower salinity tolerance limit is considered. Manila clam larvae are able to tolerate salinities as low as 12 psu (Katsuyuki Numaguchi, 1998) and the tolerance experiment reported in this chapter also suggests that larvae can survive exposure for a period of up to 24 hours at salinities as low as 10psu with low mortality. The halocline experiment also demonstrated that larvae will actively swim in to salinities as low as 14psu disproving the argument that the larvae are unable to swim at salinities below 18psu.

The lack of a relationship between salinity and the swimming velocity of actively swimming larvae could be because all of the salinities in which the Manila clam larvae were actively swimming were all within the optimum range for this species (Katsuyuki Numaguchi, 1998). Larvae were also swimming at a slower velocity than they were capable of so this suggests larvae were maintaining position within these conditions as opposed to trying to avoid them. This could suggest that the larval response to suboptimum conditions is to suspend swimming altogether rather than increase or decrease swimming velocity (Hidu &Haskin, 1978).

5.4.4 General conclusions

Different native populations of adult Manila clams have different tolerances to reduced salinities (Numaguchi, 1998; Tezuka et al., 2013) which may be a result of population diversity across their native range. The lower salinity tolerance level for Manila clam larvae cultured in the UK is the same as that of the population of Manila clams in the USA (Elston et al., 2003). This could be due to the fact that the brood stock for the UK population was imported from North America (Spencer et al., 1991). The populations of Manila clams in the UK may have a reduced salinity tolerance range compared to the native populations. This may have led to a reduction in genetic diversity in the UK population and subsequently a reduction in salinity tolerance.

Salinity did not influence swimming velocity, but did influence whether larvae actively swam up in to the water column or remained on the bottom of the cuvette with closed

valves. This suggests a behavioural response to sub optimal salinities. Oyster larvae have been observed to sink to the bottom in estuarine environments during the ebb tide and swim upwards during the flood tide (Wood & Hargis, 1971). This allows the larvae to be retained in the estuary system and not be washed out to sea. A similar behavioural response may therefore be present in Manila clam larvae.

Manila clam larvae have exhibited behavioural responses to salinity in order to maximise dispersal and future settlement success, by adjusting swimming behaviour to different conditions (Tezuka et al., 2013). Larvae actively swam into reduced salinities, in order to ensure that larvae remain in conditions suitable for adult survival and growth. This could be because adult Manila clams are often found in estuarine environments (Cigarria & Fernandez, 2000) which are characterised by reduced salinities (McLellan, 1965). The two populations of Manila clams studied in chapter 4 of this thesis; Holton Mere (intertidal and characterised by low salinity) and the Lease bed population (subtidal, characterised by fully marine conditions) exhibited different condition indices, with the subtidal population having a consistently higher condition index than the intertidal population. This could suggest that the targeting of reduced salinities by larvae may in fact be driven by an increased chance of survival of juveniles in the intertidal zone compared to the subtidal due to reduced levels of predation. An example of this is the bivalve *Macoma balthica* in the Dutch Wadden Sea. Juvenile *Macoma* are often found in the high intertidal zone where predation is reduced, before moving to the low intertidal zone once they have reached sufficient size to reduce the chance of being predated upon (J. Beukema, 1993; Bouma et al., 2001).

The swimming velocities data generated by this study can be used in hydrodynamic modelling to help predict the future spread of the Manila clam around the coast of southern England. The acclimation time of 15 minutes used in this experiment should not be an issue as larvae were actively swimming at salinities of 18psu and above. If no larvae had been swimming at any of the salinities after 15 minutes then the data would prove unreliable, however the response of the larvae to suspend swimming at salinities below 18psu should be classed as a response to these salinities. The larval swimming velocities and suspension of swimming below 18psu could be used as a parameter in models used to determine potential dispersal of this species. Future models used to

determine the spread of Manila clams in the UK should use the data generated by this thesis to characterise larval behaviour due to the fact that the work by Ishida (2005) calculated the maximum velocities at larval age as opposed to larval swimming velocity in response to salinity.

Finding and settling in a suitable habitat is not the only problem that larval bivalves encounter. Once the larvae have settled and metamorphosed into the adult stage, bivalves become vulnerable to predation by benthic predators (Hiddink et al., 2002; Hunt & Scheibling, 1997; van der Veer et al., 1998). Predation on these newly settled bivalves can have a large impact on survival and ultimately recruitment success (Hiddink et al., 2002; Hunt & Scheibling, 1997).

Chapter 6: Interactions between predators and the Manila clam and predator avoidance strategies

6.1 Introduction

Once Manila clam larvae have located a suitable habitat and metamorphosed into the juvenile stage, they become susceptible to pressure from benthic predators, such as crustaceans and fish species. High levels of predation on newly settled bivalves have the potential to adversely affect recruitment events and subsequently alter the dynamics of future generations. This highlights the importance of understanding how predator prey interactions occur.

Predation on bivalves

Bivalves are eaten by a wide range of organisms including: polychaete worms (Ambrose , 1984), shrimps (Cardoso et al., 2009; Oh et al., 2001), crabs (Mistri, 2004), fish (McArthur, 1998), terrestrial mammals (Carlton & Hodder, 2003) and birds (Caldow et al., 2007; Zach, 1979). As bivalves increase in size, the predators that feed upon them change, from polychaete larvae that feed upon the larval stages (Johnson & Brink, 1998), to wading birds feeding upon the adult bivalves (O'Brien et al., 2005; Ward, 1991).

Bivalve larvae are subject to predation from a wide range of both pelagic and benthic predators. The planktonic larval stages of bivalves are subject to selective predation by larval polychaete worm (Johnson & Brink, 1998) and fish species (Esteves et al., 2000). Small fish species such as *Gasterosteus aculeatus* and the scyphozoan *Cyana capillata* are also known predators of bivalve veliger larvae (Short et al., 2013). Bivalve larvae are also subject to predation by benthic invertebrates including adult bivalves (Lehane & Davenport, 2004; Troost et al., 2008). Adult bivalves have been observed to filter out bivalve larvae from the water column with the same efficiency as algal cells (Troost et al., 2008). This implies that adult bivalves may be major predators of bivalve larvae. Once larvae have settled out from the water column and metamorphosed into juveniles, they become vulnerable to a new suite of predators.

Epibenthic predators such as crustaceans have a large impact on the success of recruitment events (Hiddink et al., 2002). The brown shrimp *Crangon crangon* feeds upon juvenile bivalves such as *Macoma balthica, Mya arenaria* and *Cerastoderma edule* (Andresen & van der Meer, 2010; Beukema & Dekker, 2005; Cardoso et al., 2009; Hiddink et al., 2002; Oh et al., 2001). In the Dutch Wadden Sea, predation by *Crangon* has accounted for high levels of mortality in juvenile bivalves (van der Veer et al., 1998). The gut contents of *Crangon* from the Dutch Wadden Sea regularly contain juvenile bivalve shell fragments, and bivalves make up a large proportion of their diet (Hiddink et al., 2002; van der Veer et al., 1998). Due to their small size, *Crangon* are only able to prey on bivalves up to 2.5mm in shell length but preferentially feed upon bivalves under 0.5mm in shell length (Hiddink et al., 2002). High densities of *Crangon* have been found to negatively affect recruitment in bivalve species (Beukema & Dekker, 2005; Hiddink et al., 2002).

Crabs are also major predator of bivalves and can be pest species on commercial clam beds (Cigarria & Fernandez, 2000). The predation rates on commercial beds can be high enough to warrant that methods such as using plastic netting have been developed in order to prevent the crabs from getting access to the bivalves (Munroe & McKinley, 2007a, 2007b; Spencer et al., 1992). The success of crabs, and in particular the European shore crab, *Carcinus maenas*, as predatory species can be put down to its versatility in both detecting and consuming prey. *Carcinus maenas* is able to detect prey items by using both chemical and tactile stimuli (Elner & Hughes, 1978). Once a bivalve has been detected the crab then determines the most suitable method in order to access the flesh inside (Ameyaw-Akumfi & Hughes, 1987; Elner & Hughes, 1978). Smaller prey items are broken open using the crusher claw, whilst larger prey items are broken open by the crab first chipping away at the shell by the ligament to create a hole. Once a hole is created, the crab inserts the tip of the crusher claw and carries out a scissoring motion to weaken the muscles holding to the shell together. Once the muscles are weakened, the tip of the second claw is inserted and the crab then uses its strength to pry the bivalve apart before consuming it (Ameyaw-Akumfi & Hughes, 1987; Elner & Hughes, 1978). Smaller prey items are preferred by shore crabs to larger prey items due to the handling time, with larger items are discarded in favour of smaller prey (Ameyaw-Akumfi & Hughes, 1987; Elner & Hughes, 1978). As with shrimp, there is a size refuge in which

bivalves are safe from predation by crabs. A minimum shell length of 25mm has been suggested as a size at which clams are no longer viable prey items for crabs due to increased handling time (Cigarria & Fernandez, 2000; Mistri, 2004).

Once bivalves reach a size refuge to avoid predation by crabs, they then become vulnerable to predation by a range of bird species, including: oystercatchers (Caldow et al., 2007; Meire et al.,1994), gulls (Ward, 1991), crows (O'Brien et al., 2005) and sea ducks (Lewis et al., 2007). In British Columbia, bivalves make up a large proportion of the diet of overwintering birds, and the bivalve population can be seriously depleted by these predators (Lewis et al., 2007). Different species of bird often utilise different methods in order to open the shell to access the flesh inside. These methods vary, from using the beak to open the shells by oystercatchers (Caldow et al., 2007) to dropping whole clams on to hard substrates by crows and gulls (O'Brien et al., 2005; Rutten et al., 2006; Zach, 1979).

Birds are not the only non-aquatic organisms known to feed upon bivalves, terrestrial mammals have been reported to actively predate upon bivalves in a number of regions around the world (Carlton & Hodder, 2003). A review of the literature by Carlton and Hodder (2003) described a diverse range of mammals from a wide range of locations feeding upon bivalves. These species included: baboons (*Papio ursinus*) from South Africa, rats (*Rattus rattus*) in Chile, Italy and Norway, coyotes (*Canis latrans*) in Mexico, arctic foxes (*Alopex lagopus*) in Greenland, black (*Ursus americanus*) and grizzly bears (*Ursus arctos*) in North America, racoons in North America and the domestic pig (*Sus scrofa*) in North America (Carlton & Hodder, 2003). These mammals have been observed to actively feed upon bivalves, but this is not to say that other species do not also feed upon bivalves.

Predator avoidance strategies of infaunal bivalves

In order to reduce the risk of predation, many species of bivalves have developed the ability to detect the presence of a predator. The predominant method is detecting chemical cues in the water released by predators and injured prey items (Freeman, 2007; Griffiths & Richardson, 2006; Romano et al., 2011; Smee & Weissburg, 2006), however some species also detect changes in light intensity and vibrations (Wilson et

al., 2012). The responses of bivalves to the presence of a predator are diverse and often depend upon the habitat they occupy.

Epifaunal species such as mussels have developed many strategies to reduce the impact of predation including; aggregating in large numbers (Reimer & Tedengren, 1997; Wilson et al., 2012), thickening of the byssus (Cheung et al., 2004; Reimer & Tedengren, 1997) and increasing the shell thickness (Freeman, 2007; Smith & Jennings, 2000). Infaunal bivalves have developed several strategies in order to reduce the risk of predation. Closing of the valves is used by many species including *Mercenaria* to reduce the risk or predation, however using this method combined with prolonged exposure to predators can influence the growth and survivorship of this species (Nakaoka, 2000; Smee & Weissburg, 2006). Burial and burial depth is another method reported to increase the chances of avoiding predators and reduce the level of predation (Zwarts & Wanink, 1989). The depth to which an individual is buried influences the time required by predators to excavate it and increases the chances of it being discarded (Haddon et al., 1987; Zwarts & Wanink, 1993).

Under experimental conditions, the bivalve *Macoma balthica* was observed to burrow deeper into the sediment when exposed to seawater in which crabs had been feeding (Griffiths & Richardson, 2006). Increased burial depth reduced the potential of the clam becoming a prey item for the crab (Griffiths & Richardson, 2006). The burial response of *Macoma balthica,* differed from that of the common cockle, which when exposed to crabs orientated the strongest part of the shell towards the threat. This was thought to deter predators by increasing handling time (Romano et al., 2011).

The length, when extended of a bivalves siphons is known to influence the strategy used by that species to avoid predation. The length of the siphons is also a limiting factor in suspension feeding bivalves in to how deep they can burrow (Zwarts & Wanink, 1989). Organisms with longer siphons can bury deeper than those with shorter siphons. Longer siphons are able to extend further into the water column and subsequently have more access to food and oxygenated water. Some species vary their depth in the sediment in relation to food availability (Lardies et al., 2001; Zwarts & Wanink, 1989). Burial depth can reduce the growth of some suspension feeding bivalve species, due to limited food availability because the siphons are at the limit of their range (de Goeij & Luttikhuizen,

1998). This often leads to a compromise in burial depth and safety in order to optimise the access food availability (de Goeij & Luttikhuizen, 1998).

The Manila clam, interactions with predators and burial behaviour

In its native distribution, the Manila clam is an important prey item for a variety of species including: gastropods (Hasegawa & Sato, 2009), starfish (Ponurovsky & Selin, 1988), octopus (Ebisawa et al., 2011) and fish (CABI 2014). Its introduction to new geographical regions around the world has led to it becoming an important prey item for many species that it had not previously encountered.

In European waters the Manila clam is preyed upon by: the European shore crab *Carcinus maenas* (Spencer et al., 1992), the Mediterranean shore crab *Carcinus aestuarii* (Mistri, 2004), oystercatchers *Haematopus ostralegus* (Caldow et al., 2007; Cigarria & Fernandez, 2000), trigger fish *Balistes capricus* (Cigarria & Fernandez, 2000), and gilt head bream *Sparus auratus* (Cigarria & Fernandez, 2000).

Crabs are a predator of spat-sized clams but are also able to consume larger individuals (Mistri, 2004; Spencer et al., 1992). The Mediterranean shore crab, *Carcinus aestuarii*, is able to predate upon Manila clams with shell lengths up to 25mm. Above this size the shells become too hard to break open (Mistri, 2004). The European shore crab, *Carcinus maenas* is able to predate upon larger clams, up 30-40mm in shell length (Spencer et al., 1992). Burial by Manila clams reduces the levels of predation by crabs (Dudas et al., 2005; Mistri, 2004). When both Manila clams and varnish clams were prevented from burying, the red rock crab *Cancer productus* preferentially preyed upon varnish clams *Nuttallia obscurata* instead of Manila clams (Dudas et al., 2005). Varnish clams were consumed due to the ease of breaking open the shell as opposed to that of Manila clams (Dudas et al., 2005). When allowed to bury however, Manila clams were favoured due to the shallower burial depth than varnish clams and were consequently consumed at a higher rate (Dudas et al., 2005).

Substrate type influences survival rates, with clams that have burrowed in sand-gravel beds having a higher survival rate than those buried in mud, due to increased substrate stability and reduced predator foraging efficiency (Cigarria & Fernandez, 2000). This is

not due to reducing burial ability as sediment with a grain size of up to 2mm in diameter has no effect upon burial behaviour in adult Manila clams (Shin et al., 2002).

Substrate type is not the only factor that can influence survival in Manila clams. Low salinities can inhibit burial and reduce filtration rates in adult Manila clams. At salinities below 10.8psu, Manila clams are no longer able to filter or actively bury (Nakamura et al., 2005). The reduced filtration and reduced ability to bury, will influence the survival rate due to exposure to predation. The burial response of Manila clams to reduced salinity was derived from experimenting with adult clams that had a shell length larger than 30mm. This chapter aimed to determine the burial response in relation to salinity in juvenile Manila clams.

Salinity is not the only factor that can influence burial behaviour and potentially survival. Water-borne pollutants and sediment contaminants, can delay, modify or stop burial completely in the Manila clam (Matozzo et al., 2004; Shin et al., 2002). Interestingly Manila clams infected with *Perkinsus* do not show any differences in their burial time compared to uninfected clams (Yoshinaga et al., 2010).

In areas where they have been introduced, Manila clams have become an important part of the diet of wading birds, especially in North America (Lewis et al., 2007; O'Brien et al., 2005) and the UK (Caldow et al., 2007). In Baynes Sound in British Columbia they accounted for 52% of the total dry faecal mass from surf scoters *Melanitta perspicillata* (Lewis et al., 2007). No comparable studies have been undertaken in the UK. However mathematical modelling has predicted that the introduction of the Manila clam to Poole Harbour would reduce the winter mortality of overwintering birds (Caldow et al., 2007).

Potential predators of the Manila clam in Poole Harbour and the Solent region

In the UK the Manila clam is only likely to be subject to predation by aquatic and avian predators, not including man. Of all the terrestrial mammalian predators that are currently known to predate upon bivalves, only the black rat and the more common brown rat are likely to encounter Manila clams in the UK. As currently there is no evidence of this species feeding upon the Manila clam in the UK, the focus of this study will be on aquatic predators in particular *Carcinus maenas*.

The Manila clam population in Poole Harbour is subject to fishery pressure from both licensed and unlicensed fisherman (Jensen et al., 2004). The clams are removed from the sediment using a pump scoop dredge that liquefies the mud and collects any bivalves in the dredge (Jensen et al., 2004). Any clams that are below the legal minimum size limit of 35mm are returned to the water. Because clams under 35mm are returned, it raises the question of whether these clams are safe from predation once returned.

In order to reduce the chances of being eaten, the clams have to burrow into the sediment (Mistri, 2004). Burial behaviour in the common cockle (Romano et al., 2011) and *Macoma balthica* (Griffiths & Richardson, 2006) is altered by presence of a predator, but it is not known if the presence of a predator influences burial in the Manila clam. This study set out to determine whether the presence of a predator influenced burial.

Spencer *et al* (1992) investigating the size at which Manila clams were no longer prey items for the European shore crab, used *C. maenas* specimens with a maximum carapace width of 70mm. The clam population at Holton Mere in Poole Harbour is unlikely to be exposed to crabs as large 70mm. This is because crabs of this size are often the red colour morph of *Carcinus maenas*, which has a longer inter moult stage and larger chelae (Lee et al., 2003). Red colour morph crabs are also more commonly found in the subtidal regions rather than intertidally or in estuarine environments (Lee et al., 2003). The larger red crabs have a narrower tolerance range to environmental conditions such as salinity, than the smaller green morph crabs (Lee et al., 2003). The Holton Mere population of Manila clams is located in an estuarine environment, which means that they will not be exposed to the larger red morph specimens of *Carcinus maenas*. This raises the questions of, at what shell length does the Holton Mere population reach a size refuge from predation by *Carcinus maenas* and does salinity influence predation rates? This study set out to answer these questions.

6.2 Materials and Methods

Effect of reduced salinity on the burial behaviour of juvenile Manila clams

The Manila clams used in this experiment were provided by Seasalter Hatcheries in Kent. When not being used for experiments the clams were housed on a water table with a continuous flow of unfiltered seawater (salinity 32 psu ±1, temperature 10°C ±2). The clams were checked on a weekly basis to remove any dead or dying clams to maintain the health of the stock. The clams used in the experiments had a size range of between 7.5-19mm in shell length. Juvenile Manila clams were used in the experiment as they exhibited greater vigour in reburial compared to adult clams in an initial trial experiment.

The clams used in the experiment were housed overnight in a 4 litre glass aquarium containing full-strength aerated seawater and sediment maintained at 15°C in a temperature controlled room. Only clams that had buried during the acclimation period were used in the experiment as any unburied clams were deemed to lack vigour.

The experiment was undertaken in a 15°C temperature controlled room, where the un-filtered seawater and distilled water used to create the experimental salinities were allowed to acclimate for a period of 24 hours. The salinities tested in this experiment were: 34, 32, 30, 28, 26, 24, 22, 20, 18 and 16 practical salinity units (psu). The minimum salinity of 16 psu was selected based on the work by Numaguchi (1998) who reported that the optimal range of salinities for Manila clams was 18-34psu. The lowest value of 16 psu was used as a salinity outside of the optimal range. A refractometer and a salinity probe were used to measure the salinities to ensure they were of the correct values. The response of 30 clams to each of the experimental salinities was tested, with a total of 300 clams exposed to the ten salinities.

The vessels used in this experiment were 600ml glass beakers filled with 30mm of sediment with a grain size of between 180 to 250 microns. The experimental water was added to the beaker and allowed to settle and a single clam was placed in to each beaker. At the end of the experiment the clams were removed and the water was drained.

Once the experiment had started, the clams were observed every 15 minutes for a period of 90 minutes. The length of time for the experiment was determined from preliminary trials in which individual clams were observed for a time period of four hours. It was observed that there were no changes in behaviour after 90 minutes in these trials. A similar time response was also recorded with another study investigating burial in the Manila clam (Mistri, 2004).

The clam's burial response to the different salinities was classified into three types of behaviour: a) non- buried (clam located on the surface of the sediment, with no attempt made to bury), b) partially buried (shell partially buried in the sediment, but at least 50% exposed) and c) fully buried (less than 10% of the shell exposed) (see figure 6.1).

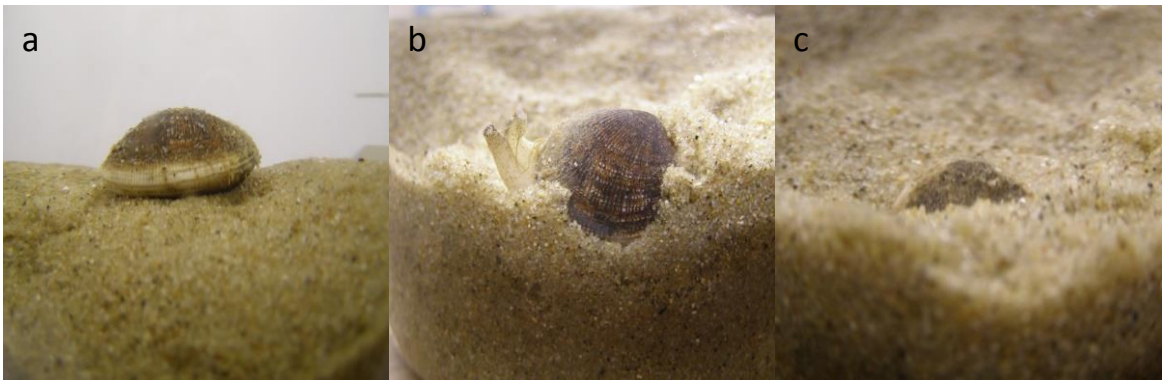

Figure 6.1: The three different burial behaviours observed in the experiment investigating the effect of salinity upon burial behaviour. a) non-buried, b) partially buried, c) fully buried.

Burial response to predator presence/absence

As with the previous burial experiment, the burial response result would be classed as: non-buried, partially buried or fully buried. This experiment was designed to be similar to the one carried out by Romano *et al* in 2010, which looked at the effect of the presence of the shore crab on the burial behaviour on the common cockle *Cerastoderma edule* (Romano et al., 2011). The treatments that the clams were exposed to were: no crab, a caged crab and a non-caged crab (Romano et al., 2011). The outcome of the experiment was whether the clams had attempted to bury or not.

The experiment was carried out in a temperature controlled room maintained at 15°C, using full strength unfiltered sea water (32psu). The experiments were conducted using

nine 12 litre glass aquaria, with each of the treatments replicated three times. This meant that for each time the experiment was run, three tanks contained a caged crab, three tanks contained a free roaming crab and three tanks had no crab. The aquaria were filled to a depth of 50mm with sand (collected locally with a mean particle size of between 250 and 500 microns) and then topped up with seawater to a depth of 10cm above the sand.

At the start of the experiment six juvenile Manila clams were added to each tank containing one of the three treatments (so 18 clams were exposed to each treatment, 3x6). The experiment was repeated three times giving a total of 54 clams exposed to each treatment (3x18).The clams were then observed every 15 minutes over a period of 90 minutes and the burial status recorded as: non-buried, partially buried or fully buried based on the criteria from the burial response to salinity (see figure 6.1).

The clams used in the experiment had maximum shell length of between 13 and 20mm. The size of the clams used in the experiment meant that the sediment depth did not limit burial. The clams were maintained in a glass aquarium overnight with sediment and full strength seawater maintained at 15°C. Only clams that had actively buried in the holding tank were used in the experiment. Unhealthy clams or those that did not open their valves were discarded.

The crabs used in the experiment were all female (to reduce the number of clams consumed) and had a maximum carapace width of between 44 and 48mm. For the treatments involving caged crabs, a cage was constructed using plastic mesh with a 10mm mesh size. The dimensions of the cage were: 85mm x 85mm x 85mm, the crab was able to move around in the cage, but was unable to feed upon the clams in the aquarium. The un-caged crabs were allowed to freely roam around the aquarium. Each of the treatments had three replicates, to give a total of nine aquaria used for each experimental run (see figure 6.2).

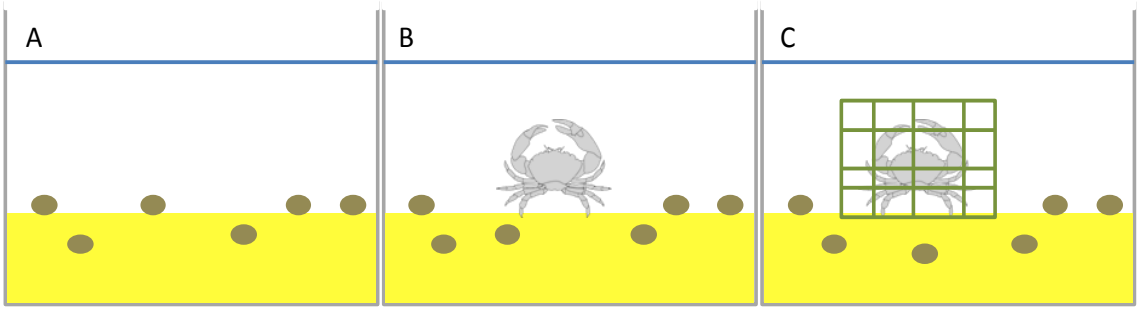

Figure 6.2: The experimental setup for the experiment investigating the effect of predator presence on burial in Manila clams. The brown ovals are juvenile Manila clams, six were placed into each tank. Tank A has no predator, tank B has a free roaming crab and tank C has a caged crab. Each treatment was replicated three times.

After each experiment, the water from each tank was completely drained to prevent the excretions from the crabs from contaminating the water and potentially affecting future replicates.

Interactions between the European shore crab and juvenile Manila clams

Experiments were undertaken with juvenile Manila clams ranging in size from 1-20 mm maximum shell length. All the clams used in these experiments were provided by Seasalter Hatcheries in Kent. All clams used in the experiment were of good health and vitality and no moribund individuals were used. These sizes were used as they were vulnerable to predation by the common shore crab *Carcinus maenas* based on the literature and were readily available from the hatchery (Mistri, 2004). When not used in the experiments, the juvenile clams were kept on a water table with a continuous through-flow seawater system at a salinity of 34psu ± 2psu and a temperature of 10°C ±2°C. The seawater was enriched with 20ml of concentrated phytoplankton and dissolved organic matter in the form of Julian Sprungs Marine snow™ on a weekly basis. To ensure that the juvenile clams were in good health, any dead clams were removed at regular intervals to prevent the contamination of the water.

Carcinus maenas specimens were collected from two locations: Canoe Lake in Portsmouth, using hand lines and by hand from outside the Institute of Marine Sciences in Langstone Harbour. The crabs were kept in a through-flow seawater system at a

salinity of 34psu ± 2psu and a temperature of 10°C ±2°C and fed a diet of crushed bivalves when not being used in experiments. Any dead or dying individuals were removed from the tanks to maintain the health of the other individuals and to prevent the cannibalism of dead or dying individuals.

Effect of crab sex and clam size on the predation of Manila clams by Carcinus maenas

The crabs were starved for 24 hours before the start of the experiment and were randomly assigned to tanks containing full strength seawater maintained at 15°C. All the crabs used in the experiment had a maximum carapace width of between 45 and 51mm. Only fully-intact crabs were used in the experiments-those with claws either missing or in the process of regenerating were rejected.

Experiments were conducted with the following clam size classes: 18-20mm, 14-16mm, 10-12mm, 8mm and 4-5mm maximum clam shell length. For each size class of clam 30 crabs: 15 male and 15 female were exposed to 10 clams. This gave a total of 30 crabs exposed to 300 clams of each size class. Each crab was exposed to all of the clam sizes used in the experiment. Hunger levels were standardised between each exposure by starving the crabs for a period of 24 hours.

The crabs were placed individually into 10 litre glass aquaria containing 5 litres of unfiltered seawater in a temperature controlled room maintained at 15°C. They were allowed to acclimate to the tanks for one hour before the start of the experiment to reduce the effect of handling stress upon the individual crabs. Once in the aquaria the crabs were not disturbed except to add the clams at the start of the experiment.

To each aquarium ten juvenile Manila clams, all of the same size class were added as the prey items for the crabs. Each experimental run lasted for a total of 60 minutes and at the end of the experiment the crabs were removed from the tank and the number of intact clams remaining was counted. The tanks were observed at 30 and 60 minutes to determine the number of clams consumed.

Effect of salinity on the predation of Manila clams by Carcinus maenas

Experiments were undertaken in ten 10 litre glass aquaria filled with 5 litres of water of the experimental salinity maintained at 15°C. The salinities tested were; 34, 26, 20, 14,

8 and 4psu. These salinities were chosen because they represented conditions that occur in the Manila clam's natural estuarine habitat. Thirty crabs were exposed to each of the salinities and the number of clams consumed was recorded.

The clams used in this experiment were of the 8-10 mm size class. Ten clams were added to each aquarium at the start of the experiment. Individual experiments lasted for a total of 60 minutes and observations were taken after 30 and 60 minutes. Both male and female crabs in equal numbers were used and were starved for 24 hours before each exposure to the experimental salinity to standardise hunger. The crabs had a maximum carapace width of between 45 and 51mm.

Effect of temperature on the predation of Manila clams by Carcinus maenas

To investigate the effect of temperature on predation rates, the same experimental set up was used as in the salinity experiment, with the temperature being the variable instead. Full strength seawater with a salinity of 34psu was used with the same sized crab and clam size classes as in the salinity experiment. The crabs were allowed 24 hours to acclimate to the experimental temperature, which also acted as a starvation period.

All exposures were undertaken in the same temperature controlled room to prevent any additional factors being introduced. The experiments were run in series starting with the lowest temperature first and culminating with the highest. The experimental temperatures were; 3, 6, 9, 12, 15, 18, 21 and 24°C. The crabs were observed after 30 and 60 minutes and any surviving clams were removed from the aquaria and counted.

Crab claw morphology

To investigate whether claw morphology had an influence on the ability of male and female crabs to predate upon Manila clams, morphometric measurements were recorded for the left and right claws for 15 male and 15 female *Carcinus maenas* specimens. Specimens were collected from the intertidal zone in Langstone Harbour, Portsmouth, adjacent to the Institute of Marine Sciences. Specimens were sorted in situ and only intact crabs (those with both claws fully intact, and not in the process of regeneration) were collected. The carapace width of the crabs collected was measured at its broadest point and only individuals with a carapace width of between 45-51mm

were retained for claw measurements (this was to reflect the size range used in the predation experiments).

Crabs were placed on ice for 30 minutes before measuring commenced in order to slow them down and make measuring easier. All measurements were taken using digital callipers and were measured to the nearest 0.01mm. The crabs were sexed, then the maximum carapace width was recorded before measuring the dimensions of the claws. The following claw dimensions were measured: dactylus length, the length of the top of the propodus, the bottom length of the propodus, height of the propodus and the width of the propodus (see figure 6.3). Once the claw measurements had been recorded, they were divided by the carapace width in order to standardise these values across all of the crabs measured.

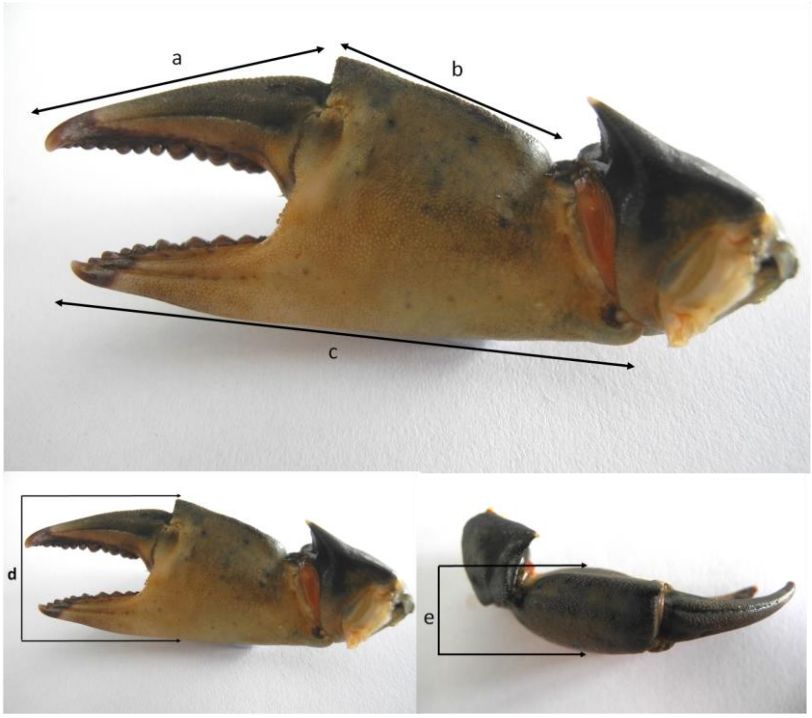

Figure 6.3: The Morphological measurements recorded for both left and right claws for male and female crabs. The claw segments measured where; a) dactylus length, b) top length of the propodus, c) bottom length of the propodus, d) height of the propodus and e) width of the propodus. All segments were measured to the nearest 0.1mm.

Statistical analysis

The burial experiments investigating the effect of salinity on whether clams attempted to bury or not was analysed using binary logistic regression. If a clam attempted to burrow it was classed as a 1 and if it did not attempt to bury it was classed as a zero.

The experiments investigating the effect of crab presence/absence on clam burial were analysed using chi squared test of association due to the fact that the experiment generated counts based on categories.

The percentage data generated by the predation experiments were first arcsine transformed for normality before statistical analysis. The experiment investigating the effect of crab gender and clam size was analysed using an ANOVA general linear model. The experiments investigating the effect of temperature and crab sex on predation was analysed using an Generalized linear model with a logit function, whereas the experiment investigating salinity and crab sex was analysed using an ANOVA general linear model.

The standardised crab claw measurements were first tested for normality and found to be non-normally distributed. No- transformation was carried out and the standardised values were analysed using the non-parametric Mann-Whitney U test.

6.3: Results

6.3.1 Influence of salinity on burial behaviour in juvenile Manila clams

Salinity had a significant effect upon whether Manila clams attempted to burrow into the sediment, when analysed using a logistic regression model. The logistic regression model was statistically significant, (χ^2 =115.307, d.f=9 p <0.05). The model explained 42.7% (Nagelkerke R^2) of the variance in whether clams attempted to burrow or not and correctly classified 75.6% of cases (see figure 6.4). As salinity decreased from 34psu down to 16psu, clams were less likely to attempt burial, with clams 20 times less likely to burrow at 18 psu when compared to 34psu.

At a salinity of 16psu none of the 30 clams exposed were observed to bury in to the substrate. Several of these clams were observed to be gaping, but no attempt was made to bury. At a salinity of 18psu 15% of the clams did begin to bury, but it was not until 26 psu that 50% of the clams exposed would attempt burial. The salinity with the highest percentage of clams that were fully buried was at 34psu with 73% of the clam buried.

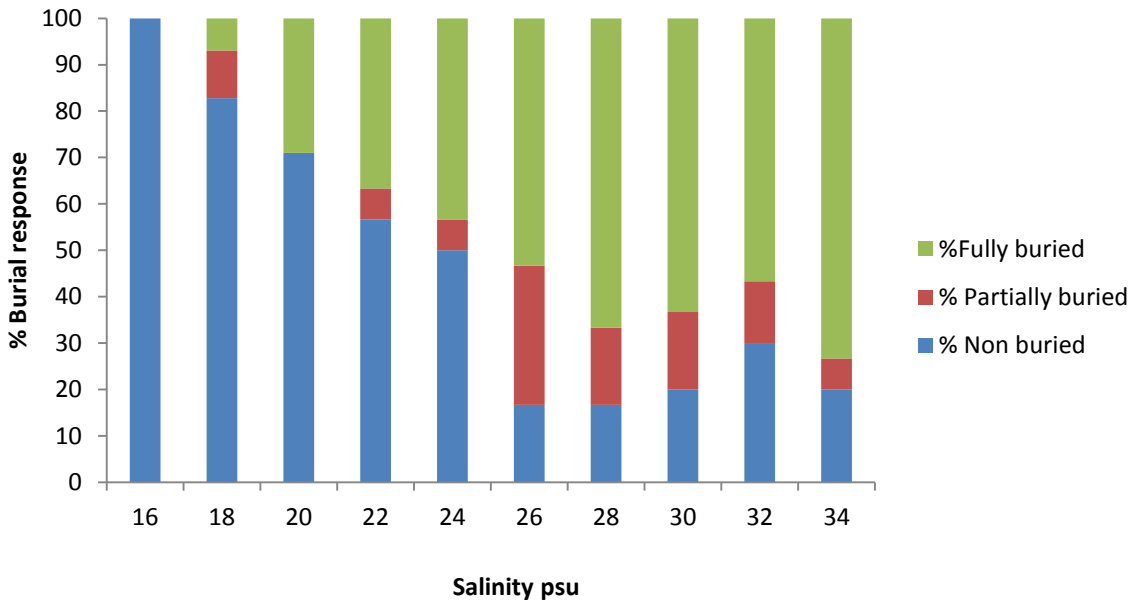

Figure 6.4: The mean percentage behavioural response of Manila clams to different salinities (n=30). The responses to the salinities are categorised as: fully buried, partially buried and non-buried.

6.3.2 Juvenile Manila clam burial response to predator presence/absence

There was no significant difference in the number of clams that attempted to bury in response to the presence or absence of a crab (χ^2, 6.815, d.f.= 4, p value > 0.05) (see figure 6.5).

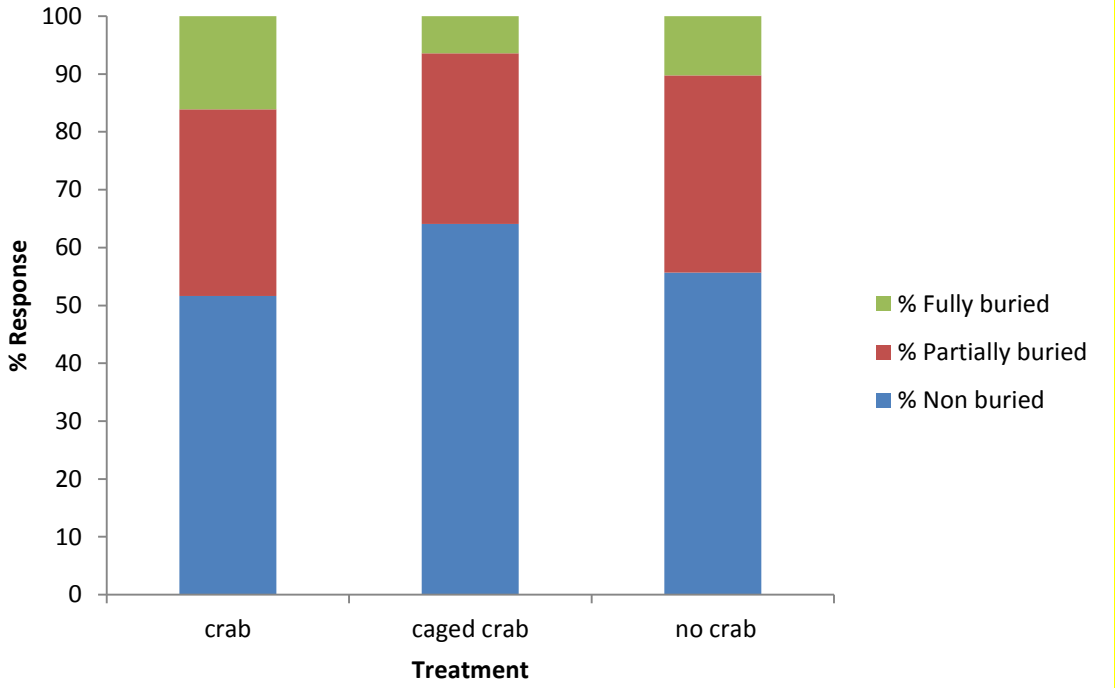

Figure 6.5: The proportion of juvenile Manila clams that attempted to bury in response to the presence or absence of a predator (n=54). The responses to the salinities are categorised as: fully buried, partially buried and non-buried.

6.3.3 Interactions between clam size and crab sex in determining predation of Manila clams by Carcinus maenas

There was a significant effect of crab gender (ANOVA general linear model, F=7.16, d.f:1, P value= 0.008) and clam size (ANOVA general linear model, F=26.85 d.f: 4, P value=0.00) on the number of clams consumed by crabs. There was no interaction between crab gender and clam size in determining the number of clams eaten (ANOVA general linear model, F=16, d.f:4, P value=0.179) (see figure 6.6).

As the clams increased in shell length, the number of clams eaten by the crabs decreased. Male and female crabs consumed between 73-79% of clams between 4-5mm, 60-67% at 8mm, down to 6-9% at 18-20mm.

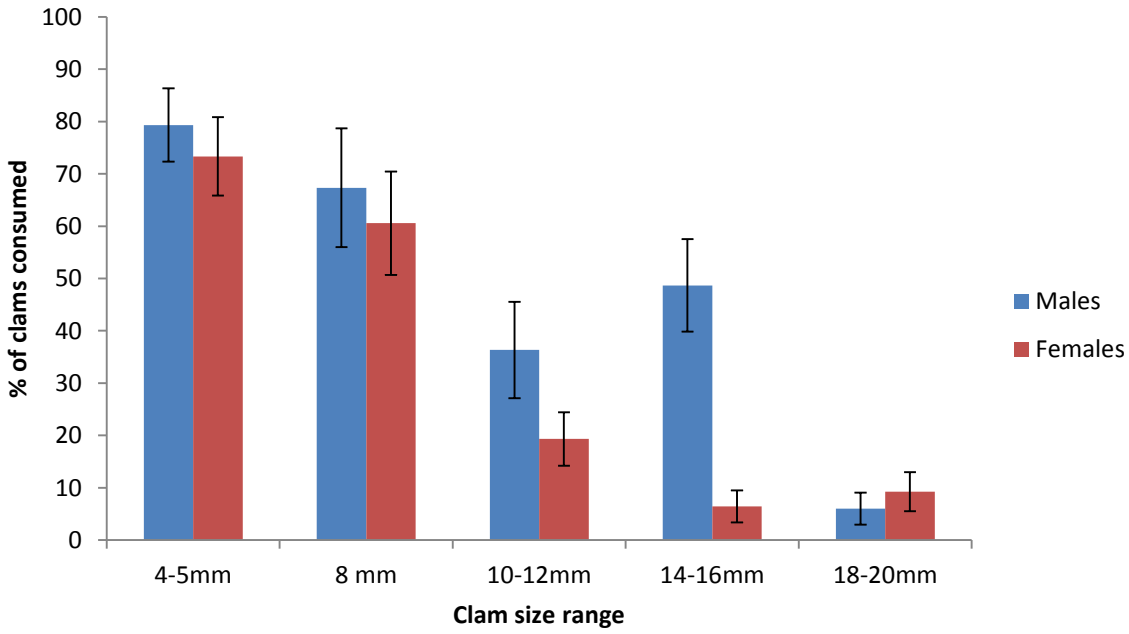

Figure 6.6: Percentage of clams of different size classes consumed in one hour by male and female common shore crabs (mean ±SE, n=30, 15 male, 15 female).

As the size of the clams increase, the technique used by the crabs to break open the shells changed. Crabs picked up the smallest clam size class and then passed them straight into the mouthparts where they were broken up and then consumed. The crabs were able to use this method until clams reached a size of 8mm. This technique had also been observed in other studies, which found that small bivalves were placed whole in to the mouthparts of green shore crabs (Ropes, 1968; Seed & Hughes, 1995).

Once clams reached a size of 10-12 mm, the crabs used a different technique in order to break open the shells. The crabs would initially try and feed the clams straight in to the mouth parts whole, but when they were unable to break open the shell, they were then passed back to the claws where the crabs would then break the clams in half asserting pressure on the shell until it cracked (see figure 6.7).

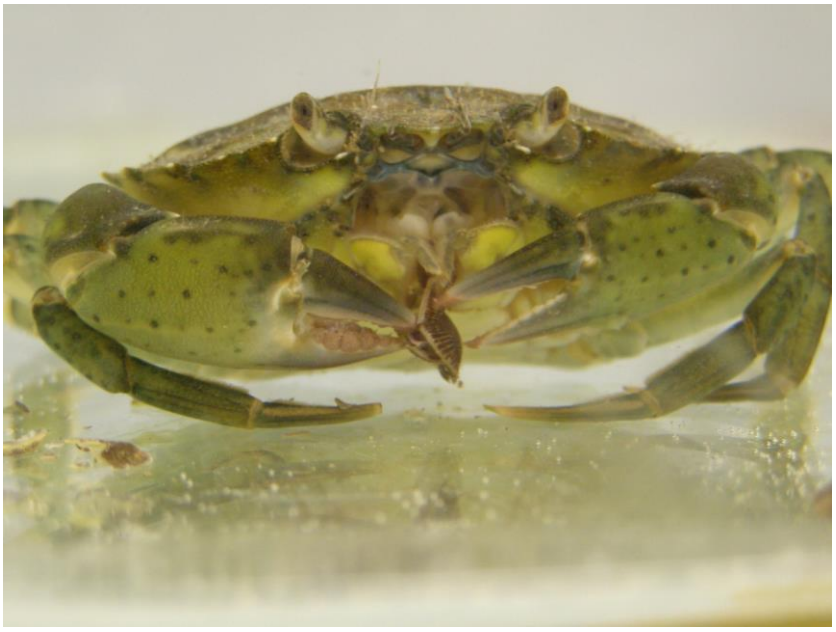

Figure 6.7: European shore crab consuming a juvenile Manila clam, the claws had been used to break open the shell before passing it into its mouthparts.

Clams above 10-12mm in size, elicited different responses from individual crabs in order to break open the shell. Clams that were too large to be fed straight into the mouthparts or broken in half by the claw strength alone, were broken in a range of different ways. One crab was observed to hold the clam in its claws and then assert pressure on one of the valves from just above the ligament, pushing it so that the ligament broke and the top valve slid away from the bottom one and exposed the flesh inside. Another technique used by the crabs on the bigger clams was to either to chip a hole in one of the valves to allow it to insert a claw, or wait patiently until the clam opened up enough for it to slide the tip of a claw in. Once the tip had been inserted, it then used its strength to prize the clam open and get to the flesh inside.

6.3.4 Interactions between salinity and crab sex in determining predation of Manila clams by Carcinus maenas:

Salinity had a significant effect upon the number of clams eaten by crabs over 60 minutes (ANOVA GLM, d.f; 4, F= 5.74, p value <0.05). The highest percentage of clams were consumed at 20 and 26 psu (see figure 6.8). There was a reduction in the number of clams eaten at 34 psu. Subsequently the results at 34 psu were excluded from the statistical analysis as they were deemed to be anomalous.

Crab sex also had a significant effect upon the number of clams eaten after 60 minutes (ANOVA GLM, d.f; 1, F=31.27, p value <0.05). With male crabs eating more clams than female crabs across all of the salinities tested. There were no interactions between crab gender and salinity in the number of clams consumed over the duration of the experiment (ANOVA GLM, d.f; 4, F=0.32 p value =0.866).

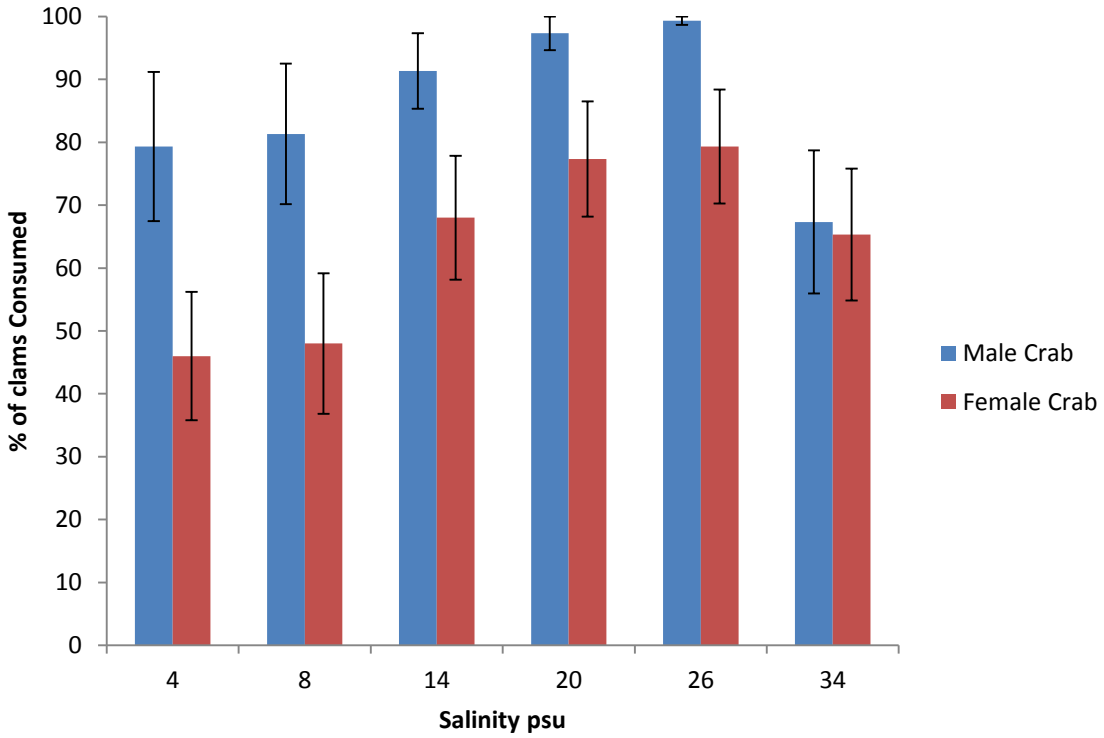

Figure 6.8: The mean percentage of clams (±SE, n=30, 15 male, 15 female) consumed after one hour at a range of salinities by both male and female crabs.

6.3.5 The effect of temperature on the predation rate of Manila clams by Carcinus maenas

There was a significant effect of temperature upon the number of clams consumed by crabs (Generalized linear model (χ^2 (7) = 46.5, p <0.05)). The general trend was that as temperature increased so did the percentage of clams consumed. However there were anomalous results for both male and female crabs at 20.4°C, these were retained in the analysis.

Crab sex did not have a significant effect upon the number of clams consumed, with (Generalized linear model, (χ^2 (1) = 0.00, p value >0.05)). There were no interactions between temperature and crab sex in determining the number of clams consumed (Generalized linear model, (χ^2 (7) = 5.106, p value=>0.05).

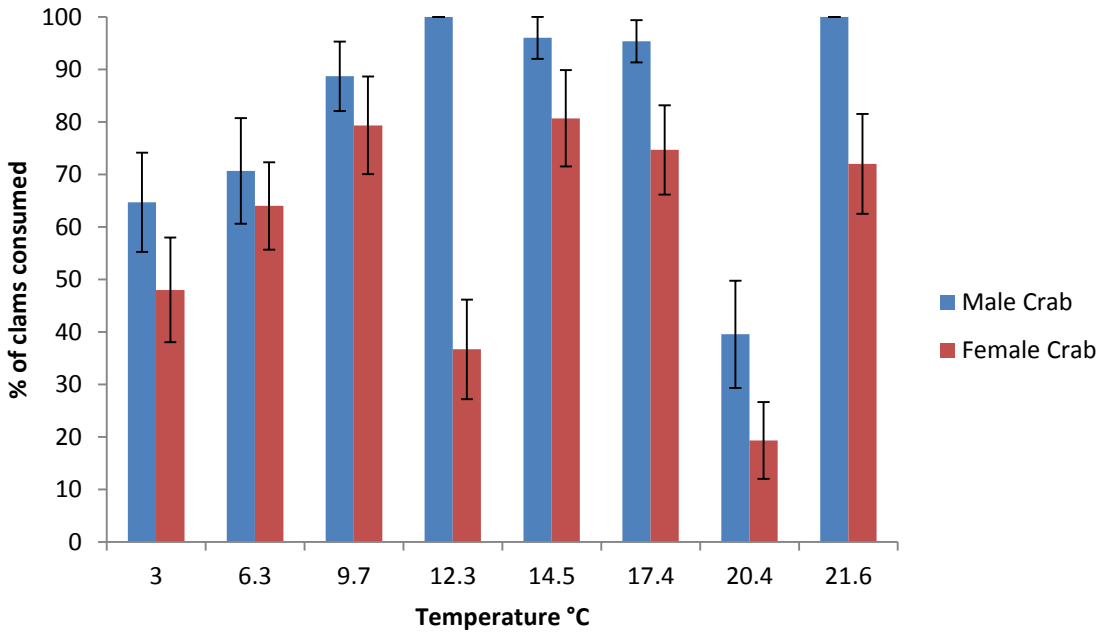

Figure 6.9: The mean percentage of clams consumed after one hour (±SE, n=30, 15 male, 15 female) at a range of temperatures by both male and female crabs.

6.3.6 Crab claw morphology measurements.

There was a significant difference between male and female crabs in all of the standardised measurements for the right claw, however there were no significant differences for the left claw (see figure 6.10). For the right claw there was a significant difference in dactylus length between males and females (Mann-Whitney U test, W=129, p value=0.0004). For the right claw there was a significant difference in the length of the top of the propodus between males and female crabs (Mann-Whitney U test, W=303, p value=0.0007). For the right claw there was a significant difference in the bottom length of the propodus between males and female crabs (Mann-Whitney U test, W=108, p value=0.000). For the right claw there was a significant difference in the height of the propodus between males and female crabs (Mann-Whitney U test, W=134, p value=0.0010). For the right claw there was a significant difference in the width of the propodus between males and female crabs (Mann-Whitney U test, W=322, p value=0.000).

For the left claw there was no significant difference in dactylus length between males and females (Mann-Whitney U test, W=227, p value=0.9478). For the left claw there was no significant difference in the length of the top of the propodus between males and female crabs (Mann-Whitney U test, W=258, p value=0.1651). For the left claw there was no significant difference in the bottom length of the propodus between males and female crabs (Mann-Whitney U test, W=169, p value=0.0771). For the left claw there was no significant difference in the height of the propodus between males and female crabs (Mann-Whitney U test, W=237, p value=0.6157). For the left claw there was no significant difference in the width of the propodus between males and female crabs (Mann-Whitney U test, W=184, p value=0.2657).

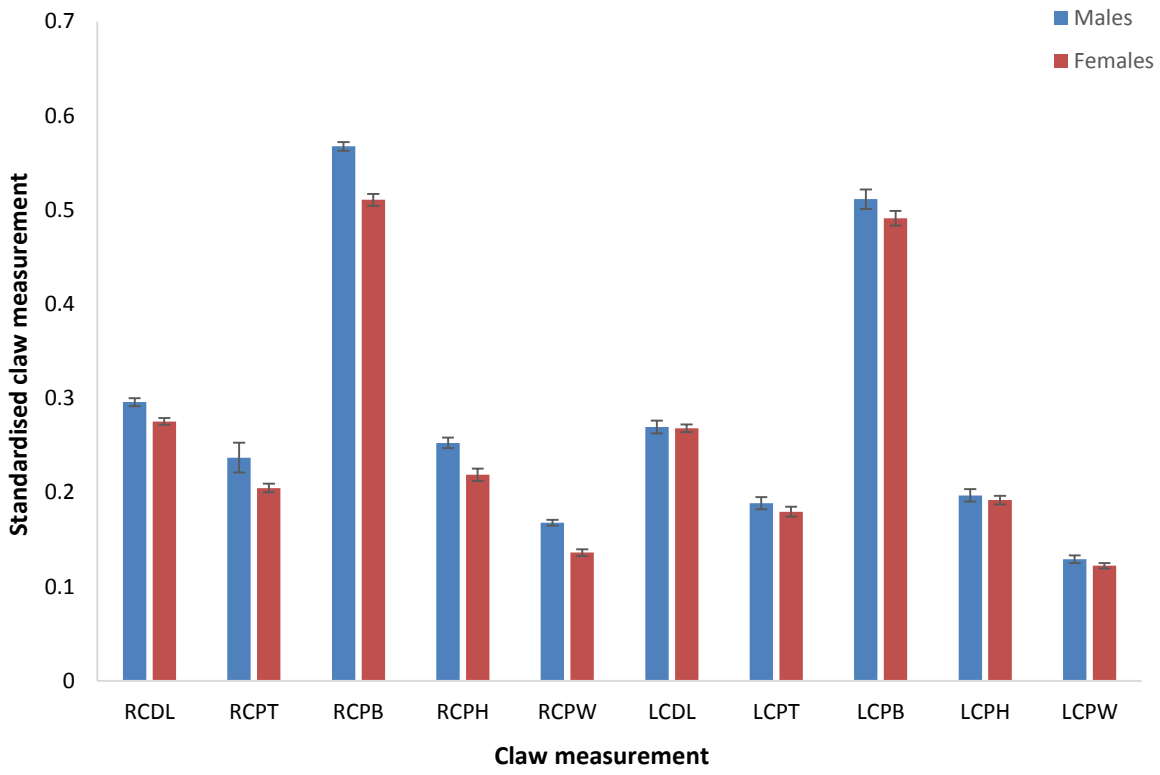

Figure 6.10: The left and right crab claw dimensions for both male and female crabs standardised against carapace width (Mean ± SE, n=15). The measurements included were: RCDL; right claw dactylus length, RCPT; right claw propodus top length, RCPB; right claw propodus bottom, RCPH; right claw propodus height, RCPW; right claw propodus width, LCDL; left claw dactylus length, LCPT; left claw propodus top length, LCPB; left claw propodus bottom, LCPH; left claw propodus height and LCPW; left claw propodus width.

6.4 Discussion

6.4.1 The effect of salinity on burial behaviour

The non-burial by clams at salinities below 18psu was a behavioural response to environmental stress. It is unlikely that handling stress would prevent the clams from burying, as it was only at the lower salinities in which burial was inhibited. If handling stress was influencing burial behaviour it would affect clams at all of the salinities not just at the lower end of the range used in the experiment. Stressful environments are known to inhibit burial in Manila clams (Matozzo et al., 2004; Shin et al., 2002). The salinities examined in this study were all above the reported lower tolerance limit of 15psu for Manila clams (Elston et al., 2003). The clams did periodically open their valves in order to test the water at salinities of 16psu, but none of these individuals attempted to bury. One of the responses Manila clams use when exposed to stressful conditions, such as elevated temperatures is to close their valves (Anacleto et al., 2014). This could suggest that closing the valves and not attempting burial is a response to unfavourable environmental conditions. The periodically testing of the water, could be an adaptive response to the estuarine environments in which Manila clams often inhabit. Salinity fluctuates on a tidal basis in estuarine environments, so like the larval response to low salinity, the juvenile clams could be waiting for the flood tide to increase the salinity before attempting to bury into the sediment.

The burial response to salinity could suggest that the population of Manila clams from which the juvenile clams were taken function best at salinities between 26-34psu. The lowest salinity at which clams buried in this study is considerably higher than that reported for natural populations of Manila clams. Nakamura (2005) reported that Manila clams would bury at salinities as low as 10 psu, whereas the lowest salinity found in this study was 18psu. The salinities at which the clams were kept prior to the experiments should not affect the results, as Nakamura maintained the clams at a salinity of 30 psu prior to experiments, whilst this study maintained them at 32 psu. This could suggest that the Manila clams that were imported into the UK for aquaculture have a difference tolerance range than the population studied by Nakamura, rather than an adaptation to the holding conditions prior to the experiment.

It has also been reported that different pedigrees of Manila clams have different tolerances to environmental conditions (Liu et al., 2011; Wu et al., 2011). The Liangdao red pedigree have the highest tolerance range to environmental conditions, including temperature (Liu et al., 2011). Three of the different pedigrees of Manila clams can be seen in figure 6.11.

Figure 6.11: The different pedigrees of Manila clams found in China. A) represents the white, B) the Liangdao red and C) represents the Zebra pedigree of Manila clams (Image taken with permission from Liu et al. 2010).

This raises the question of which pedigree of Manila clams were imported to the UK from North America for the initial aquaculture trials? The scientific trials by MAFF reported that Manila clams would be unable to reproduce due to the incompatibility of the Manila clams reproductive cycle with the seawater temperature regime (Spencer et al., 1991). Once the MAFF trials had been completed, the brood-stock created for the scientific trials was provided to hatcheries in order to create a brood-stock for aquaculture (Spencer et al., 1991; Utting et al., 1991). If the brood-stock was comprised of a mixture of pedigrees (with their related variations in tolerances) it could explain why some populations (those comprised of the more tolerant strains) became established and some disappeared. Once introduced, if only the more tolerant pedigrees (Especially the red strain) were able to reproduce, it could eventually lead to the populations being composed of this more tolerant strain. Anecdotally, the Manila clams present in the UK, show a marked similarity to the more tolerant red strain of Manila clams as seen in figure 6.11. This could suggest that the current surviving populations of Manila clams are descended from the more tolerant red pedigree, however genetic analysis would be required to confirm this theory.

Clam size also has an effect upon burial, juvenile Manila clams (shell length <20mm) take less time on average than adult clams (shell length >30mm) to bury. Matozzo (2004) reported that it took four hours for 50% of the clams (30-35mm shell length) kept at the control to burrow in to the sediment, whereas this study found that after two hours 73% of individuals were fully buried at the control conditions. A preliminary experiment also found that juvenile clams (<20mm) buried faster than larger individuals. This suggests that environmental assays in the future should use juvenile bivalves as opposed to larger individuals, in order to obtain more sensitive assays and faster results. Reasons why juvenile Manila clams bury faster than adult Manila clams could be due to their smaller size or a higher vulnerability to predation. Juvenile clams are more susceptible to predation than larger clams due to their size, which implies that burial is more important to the survival of smaller individuals than larger individuals.

6.4.2 Effect of predator presence on burial behaviour

There was no significant difference in the burial behaviour for juvenile Manila clams in the presence or absence of a predator. The low proportion of clams attempting to bury may be due to a lack of condition in the experimental clams. The fact that the proportion of clams burrowing is lower than in the salinity trials, may be a result of handling stress on the clams or lower condition levels. Stress and lower condition may have led to a reduction in the clams' ability to bury. To accurately determine whether the presence or absence of predators has an effect upon clam burial behaviour, this experiment would have to be repeated with fresh juvenile clams in order to ensure that condition did not have an effect upon their behaviour.

6.4.3 Size refuge at which clams are safe from predation by *C. maenas*

The high levels of predation on clams under 5mm could suggest that crabs are a major predator of juvenile Manila clams and may influence the recruitment success of the Holton Mere population. Crustaceans and in particular crabs are also known to affect

the recruitment success of other bivalve populations such as *Macoma balthica* in the Dutch Wadden Sea by high levels of predation (van der Veer et al., 1998).

Crab sex was found to have an effect upon the number of clams consumed. This could be due to the fact that male crabs have larger crusher claws than female crabs (Berrill & Arsenault, 1982) and are able to break open the shells easier (Smith, 2004). It is interesting to note that there is only a difference in the crusher claw morphology, between males and females and that there is no significant difference in cutter claw size. This fact, backed up by the observations of the feeding behaviour of crabs suggest that the use of the crusher claw to break open the shells is the most important method of predation upon clams by *Carcinus maenas.* Further experiments could be undertaken where the crabs are standardised by crusher claw morphology rather than carapace width. This could potentially answer the question on whether it is truly claw morphology that influences the number of clams eaten, or whether there is some other gender related factor that is influencing predation rates.

The methods used by the crabs in this study, to predate upon the different sized clams are consistent with those reported in the literature. This is with the mouthparts and the crusher claw used to access the flesh of the smaller clams, whilst sections of the shell were chipped away before the tips of the claw inserted into the larger clams (Ameyaw-Akumfi & Hughes, 1987; Elner & Hughes, 1978).

Male crabs are able to predate upon on clams up to a shell length of 16mm with moderate levels of success, which based on the clam growth rates calculated in chapter 3 implies that Manila clam spat are susceptible to predation by crabs for 180 days following initial settlement and metamorphosis from the larval stage. Crabs are still able to predate upon clams larger than 16mm but the levels of success were low.

Once clams reach a size of 20mm, they should stand a reduced chance of becoming a prey item to crabs. This is due to the fact that shells become too thick for the crabs to crush effectively (Mistri, 2004) so other methods would be required to access the flesh, which would increase handling time and the chance of the clam being discarded as a prey item (Ameyaw-Akumfi & Hughes, 1987). The size refuge of 20mm is consistent with

that at which Manila clams are no longer considered viable prey items for *Carcinus aestuarii* (Mistri, 2004*)*.

The size of 20 mm, is smaller than that described by a previous study which found Manila clams were vulnerable up to a size of 30-40mm from large specimens of *C. maenas* (Spencer et al., 1992). Clams above 30mm were still viable prey for large crabs with a carapace width >70mm (Spencer et al., 1991). Crabs of this size are rare at Holton Mere and throughout Poole Harbour (Stephanie Deane, personal communication) so clams could reach a size refuge once they reach 20mm. This is because *C. maenas* above 50mm in carapace width are mainly subtidal and not as tolerant to variations in environmental conditions (Lee et al., 2003). Clams at other locations in the harbour are more likely to be eaten by larger crabs, but the Holton Mere population would only be exposed to smaller, more euryhaline crabs.

6.4.4 Influence of salinity on predation of clams by crabs

As the salinity decreased, the number of clams consumed also decreased, however even at low salinities crabs were still able to feed upon the clams. This is due to the fact that *Carcinus maenas* is able to osmoregulate and can tolerate salinities as low as 11psu for prolonged periods of time (Lee et al., 2003; Ropes, 1968). The crabs at the lowest salinities were still able to feed, but were at the extreme of their tolerance levels so this may have affected the rates of predation. The fact that crabs can predate upon clams at salinities as low as 4psu indicates that the Manila clam will be vulnerable to predation by *C. maenas* across the whole of its habitat range.

The result of a drop in predation at 34psu were removed from the analysis as they appeared to be anomalous. This assumption was based on the results of the temperature experiment, which were undertaken at 34psu, where male crabs consumed 100% of the clams at 12.3°C and 21.6°C. If predation rates are suppressed at 34 psu, then this would also be evident in the temperature experiment as this was carried out using seawater at 34psu. This was not the case which suggests that the reduced predation rate at 34psu is more likely to be the result of other factors, such as

a reduction in the hunger of the crabs as opposed to a response to the salinity. As a consequence of this, if the experiment was repeated, a longer starvation period of three days should be implemented in order to ensure that the hunger of the crabs does not influence the results.

It is interesting to note that crab sex also had a significant effect upon the number of clams consumed. This is noteworthy because the size class of clam used in the experiment was chosen to ensure that clam size did not influence the levels of predation. This could suggest that another factor may have caused the female crabs to consume less clams than male crabs. A potential explanation for the reduced levels of predation could be linked to the size of the claws of female crabs compared to males and the time required to break open each clam. As each experimental run only lasted for one hour, it could be that the female crabs did not have enough time to break open all of the clams, as each clam could potentially take longer to open as they had to use different methods to access the flesh inside because of their smaller claws. Reduced tolerances to salinity by female crabs compared to males can potentially be ruled out, as the model did not detect any interactions between salinity and gender and the results followed the same pattern for both male and female crabs, with the percentage of clams consumed reducing with salinity. Future experiments investigating the different techniques used to open up bivalves by similarly sized male and female crabs could determine whether handling time of prey items are influenced by claw size. A follow up experiment using claw size, rather than carapace size to standardise the sizes between sexes would also allow conclusions to be drawn as to whether it is in fact claw size or another factor that causes reduced levels of predation between male and female common shore crabs.

6.4.5 Influence of temperature on predation of clams by crabs

Seawater temperature was the only factor found to influence the number of clams that the crabs consumed. The sex of the crab did not influence the numbers eaten and there were no interactions detected between water temperature and crab sex. The lowest

temperatures examined in this study coincided with the lowest number of clams consumed by crabs. This could be due to the low temperatures reducing the metabolic activity of the crabs, which reduces the feeding rate (McGaw & Whiteley, 2012). The drop in predation rates at temperatures below 6.3°C found by this study was also reported by a study in New Hampshire which found that *C. maenas* activity reduced at temperatures below 7°C (Ropes, 1968). In the study carried out by the author, crabs did continue to feed at low temperatures and male crabs consumed 63% of the clams at 3°C. This is noteworthy because crabs often migrate to deeper waters once temperatures drop to 2°C or below (Ropes, 1968). This raises the question of, if crabs migrate to deeper waters at temperatures of 2°C and below, will crabs predate upon juvenile clams over the winter, due to the intertidal nature of the Manila clam? As this experiment was carried out under artificial conditions, i.e. no substrate and short term exposure to reduced temperature (24 hours) field studies could be carried out to determine what crabs are feeding upon during periods with reduced seawater temperatures. This could be investigated by capturing crabs during the winter period and using molecular techniques to analyse their gut contents and find out what they are consuming.

The reduced predation rates at lower temperatures suggest that a winter with an average sea temperature of 7°C or below could be beneficial to the survival of juvenile Manila clams. This is because it would reduce the level of predation and allow more individuals to survive the winter. Temperatures of 7°C would not cause mortalities of Manila clams as juvenile clams are also able to survive at low temperatures, and increase body mass at temperatures as low at 6°C (Laing & Child, 1996). Temperatures of 7°C and below would also be beneficial to clams as the minimum threshold for gametogenesis is 8°C (Mann, 1979). This would allow the clams to overwinter without entering metabolic debt by producing gametes. The reduced predation by crabs and the ability of Manila clams to increase mass at low temperatures, suggest that winter may be an important season for Manila clam populations, with growth being able to occur at a slower rate but with reduced levels of predation.

The reduction in the number of clams consumed at 20.4°C was probably an anomalous result rather than a true response to the temperature. This is because the number of

clams consumed by male and female crabs at 21.6°C was comparable to that at 17.4°C. However when the data was statistically analysed with the results from 20.4°C omitted, the results of the analysis were the same, with temperature found to be the only factor to influence predation, and no interactions detected. The reduction in predation at 20.4°C could in fact be due to reduced hunger levels with the crabs sated from the previous temperature experiments, with the hunger levels returning by the 21.6°C exposure. As with the salinity experiment if repeated, the starvation period should be increased from 24 hours to three days in between experimental exposures, this would ensure that the crabs were unable to become sated in between exposures. Repeating the experiment and increasing the number of temperatures examined would give a more complete picture and allow stronger conclusions to be drawn on the influence of temperature on predation rates.

All of the predation experiments in this study had the same basic set up, with an aquarium containing either a male or female crab, ten juvenile Manila clams and five litres of seawater, at either the experimental temperature or salinity to be examined. The absence of any form of substrate in the aquaria ensured that Manila clams were not able to burrow to avoid being detected by crabs. This meant that the experiments did not mimic natural conditions. However as the aim of the experiments was to assess the ability of the crabs to feed under the experimental conditions, as opposed to forage, it was deemed acceptable to use bare aquaria. If the aquaria contained substrate, different results may have been recorded, and future experiments to investigate the influences of temperature and salinity on foraging behaviour may produce interesting results and should be attempted.

6.5 General conclusions

The lack of large mammalian predators in the UK, suggest that the main predatory threats to the Manila clam throughout UK waters are: zooplankton, small fish species, adult bivalves, crustaceans, birds and man. Of these organisms the highest levels of predation would be by crustaceans such as crabs on the juvenile clams. Predation upon

spat clams by crabs has the potential to influence the success of recruitment events in the UK. A small spat fall may be decimated by crabs and may result in a low number of recruits to the population. High losses of recently settled juvenile clams have also been reported in other locations, notably in North America (Ruesink et al., 2013). The estuarine habitat of the Manila clam does not influence predation, as crabs are able to tolerate lower salinities than clams.

The intertidal habitat of the Manila clam may reduce the levels of predation upon juvenile clams by aquatic predators due to lower immersion times than in subtidal habitats. The intertidal habitat would expose clams to terrestrial predators such as birds, but these predators target larger individuals as opposed to juveniles. This could imply that smaller individuals inhabit the intertidal zone as an adaptive response to reduce predation by subtidal predators which typically prey upon smaller individuals. This is further evidenced by the condition indices of the intertidal (Holton Mere) and subtidal (lease beds) populations in Poole Harbour. The subtidal population has a higher condition index than the intertidal population. This raises the question of whether subtidal predators influence the distribution of the Manila clam in Poole Harbour by high levels of predation upon spat. To answer this question, further experiments need to be undertaken to determine whether predation by subtidal predators has the ability to determine the distribution of a species.

Chapter 7: General Discussion

The main conclusion that can be drawn from this study is that the Manila clam has become successfully naturalised in British coastal waters, and has a wider distribution than reported in the literature. Although it has become naturalised, it is at the extreme of its tolerances for temperature, salinity and food availability, as evident from the low densities and low condition index of the Holton Mere population, as well as its inconsistent reproductive success. There is also evidence to suggest that predation pressure from aquatic predators may have an influence on the distribution of the Manila clam and currently restrict it to the intertidal zone. There are also remarkable parallels between the introduction and subsequent naturalisation of the Manila clam and the Pacific oyster twenty years earlier.

7.1 Parallels between the naturalisation and dispersal of the Manila clam and Pacific oyster

There are many parallels between the introduction and subsequent spread of the Manila clam and the Pacific oyster in European waters. Both species are naturally distributed in the Western Pacific and were introduced into European waters from North America for the purpose of aquaculture (Humphreys, 2010; Spencer et al., 1991; Troost, 2010). The introduction and subsequent naturalisation of both the Manila clam and Pacific oyster followed exactly the same process. Both species were deemed ideal as aquaculture species for European waters, as they would be able to grow to marketable size but would be unable to reproduce due to an incompatible temperature regime with their reproductive cycles (Spencer et al., 1991; Troost, 2010; Utting et al., 1991). This was proven to be incorrect and both species managed to reproduce and naturalise within several years of their introduction.

The Pacific oyster was introduced into the Dutch Wadden Sea in 1966, with the first spat fall occurring in 1971, with larger spat falls observed in 1976 and 1982 (Troost, 2010). Increasing seawater temperatures, and in particular warmer summers have been suggested as the cause for these successful reproductive events (Diederich et al., 2005).

The fact that the Pacific oyster was able to successfully reproduce, contrary to scientific opinion should have been used as part of the decision making process when introducing the Manila clam. Based on this, the Manila clam should not have been allowed to be cultured in the UK, due to the risk of the species becoming naturalised. However the Manila clam was introduced and has subsequently spread out from the initial areas of introduction, initially to locations close to the aquaculture sites (John Humphreys et al., 2015). This also occurred with the Pacific oyster, with areas adjacent to culture areas being colonised first (Diederich et al., 2005; Troost, 2010).

Although the Manila clam and Pacific oyster have managed to reproduce and establish feral populations, successful recruitment does not occur on an annual basis. In the Northern Wadden Sea, the Pacific oyster experiences patchy recruitment, with successful recruitment only observed in 6 out of 18 years. The Holton Mere population also experiences patchy recruitment, with a successful recruitment event only occurring in one of the four years examined in this study (in 2010). This could suggest, that like the Pacific oyster, the Manila clam experiences inconsistent reproductive success and relies upon years with good recruitment to and replenish its numbers, rather than having regular recruitment events.

This reliance on warmer years for reproductive success could be argued as a potential weakness, when coupled with the high levels of exploitation at Holton Mere. However the high fecundity of Manila clams (mature females produce on average 5-8 million eggs (Utting et al., 1991)) suggest that a successful recruitment event , as seen in 2010, could quickly replenish the population and makes it unlikely that the clams will disappear from Holton Mere. The patchy recruitment by Pacific oysters in the North Wadden Sea did not prevent the oysters from expanding their range from the intertidal to subtidal zone (Diederich et al., 2005), which could suggest that intermittent recruitment does not prevent the continued existence of a population.

7.2 Naturalisation of the Manila clam along the South Coast of England

The current distribution of Manila clam populations along the South and south eastern coast of England is confined to areas of human introduction and marginal habitats. This distribution is maintained by high levels of exploitation, reduced reproductive success and competition from already established and diverse community structures.

The initial naturalisations of the Manila clam may have occurred in areas with inhospitable conditions, such as areas of reduced salinities and low immersion times. These areas of marginal habitats are found at: Holton Mere in Poole Harbour, the Medina Estuary on the Isle of Wight and the North West corner of Portsmouth Harbour. All of these areas are characterised by reduced salinities and intertidal habitats. The tolerance of Manila clams to reduced salinities and exposure to air (both larval and adult), may have provided it with the competitive edge over local species such as palourdes in order to become established. Larvae of the Manila clam are also reported to be capable of settling in areas of reduced salinities (Tezuka et al., 2013) which would also ensure that areas of reduced salinity would be settled first. It has also been reported that tolerance to low salinity is an important factor in determining the success of a marine invasion (Miller et al., 2007). This suggests that the Manila clams' large tolerance range to salinity has aided its establishment in marginal localities.

The suboptimal conditions found at Holton Mere could suggest that the establishment of the Holton Mere population is the result of a higher rate of survival of larvae in this location. The marginal nature of the environment may have aided in the survival of larvae, due to a reduction in larval predation caused by the higher exposure times and reduced salinities reducing the number of active predators. Marginal environments are also known to have reduced species diversity which allows invasive species to become established (Levinton, 2001), p419,430).

Although it has become established in these marginal areas, these are not ideal habitats that enable the Manila clam to thrive. Evidence for this can be found by comparing the condition index of the population of Manila clams from Holton Mere and that of the population from the lease beds within Poole Harbour. The average condition index of the Lease Bed clams is higher than that of the Holton Mere population. The difference

in environmental conditions between the two locations is immersion time and salinity. The clams from the lease beds are only exposed at extreme low tides and do not experience salinities as low as the Holton Mere population do.

The recruitment of new individuals at Holton Mere may in fact rely heavily upon larvae produced by the larger, higher conditioned clams found on the lease beds as opposed to clams from Holton Mere. This does not mean that the Holton Mere population does not contribute to its replenishment, a large proportion of new recruits may come from populations around the Harbour. The simulation by Herbert et al (2012) predicted that larvae from the Lease beds and Holes Bay clam populations could end up at Holton Mere when targeting salinities of 17psu. The simulation also predicted that a high proportion of the larvae released by the Holton Mere population would remain in the vicinity of Holton Mere. This could suggest that the population at Holton Mere plays a significant role in providing larvae for its own recruitment. However the low condition index and density of the Holton Mere population does raise the question of how much does it contribute to recruitment in terms of larval supply? It is likely that the Holton Mere population does self-replenish to a certain extent, but that it also gets a significant supply of larvae from other populations around the Harbour.

This raises the further question of what would happen to the Holton Mere population if the Manila clams located elsewhere in the Harbour were removed? The answer to this question is that the population at Holton Mere would probably continue to exist, but at reduced densities due to the reduced larval supply. The population would also be under increased risk of over-exploitation by legal and illegal fishing pressure, however as the number of “legal sized” clams reduces the fishing pressure should reduce as well, potentially giving the population time to recover. The scenario of the Manila clam being removed from all locations throughout the Harbour except Holton Mere is however purely hypothetical, due to the number of existing populations throughout the Harbour.

7.3 Interactions between Manila clams and native predators

Predation by crabs and shrimps has been reported to influence the success of reproductive events of *Macoma balthica* by predating upon the newly settled spat (Hiddink et al., 2002). The reduced salinity and intertidal nature of Holton Mere could decrease the levels of predation by crabs and other aquatic predators on the newly settled larvae. This is because the reduction in immersion time in the intertidal habitat would reduce the time that the small clams are exposed to aquatic predators and thus reduce the rates of predation. This phenomenon of reduced predation on juvenile bivalves located high in the intertidal zone has previously been observed for *Mytilus edulis* in the Menai straight in Wales (Caldow et al., 2004). This was because aquatic predators such as crabs and starfish are the main predators upon juvenile mussels and these are restricted to the sub-tidal zone (Caldow et al., 2004). Clam larvae may settle in other areas of the harbour with higher average salinities and longer immersion times, but at these locations the newly settled larvae may be under higher predation pressure from aquatic predators. This could explain why the naturalised populations are found in areas of marginal habitat.

Salinity tolerance may be the main factor in determining the initial distribution of the Manila clam, but once it has become established in an area, predation and reproductive success control the dynamics of the population. The inconsistent nature of recruitment, leading to the dominance of older age cohorts means that fishing pressure can have an impact upon the population at Holton Mere, with larger individuals specifically targeted by fisherman. Larger individuals have a higher quality and quantity of gametes than smaller individuals (Tumnoi, 2012). The removal of these larger, more fecund individuals may result in fewer larvae, due to reduced gamete production and fertilisation success. Fertilisation success may be affected due to lower densities of gametes ejected into the water column in broadcast spawning events by the remaining clams. This means that there are potentially fewer larvae settling out on to the substrate so predators could have a greater impact on the recruitment event by consuming a higher proportion of the settling larvae.

7.4 Exploitation of Holton Mere population of Manila clams

Highly exploited populations of clams such as Holton Mere are able to persist, even under high levels of fishing pressure due to the high fecundity of clams and the supply of larval recruits from localised brood stock populations. Manila clam populations are unlikely to disappear, due to the economic benefits of their presence to local fishermen. The fishermen have an economic interest in ensuring that the clams do not disappear (see monetary value of landings in chapter 3). An example of fishermen ensuring that the populations persist is that some have been reported to have laid spat clams in areas to create new, and maintain current fisheries (personal communication, Sloyan Stray, MMO). These factors, both economic and the adaptive physiology of the clams themselves suggest that the Manila clam is now here to stay.

The Holton Mere population of Manila clams is unlikely to reach the densities reported for the Venice Lagoon population, due to the marginal habitat that exists at Holton Mere and the levels of exploitation by fishermen. The Holton Mere population does not have a high enough level of recruitment to sustain the levels of exploitation it currently experiences and increase in population density. The fishing effort is controlled by the density of clams rather than the density controlled by the fishing. This is because as soon as the density of legal sized clams increases so does fishing effort, and when the density decreases so does fishing effort. This displays a typical predator-prey system with a time lag, whereby fishing effort increases after the population density increases, before reducing again once the population of "legal" sized clams has reduced. This suggests that unless the Holton Mere fishery is intensively policed, it will never reach the densities recorded elsewhere.

7.5 Conservation implications of the Manila clam

The status of the Manila clam as a non-indigenous species, raises the question of whether it should be preserved and managed at Holton Mere and indeed the rest of Britain, or allowed to be exploited at the current high levels. There are arguments for and against the preservation of this species. A major argument against preservation is the Manila clam's status as a non-native species, and its potential to disrupt ecosystems.

It could be argued that as it is not native to the UK then it should not be conserved. This argument is valid if the species in question provides no environmental or economic benefits to the environment it has colonised and if its removal causes no further disturbance. Based on these criteria, the Manila clam should be conserved and managed, due to the benefits of its presence to overwintering wading birds as a food source, its presence reduces the predicted winter mortality for bird species (Caldow et al., 2007). The continued presence of the Manila clam has a benefit to the local economy and is worth hundreds of thousands of pounds each year. Another compelling argument for the continued management of the Manila clam fishery in Poole Harbour is the habitat disturbance caused by fishing methods, most notably the pump scoop dredge (Parker & Pinn, 2005). If left un-regulated this practice has the potential to degrade habitats and reduce species diversity, which suggests the importance of the management of the fishery. The benefits to the local economy and ecosystem provided by the Manila clam, coupled with the fact that it has been naturalised in Poole Harbour for over 20 years suggests that it should be managed in the same way as local species.

7.6 Potential further dispersal of the Manila clam linked to predicted climate change

Since the 1980's the average seawater temperature around the globe has increased at an unprecedented rate, with the largest increases occurring in more northern latitudes (Philippart et al., 2011). The average seawater temperature in the North East Atlantic has increased by 1°C between 1975 and 2005 and is predicted to increase by a further 2°C within the next 50-100 years (Hiscock et al., 2004; Philippart et al., 2011). Storm events, increased rainfall and earlier springs are also occurring on a more regular basis (Philippart et al., 2011). Rising average seawater temperatures, linked with habitat disturbance caused by more regular storm events and increased rainfall could provide the catalyst for the more rapid expansion of the Manila clam, not just along the coastline of Britain, but throughout Europe as well.

Increasing seawater temperatures have already been linked to the northward expansion of "Southern, warm water adapted species" and the contraction of the range of "Northern, cold water adapted species" (Barry et al., 1995; Perry et al., 2005; Philippart et al., 2011). In British coastal waters, two southern gastropod species, *Osilinus lineatus*

and *Gibbula umnilicalis*, have increased their geographic range northwards based on an average seawater temperature increase of 1°C (Mieszkowska et al., 2007; Mieszkowska et al., 2006). This could suggest that an increase of 2°C in average seawater temperature would be enough to facilitate the further northwards spread of the Manila clam along the coastline of Britain, and potentially Europe as well.

Rising seawater temperatures alone would not be enough to facilitate the further spread of the Manila clam. Increasing average seawater temperatures and more regular storm events may disturb established ecosystems and provide the Manila clam with the opportunity to colonise these newly-disturbed habitats. Higher average seawater temperatures, may lead to the vacation of ecological niches currently occupied by indigenous colder water adapted species, creating an opportunity for the Manila clam to colonise these habitats.

An example of a habitat that the Manila clam could become established in after a disturbance event is the Dutch Wadden Sea. The seawater temperature regime in the Wadden Sea, is compatible with Manila clam physiological processes but it has not currently established here in large numbers, although it is now present (Oliver Jewell, personal communication). This may be due to high levels of predation on settling clam larvae (Hiddink et al., 2002) or a high level of species diversity. A shift in temperature regime, or increased storm events may be enough to disturb the conditions enough in the Wadden Sea and provide the Manila clam with the opportunity to increase its presence.

7.7 Predicting the further spread of the Manila clam

The information contained in this thesis could be used to develop a modelling tool to identify areas that are vulnerable to invasion by the Manila clam. This can be broken down into a two-step process. The first step in predicting the spread of Manila clams would be to categorise and score potential sites on their compatibility with the life history of the Manila clam. This would be based on the environmental and physical properties of the locations and the proximity of these sites to other populations of Manila clams.

Stage 1 categorising sites for susceptibility to invasion

The environmental conditions that would be most vulnerable to invasion by the Manila clam would include intertidal soft sediments, located in estuarine areas. Areas of low species diversity are also more likely to be colonised than areas of high diversity, due to reduced competition (Levinton, 2001) p419,430). This information could be used to create a scoring system, with the higher the score the more likely the environment to be invaded. The scoring system could be as follows (See table 15):

Therefore a low risk category site would be a subtidal location with rocky substrate, with a salinity above 32psu, high species diversity and maximum summer temperatures below 14°C, this would score 0. A medium risk site could be an intertidal location with a sandy/mud substrate, with a salinity above 32psu, high species diversity and a maximum summer temperature between 14-18°C, which would score 20. A high risk category site would be an intertidal location, with a sandy/mud substrate, with reduced salinities, low species diversity and a maximum summer temperature above 18°C, which would score 50. Once sites have been designated as having a low, medium or high risk of invasion by Manila clams, the next step would be to consider potential dispersal mechanisms.

Table 15: A point scoring system to determine the potential likelihood of a site being successfully invaded by Manila clams. The scoring system has been split into three main categories, environmental and biological features. These categories have then been given arbitrary scores between 0 and 10, with 10 being the most compatible with Manila clam physiology and therefore most susceptible to invasion. The highest possible score is 50, so a location with a score of 50 would be highly susceptible to colonisation by Manila clams, whilst a location with a score of 0 would be least likely to be successfully colonised by Manila clams.

Category	*Variable*	*Classification*	*Score*
Environmental	Sediment type	Mud/Sand	10
		Gravel	5
		Rock	0
	Salinity regime	16-24 psu	10
		24-32 psu	5
		>34 psu	0
	Immersion time	Low	10
		Medium	5
		High	0
	Maximum average temperature	>18°C	10
		14-18°C	5
		< 14°C	0
Biological	Species diversity	Low	10
		Medium	5
		High	0

Stage 2 identifying potential methods of dispersal

The methods by which Manila clams may undergo further dispersal can be broadly categorised into either natural (through the process of larval dispersal) or anthropogenic (through human activity) in nature. Thus two separate models could be used to predict the spread of Manila clams, one using anthropogenic methods and the other using natural dispersal methods. These could eventually be combined to create a more complex model, but here I have described them singularly.

The Manila clams status as a non-native species highlights the importance of human activity in its initial introduction into the British Isles, where it was introduced for the purpose of aquaculture (Humphreys et al., 2015; Jensen et al., 2004). Anthropogenic methods of dispersal may still be the most important factor in currently predicting the spread of Manila clams around the south and South East coast of England (Humphreys et al., 2015). Anthropogenic dispersal played an important part in the dispersal of Manila clams in British Columbia where fisherman introduced Manila clams to locations in order to create fisheries (Bourne, 1982). The distribution of Manila clams in British Columbia also suggested human-driven dispersal as opposed to natural dispersal, due to the patchiness of populations and the presence of populations that were isolated from one another by large geographic distances (Bourne, 1982). This could suggest that when currently considering dispersal methods in the UK, human activity is the most important factor to consider.

The main human activity that could influence dispersal, is fisherman laying juvenile clams in order to create a fishery. In order to use this information to predict spread, it is important to consider the location of fish markets and wholesalers, places where fisherman can sell their catch. This is because it will allow fisherman to increase profits by reducing the distance they need to travel with their catch. With this in mind, proximity to a fish market can help identify areas that are potentially liable to be seeded with clams. An example of where this has occurred already, is the Medina Estuary on the Isle of Wight. The Medina Estuary lies within 17km sailing distance of Viviers UK Ltd, a fish market located in Old Portsmouth. It has been suggested that clams were seeded into the Medina Estuary in order to create a new fishery. An application for the classification of this population and suitability as a fishery fit for human consumption has been submitted by the owner of Viviers UK Ltd (Sloyan Stray, MMO, personal communication). With this in mind, Estuaries with a suitable habitat and within 20km of a fish wholesaler should be considered locations that could be colonised by Manila clams in future.

Although human activity is likely to be the main factor currently driving Manila clam dispersal along the South and South-east coast of England, it is not the only way in which clams may spread. Natural dispersal during the larval stage may also contribute to the

spread. Natural dispersal can been modelled by incorporating larval behaviour with hydrodynamics, coastal geomorphology and environmental factors such as salinity and water temperature. Models have already been used to predict the spread of Manila clams throughout Poole Harbour (Herbert et al., 2012), King scallops in the English Channel (Nicolle et al., 2013) and Pacific oysters in Chesapeake Bay in North America (North et al., 2008). The model that predicted the spread of Manila clams in Poole Harbour used an existing hydrodynamic model and the behavioural responses of Manila clam larvae to various salinities (Herbert et al., 2012). The larval behaviours used in the model were: swimming velocity in response to salinity and the salinity at which larvae actively swam (Herbert et al., 2012). The results from the larval chapter investigating the influence of salinity of swimming behaviour could be used to parameterise future models, rather than using the results taken from the literature, this was the approach previous used by Herbert *et al.* (2012).

The approach used by Herbert *et al.* (2012) could be used to model the dispersal of larvae from existing populations of clams using existing hydrodynamic models and the behavioural responses of larvae to salinity reported in this thesis. The models should also take into account costal geomorphology, as some features may influence the retention of larvae in estuarine systems. In the St Lawrence Estuary in Canada, embayments and headlands influenced the retention of larvae, with higher densities recorded in embayments compared to adjacent areas (Archambault et al.,1998). The models should be run for a period of up to two weeks, as this is the average duration of the larval stage (Ruesink et al., 2013). If larvae do not reach an area of suitable habitat (one that has been identified in stage one) within two weeks it is unlikely that they will settle here in sufficient numbers to establish a new population. Once the models have been run and the locations that are susceptible to invasion by Manila clams have been identified, the locations should then be sampled in order to validate the results.

7.8 New information gained from this study

Distribution and dispersal along the South and south eastern coast of England:

- The Manila clam is more widely distributed along the coastline of South and Eastern England than currently reported.
- Dispersal of the Manila clam in British waters is mainly driven by human agency rather than natural dispersal

The Poole Harbour population:

- The Poole Harbour population has highly variable recruitment
- Temperature is the main driver of Manila clam condition index in the Poole Harbour population

Predation upon the Manila clam:

- Crabs are able to predate upon clams at salinities as low as 4psu
- Crab continue to feed at temperatures as low as 3°C
- Male crabs are able to feed upon clams under a greater range of conditions than female crabs

Manila clam tolerance to reduced salinity:

- Salinity tolerance is the most important factor in determining initial colonisation of habitats by the Manila clam
- Below salinities of 16psu adult clams will not bury into sediment
- Manila clam larvae will selectively swim into lower salinity water until it reaches 18psu
- Manila clam larvae will not swim at salinities below 18psu
- Manila clam larvae respond to suboptimal salinities by not swimming and remaining closed. This is a behavioural response rather than a physiological one as salinities in which the larvae do not swim are not lethal.
- Larvae can tolerate salinities down to 10 psu for up to 24 hours with low levels of mortality

7.9 Potential for further studies

Mapping of further changes in the distribution of the Manila clam

A new study to map the current distribution of the Manila clam is important because this study marks the distribution up until 2010. Since this study, natural dispersal may have begun to occur and new populations may have also been seeded by fisherman in areas that previously did not have a presence of Manila clams. More attention also needs to be paid to the populations in Suffolk and Essex because there is more potential for dispersal in these counties than along the south coast, due to the interconnected nature of the estuaries in Essex and Suffolk.

Condition of the UK populations of Manila clams

The fact that the Manila clam population in Poole Harbour has a lower condition index than natural populations raises the questions of whether there are differences in condition across the populations of Manila clams in the UK. A study comparing the condition index of the populations in Southampton water, Poole Harbour, the Medina Estuary, Portsmouth Harbour and Langstone Harbour would provide information on the physiological state of populations across the Solent region. It would also allow for the determination of whether there is a geographic gradient of clam condition from Poole Harbour in the West to Langstone Harbour in the East. If a gradient does exist, it could be the result of differences in environmental conditions such as temperature across the region, with higher average water temperatures typically recorded in Poole Harbour in the West compared to than in Portsmouth Harbour in the East. The presence of condition index gradient would also allow for the prediction of future spread based on clam condition.

The use of condition index as an indicator of spawning activity would also allow for the reproductive cycle of each population to be estimated and determine whether spawning occurs during the same time period across all the populations.

Modelling larval dispersal in the Solent region

The modelling of Manila clam larval dispersal in Poole Harbour (R. J. H. Herbert, Willis, et al., 2012) and its subsequent validation by sampling, make modelling an important tool for further studies. Modelling the dispersal of Manila clams throughout the Solent region using larval behaviour, hydrodynamics models, seawater temperature regimes and calculating potential spawning times could help predict further spread and also pinpoint areas for future sampling. It would also enable the current distribution of Manila clams to be categorised as either the result of human interactions or natural dispersal. It would also allow the potential spread of future invasive organisms to be predicted based on larval behaviour and area of initial introduction.

Genetic studies to investigate diversity of the UK population detect and hybridisation between Manila clams and the local palourde Tapes decussatus

There have been some genetic studies investigating the relationship between European populations of Manila clams. Genetic analysis of two populations in Italy, one in the Sacca di Goro Lagoon in the Adriatic sea and the other in the Gulf of Olbia off the coast of Sicily found that these two populations, although geographically isolated were part of one metapopulation (Mura et al., 2012). This raises the question of whether the UK populations of Manila clams are genetically diverse or whether they are also part of one larger metapopulation?

In China there are three pedigrees of Manila clam, the white, the Liangdao red and the Zebra pedigree. These pedigrees have different tolerance ranges to environmental conditions in China which raises the question of whether a particular pedigree dominates the populations in the UK. A study to investigate which pedigrees are present may also help to explain why the Manila clam became naturalised against all predictions by the scientists at the time.

A recent study has also discovered hybridisation between Manila clams and the local palourde species in north western Spain (Hurtado et al., 2011). This raises the question of whether the same event could occur in British waters. It is important to determine whether hybridisation has occurred in the UK, because if it has, it could lead to the eventual extinction of the local species. To determine whether hybridisation has

occurred in the UK, the morphological and genetic characteristics of both Manila clams and palourdes needs to be investigated from locations where they coexist. Individual clams with both Manila and palourde-like characteristics would undergo genetic analysis to determine whether hybridisation has occurred.

The issue of hybridisation with the local palourde species also raises the question of whether there would be any benefit to both species from such a hybridisation. Would hybridisation result in a clam that has the growth rate and environmental tolerances of the Manila clam with the resistance to local pathogens and reproductive ability of the Palourde or would it result in hybrid that has a reduced level of fitness? To answer this question further experimentation with artificially created hybrid clams would need to be undertaken, however the ethical ramifications of creating such a "super-clam" would probably prevent this from happening.

Development of the use of the Manila clam in sewage treatment and polyculture

The Manila clams large tolerance range to both temperature and salinity coupled with its ability to efficiently filter out particles from the water column (Yasuo Nakamura, 2001) suggest that it may be a suitable organism for sewage treatment and polyculture systems. Developing the use of Manila clams in tandem with fish cages in fish farms may reduce levels of eutrophication by the removal of uneaten feed and faecal matter produced by fish by the filter feeding clams. The same principle could also be employed in sewage treatment plants, using a bed of clams to remove particulate matter. Developing the use of Manila clams as a form of biological filter could utilise the filter feeding abilities of the Manila clam in order to reduce impacts of aquaculture and sewage treatment on the natural environment.

Reports and Publications

Marine Management Organisation Project FES 228 'Does Phytoplankton abundance control shellfish success in Poole Harbour'

Humphreys J., Harris M.R., Herbert R.J., Farrell P., Jensen A. and Cragg S.M. (2015) Introduction, dispersal and naturalization of the Manila clam Ruditapes philippinarum in British estuaries, 1980–2010. *Journal of the Marine Biological Association of the United Kingdom*, 1-10

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