

**Community ecology and population dynamics of the European
native oyster (*Ostrea edulis*) in Essex, UK:**

**A baseline for the management of the Blackwater, Crouch, Roach
and Colne Estuaries Marine Conservation Zone**

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*“O Oysters,” said the Carpenter,
“You’ve had a pleasant run!
Shall we be trotting home again?”
But the answer came there none -
And this was scarcely odd, because
They’d eaten every one.*

Part of “The Walrus and the Carpenter” in Alice Through the Looking Glass

By Lewis Carroll

(Predation and fishing mortality on oysters)

Summary

Following the designation of the Blackwater Crouch Roach and Colne Estuaries Marine Conservation Zone (hereafter BCRC.MCZ) in 2013, there is now a legal obligation to “protect and enhance” remaining stock of the European native oyster, *Ostrea edulis*. Despite this, little is known about the current distribution, abundance and status of the *O. edulis* populations within this protected area, with no studies documenting epibenthic species associations with naturally occurring native oyster densities. Widescale dredge surveys were used to assess the current status *O. edulis* over a 5-year period between 2014 and 2018 and associated species between 2016 and 2018 across the BCRC.MCZ. Between 2016 and 2018 surveys were completed biannually in post-winter (February/Post-winter) and post-summer (September/October) to assess seasonal variation in these distributions. Associations between increasing natural densities of native oyster and increasing epibenthic species richness have been observed across the BCRC.MCZ, however, these associations are suppressed and even reversed in areas which also support high densities of the non-native *Crepidula fornicata*. In addition, a novel experiment was designed to monitor the growth rates and survival of *O. edulis* with individual oysters monitored for a maximum of 18 months across three sites within the BCRC.MCZ and four sites within areas designated for the mariculture of native oysters. Data from both studies was then used to create an Integral Projection Model to assess the current status of native oyster populations, assess limiting factors to population growth, and make future projections of these populations to drive management and restoration decisions. Site-dependent restoration techniques are recommended with strategies to increase adult oyster survival and juvenile recruitment recommended.

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Chapter 1:

Factors affecting the recovery of the European native oyster (*Ostrea edulis*), with a focus on the Essex estuaries – A review.

In this PhD I have investigated how populations of native oysters and associated communities in Essex are composed in terms of size and life stage, how these communities are affected seasonally and how growth rates and survival differ in different areas around the Essex estuaries. This PhD then used this data to make management recommendations for the restoration and sustainable management of the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone via the development of a population model.

As with any edible product, sustainable harvest is key to developing long term food security. Oysters may not be a food source which is consumed on a mass-scale such as other types of shellfish, with *O. edulis* only accounting for 0.2% of the global farmed oyster production (FAO, 2004). However, oysters have a history of providing vital protein and minerals to poorer populations up to the industrial revolution when populations were becoming severely depleted (MacKenzie *et al.*, 1997b).

1.1 An introduction to oyster restoration

Once common in coastal areas around the world, it is now estimated that approximately 85% of oysters and oyster reefs have been lost (Beck *et al.*, 2011). Declining water quality, anti-fouling paints (in particular tributyl tin – TBT), over fishing, disease, climate change and increased levels of sedimentation within rivers all have negative effects on oyster reproduction and growth rates (Laing *et al.*, 2006). Recent years has seen a resurgence in oyster restoration and the wide variety of different ecosystem services provided by oysters are being recognised (Beck *et al.*, 2011). These studies

assessing services such as water filtration, reducing chlorophyll *a* concentrations (Nelson *et al.*, 2004), providing habitat for many animals, and providing coastal defences (Grabowski & Peterson, 2007) have largely been directed towards rock oyster species such as *Crassostrea virginica* and *Crassostrea gigas* (sometimes *Magallana gigas*) with fewer studies to date associated with flat oyster restoration. As such, ecosystem services have been largely assumed between various oyster species and genera. There is now a growing call to address species-specific benefits and associations to oyster restoration, particularly when species can differ so greatly, such as the faster growing, multi-dimensional-reef forming species such as the eastern oyster vs slower growing, subtidal flat oysters (Gillies *et al.*, 2017).

1.2. Oyster beds and the importance of oyster fisheries

Ostrea edulis oysters are flat oysters, with a native range from Norwegian Sea down to the Bay of Agadir on the Atlantic coast of Morocco and as far east to the Black Sea (UKBAP, 1999; Morga *et al.*, 2009). This species of oyster is commonly known by many names including the native oyster, edible oyster, European flat oyster or Colchester native. They are thought of as ecosystem engineers, creating sessile shell deposits on which other species may colonise (Smyth and Roberts, 2010). Individual oysters and the beds they create are said to be a “Nationally important Marine feature” found around the UK coastline but predominantly in southern areas (Laing *et al.*, 2006; Figure 1.1). Following extensive decreases in populations on all UK coastlines attributed to disease, anthropogenic and natural coastal change and overexploitation, *O. edulis* were listed as a Biodiversity Action Plan (MacKenzie *et al.*, 1997a)(BAP) species with a Species Action Plan (SAP) making it an important species for conservation in the UK (Lenihan & Peterson, 1998; Laing *et al.*, 2006; Figure 1.1). The status of European native oysters was updated a “Priority Species” in the UK post-2010 Biodiversity Framework (Laing *et al.*, 2005; 2006; JNCC & DEFRA, 2012). *O. edulis* is an economically important species and despite constituting less than 0.2% of the global farmed oyster production, the market was valued at USD 24.3 million in 2002, on average costing 3 to 5 times more in weight than the more widely produced *Crassostrea gigas* (sometimes *Magallana gigas*), the Pacific rock oyster (FAO, 2004). Being

the native UK oyster, *O. edulis* is seen to have both significant cultural and economic value to British and European waters and attempts to recover populations have been made

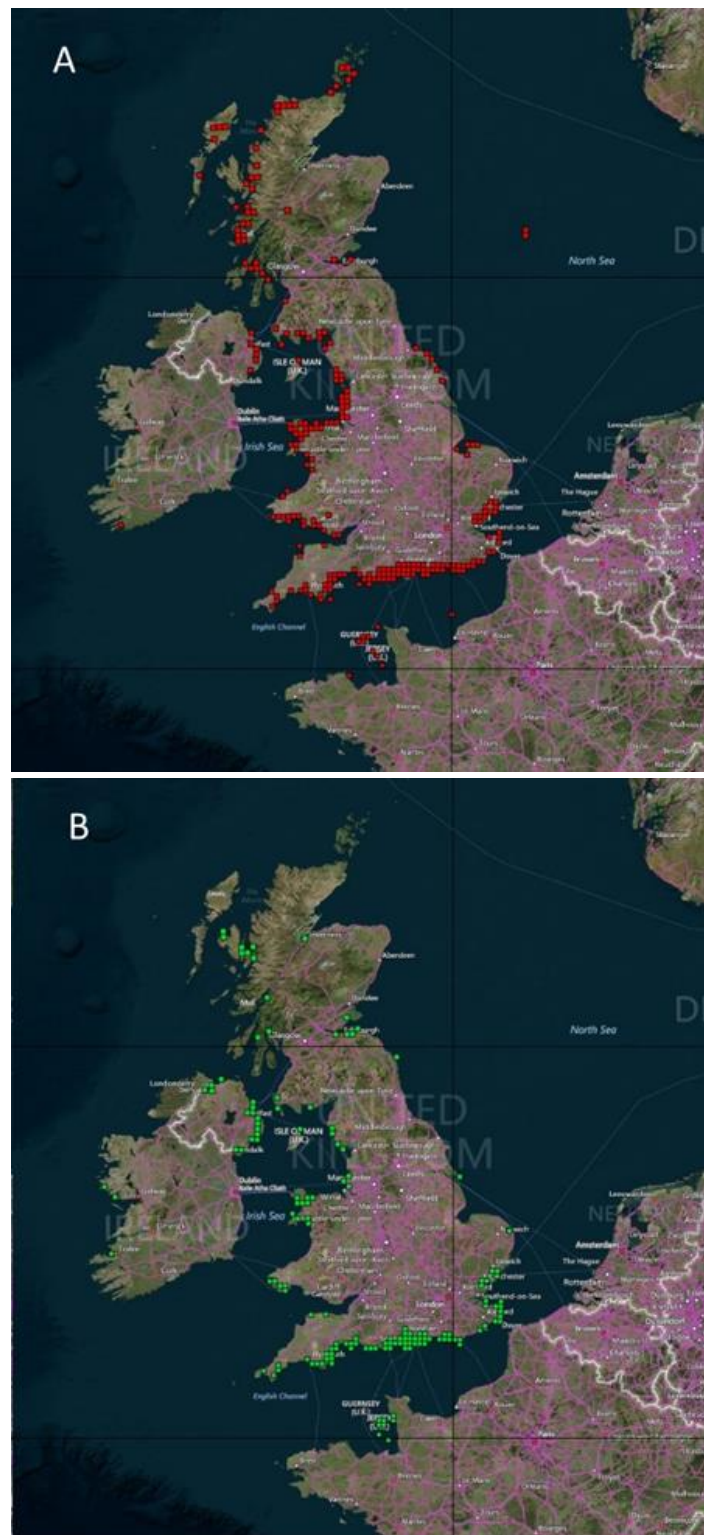


Figure 1.1 A: *Ostrea edulis* records for the year range 1600-2006. B. *Ostrea edulis* records for the year range 2006 - 2016 (NBNGateway, 2016).

repeatedly with limited success (MacKenzie *et al.*, 1997a; Kennedy & Roberts, 1999). In addition to the economic benefits of commercial oyster fisheries, oysters of all species are seen to be beneficial to water quality, this is through the fixation of carbon through shell production (Hammen and Wilbur, 1959), the filtering and removal of large quantities of organic and inorganic particulate matter thus increasing water clarity (Nelson *et al.*, 2004) in addition, through the formation of shell which may act as a hard substrate useful for other species to attach onto or use for refuge (Korringa, 1951; Smyth and Roberts, 2010). Due to the wide variation in size and densities of oyster beds around Europe, in 2009 a review of available data classified an oyster bed to have to have a “specific density” of oysters of 5 oysters m⁻² (Haelters & Kerckhof, 2009). This specific density is a contentious issue as many surveys investigating oyster density in areas where oysters have remaining strongholds have densities far below this threshold (Kennedy & Roberts, 1999; McGonigle & Scott, 2012). Native oyster beds are specifically defined as a habitat and are subject to differing protection to that of “native oysters” which, as individuals, may be harvested within sustainable fishery guidelines. This creates conflict over how an oyster bed should be defined, with positives and negatives for both high and low-density definitions. Low densities can be associated with decreased likelihood of fertilization and reduced filtration capacity in contributing to nutrient cycles (Newell *et al.* 2006; Guy *et al.* 2018). High densities are associated with increased competition for resources and increased susceptibility to diseases such as *Bonamia* (Doonan *et al.*, 1999).

In addition, due to the large geographic range and array of factors influencing survival rates, what may be a suitable level of bed density, biomass size distribution or productivity in one oyster population may be different to that of another. It is therefore important to determine how current native oyster populations range in density and how potential benefits may vary with these natural densities.

1.3. A history of oysters in Essex

Oyster cultivation was thought to have first been established by the Romans around 2000 years ago in Naples (Andrews, 1948; Spencer, 1990). The Romans were then thought to have developed an

oyster fishery in Colchester, Essex, UK, with large amounts of oyster shell being discovered in archaeological digs (Colchester Archaeological Trust, 2001). Consumption grew and peak production in the UK oyster industry occurred around the mid 1800's (MacKenzie *et al.* 1997b). The first documented decline of the UK Native oyster was thought to have first been brought about due to the industrial revolution. Oysters were able to be shipped inland and to London quickly via the newly established railways (Utting & Spencer, 1992; Edwards, 1997). This demand was satisfied with the use of motorised fishing vessels, with hand-pulled dredges largely discarded in all but the Fal fishery in Cornwall (Long *et al.*, 2017). By the middle of the 19th century most oyster fisheries were becoming severely depleted due to continuous over dredging and no closed season. Parliamentary Select Committees were set up and in 1877 legislation was passed to ban the sales of oysters from 14 May to 4 August each year, to conserve spawning stocks (Laing *et al.*, 2006). In around 1870 trade began in shipping live American oysters (*Crassostrea virginica*) across the Atlantic, with oysters re-laid in UK beds for fattening before harvest. Despite American oysters being unable to breed in the UK waters, and they themselves not present in UK today, the American slipper limpet *Crepidula fornicata* (L.) and the American tingle (*Urosalpinx cinerea*) were thought to have been brought across at this time with these species thought to be competitors and predators of oysters respectively (de Montaudouin *et al.*, 1999; Hancock, 1954; Orton, 1912). *C. fornicata* and *U. cinerea* remain a problem today (Hancock, 1954; 1955; Utting & Spencer, 1992; Kamphausen, 2012). After the American oyster, the the government encouraged the import of the Portuguese oyster *Crassostrea angulata* between 1926 and 1960, however this species did not proliferate due to highly limited spawning within the cooler waters of the UK. Trade then stopped in the 1960's due to viral gill disease (Utting & Spencer, 1992).

Populations of native oysters then grew during the 1950s with peak *O. edulis* output of the 20th century occurring in 1961 of 30,000 tonnes worldwide (FAO, 2004). However, severe winters of 1962 resulted in a further crash with subsequent recruitment failure during the 1970s now attributed to the use of tributyl tin (TBT) (Crisp, 1964; Laing *et al.*, 2005).

The Pacific oyster, *Crassostrea gigas*, was introduced into the UK in 1965 after various assessments into the impact it would have on the UK coastline. *C. gigas* is still cultivated on a large scale today

(Herbert *et al.*, 2012). Pacific oysters were previously cultivated from hatchery reared stock with temperatures in the UK waters previously assumed to be too cold for Pacific oysters to reproduce. This species was not thought to pose a significant threat to the native oyster via competition as it is more commonly found in shallow intertidal waters with native oysters thought to be dominant in subtidal areas (Troost, 2010; Tully & Clarke, 2012). However, temperatures have since risen from when Pacific oysters were first introduced, and reproductively active oysters have been found around the south coast of England with recent discoveries of feral *C. gigas* as far north as Scotland (Jones *et al.*, 2013; Smith, 2014), and self-sustaining populations found around Essex (personal communication, 2018).

Also, in 1965, when *C. gigas* were first introduced, implementation of the Molluscan Shellfish (Control of Deposit) Order came in to force. This controlled the import of molluscs from other countries and was primarily implemented to prevent further introduction of disease (and the development of hatchery seed). This remains in place today with stringent checks and limitation to the further movement of any live shellfish stock from overseas.

In the Essex estuaries there is a strong history of research on *O. edulis*, and following “an unexplained mass mortality” in Essex in 1920-1921 (Mistakidis, 1951), and a series of bad storms in 1939-1940 and 1946-1947, a Shellfish Research Station at Burnham on Crouch opened in 1947 by the Ministry of Agriculture, Fisheries and Food. This facility had the primary aim of investigating the decline of the East coast oyster fisheries and to implement management to create the conditions for the local restoration of the oyster beds. Work focused on factors affecting settlement and spawning processes, conditions and community structure of bottom fauna, predator and competitor interactions and also cultivation processes (Mistakidis, 1951). Today, a series of private grounds maintain the cultivation of oysters in the Essex estuaries. Harvests now consist primarily of *C. gigas* from small, dense, intertidal beds with a smaller yield of cultivated native oysters from large, lower density subtidal areas. The “wild oyster grounds” i.e. grounds generally owned by The Crown Estate and not privatised, remained to be fished at a low level until 2015 when a bylaw came into act between the dates of 31st May 2015 and 31st May 2018, now extended to May 2020 within the newly established Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone (BCRC.MCZ) (KEIFCA, 2018).

The BCRC.MCZ is located on the Essex coast covering an area of 284 km², designated to protect intertidal mixed sediments, native oysters and native oyster beds (UKGovernment, 2013). The MCZ boundary overlaps many other designated areas such as Sites of Species Scientific Interest, the Essex Estuaries Special Area of Conservation and Mid-Essex Coast Special Protection Area (Figure 1.2, Defra, 2013).

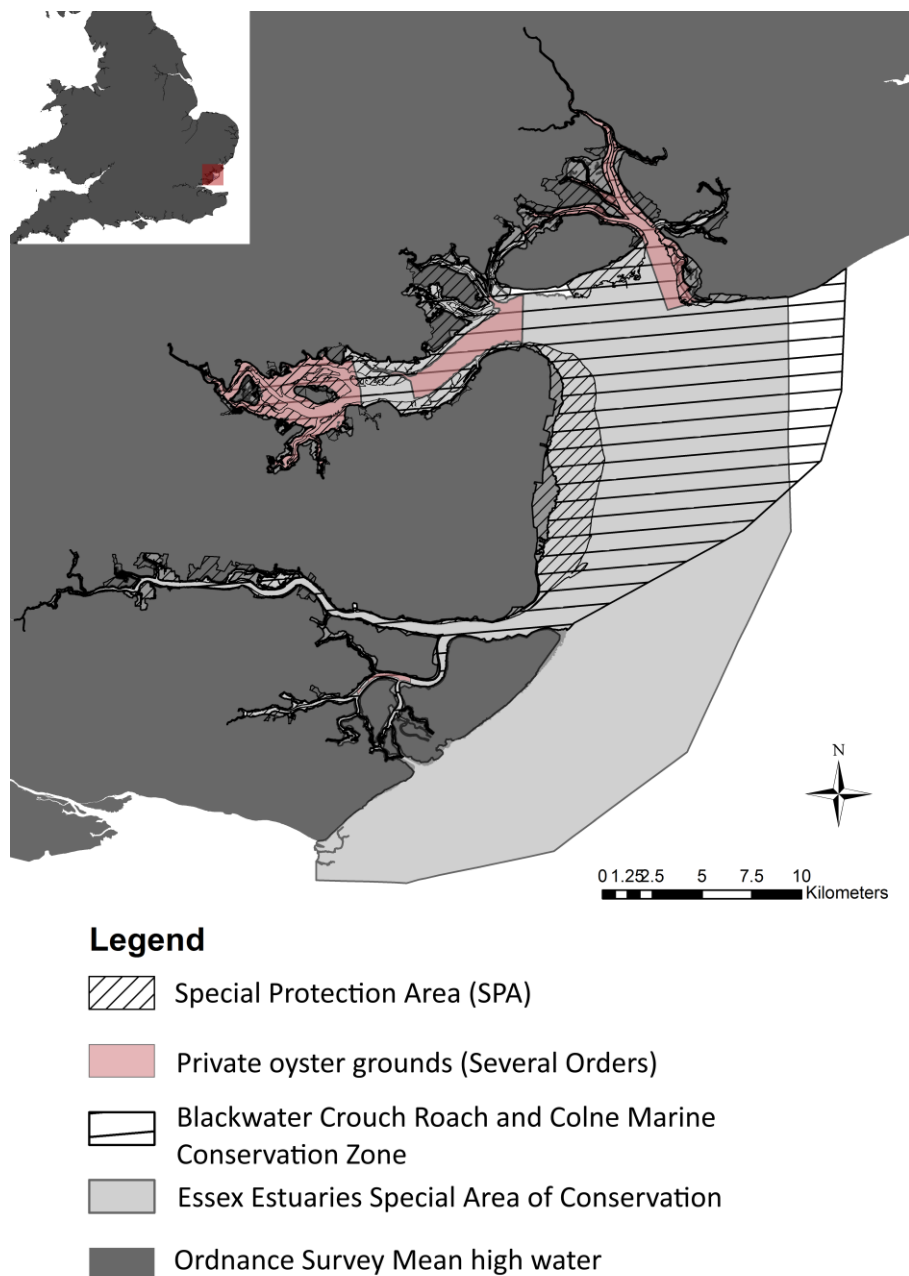


Figure 1.2. Map of Essex highlighting the overlapping protected areas in the marine and coastal environment. (Created in ArcMap 10.6.1).

Prior to designation of the BCRC.MCZ, harvesting of wild native oysters in “public” or state-owned areas of Essex (i.e. the BCRC.MCZ area) was restricted under the Kent and Essex Inshore Fisheries and Conservation Authority (KEIFCA) ‘Shellfish Beds’ byelaw. This byelaw permitted KEIFCA to restrict the date, time and duration of native oyster harvesting, specify the type of fishing equipment used and require log books to be maintained along with daily reporting to fisheries officers. KEIFCA also have an oyster byelaw that sets out a minimum shell size of 70 mm for harvesting. Between 2012 and 2014 harvesting of native oysters was permitted for a maximum of 2 weeks per year. In May 2015, KEIFCA implemented a 3-year temporary closure, with the potential to extend, under the ‘Shellfish Beds’ byelaw which prohibits the taking or disturbing of native oysters or native oyster beds in the BCRC.MCZ area (KEIFCA, 2010). A unique feature to this MCZ is that there is now a legal obligation to not only protect, but restore native oyster beds, with the long-term plan for recovery also incorporating the culturally significant fisheries of native oysters.

1.4. General biology of *Ostrea edulis*

O. edulis is a viviparous, protandric, hermaphroditic bivalve (Orton, 1927). Life expectancy may be up to 20 years with the usual life span between 5-10 years (Richardson *et al.*, 1993; FAO, 2004a; Roberts *et al.*, 2010). Depending on geographic location, spawning tends to occur once water temperatures reach a prolonged 14-16°C with the potential for multiple spawning events to occur within the same summer season (FAO, 2016). Within the UK, there is an average of two spawning events each summer with oysters changing sex after each spawning event (Walne, 1974; Wilson & Simons, 1985).

To reproduce, males will release sperm into the water column which is then drawn into female oysters to internally fertilise eggs (Horst, 1882). Larvae develop internally for approximately 10 days (Woolmer *et al.*, 2011), in this time “ripe” females can be observed through the presence of “white sick” which turns grey and then black as the larvae develop (Younge, 1960). Being a partial broadcast spawner means *O. edulis* has been used to assess Allee effects with brood sizes found to decrease when oysters are over 1.5m apart (Guy *et al.*, 2018). After being internally brooded larvae are expelled into the

plankton for approximately 10 days where they are known as veliger larvae. During this plankton stage, veliger larvae will grow a “foot” and become pediveligers, at this point oysters settle onto suitable substrate, such as clean shell, where they are thought to move using their pediveliger foot to select the best settlement positions (Laing *et al.*, 2005). Once settled, pediveligers undergo metamorphosis over 3-4 days becoming sessile, resulting in settled oysters or spat (Laing *et al.* 2005). Sexual maturity in *O. edulis* is thought to occur in the UK between 3-4 years of age (Cole, 1941; Millport, 2006), with some reports that functional males have been recorded at 1 year (Cole, 1941; Walne, 1974; Millport, 2006). Other reports however state that *O. edulis* will reach maturity at 35mm length (Gravestock *et al.*, 2014) or approximately 1 year of age (Richardson *et al.*, 1993). Few oysters have been found to be female at 2 years of age or 40mm in shell length however fecundity is relatively low until the oyster is at least 4 years old (Cole, 1941).

1.5. Oyster growth and survival

The speed at which an oyster grows to a harvestable size is of great importance in oyster fisheries; the faster the growth rates, the sooner an oyster can go to market and thus be more beneficial financially. There are a wide variety of factors influencing oyster growth, these factors include but are not limited to genetics, disease prevalence and susceptibility, temperature, salinity, food availability, pollutants including CO₂ through ocean acidification, predation, competition (both inter and intraspecific) and sedimentation (Mann, 1979; Laing & Millican, 1986; Robert *et al.*, 1988; Richardson *et al.*, 1993; Berntsson *et al.*, 1997; Rees *et al.*, 2001; Sanford *et al.*, 2014). There are tradeoffs for fast and slow growth in oysters with fast growing oysters found to have thinner shells than slower growing oysters, leaving them more susceptible to predation (Shaw *et al.*, 2007). In addition to increased predator protection from thicker shells, slow growing and thicker shelled oysters may be more resistant to the effects of ocean acidification (Waldbusser *et al.*, 2016).

Geographic variation in growth and survival

High variation in growth rates, mortality and disease susceptibility has been observed between populations of native oysters collected from around Europe and raised under the same conditions in trays in Northern Spain. This has highlighted the potential for populations to be extremely locally adapted to specific temperatures and conditions (da Silva *et al.*, 2005). Within the UK, native oysters are found to have variable growth rates around different geographic locations, with oysters from the Blackwater growing at a faster rate and to a larger size than populations in the Fal and the Solent (Richardson *et al.*, 1993). Variation within a single area has also been well studied in contained trays or trestles of oysters with notably “fast growing” and “slow growing” oysters inhabiting a single site, resulting in a wide range of possible growth rates (Davis & Calabrese, 1969; Leitao *et al.*, 2001).

Genetics

Whilst differences in growth rate from different locations may in-part be due to being adapted to different conditions, such as temperature, it is known that within a population you will find both fast and slow growing individual, with speed in growth rate can be selected for or against (Toro & Newkirk, 1990).

Aneuploidy is the presence of an abnormal number of chromosomes. It is common in bivalves with the effects said to be less harmful in plants and lower animals than found in mammals and other higher mammals, it is influenced by a variety of biological and chemical agents with pollutants such as hydrocarbons said to increase its prevalence in blue mussels (Dixon, 1982). Aneuploidy is said to directly reduce individual growth rates of *C. gigas* however there is little information regarding the effects of aneuploidy on *O. edulis* (Thiriot-Quiévreux, 1986; Leitao *et al.*, 2001).

When restoring native oysters, it is important to ensure oysters used are adapted to the local conditions which the oyster will be subjected to (da Silva *et al.*, 2005). Genetic variation was previously thought to be minimal in *O. edulis*, and oysters were freely moved around the country and between countries

however, recent research has highlighted the intrapopulation genetic variability of oyster populations across Europe which may need to be considered in any future movement of the native oyster (Launey *et al.*, 2002).

Pollutants

Oysters are particularly sensitive to impacts from various pollutants with *O. edulis* listed as having high potential to be used in bioindicator programs to assess pesticide contamination in estuaries and near shore areas (Valbonesi *et al.*, 2003). Oysters can be impacted in many ways from anthropogenic pollutants with effects ranging from tainting of the meat to be inappropriate for human consumption to reduced growth, reproductive output and increased mortality (Thain, 1986; Hassard *et al.*, 2017). The now banned tributyl tin (TBT) has been attributed to a decline in *O. edulis* throughout the 1970s and 1980s. The impact of this anti-fouling paint on oysters was first observed in the 1980s in France with highest contamination levels observed in harbours and marinas (Alzieu *et al.*, 1990). Since then, the effects of TBT on various species of oyster has been explored with different responses observed between *C. gigas* and *O. edulis*.

In addition to impacts from coastal run-off and antifoulants, ocean acidification from increased atmospheric CO₂ is reducing carbonate availability to growing oysters and has been found to negatively impact oyster populations worldwide (Barton *et al.*, 2012; Sanford *et al.*, 2014). In some areas of the US reductions on ocean pH has been attributed to up to 70% decline in larval survival in oyster hatchery rearing, with oyster shell dissolving at a faster rate than the oyster can form the shell (Clements & Chopin, 2016). In addition, oysters in acidified waters produce thinner shells which, in turn, results in higher mortality rates from predation (Sanford *et al.*, 2014).

Disease

Disease, specifically bonamiasis and marteiliasis (otherwise called Abers Disease), said to be accountable for the 93% drop in *O. edulis* production in France, is a serious issue for native oyster production (Laing *et al.*, 2014). In Europe, there are five main diseases of concern for *O. edulis*. These are a herpes-like infection caused by a virus called OHSV-1, a fungus shell disease *Ostracoblabe implexa*, the protozoan diseases, bonamiasis (*Bonamia ostreae*), marteiliasis (*Marteilia refringens*) and Denman island disease (*Microcytos mackini*) (Table 1.1). Out of these, the disease with primary cause for concern within the UK is that of bonamiosis (*Bonamia*), with the Essex estuaries testing positive for the presence of the protozoan parasite. This area spans from Landguard Point to the north down to North Foreland Lighthouse at the south and includes the BCRC.MCZ (DEFRA, 2016).

Table 1.1: Summary of the 5 main diseases of concern for oysters in Europe.

Disease	Caused by	Species	Symptoms	In UK?	Species at risk	Reference
Bonamia	Protozoan parasite	<i>Bonamia ostreae</i>	Can be asymptomatic also causes gill and mantle lesions and yellow discolouration	Yes	<i>Ostrea edulis</i>	MacKenzie <i>et al.</i> , 1997a; Hoare, 2004;
Denman Island Disease	Protozoan parasite	<i>Microcytos mackini</i>	Green abscess-like lesions in mantle and palp tissues	No but of concern	<i>Crassostrea gigas</i>	Gagné <i>et al.</i> , 2008; Hine <i>et al.</i> , 2001
Dutch shell disease or shell wasting disease	Fungus	<i>Ostracoblabe implexa</i>	Black lesions and malformation of the shell	Yes	<i>Ostrea edulis</i> , <i>Crassostrea gigas</i> , <i>Saccostrea cucullata</i> and <i>C. angulate</i>	Cole & Hancock, 1954
Oyster herpesvirus-1 microvariant (OsHV-1 μ var)	Virus		No direct symptoms observed	Yes	Thought to only affect <i>Crassostrea gigas</i>	FAO, 2004; Cefas, 2015; Rodgers <i>et al.</i> , 2015
Aber disease or Marteiliasis	Protozoan parasite	<i>Marteilia refringens</i>	Loss of pigmentation, cessation of shell growth, occasionally shrunken and slimy tissues	No - but of concern	<i>Ostrea edulis</i>	Marine Scotland, (2010)

Bonamiosis transference is thought to increase in areas with oyster densities over 1.26 m⁻² (Doonan *et al.*, 1999; Flannery *et al.*, 2014; Cranfield *et al.*, 2005). Susceptibility to bonamiosis is thought to be genetically linked and attempts have been made to breed “*Bonamia* resistant” oysters which have been

achieved with limited success (Morga *et al.*, 2012). Oysters in some areas are able to survive longer, reach marketable size and are able to reproduce however oysters in these areas are said to eventually succumb to bonamiosis and a cure has not been fully recognised with research ongoing (Ronza *et al.*, 2018). Other research has found that faster growing oysters may test positive for *Bonamia* sooner, therefore oysters may appear to grow at slower rates over time in populations which harbour this oyster parasite (Cáceres-Martínez *et al.*, 1995).

Predators and competitors

Despite many attempts to replenish oyster stocks there have been a few major issues preventing successful restoration even once fishing effort is ceased or reduced to low level. These include but are not limited to predators, competitors, diseases and “forms which attack the shell” (Hancock, 1969). The presence of high populations of predators not only reduces oyster survival through the direct consumption of oyster meat but also may reduce growth rates of oysters due to the reduced feeding time availability due to repeated failed predation attempts. This may be due to needing to stay clamped closed for a longer than optimal time due to predators attempting to open oyster shells or due to the extra energy required to invest in repairing shell which may become chipped or broken due to physical action from attempted predation events.

In the UK, the main oyster predators are assumed to be the American tingle (*Urosalpinx cinera*), The European rough tingle (*Ocenebra erinacea*), starfish (primarily *Asterias rubens* and *Crossaster papposus*), the shore crab (*Carcinus meanas*), the purple tipped sea urchin (*Psammechinus spp.*) and the oystercatcher (*Haematopus*) (Hancock, 1954, 1955, 1958, 1969; Orton & Winckworth, 1928). These predators are known to prey on settled oysters, however relatively little work has been done to identify predatory species which feed on unsettled spat within the water column although predators are known to include jellyfish, arrow worms and benthic filter-feeders (Hancock, 1969).

Starfish are thought to be “voracious predators” of oysters in British waters, with records of them preying on UK oysters dating back nearly 200 years (Forbes, 1841). Previous studies conclude that

Asterias rubens are likely to be the most influential echinoderm oyster predator, with *Crossaster papposus* found to preferentially feed on *A. rubens* rather than oysters (Hancock, 1958). Populations of starfish can vary throughout the year with starfish abundance thought to increase between April and July and decline between September and March (Gallagher *et al.*, 2008). Numerous studies have been done to investigate the feeding behaviour of various starfish species on oysters, most notably those completed in the 1950's at the Burnham on Crouch research centre (Hancock, 1954, 1958; Mistakidis, 1951). Starfish do not feed solely on oysters and are reported to feed on slipper limpets, mussels, barnacles and *Littorina* amongst other species, sometimes preferentially (Hancock, 1958; Korringa, 1951). There is some disagreement in starfish feeding preferences with other studies suggesting that small, young starfish can cause serious damage to oyster spat and should be regarded as an influential oyster pest (Korringa, 1954). An alternative study suggested that *A. rubens* of less than 100mm will tend to devour a greater number of barnacles over oyster spat, with smaller starfish preferentially selecting a greater proportion of barnacles over alternative species (Hancock, 1955). However, once the starfish grew to a size over 100mm in radius *Crepidula* were preferred. Overall throughout the experiment using starfish of various sizes, only 24 oyster spat were consumed, 54 *Crepidula* spat, 5 adult oysters, 114 *Crepidula*, 2 *Urosalpinx* and 26,900 barnacles showing the preference of *A. rubens* for different species. When four large (radius approx. 140mm) *A. rubens* were only provided adult oysters to feed on they were observed to consume an average of 4.8 adult oysters a week and a maximum of 8 adult oysters a week in a lab experiment. These experiments highlight the variation in dietary preference of *A. rubens*. They also highlight the possible benefits starfish may have on an oyster bed, potentially consuming competitive species such as slipper limpets, oyster drills and barnacles, however these experiments were not video recorded and so natural deaths may have occurred with starfish feeding on deceased individuals (Hancock, 1955, 1969).

In order to remove starfish from oyster and mussel beds, starfish mops can be used, with varying efficiency (4-78%, average 27% \pm 3.2; Calderwood *et al.* 2016), however, these are not used within Essex, UK. Another method used to reduce starfish predation on oyster beds is through the farming of oysters in waters of reduced salinity, this is because oysters are generally able to tolerate a greater

amount of freshwater input. Reduced salinity, however, creates a trade-off in oyster production whereby oysters grow at a slower rate in areas of reduced salinity and may also die if exposed to water under 15 ppt for a period of 15 days or more (Korringa, 1952; Menzel *et al.*, 1966, Perry & Cringo, 2000).

There are two main species of oyster drill within the Essex estuaries: *Urosalpinx cinerea* and *Ocenebra erinacea* (L.). The former species is native to Europe and is otherwise known as the European sting winkle with the latter species non-native and known by a number of different names including the American whelk tingle, drill or borer. *U. cinerea* is thought to have been introduced to the UK around the same time as slipper limpets via the import of American oysters *Crassostrea virginica* (Hancock, 1954). First records of the appearance of *U. cinerea* in UK waters were made in 1920 in the River Blackwater area when they were first confused with the sting winkle *Ocenebra erinacea* (L.), (Orton & Winckworth, 1928). By the 1950s in the Essex surveys from Burnham on crouch, maximum densities of 6 *U. cinerea* m⁻² were observed with an average of 2-3 m⁻² (Mistakidis, 1951) this is in great contrast to the recent surveys performed in the area where none were found in surveys performed within the Blackwater estuary in 2014 and 2015 (Wiggins, 2014), however recent surveys using clay tiles have found populations remaining in defined, small areas associated with non-native oyster cultivation in local estuaries (unpublished data, pers. obs, 2018).

It is thought that *U. cinerea* are able to drill oyster spat and barnacles as soon as they emerge from their egg capsules with laboratory experiments observing tingles of as small as 2-3mm able to drill oyster spat of around 4-5mm in diameter (Hancock, 1954). It has also been estimated that a single *U. cinerea* could destroy approximately 59 oyster spat within a season of 5 months. *U. cinerea* are known to hibernate during the winter months and so removal of this species from oyster beds has been said to be more effective during the summer months when they move closer to the shore into shallower waters and when egg capsules may also be taken (Hancock, 1969). Due to their size, *U. cinerea* may slip through trawling nets and so smaller mesh sizes when trawling are suggested to be used for surveys. Alternatively, trapping using regularly checked tiles has been suggested to be the most efficient way of removal (Hancock, 1969).

The European rough tingle, *O. erinacea*, is also said to be a predator of oysters however this is native to the UK waters and not considered to be as voracious as the American tingle. This is also thought to target smaller oysters and spat and exhibits similar behaviour to that of *Urosalpinx cinera*, therefore if control measures are required, similar methods to those used to remove *Urosalpinx* are thought to be applicable to both species (Hancock, 1969).

Despite being a reported predator of oysters, *C. maenas*, as with starfish, will preferentially select mussels, and also cockles, over oysters. These crabs appear to reject prey if they are unable to open them after a certain number of attempts, and with oysters potentially being less profitable requiring more effort to open them overall, shore crabs will preferentially select other species (Mascaró and Seed, 2000). This is thought to be due to the faster opening times of smaller prey, however *C. maenas* have been observed to be able to open pacific oysters of up to 50-60mm SL (Dare *et al.*, 1983; Sanchez-Salazar *et al.*, 1987).

Purple-tipped sea urchins, *Psammechinus miliaris*, have been observed to kill and eat oysters by using their teeth to grind through the shell. It has been speculated however, that these urchins generally do not directly target oysters, instead consuming species living on the oyster shell (Hancock, 1892).

Despite showing population decline, Eurasian oystercatchers *Haematopus ostralegus* are thought to be the main avian predators of native oysters however are not thought to be important predators both due to the size of oysters and because the majority of populations are not exposed by low tides (Prater, 2010). Despite the name, the oystercatcher will tend to primarily feed on other bivalves such as mussels and cockles exposed at low tide (Heppleston, 2014).

The primary competitor of concern within UK waters is the slipper limpet *Crepidula fornicata*. Other competitors include barnacles, encrusting worms, sea squirts and mussels (Hancock, 1969). There is evidence that there may be some level of competition between the Pacific oyster *Crassostrea gigas* and *O. edulis* with *C. gigas* found to have a negative effect on *O. edulis* growth when both species of oysters are found within the same horizontal plane (Zwerschke *et al.*, 2018). Populations of feral *C. gigas* increasingly found in subtidal areas down to 42m depth where *O. edulis* are said to be dominant and

therefore cooccurrence of these species may be an increasingly pressing issue (Tully & Clarke 2012; Nielsen *et al.* 2017; Smaal, 2009). The first recorded spatfalls of *C. gigas* were thought to be recorded in Europe in 1975 in the Oosterschelde, Netherlands with warm summers considered the main cause of recruitment (Drinkwaard, 1999). In feeding comparisons, both oyster species have been found to preferentially filter retain algae of the same size (7-32 μ m) however *C. gigas* had a clearance rate up to three times higher than that of *O. edulis* for smaller algal species despite the lower threshold for algal size being the same for both oyster species (Nielsen *et al.*, 2017). This implies that *C. gigas* may be more versatile than *O. edulis* and it may be possible to outcompete the native oyster in blooms of smaller algal species. Even if populations of *C. gigas* are occurring in areas unsuitable for *O. edulis* there is also no evidence either way as to if *C. gigas* are consuming the spat of native oyster. The size of *O. edulis* veliger larvae is said to be approximately $147 \pm 5.0 \times 126 \pm 7.2 \mu\text{m}$ (Acarli & Lok, 2009), however with the maximum particle size able to be retained by *C. gigas* ranging between 20 and 32 μm , it may be unlikely retention and therefore consumption of *O. edulis* veliger larvae would occur (Nielsen *et al.*, 2017). However, it may be possible for veliger larvae to be killed in the filtration process and excreted as pseudofaeces as occurs with *C. gigas* feeding in the presence of blue mussel larvae size $172.7 \pm 18.1 \mu\text{m}$ (Troost, 2009).

Crepidula fornicata, otherwise known as slipper limpets, were introduced to the UK in the late 1800s with the import of the American oyster and quickly became prevalent in UK waters quickly developing a name for itself as the “oyster pest” (Orton, 1926; Mistakidis, 1951; de Montaudouin *et al.*, 1999). It was first recorded in Liverpool Bay in 1872 with records in the River Crouch, Essex dating back to 1893 where it was first thought to establish itself (Goulletquer *et al.*, 2002). This population was suspected to have spread from the Colne prior to this date when American oysters were laid in the area (Mistakidis, 1951). By 1951 slipper limpets in the Crouch had reached densities of over 1000 individuals m^{-2} with an average density of 445.5 limpets m^{-2} (Mistakidis, 1951). Slipper limpets are recognised competitors to oysters, however no studies appear to have assessed the ability for *C. fornicata* to consume *O. edulis* larvae to date. *C. fornicata* are primarily thought to compete with *O. edulis* for food, space and hard shell on which their planktivorous juveniles settle. *C. fornicata* remains

Table 1.2. Net interactive effects of slipper limpets on oyster beds.

Effect	+ve	-ve	Why	Oyster species	Reference
Filter feeder creating 'mussel mud' or pseudofeaces		X	Degrades oyster grounds and hinders recruitment	<i>Ostrea edulis</i>	OSPAR, 2009
Dead shells provide hard substrate	X		Creates substrate for spat to settle on	<i>Ostrea edulis</i>	OSPAR, 2009; Orton, 1926
Trophic competition with oysters		X	Both fighting for the same food source	<i>Crassostrea gigas</i>	Montaudouin <i>et al.</i> , 1999
Spatial competition		X	Both fighting for the same space on the ocean floor	<i>Crassostrea gigas</i> , <i>Ostrea edulis</i>	Montaudouin <i>et al.</i> , 1999; Walne, 1956
Causes constant diatom production, preventing toxic algal blooms	X		effective silicate pump freeing silica for diatom blooms	<i>Not specified</i>	Ragueneau <i>et al.</i> , 2002
May provide protection against <i>Asterias rubens</i> predation	X		Appears to be more attractive to common starfish predators	<i>Ostrea edulis</i>	Hancock 1955,1956; Thieltges <i>et al.</i> , 2002)
Increased labour of dredging (particularly when by hand such as Fal)		X	Increased shell biomass results in greater hauls which have to be thrown back	<i>Not specified</i>	Orton, 1926
Oysters require cleaning before sale to rid them of limpet chains		X	Decreased aesthetic appeal and reduced accuracy in weight	<i>Not specified</i>	Orton, 1926
Impossible to remove from oyster beds entirely		X	Due to prolonged breeding season and the free-swimming larval stage in its development	<i>Not specified</i>	Orton, 1926
Doesn't predate directly on the oyster and competes with other oyster competitors	X		Such as tunicates, other bivalves and other plankton feeders	<i>Not specified</i>	Orton, 1926
Able to remain at the surface of benthos under low-levels of sedimentation	X		Maintains hard substrate availability in areas where dead shell may become buried over time due to sedimentation	<i>Not specified</i>	(Powell-Jennings and Callaway, 2018)

to be a problem for oyster fishing and farming in the UK today however there is some disagreement on the extent to the problem they cause, with some oyster growers highlighting their importance in oyster

recovery through the provision of shell in areas where suitable settlement substrate for oysters may be limited (personal communication). Here, *C. fornicata* are potentially able to remain at the surface of sediment following low levels of sedimentation (Powell-Jennings & Callaway, 2018). Effects of *C. fornicata* are described in Table 1.2. *C. fornicata* only tend to thrive in southern areas of the UK where water temperatures are high, however, there have been a number of management plans put in place in the south of England (Fitzgerald, 2007; Rayment, 2008). Where slipper limpets are high in abundance, despite their shell creating cultch for the settlement of oyster spat, blue mussel shells are recommended to be used preferentially for clutching due to the reduced lifespan of the shell, resulting in it crumbling after 2 years. This reduces the likelihood of long chains of slipper limpets developing (Korringa, 1946; Mistakidis, 1951). Alternatively, removable cultch is recommended such as bags of shell, tiles and bundles of birch (Korringa, 1946).

1.6. Stock restoration

The loss of available standing stock of oysters may be a limiting factor when attempting to restore native oyster populations therefore re-stocking is seen as an effective strategy in some areas (Laing *et al.*, 2006). Once population densities fall below a critical threshold, as with many other species, oysters are likely to be subjected to an Allee effect whereby too few individuals are present to result in successful reproduction (Courchamp *et al.*, 1999). *O. edulis* are found to be influenced by density dependent reproduction with significant reductions in brood size observed when oysters are more than 1.5m apart due to the requirement of internal fertilisation (Guy *et al.* 2018). In addition to reproductive benefits to maintaining a minimum density of oysters, as ecosystem engineers, oysters may increase shell budgets and subsequently increase their own settlement success, resulting in a positive feedback by creating their own suitable habitat (Cuddington *et al.*, 2009). When increasing oyster populations there are three primary overarching ways of achieving this:

1. Management of natural populations by increasing populations via habitat management, such as ensuring the availability of shell for oyster settlement, alone;

2. Increase of populations through mariculture where oyster spat are collected from the wild, grown to a larger size to decrease predation risk and mortality of juveniles through other mechanisms such as smothering due to increased sedimentation then released;
3. Take adult individuals and spawn them in captivity through aquaculture allowing the spat to settle, grow to a large enough size before planting them into the wild. This may allow the selective breeding of disease resistant oysters (Culloty *et al.*, 2004; Elston *et al.*, 1987; Flannery *et al.*, 2014; Naciri-Graven *et al.*, 1998) however great care must be taken to ensure a large enough genetic diversity is achieved to prevent inbreeding in future wild populations after the oysters have been seeded (Laing *et al.*, 2006).

Option 1 is largely through a combination of water quality management, cessation or control of fishing, control of dredging and sedimentation upstream and, thought to be most important, the management of cultch (see below). Control of fishing and other activities may largely be accomplished with the use of Marine Protected Areas (MPAs) such as the Marine Conservation Zone (MCZ) designated in Essex in 2013 which are also thought to be largely beneficial to increase populations (Roberts *et al.*, 2003; Natural England, 2015). If managed correctly, MPAs can increase the output of oyster spat allowing for a sustained breeding population seeding other fished areas (Goulletquer & Moine, 2002; Mroch III *et al.*, 2012; Deane, 2015). In addition, the continuation of the use of nationally used closed seasons, such as the closure in place between 14th May and the 4th August are essential for the preservation of breeding populations (Laing *et al.*, 2006).

Options 2 and 3 generally would also require habitat and fishing management to prevent a population crash however these options may be able to be designed to match the Cost-Benefit Analysis. Previous studies have indicated that the most cost effective way to carry out a native oyster restoration program would be to import half-grown native oysters from another, disease free area (Laing *et al.*, 2006). However, this only applies if the main limiting factor is lack of adult stock. Where adults are present, but the population recovery is limited by habitat, relaying of more adults is unlikely to result in recovery unless adults are relayed in sufficient numbers to result in the alteration and creation of habitat.

Hatchery rearing techniques were largely first developed in the 1960s and 1970s with greatly varying rates of survival recorded past metamorphosis (between 50% and 90%) It does however, require very high levels of seed to be financially viable with relayed oysters often suffering increased susceptibility to disease (Laing *et al.* 2005).

Cultch management

Cultch management is seen by some as essential for the restoration of native oysters (Laing *et al.*, 2006). Cultch is generally made up of hard pieces of shell which provide the essential hard substrate on which oyster spat settle. In areas where oyster beds have been over-fished, or sedimentation levels are high, oyster spat may have little to no shell or gravel remaining on which to settle. Even where they do in summer, further sedimentation in winter may result in smothering and high mortality of newly settled spat (Tully & Clarke, 2012). In order to increase or maintain cultch availability two main management techniques may be applied. These are; harrowing oyster beds to reduce sedimentation and the laying of cultch (or clean shell) to promote settlement above any sediments.

Laying of cultch

In areas lacking shell availability the laying of cultch in the form of broken oyster shell and or mussel shell has previously been found to be highly beneficial to oyster spat settlement (Barry, 1981; Tully & Clarke, 2012). Previously the laying of cultch has provided highly variable levels of success with timing of when to lay cultch essential in its effectiveness. Laying cultch relies on there being sufficient naturally occurring spat within the water column which may not necessarily be the case in sparse oyster beds where fertilisation levels are low or in areas with limited standing stock (Korringa, 1946).

Harrowing

Harrowing is the pulling along of an implement such as an oyster or scallop dredge with the bag removed or a grass or agricultural harrow along the sea bed (Bromley *et al.*, 2016). This technique is designed to uncover new shell by bringing it to the surface and remove epifauna. It is a widely used technique however there is some disagreement on its effectiveness (Bromley *et al.*, 2016; Haelters & Kerckhof, 2009). It is fairly accepted in most areas that well dredged oyster grounds do not require harrowing as the constant disturbance of the substrate by oyster dredges perform the same effect as harrowing (Woolmer *et al.*, 2011). Harrowing is said to be most effective when performed just before the spawning season preventing a build-up of sediment and pseudofaeces before the spat are to settle.

Harrowing is part of traditional oyster techniques and is perceived to benefit oyster grounds with annual harrowing taking place within the Tollesbury and Mersea Several Order (KEIFCA, 2015b). In the Fal estuary, gentle chain harrowing is deemed to be part of “usual” activities and will not require comprehensive assessments for it to take place and in the Blackwater fishery in Essex harrowing was included in licencing requirements (Fitzgerald, 2007; Bromley *et al.*, 2016). Harrowing, along with frequent dredging is also said to maintain an equilibrium in substrate in areas of high *Crepidula fornicata* density where high volumes of pseudofaeces increases the risk of smothering to oyster spat (Hancock, 1955). However, harrowing and use of chains has also been associated with the spread of slipper limpets and the maintenance of low quality benthic habitats, nevertheless no quantitative studies have been published (Fitzgerald, 2007). The effectiveness of harrowing has therefore been called into question and is currently being tested by the Kent and Essex Inshore Fisheries Conservation Authority (KEIFCA). So far, only preliminary results have been released, early results indicate that although harrowing causes changes to the seabed, these changes are short-lasting in exposed areas and longer lasting in more sheltered areas. Oyster spat settlement has yet to be quantified here however local oyster growers argue that the timing of harrowing and amount of effort used to harrow different grounds are highly important (KEIFCA, 2015; pers. com. 2017). The first recognised study into the effectiveness of harrowing was completed in 1972, investigating spat settlement in the Fal and Crouch rivers despite harrowing being widely used prior to this. Waugh (1972) found that harrowing was only effective if

shell brought to the surface can remain sediment free until spat settle and that overall, harrowing had little effect, with harrowed areas quickly becoming silted, thus resulting in decreased spat settlement. In comparison, fallowed (i.e. completely untouched, not harrowed or dredged) grounds showed increased level of spat settlement in the same area (Tully & Clarke, 2012, Waugh, 1972). A more recent study in Lough Foyle, Northern Ireland, found a significant difference in number of oyster spat settled in non-harrowed areas as opposed to harrowed with non-harrowed areas seen to be better for oyster settlement. In this study only 12 oyster spat were collected (nine from harrowed and three from non-harrowed), therefore, as this shows such low spat settlement that it is difficult to draw firm conclusions from settlement alone. It is important to note here that silt build-up in the experimental area is unlikely to be the cause of low settlement in the Bromley study. It is widely accepted that harrowing is not a “one size fits all” technique and should be used only in certain areas where silt removal prior to seeding is planned (Cole, 1956).

Regular chain harrowing has also been used by the Mumbles Oyster Company for the breaking up of chains of slipper limpets (Syvret & Woolmer, 2015). Whilst this is also a proposed technique in the Port of Truro Oyster fishery, it is under-studied and may require further research (Fitzgerald, 2007). Harrowing has been suggested to break up slipper limpet chains breaking the “female” base from the “male” top. This would in theory reduce reproductive output if sufficient chains are broken. Alternatively, the breaking of these chains may allow for recruitment of other slipper limpets therefore creating two separate chains over time thus doubling the potential reproductive output (Fitzgerald, 2007). In addition to the control of the non-native slipper limpet, harrowing has also been used in the Fal Estuary for the removal of excessive amounts of seaweed (Woolmer *et al.*, 2011).

Overall, many studies imply that harrowing should only be undertaken with precaution, in areas where oyster shell is available beneath the surface and only in areas of lower levels of sedimentation, just before breeding seasons. Harrowing can be highly detrimental to benthic fauna and flora and in some cases can increase the likelihood of siltation and smothering of spat. In addition to this, harrowing may not be required in areas which are already frequently dredged as this fishing technique likely provides

a similar level of benthic alteration to that of harrowing with unclear effects on populations of *C. fornicata* (Tully & Clarke, 2012; Bromley *et al.*, 2016).

Fallowing

An alternative approach to encouraging oyster bed growth is by allowing beds to go fallow. This involves a complete closure of oyster fishing, and cessation of dredging, harrowing and the laying of cultch. The understanding that an oyster bed may need to lie fallow for at least short periods of time was first described by Julius Nelson in 1892 for oyster beds to “rest now and then” (McCay, 1998). The comparison of active cultch management as opposed to allowing beds to fallow was investigated by Waugh (1972). Here, increased spat settlement was found in fallowed areas as opposed to harrowed areas implying a likely benefit from the total cessation of human intervention in some areas (Tully & Clarke, 2012).

1.7. Active management in Essex

Alteration of the sea bed, either through cultch management or the increased proliferation of the native oyster is of particular contention within the Essex estuaries due to the majority of the MCZ overlapping a Special Area of Conservation (SAC). This SAC was designated on 1st April 2005, also incorporating six of the Special Protection Areas (SPAs) within the Essex estuaries. Protection of the benthic environment in the form of mixed sediments is part of the SAC’s primary objectives and thus may act in conflict to the requirement to protect *O. edulis* due to the potential need to alter the benthos in some areas in the form of cultch laying and or harrowing. Subsequently, in November 2015 Natural England put a hold on the use of cultch to increase the availability of hard substrate to oyster spat, a technique deemed essential by some in oyster restoration (Laing *et al.*, 2005). To combat the potential conflict which arises from the different apparent priorities of the SAC and BCRC.MCZ a community group called the Essex Native Oyster Restoration Initiative (ENORI) has been working to designate specific

areas within the BCRC.MCZ specifically to attempt various methods of active native oyster restoration through a community driven voluntary no-take zone called the Blackwater Conservation Box (Zoological Society of London, 2018; Mcleod *et al.*, 2019).

1.8. Benthic sampling methods

When assessing oyster communities there are five primary methods for collecting data. These are dive surveys, camera surveys, grab sampling, dredge surveys and patent tongs. Patent tongs have previously proved very effective at assessing oyster density in the Chesapeake Bay area. This technique, however, has low efficiency in assessing densities of individual oysters below 25 oysters m^{-2} (Schulte *et al.*, 2018). Grab size is, nevertheless, much larger than a standard van Veen or day grab (1.03m^2 vs 0.1 or 0.25m^2) therefore may provide useful if future surveys require quantification of patchy oyster landscapes.

Grab sampling has been tested in previous surveys and has proved effective at providing information regarding infaunal communities (Cameron, 2017). However, as with quadrat sampling, this method of sampling is known to under-estimate presence and densities of lower density species (Moore *et al.*, 1999). While it is possible to detect oysters in grab samples, a very high sampling effort would be required which would restrict any survey to one small geographic area due to expected low and variable *O. edulis* densities (e.g. a grab of 0.1m^2 would only be able to detect oyster densities of approximately 10 oysters m^{-2} and above. This is reduced with replication).

Whilst the use of visual analysis using dive and camera surveys would result in the lowest disturbance impact on the sea bed, and likely most accurate small-scale quantification of population and community, these methods require good and regular visibility. For the use of camera systems, a minimum of 3m visibility or 4 times the camera to subject plane distance is said to be required to create usable images of the sea floor (Davies *et al.*, 2001). High sedimentation and particulate matter within the water column has long been recognised within the Essex area and visibility suitable for camera or dive surveys is not reliably available (Sheldon, 1961). In addition, it may be difficult or impossible to confirm whether an oyster is alive, or a dead oyster which is closed, during a survey which only uses photographic or video

analysis. Diver surveys have been proposed in Essex, however this method for data collection is only suitable for smaller-scale surveys and are not cost-effective for a wide-scale survey, such as for assessing the *O. edulis* population within the BCRC.MCZ (Chai *et al.*, 1992).

Finally, dredge surveys have proved effective in obtaining information regarding distribution and relative abundance of macrofaunal benthic species. In addition, dredges are highly popular due to their ease in use and large coverage area, particularly in comparison to grab samples (Chai *et al.*, 1992; Cameron, 2017). It is well understood that dredges do not catch 100% of the flora and fauna on the sea bed and as dredge efficiency data is highly site, operator and target species dependent and this results in large errors in population estimates which need to be accounted for when analysing data. Efficiency of dredges are thought to range from 5-40% and up to 60% in some areas (Chai *et al.*, 1992; Eleftheriou & Moore, 2013; Taylor *et al.*, 2014).

1.9. Estimating population changes

In order to assess a population and how populations are likely to change with future management techniques, models are often used to predict population changes. A large amount of previous literature on population structure has been predominantly based on the Lotka-Volterra models (de Roos & Persson, 2013). These models are simple and address predator-prey and competitive interactions of species and generally rely on the number of individuals within a group or subset of organisms (Reichstein *et al.*, 2015). There are, however, limitations to these models as they do not take into account the individual variation within populations, such as in terms of growth rate, mortality, life-history stage and other factors (Schröder *et al.*, 2014). At the simplest level, a size structured model will limit juveniles to be only capable of growth or mortality and adults only capable of reproduction or mortality. This brings about great restrictions due to differences throughout life in fecundity and or disease susceptibility (Ebenman & Persson, 1988; Arzul *et al.*, 2011). Previous oyster population surveys focus on the number of oysters without taking into account the size of the oyster (Kennedy & Roberts, 1999). This gives a good idea on the absolute population number but does not give an estimate of biomass, fecundity or population of oysters at or above harvestable size. Assessing individual based growth,

survival and fecundity through the use of matrix or Integral Projection Models (IPMs) will allow for more accurate estimations of population change and aid identification of which life stages have most influence in population growth. This in turn allows for more targeted conservation and restoration efforts with efforts able to be focused on life stages that have the greatest impact on population change.

1.10. Conclusion and key knowledge gaps

Despite maintaining a closed fishing season to conserve *O. edulis* breeding stocks, native oysters are said to only reproduce sporadically (Laing *et al.* 2005). This, combined with the high longevity of this species, reaching approximately 5 years to reach marketable sizes, and the susceptibility of oysters to mortality from natural and anthropogenic events such as storms, increased sedimentation, predators, competitors and disease, has left this species highly susceptible to overfishing (de Montaudouin *et al.*, 1999; Korringa, 1946; Tully & Clarke, 2012). It is therefore proposed that if populations around the UK are brought back to historic levels, similar harvest levels to those recorded in the pre-war years will never be able to be achieved due to the likelihood that these surpassed the sustainable harvest levels (Laing *et al.*, 2006). Controlled management of *O. edulis* can increase population number and result in the production of small-scale fisheries as proven in Denmark where landings were transformed from effectively zero in 2000 to over 900 tonnes by 2003 (Laing *et al.*, 2006). The further understanding of predator, competitor and disease dynamics, combined with effective population modelling and understanding of how populations are composed in terms of life stages will allow for more precise fishery and conservation management. Due to the wide variation in types of habitat and temperature range in which *O. edulis* resides, a site-specific management technique should be applied where available, particularly regarding areas where *Bonamia* and other diseases are present.

Current recognised issues for limiting the restoration of oysters in Essex are the need to understand the distribution, abundance and density of native oyster populations so that informed restoration can take place. This lack of knowledge about the distribution and abundance of current populations mean targeted restoration projects and quantifying restoration “success” is not possible. Once populations are

mapped and estimated, understanding what may be limiting recovery in terms of life stage most at risk will help to further direct restoration by influencing the type of restoration required (i.e. stock vs habitat-based management). Reduced or limited amount of standing stock influences the ability of *O. edulis* to efficiently reproduce due to broadcast spawn with sperm becoming too diluted within the water column before it is drawn in by the females (KEIFCA, 2013; Mroch III *et al.*, 2012). In addition to this, the presence of *Bonamia* disease may limit the maximum density of oysters possible on these grounds, with disease transfer rates higher in more densely populated areas (Cranfield *et al.*, 2005). If settlement is occurring throughout Essex, it is possible that predators such as starfish may be moving in to oyster areas and consuming spat. Understanding the distribution and seasonal movements of primary predators and competitors is therefore required to assess if there are predictable movements of key associated species which may help to direct restoration by protecting oysters from predators at various life stages, such as occurs in France with protection from starfish (Barthelemy *et al.*, 1991).

The designation of the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone makes clear separation of *O. edulis* as a species and *O. edulis* as a bed or habitat. Previous research has highlighted the increase in species epifaunal diversity associated with live oysters over dead shell and other types of hard substrate, however this fails to assess how naturally occurring native oyster densities may be associated with epibenthic species richness (Smyth & Roberts, 2010). This is required to understand how oyster density may be associated with species richness and will help identify potential ecosystem services oyster beds may provide as opposed to low-density, free-living individuals.

These areas of research, combined with the clarification of how the BCRC.MCZ and other designations are to work with each other, are of paramount importance to ensure the continued survival of Essex native oysters and will be the focus of this PhD.

1.11. Thesis rationale and aims

While the ecology and biology of *O. edulis* has been well studied over the past century, largely due to the research station at Burnham on Crouch, more recent years has largely focused on specific estuaries

within Essex or on disease transference, with a single widescale distribution survey completed in 2012 (Allison, 2018). Due to the protected area designation there is now a legal obligation to protect and restore native oysters within the BCRC.MCZ.

This project has been developed due to the need for a comprehensive study of the current status of the *O. edulis* population following designation of the BCRC.MCZ after the cessation of fishing for native oysters in this area. This is to establish a baseline estimate of what the current biomass and population number of native oysters are and to give an understanding of what it might mean for oysters to be “restored” within Essex. In addition, assessing current distribution, abundance and seasonal movements of key associated species will enable more accurate predictions on which species may be affected by any restoration projects.

The aims of this thesis were to:

1. Establish a baseline of the current status and distribution and abundance of *Ostrea edulis* within the newly established BCRC.MCZ along with seasonal changes in distribution and abundance of key associated species, capturing data to see how this baseline changes over time.
2. Investigate potential species richness associations of varying natural densities of *O. edulis* and make predictions on how associated species may change with increasing native oyster density through successful restoration efforts or declining native oyster density through fishing or other population change.
3. Assess individual growth and mortality rates of native oysters of various sizes at densities known to occur naturally throughout Essex.
4. Predict future population changes and determine the sensitivity of each oyster life history stage to different management interventions and species interactions.

These aims have been structured into the following chapters with all methods presented in each respective chapter:

Chapter 2: Native oysters and their community through space and time – a multi-annual study of benthic community ecology in the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone.

Following the designation of the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone (BCRC.MCZ) there is now a legal obligation to protect and restore the native oyster in Essex, however the actual distribution and abundance of *O. edulis* and key associated species were unknown. Biannual dredge surveys in the BCRC.MCZ were completed between 2014 and 2018 to provide abundance and distribution estimates of key associated species. This chapter establishes the baseline over the distribution and abundance of the native oyster and associated primary predators and competitors. Estimates of population and biomass along with distribution maps are provided to highlight annual and seasonal changes in *O. edulis* count and biomass, *C. gigas* count, *A. rubens* count and *C. fornicata* biomass. Variation in shell morphology throughout the BCRC.MCZ are addressed. This chapter forms the basis on the starting point for *O. edulis* biomass availability at the start of the BCRC.MCZ designation and will be used to assess if or when restoration of European native oysters in Essex has been “achieved”.

Chapter 3: Density and seasonally dependent associations of biodiversity with the European flat oyster (*Ostrea edulis*): evidence for marine planning.

Species specific benefits of oyster restoration need to be addressed to understand the implications of native oyster restoration on the wider marine benthic community. This chapter uses data from the aforementioned biannual dredge surveys between 2014-2018 to investigate links between natural densities of *O. edulis* (0-4.2m⁻²), and the prevalence of other dominant habitat features such as non-native slipper limpet (*Crepidula fornicata*), shell availability and resultant epibenthic species richness.

Chapter 4: A novel method for tracking growth rate and survival of individual native oysters at low density

Identifying variation in growth rate and survival of oysters throughout the Essex estuaries is essential in both pinpointing the most suitable areas for restoration and highlighting areas which may be most at risk of population decline or collapse. In addition, understanding growth rate and survival will assist in highlighting which life stages of the European native oyster are most at risk of mortality and what life-stage should be primarily focused on for restoration efforts.

Individual, marked native oysters of naturally occurring size distribution were followed over the course of between 12-18 months at sites where oysters are locally adapted in the BCRC.MCZ or where oyster growers have relayed for fattening and growth in private oyster growing areas. Growth and survival rates of individual native oysters were monitored four times a year in relation to geographic and size variation.

Chapter 5: Demographic modelling of *Ostrea edulis* in Essex, UK.

Variation in growth rates and survival have been observed in the European oyster throughout the BCRC.MCZ, however, little is known about how these vital rates translate into population changes. In this Chapter, the demographics of *Ostrea edulis* within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone are used to develop an Integral Projection Model (IPM), with predictions on future populations given current levels of growth, survival and recruitment. In addition, various management interventions are addressed with their impact on sub-populations within the BCRC.MCZ modelled.

Chapter 2:

Native oysters and their community through space and time – a multi-annual study of benthic community ecology in the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone.

2.1. Introduction aims and hypotheses

The UK native oyster, *Ostrea edulis*, is listed as a Priority Species in the UK post-2010 Biodiversity Framework, with populations having declined across its geographic range. In 2013 the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone (hereafter the BCRC.MCZ) was designated due to the presence of European native oysters (*Ostrea edulis*) and native oyster beds. This designation means that there is now a legal obligation to protect and conserve native oysters in this area with a no-take byelaw of *Ostrea edulis* enforced from the 31st May 2014. Historically, native oysters having been well studied however, the vast majority of studies have taken place either on fished grounds or in laboratory facilities. This new designation provides a unique opportunity to study why they declined and their current ecology whilst fishing is limited. New efforts are therefore under way to understand the reasons for decline of this oyster.

While there has been characterisation of densities and distribution of native oysters within the BCRC.MCZ in 2012 in order to develop the evidence for MCZ designation (Allison & Underwood, *in press*), no study has coherently mapped full distributions of native oysters and associated species over time to assess current trends in oyster population abundance and associated community dynamics. Estimating population sizes and understanding seasonal changes via individual births, deaths and movement is key for the management of protected areas, both in terms of restoration and conservation activities but also in terms of fishery management.

Regular monitoring and estimation of oyster populations has occurred elsewhere, for example in Lough Foyle, with regular surveys dating back to 2008 and occasional surveys dating back to 1991 (McGonigle & Scott, 2012). These surveys have enabled active management of the native oyster fishery, only opening when stocks are deemed sufficient and when the population shows sufficient distribution of age classes, however these fall short of making future predictions on population growth or recovery due to the lack of information on the range of survival, growth and reproduction probabilities for the populations (Loughs Agency, 2018). In addition, other methods of population assessment depend on Catch Per Unit Effort (CPUE) which are notoriously difficult to use for assessing abundance of stocks (Maunder *et al.*, 2006).

In order to fulfil the objectives and requirements of the BCRC.MCZ designation, to “protect and restore” native oyster stocks, population assessments, analysing current spatial distribution, abundance, biomass, and size frequency distribution of native oysters within the protected area is required. This will direct conservation and restoration efforts accordingly to the right activities and establish a starting baseline from which to make predictions on, if or when it may be possible to re-open a native oyster fishery and if so, what level of extraction may be deemed sustainable.

Size frequency distribution of populations

When a species is targeted for restoration or for harvest, understanding the size, age or stage structure of the target population is central for making informed management decisions (Moore *et al.*, 2016). While solely basing management decisions on size structure and abundance alone are recognised to be insufficient for sustainable population management, understanding these metrics are a vital place to start in regards to sustainable population management (Pope *et al.*, 2010).

Size frequency plots of a population can help to identify the life stage at which bottlenecks to population growth are occurring. If a population is formed of a high level of biomass of small or juvenile individuals, population growth is likely limited by development and may be indicative of an over-exploited adult population (Pope *et al.*, 2010), whereas a population composed of a high proportion of adult individuals is likely limited by reproduction whereby too few adults are reproducing successfully

(Persson & de Roos, 2013). Reproduction limitation of a population may occur due to a variety of reasons, firstly, populations limited by reproduction may be experiencing an Allee effect where density of adults is insufficient for successful reproduction to occur (Courchamp *et al.*, 1999). Secondly, the target population may be resource limited. This can include food limitation whereby adults are not obtaining enough energy to reproduce, adults out-competing juveniles which are born, or a lack of juvenile-specific food resource or lack of habitat suitable for juvenile growth (Persson & de Roos, 2013). When adults are out-competing juveniles, restricting successful juvenile development, culling of adult individuals has been observed to increase population abundance when populations are experiencing negative adult density dependence (Schröder *et al.*, 2014). Whilst it is highly unlikely that such intra-stage competition between adults and juveniles is occurring in native oysters, that does not mean that the success of one age, stage or size class is not more limiting to population recovery than another (Persson & de Roos, 2013).

Variation in shell shape

Variation in relative shell morphology is highly important to both understanding other elements of the structure of a population. Deeper oysters potentially grow at slower rates than shallower shelled oysters (Orton, 1938), with shape and depth also influencing marketable oyster quality (Cole & Waugh, 1959). Oyster dimensions are key in the grading and legal landing of *O. edulis* in the context of oyster fishing, with grade for sales generally dictated by total weight and longest length of the oyster and legal minimum landing size by shell diameter. In Essex there is a minimum landing size of 70mm for *O. edulis* (Kent and Essex Inshore Fisheries and Conservation Authority, 2010). In addition, quality of oyster is also assessed by a condition index (CI) and meat yield (MY) which are influenced by the relative volume of the shell and meat (Equation 2.1 and 2.2, Acarli *et al.*, 2011).

$$CI = [dry\ meat\ weight\ (g)/dry\ shell\ weight\ (g)] * 100 \quad [Equation\ 2.1]$$

$$MY\ (\%) = [wet\ meat\ weight\ (g)/total\ weight\ (g)] * 100 \quad [Equation\ 2.2]$$

This means pinpointing any trends in predictable shapes of oyster may also help to promote recovery of oysters of desirable shapes, if recovery objectives are also for a marketable fishery. Therefore, in addition to length measurements width and depth measurements will be taken in this study.

Aims

The aim of this chapter is to determine the sub-site characteristics, size structure, spatial distribution and abundance of *O. edulis* populations and key associated species in their ecological community within the BCRC.MCZ across years and between seasons. This is to provide the evidence to assist policy makers in establishing baseline native oyster abundances in the BCRC.MCZ and evaluate how abundances are changing spatially and temporally – in order to set targets for a restoration management plan. In addition, providing estimates of abundance and spatial distribution of key associated species will help to track potential proliferation of potentially competitive non-native species such as *Crepidula fornicata* and *Crassostrea gigas*, and assess any predictable movements of predators such as *Asterias rubens*. I therefore, undertook a seasonally and spatially extensive study on European native oysters in the BCRC.MCZ.

2.2 Method

An initial exploratory dredge survey was carried out in August 2014 across the “public” areas of the BCRC.MCZ by the Kent and Essex Inshore Fisheries Conservation Authority (CASE collaborators on the current project) – i.e. ignoring areas covered by several order designations whereby the active mariculture and harvesting of *O. edulis* and *Crassostrea gigas* occur (Wiggins, 2014). The use of a dredge was necessary to assess the abundance of *O. edulis* over an extensive area for baseline data on which to develop restoration and conservation objectives and gain a clear and reliable understanding of the distribution and abundance of native oysters and associated benthic community ecology. Alternative sampling was explored, e.g. grab sampling, and found to not capture the observed and known densities of subtidal macrofauna. This was due to the small coverage of grab samples on the sea bed (Eleftheriou & Moore 2013; Cameron, 2018) (for more information see Chapter 1: Benthic sampling methods). In

Table 2.1. Dimensions of the dredge used in the oyster surveys.

Width of aperture (width of coverage)	1200mm
Overall length of dredge and bag	1800mm
Length of bag	550mm
Bag mesh size top and bottom	40mm diameter ring
Blade type	ladder blade 22mm spacing
Dredge type	Rear opening with drive plate

the 2014 survey, the BCRC.MCZ was divided into a sample grid comprising over 95% of the MCZ with the remaining 5% either inaccessible due to boat moorings, low water or private ownership of the seabed. Grid rectangles measured 0.5' latitude and 1' longitude (927x1170 metres) (Figure 2.1) (Wiggins, 2014). The 12m survey and enforcement vessel "Tamesis" was used to undertake the survey where a 1.2m ladder dredge was pulled over the seabed for a total of 100m towed at a ground speed of 2 knots, with tow start and end points recorded resulting in a dredged area of 120m² (for dredge dimensions see Table 2.1). If *O. edulis* were found in the dredge, sample a further 4 sites were surveyed, where possible, depending on the topography of the area. The original grid square was broken up into 4 smaller sub-rectangles of 0.25' latitude and 0.5' longitude with a 100m dredge tow undertaken in each sub-rectangle in order to provide a more detailed view of oyster populations and community in the area. The sampling protocol was the same in the sub-rectangles as for the main grid square. This survey method was repeated in 2015 but only the sites where native oysters were found in 2014 were resampled (n=32) with two dredges per site, not including sites further up river estuaries which were over private grounds. All data for 2014 and 2015 was provided by KEIFCA for these years.

Between 2016-2018, I undertook more detailed surveys biannually post-winter (March/April) when sea temperatures were likely coldest and post-summer (September/October) following the *O. edulis* breeding season, following the initial exploratory annual surveys in September 2014 and 2015 (Figure 2.1; Table 2.2). This new survey design was agreed on with KEIFCA and undertaken on their vessel

Table 2.2: Recorded species and information from dredge samples (x = measurement was taken in that year).

	2014	2015	2016 onwards
Number of <i>Ostrea edulis</i>	x	x	x
Weight of <i>Ostrea edulis</i>	x	x	x
Length <i>Ostrea edulis</i>	x	x	x
Height and depth of <i>Ostrea edulis</i>			x
Number of <i>Crassostrea gigas</i>	x	x	x
Weight of <i>Crassostrea gigas</i>	x	x	x
Length of <i>Crassostrea gigas</i>	x	x	x
Total oyster shell weight	x	x	x
Number of <i>Asterias rubens</i>	x	x	x
Number of <i>Crossaster papposus</i>	x	x	x
Number of brittlestar (various spp.)			x
Number of <i>Ocenebra erinacea</i>			x
Number of <i>Ocenebra erinacea</i> shell			x
Weight of <i>Ocenebra erinacea</i>			x
Number of <i>Urosalpinx cinerea</i>	x	x	x
Weight of <i>Urosalpinx cinerea</i>			x
Live ray eggs	x	x	x
empty ray eggs	x	x	x
yolk only ray egg	x	x	x
Live weight of <i>Crepidula fornicate</i>	x	x	x
Dead shell weight of <i>Crepidula fornicate</i>	x	x	x
Cockle weight	x	x	x
Mussel weight	x	x	x
Other shell weight	x	x	x
Number of live whelks	x	x	x
Number of dead whelk shells			x
Number of hermit crabs			x
Number of spidercrabs			x
Number of <i>Carcinus maenas</i>			x
Number of <i>Cancer pagarus</i>			x
Number of <i>Necora puber</i>			x
Total shell weight			x
Other species	x	x	x

Tamesis, with myself, and 5 members of their staff. Sites where either species of oyster (*C. gigas* or *O. edulis*) were found in 2014 were surveyed in 2016 - 2018, including the 4 additional sub-sites within the grid square where possible (n=104), in some areas, land or very shallow water depth limited sub-site availability. In addition, despite oysters being present at some sites inside estuaries in 2014, only

river mouths and offshore sites were sampled from 2016 onwards, with upstream areas unavailable for resampling (Figure 2.1). Seven sites were also unable to be surveyed during post-winter 2016 due to boat repairs resulting in time constraints, however these could be sampled in subsequent surveys. All species (except *C. gigas*) were returned to the sea alive close to their collection site.

All *O. edulis* caught during all surveys were size measured (n=3649). Prior to 2016 surveys all oysters were only measured for length (i.e. umbo or hinge to tip). However, it is recognised that oysters do not necessarily grow solely umbo (hinge) to tip therefore, from 2016 onwards, length (i.e. hinge to tip), width (i.e. perpendicular to the length measurement at the widest point of the oyster) and depth (deepest point of the oyster after removing any shell and or rock debris) were measured and an approximate single metric of “area” was used to reference oyster size assuming each oyster has an elliptical shape (i.e. Equation 2.3).

$$area = (length/2)(width/2)\pi \quad \text{[Equation 2.3]}$$

In addition, due to depth measurements being taken it was possible to calculate an approximate volume of the oyster assuming a uniform depth across the entire oyster (i.e. Equation 2.4).

$$volume = area * depth \quad \text{[Equation 2.4]}$$

All bathymetry measurements used in analysis were provided from KEIFCA and extracted from chart depths with minimum depths extracted for each dredge from the start point of the dredge tow.

Species distribution maps

Species distribution of native oyster abundance (counts) and biomass between 2014 and 2018, along with the primary competitors and predators (*Crassostrea gigas* abundance, *Asterias rubens* abundance and *Crepidula fornicata* biomass between 2016 and 2018) have been mapped using ArcMap (ArcMap, 2018). Data from 2014 and 2015 were not used to estimate abundances and distributions of associated species due to potential differences in methods, staff and therefore reliability of quantification between years.

Densities of all species were interpolated across larger areas using Inverse Distance Weighting (IDW) of the raster matrix using weighted averaging, as used in the management of oyster beds in Ireland (Tully & Clarke, 2012). This works by averaging the values of data points in the local vicinity of each cell within the raster image, with 5 points used to calculate each interpolated cell due to the 5-point sample design of the dredge survey. A power value of 2 within the interpolation was used to give higher power of influence of near sites over more distant points to give a smoother surface of population density (Tully & Clarke, 2012). An output cell size of 10 x 10m was used to speed processing whilst maintaining a high level of accuracy. Ordnance survey mean high water was used to constrain calculations to prevent any point sharing across land masses. Adjusted weights and counts for *O. edulis*, *C. gigas* and *C. fornicata* were used to incorporate a 20% dredge efficiency into these estimates, following a discussion and widescale literature review performed by Natural England and KEFICA for the local area (KEFICA, 2016). Mean density or biomass of individual species m^2 were extracted from the interpolated raster images, masked by individual beds of known spatial area (specified below, Figure 2.1). It is not possible to suitably calculate standard error from IDW calculations due to the autocorrelation of the data which means I would be treating the standard deviation like a weighted standard deviation. As dredge efficiency may also be a contentious issue, with no direct assessment of efficiency able to be made at this time, all IDW models were therefore repeated to assess the effects of different dredge efficiencies. Populations were therefore also estimated using 10% and a 30% efficiency (upper and lower bounds respectively) with these dredge efficiency values used to calculate error bars of population estimates. When dredge efficiency has not been incorporated (i.e. with *A. rubens* where 100% dredge efficiency was assumed with the distribution of this species of primary importance as opposed to absolute abundance), no error bars are provided for population estimations. It is recognised however that it is unlikely that dredge efficiency for starfish is 100% with previous studies indicating approximately 10% efficiency of scallop dredges (Jenkins *et al.*, 2001).

Boundaries of individual areas sampled were established from the 2014 survey. Boundaries were created using the start point of dredges containing no oysters surrounding those areas which contained oysters. Where oyster areas were adjacent to the shore, chart-based subtidal boundaries were used to define the boundary, with the Blackwater also constrained by the edge of the private Several Order

fishery (Figure 2.1, area 1). This resulted in 7 oyster areas: Blackwater mouth (hereafter Blackwater) (Area 1 = 4,397,936 m²), Colne mouth (hereafter Colne) (Area 2 = 4,569,339 m²), Eagle (Area 3 = 4,018,615 m²), Wallet Spitway (Area 4 = 6,203,951 m²), Whitaker Channel (Area 5 = 3,722,746 m²), Crouch mouth (hereafter Crouch) (Area 6 = 1,517,306 m²) and Ray Sand (Area 7 = 15,086,911 m²) with a total of 39,516,807 m² or 23.5% of the subtidal area of the BCRC.MCZ (168,000,000 m² subtidal in MCZ, Figure 2.1).

Maps were then created from the interpolated raster images masked by individual beds. Each survey resulted in an individual map with maps also converted to video and gifs to assist in visualisation of spatial changes over time.

To assess differences in the effect of performing smaller surveys on population estimates, IDW models for *O. edulis* biomass in 2014 surveys were repeated removing dredges outside of areas not surveyed in 2016 onwards. This was due to the influence of dredge points on the borders of bed areas where no oysters were found in the 2014 survey altering the predicted densities of oysters within the masked bed areas. This allowed assessment of the impact only performing a smaller, specified survey may have on predicted population estimates.

Data analysis

All statistical analysis used dredge efficiency adjusted weights and counts per dredge (120m²) for species abundances, with weights converted to grams to maintain whole integer numbers for analysis. *O. edulis*, *C. gigas* and *C. fornicata* abundances were also adjusted to incorporate a 20% dredge efficiency, following a discussion with Natural England and KEFICA (i.e. counts and weights were multiplied by a factor of 5).

As it is not possible to calculate standard error from IDW estimations, further analysis using raw dredge data was used to assess accuracy of survey sampling methods. Here, raw data (individual measured dredge densities adjusted to 20% dredge efficiency) were re-sampled using jackknife resampling to assess the bias in estimates of average density for all sites associated any one individual site (i.e. the

impact of the removal of sites on the raw mean dredge density). Mean density with jackknifed standard error was then plotted to highlight sampling error.

Negative binomial generalized linear models (glm.nb) in the MASS package (Ripley *et al.*, 2018) and Anova using individual survey and individual oyster bed site as predictors with Tukey's HSD *post hoc* analysis were used to test differences in population changes between different surveys and sites for all measured species abundances and weights. Coefficients were also extracted from the models assessing *O. edulis* biomass and count using the summary to assess differences in trends between *O. edulis* biomass and *O. edulis* population number to track population changes over time.

Frequency distributions were used to assess changes in size distribution over time with 95% confidence intervals calculated using bootstrap resampling permutations. Data from 2014 and 2015 were only used for length frequency distribution analysis for a given year with data from 2016 onwards also used for differences between seasons and between changes in volume of oysters.

A single metric of oyster shell depth to oyster area ratio was created to analyse morphological differences in shell shape using Equation 2.5.

$$ratio = shell\ depth\ (mm)/oyster\ shell\ area\ (mm^2) \quad [Equation\ 2.5]$$

Geographic and bathymetric depth effects on the shell depth to area size ratio of individual oysters were assessed using a negative binomial glm and Analysis of Variance to assess differences in mean shape of oyster between different areas and depths of water. All statistical analysis was completed using R studio (R Core Team, 2017).

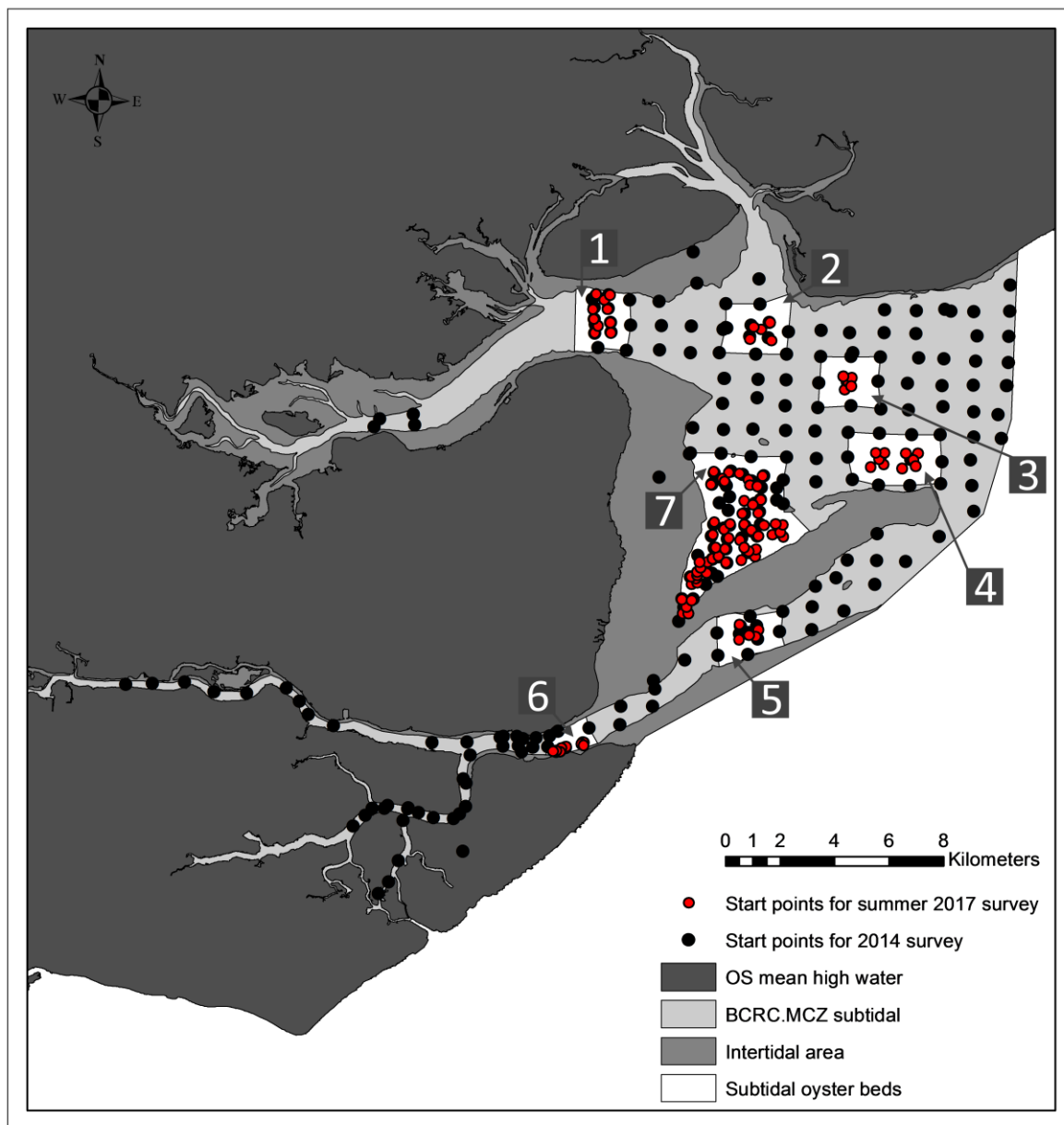


Figure 2.1. Map of the Blackwater Roach Crouch and Colne Estuaries Marine Conservation Zone including subtidal the 7 oyster areas (Blackwater (1), Colne (2), Eagle (3), Wallet Spitway (4), Whitaker Channel (5), Crouch (6) and Ray Sand (7)), start coordinates for the 2014 KEIFCA native oyster survey and start coordinates of dredge tows indicating areas sampled from 2016-2018. Actual coordinates plotted are for the September 2017 survey however all other surveys maintained similar distributions.

2.3 Results

Population estimation: Ostrea edulis

It is recognised that both biomass and count estimates are highly important to incorporate in population estimations. Negative binomial GLMMs showed differences in coefficient direction extracted between models using count vs biomass as a dependent variable, with coefficients from the Ray Sand positive when using biomass but negative when using counts (Table 2.3). These highlight potential differences in analysis and interpretation when using counts as opposed to biomass for native oysters.

Interpolated maps highlight key areas of high- and low-density native oyster biomass within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone (Figure 2.2-2.9 for animated video please visit <https://tinyurl.com/ybz4y9np>).

The biomass densities surveyed at the mouth of the Crouch are consistently high in comparison to other areas, with the Ray Sand bed highly variable (Figure 2.10). Total biomass estimation ranges from a low of 133.9 tonnes within all defined bed boundaries combined in August 2014 using data from the full KEIFCA survey, to a high of 412.5 tonnes in the post-winter 2017 survey. My most recent surveys, (i.e. only sites where oysters were found in 2014 and therefore based on the same survey design), estimates population biomass of 221.4 tonnes in September 2018 (post-summer) with estimates ranging between 221.4 tonnes and 418.3 tonnes since 2016. Overall, there appears to be an uplift in population biomass and abundance between 2014 and 2015, however following 2016 there appears to be a slight decline. From 2016 onwards, post-winter surveys consistently show increases in areas of higher density biomass in comparison to post-summer surveys (Figure 2.11), however, there is no statistical difference in the average total biomass of native oysters caught in dredges between 2016 and 2018 surveys (glm.nb, Anova, $F_{5,581} = 1.7617$, $P = 0.1177$). There was a statistical difference in the weight of oysters in a dredge between areas across the MCZ with full *Post-hoc* comparisons shown in Appendix I: Supplementary Information Table 2.2 (glm.nb, Anova, $F_{6,586} = 24.48$, $P < 0.001$).

Table 2.3. Coefficients extracted from negative binomial glm for biomass and count as dependent variables with area and year as predictor variables. Differences in the direction of the coefficient show differences in how each dependent variable changes between years. Areas with different signed coefficients are highlighted in grey.

Coefficients	Biomass				Count			
	Estimate	Std.Err	T-value	Pr(> t)	Estimate	Std.Err	T-value	Pr(> t)
(Intercept)	6.5659	0.4219	15.562	<2e-16	3.0418	0.2936	10.361	<2e-16
Season winter	0.4774	0.2833	1.685	0.091946	0.3922	0.1927	2.035	0.041808
Crouch	2.1475	0.5881	3.652	0.000261	1.5884	0.4116	3.859	0.000114
Eagle	-4.0076	0.7399	-5.416	6.08E-08	-4.1728	0.5977	-6.981	2.93E-12
Outer Colne	-1.5316	0.7367	-2.079	0.037621	-1.7627	0.5123	-3.441	0.00058
Ray Sand	0.3225	0.4629	0.697	0.486007	-0.4374	0.3202	-1.366	0.171923
Wallet Spitway	-1.0764	0.5973	-1.802	0.071533	-2.1468	0.4203	-5.108	3.26E-07
Whitaker	-1.7921	0.7747	-2.313	0.020717	-2.9972	0.5565	-5.386	7.22E-08

Error from dredge efficiency estimation has the largest range in the post-winter 2016 survey where total population estimate ranges from 0.006 kg m⁻² average density (278 tonnes in total, Figure 2.11) at 30% dredge efficiency to 0.021 kg m⁻² (836 tonnes total) at 10%. Conversely, Jackknife resampling using measured average dredge densities show similar variation estimates ranging from 0.015 to 0.021 kg m⁻² (592 to 829 tonnes), if average density is multiplied up by total area of beds (39516808m²). This confirms that extrapolation of abundance without taking into account patchiness and variation of biomass even within relatively large areas can lead to overestimation of population estimates when densities/abundances are low (278 vs 592 tonnes).

The effects of using a smaller survey on estimating populations show an increased population estimate within defined bed boundaries (133.9 tonnes using a full survey within the specified areas vs 193.0 tonnes only using areas surveyed from 2016 onwards). This is due to the removal of influence on the estimation of samples surrounding bed boundaries where no oysters were detected – e.g. density = zero. For absolute estimates for all beds and surveys see Appendix I: Supplementary Information Table 2.1.

Interpolated maps of estimated counts of individual native oysters mirror key areas of high- and low-density native oyster biomass within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone (Figure 2.13-2.20 for animated video please see <https://tinyurl.com/y9kqp353>).

The variation in abundance (count) densities surveyed closely mirror those observed with *O. edulis* biomass, with abundances on the Ray Sand highly variable and highest numbers observed at the mouth of the Crouch. Ray Sand populations range from 432,032 individuals in 2014 to 3,020,953 in post-winter 2017 survey with the Crouch populations ranging from 1,315,873 to 2,516,921 in the same years (Figure 2.21). As with biomass estimations, post-winter surveys consistently show increased areas of higher numerical abundance in comparison to post-summer surveys. This is reflected in the extracted population estimates with total populations for all areas combined ranging from 2,221,607 in August 2014 to 5,860,737 in post-winter 2017 surveys (Figure 2.21). A negative binomial GLM highlighted differences in abundance (counts) of oysters between sites (Anova, $F_{6,856} = 26.911$, $P < 0.001$). There was no difference observed between surveys and so this predictor was removed from the model. Full *Post-hoc* analysis can be found in Appendix I: Supplementary Information Table 2.3. The highest densities of oyster observed throughout this study occurred in the Blackwater area in 2015 when estimated densities reached 7.25 oysters m^{-2} over a 100m dredge tow incorporating 20% dredge efficiency (Figure 2.14), since the post-winter 2016 survey when surveys were altered, a maximum average density across a 100m dredge tow, incorporating 20% dredge efficiency of 4.04 oysters m^{-2} was observed in the Crouch in the post-winter 2017 survey.

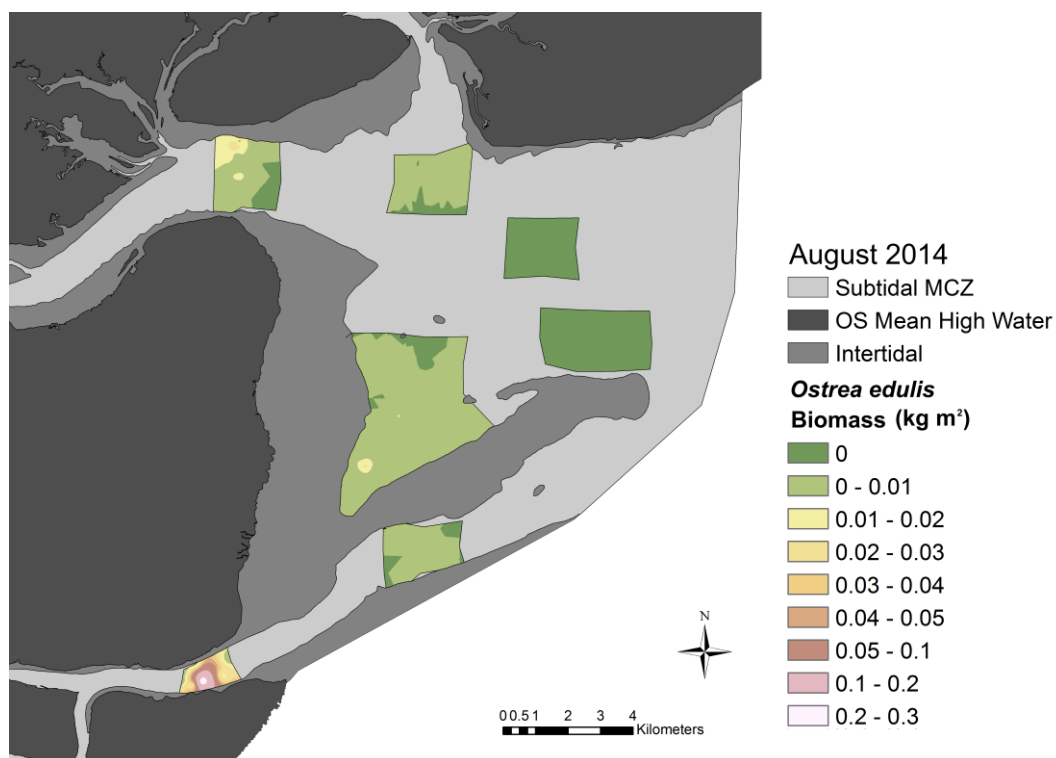


Figure 2.2. Interpolated distribution of *Ostrea edulis* biomass (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from August 2014 dredge surveys within resampled specified oyster bed areas only.

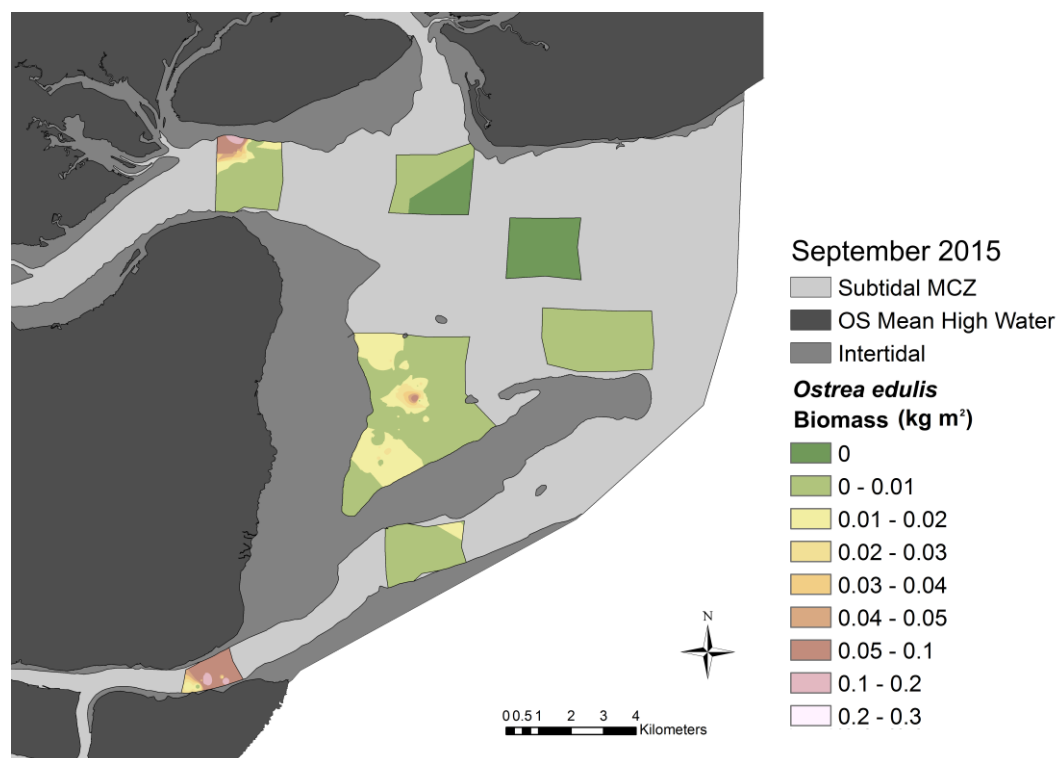


Figure 2.3. Interpolated distribution of *Ostrea edulis* biomass (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from September 2015 dredge surveys within resampled specified oyster bed areas only.

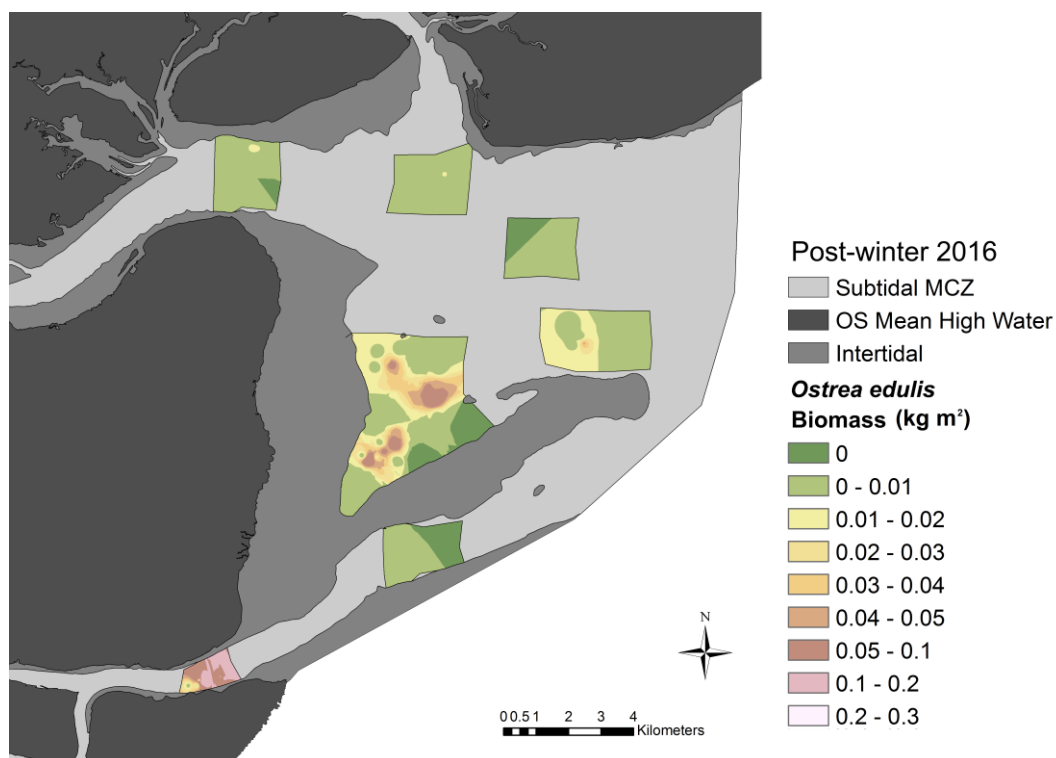


Figure 2.4. Interpolated distribution of *Ostrea edulis* biomass (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-winter 2016 dredge surveys within resampled specified oyster bed areas only.

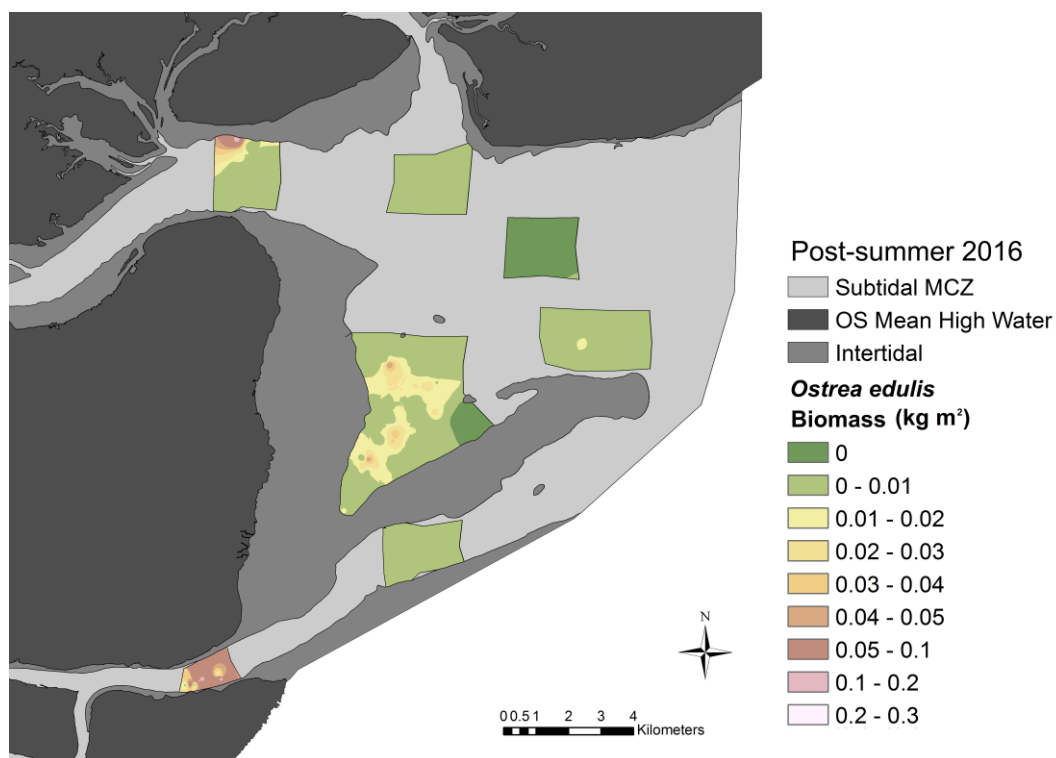


Figure 2.5. Interpolated distribution of *Ostrea edulis* biomass (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-summer 2016 dredge surveys within resampled specified oyster bed areas only.

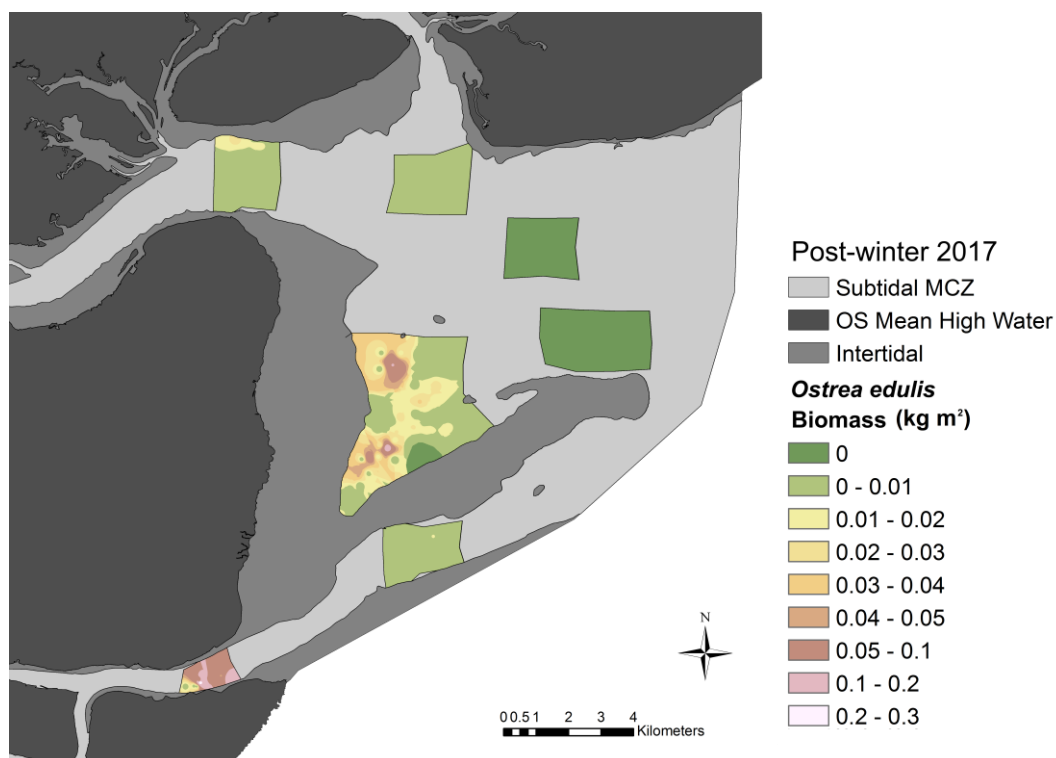


Figure 2.6. Interpolated distribution of *Ostrea edulis* biomass (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-winter 2017 dredge surveys within resampled specified oyster bed areas only.

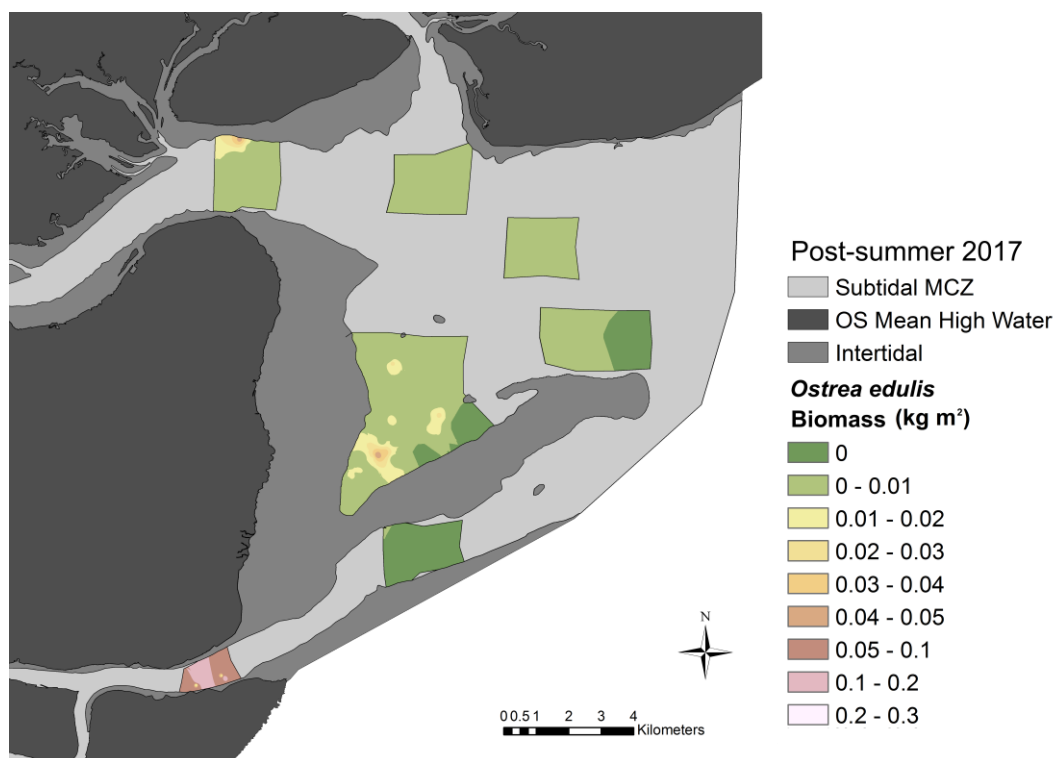


Figure 2.7. Interpolated distribution of *Ostrea edulis* biomass (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-summer 2017 dredge surveys within resampled specified oyster bed areas only.

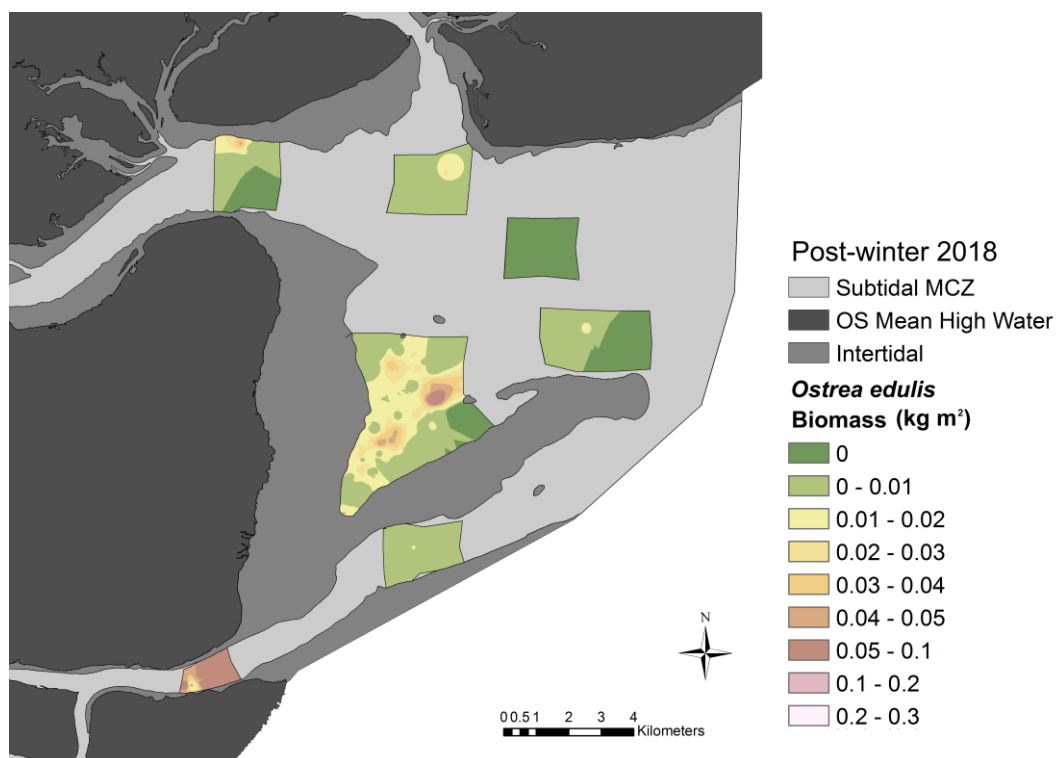


Figure 2.8. Interpolated distribution of *Ostrea edulis* biomass (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-winter 2018 dredge surveys within resampled specified oyster bed areas only.

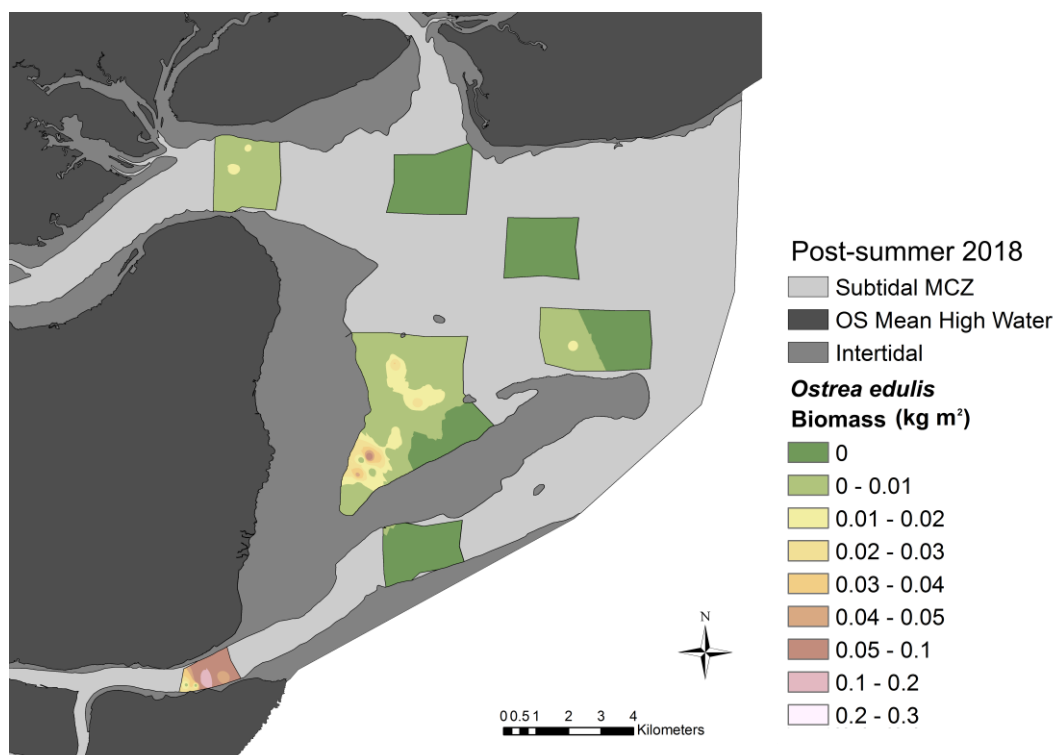


Figure 2.9. Interpolated distribution of *Ostrea edulis* biomass (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-summer 2018 dredge surveys within resampled specified oyster bed areas only.

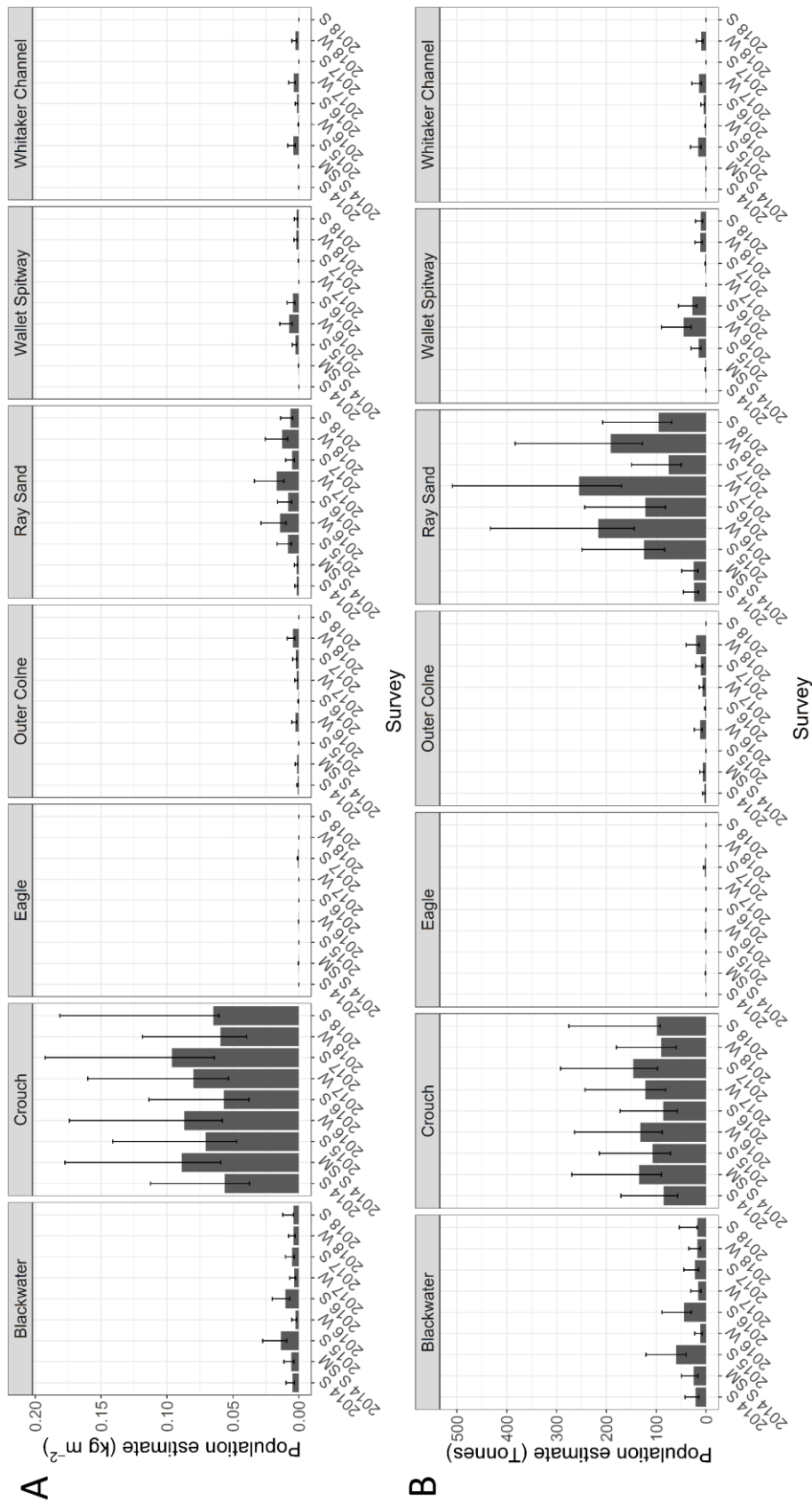


Figure 2.10. A. Average density of native oyster biomass for individual beds between post-summer 2014 (2014 S) and post-summer 2018 (2018 S) with standard deviation extracted from interpolation Inverse Distance Weighting calculations. And **B.** multiplied by bed area to give estimated population biomass. **S** specifies post-summer surveys and **W** specifies post-winter surveys. Bed areas have been specified in Figure 2.1. 1 2014 S SM indicates IDW analysis for 2014 S removing dredges which were not resampled in 2016 onwards with beds therefore not constrained by dredges where no oysters were found. Error bars show the effect of using 10% and 30% dredge efficiency with mean at 20% dredge efficiency.

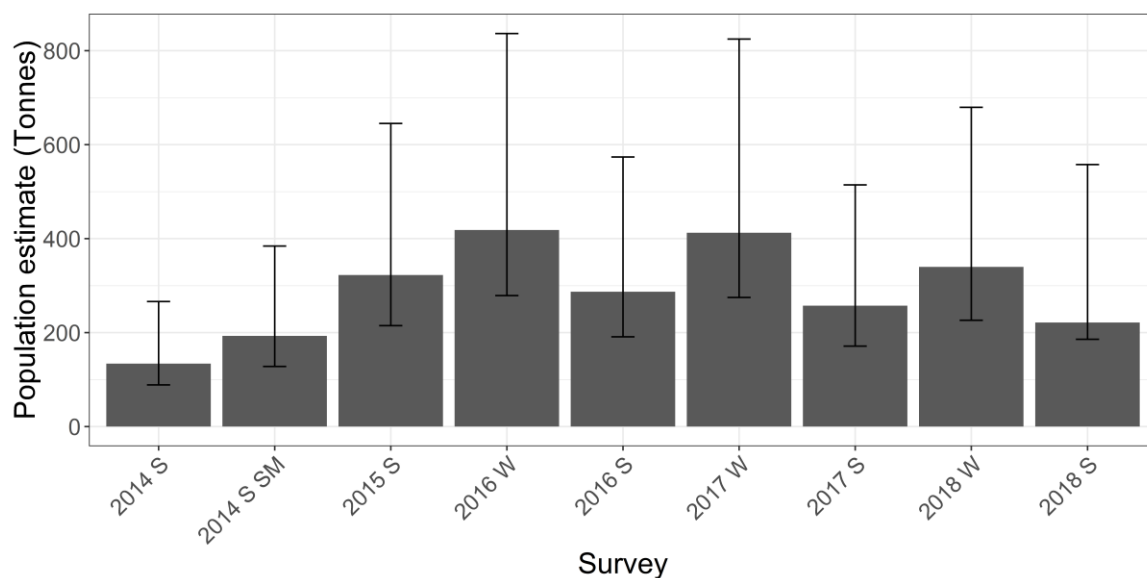


Figure 2.11. Estimated native oyster biomass for all beds combined between post-summer 2014 (2014 S) and post-summer 2018 (2018 S) with standard deviation extracted from interpolation Inverse Distance Weighting calculations. **S** specifies post-summer surveys and **W** specifies post-winter surveys. Bed areas have been specified in Figure 2.1 2014 S SM indicates IDW analysis for 2014 S removing dredges which were not resampled in 2016 onwards with beds therefore not constrained by dredges where no oysters were found. Error bars show the effect of using 10% and 30% dredge efficiency with mean at 20% dredge efficiency.

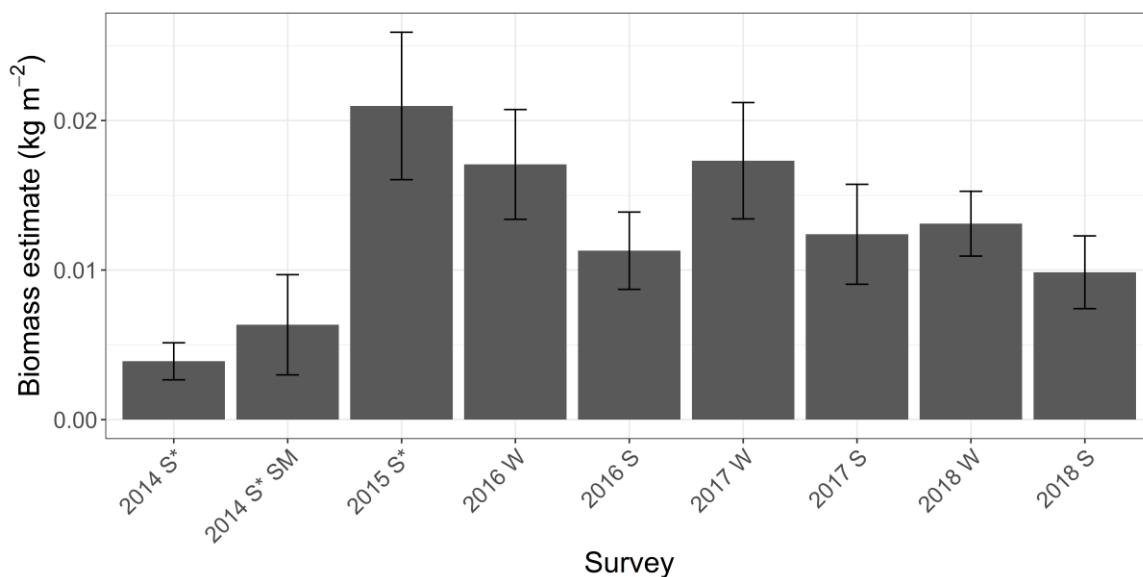


Figure 2.12. Average density of native oyster biomass (adjusted to 20% dredge efficiency) for all dredges between post-summer 2014 (2014 S) and post-summer 2018 (2018 S) with standard error calculated through Jackknife resampling. **S** specifies post-summer surveys and **W** specifies post-winter surveys. *indicates different sampling protocol, with different areas sampled, e.g. 2014 includes full MCZ where survey was highly zero-inflated. 2014 S SM indicates removal of sites not sampled in subsequent years.

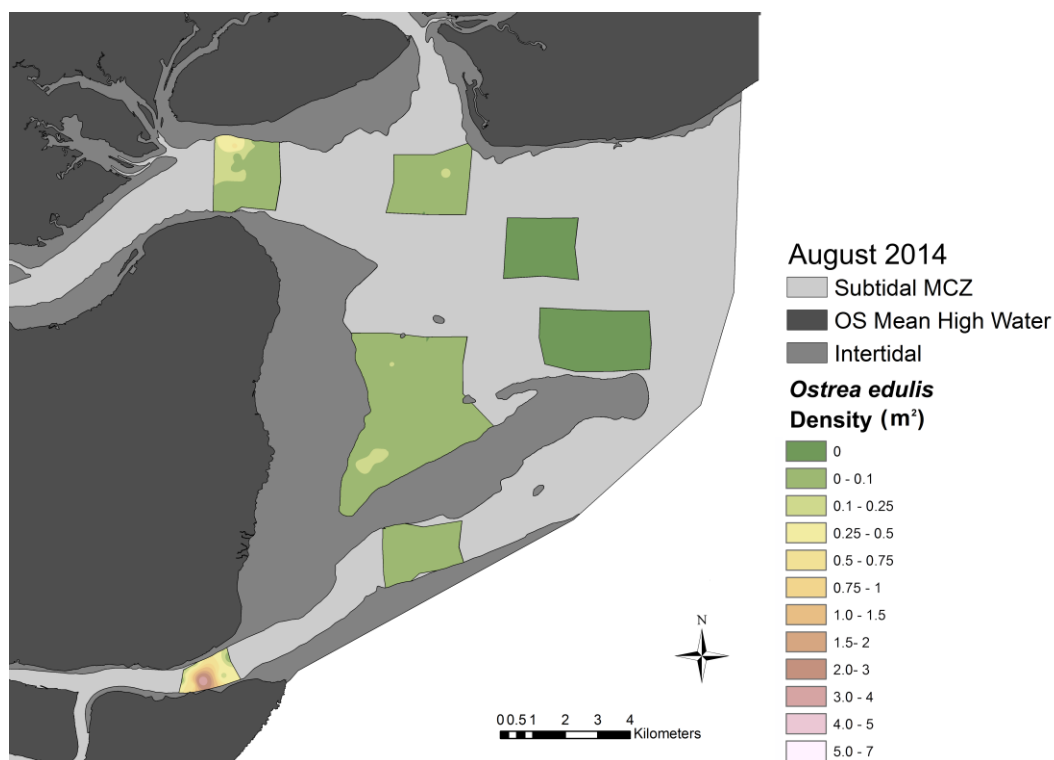


Figure 2.13. Interpolated distribution of *Ostrea edulis* counts (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from August 2014 dredge surveys within resampled specified oyster bed areas only.

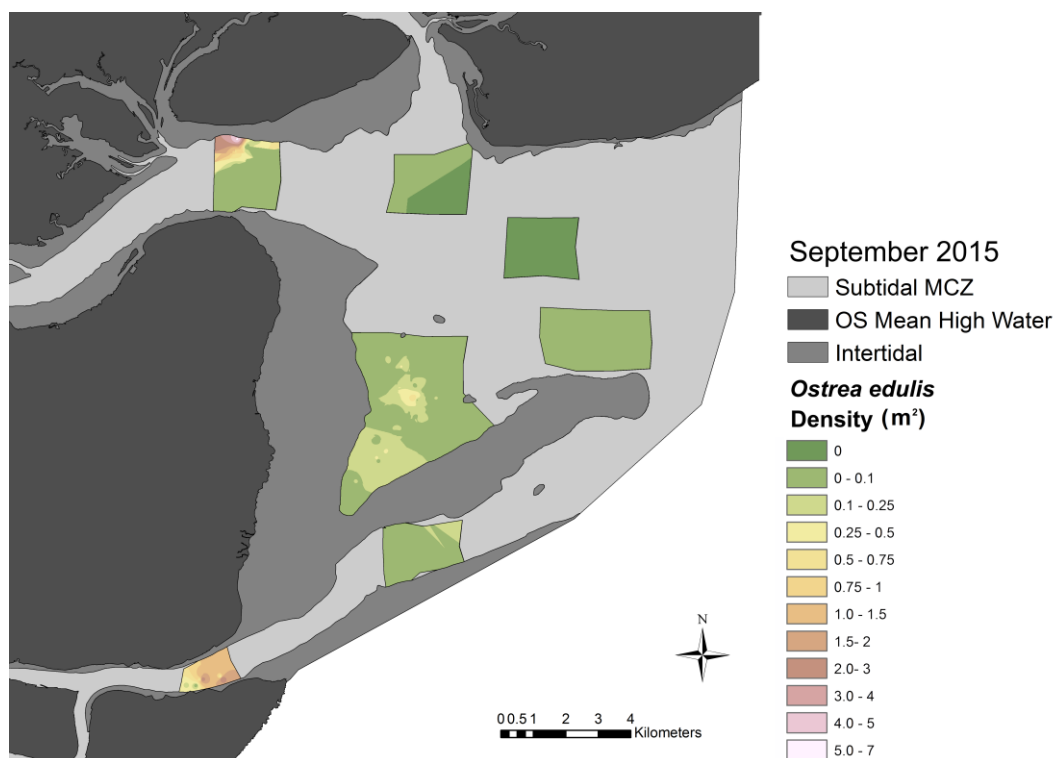


Figure 2.14. Interpolated distribution of *Ostrea edulis* counts (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from September 2015 dredge surveys within resampled specified oyster bed areas only.

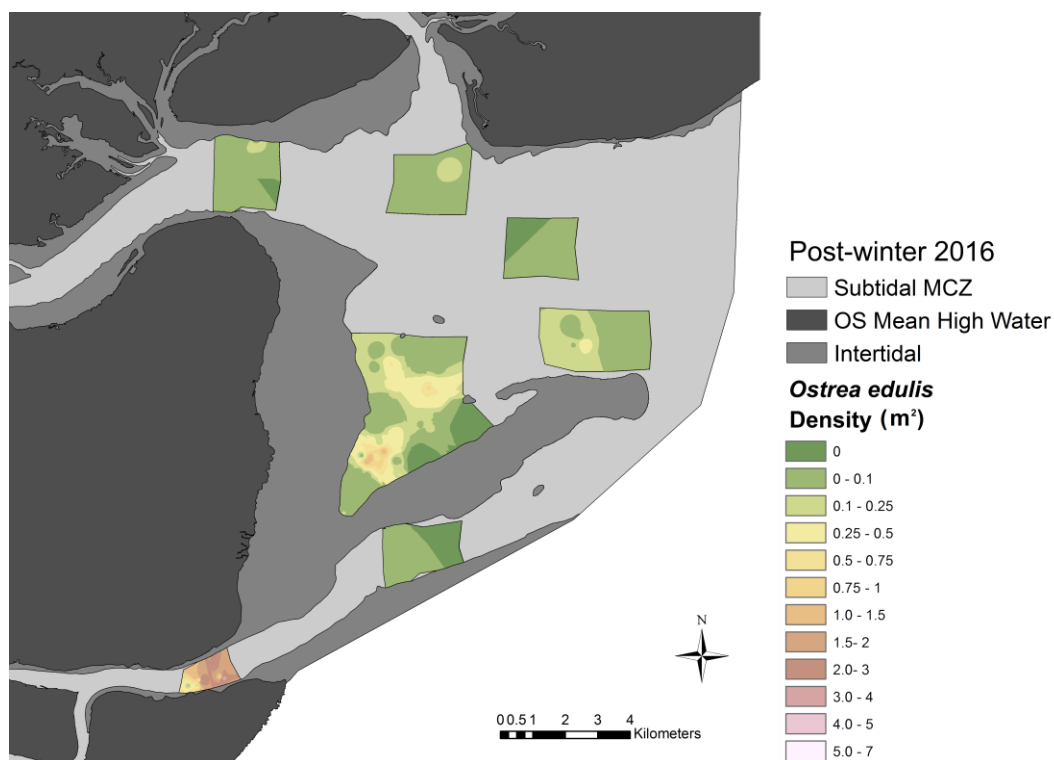


Figure 2.15. Interpolated distribution of *Ostrea edulis* counts (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-winter 2016 dredge surveys within resampled specified oyster bed areas only.

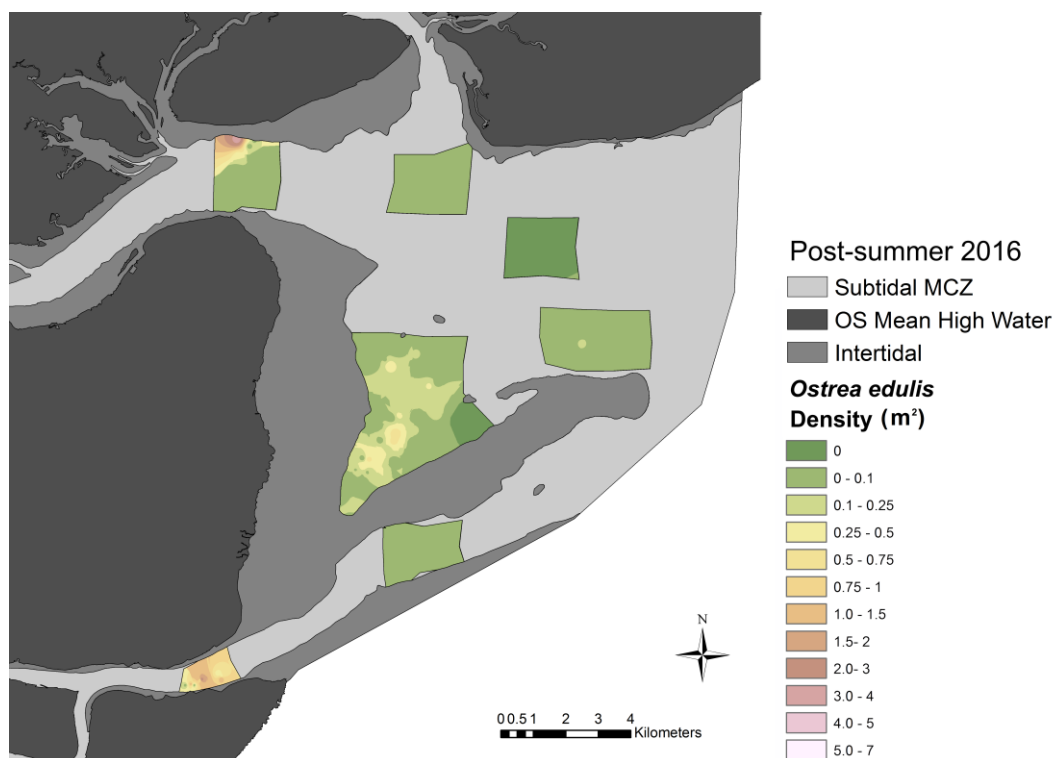


Figure 2.16. Interpolated distribution of *Ostrea edulis* counts (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-summer 2016 dredge surveys within resampled specified oyster bed areas only.

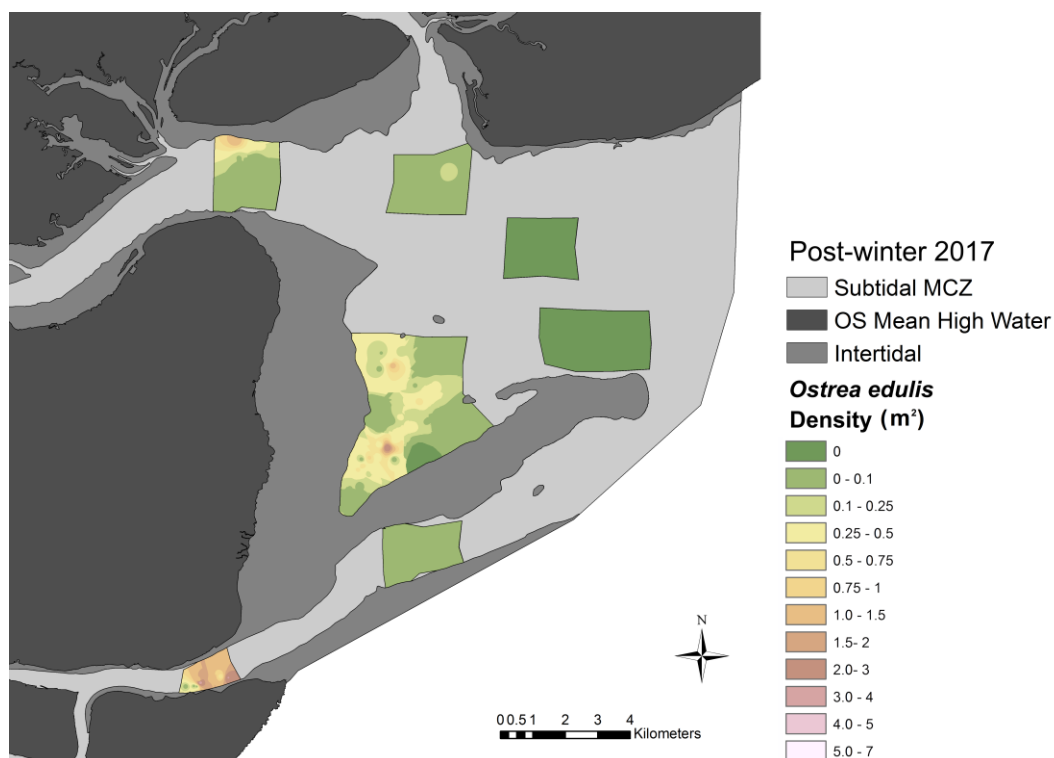


Figure 2.17. Interpolated distribution of *Ostrea edulis* counts (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-winter 2017 dredge surveys within resampled specified oyster bed areas only.

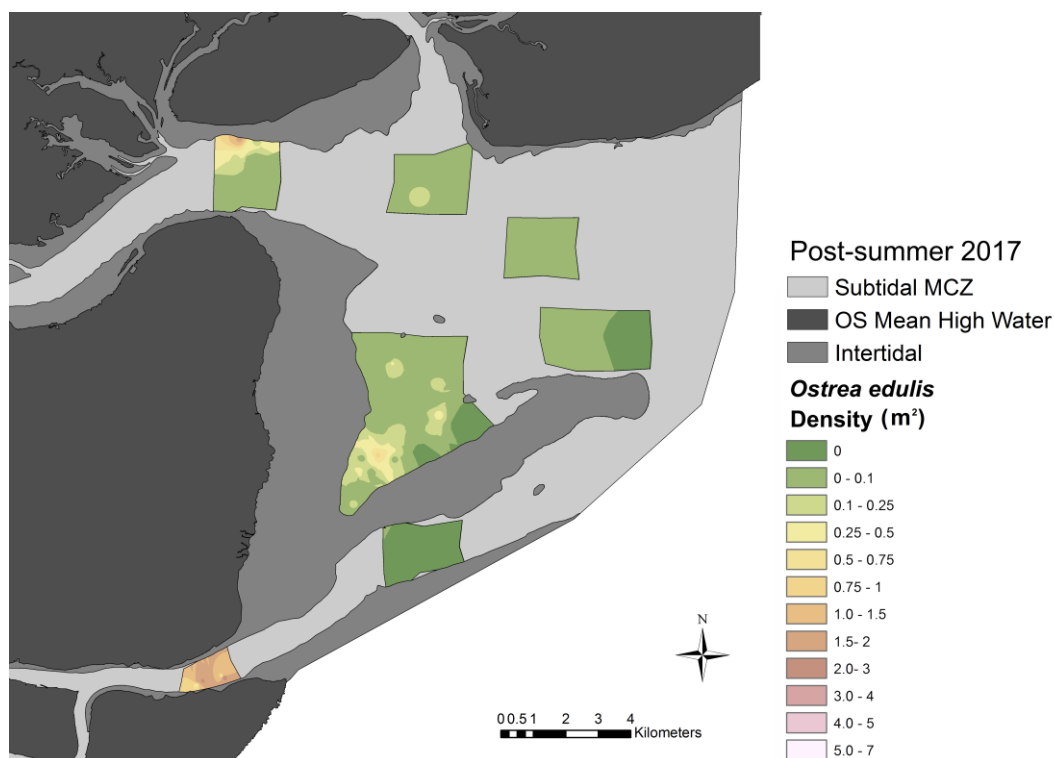


Figure 2.18. Interpolated distribution of *Ostrea edulis* counts (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-summer 2017 dredge surveys within resampled specified oyster bed areas only.

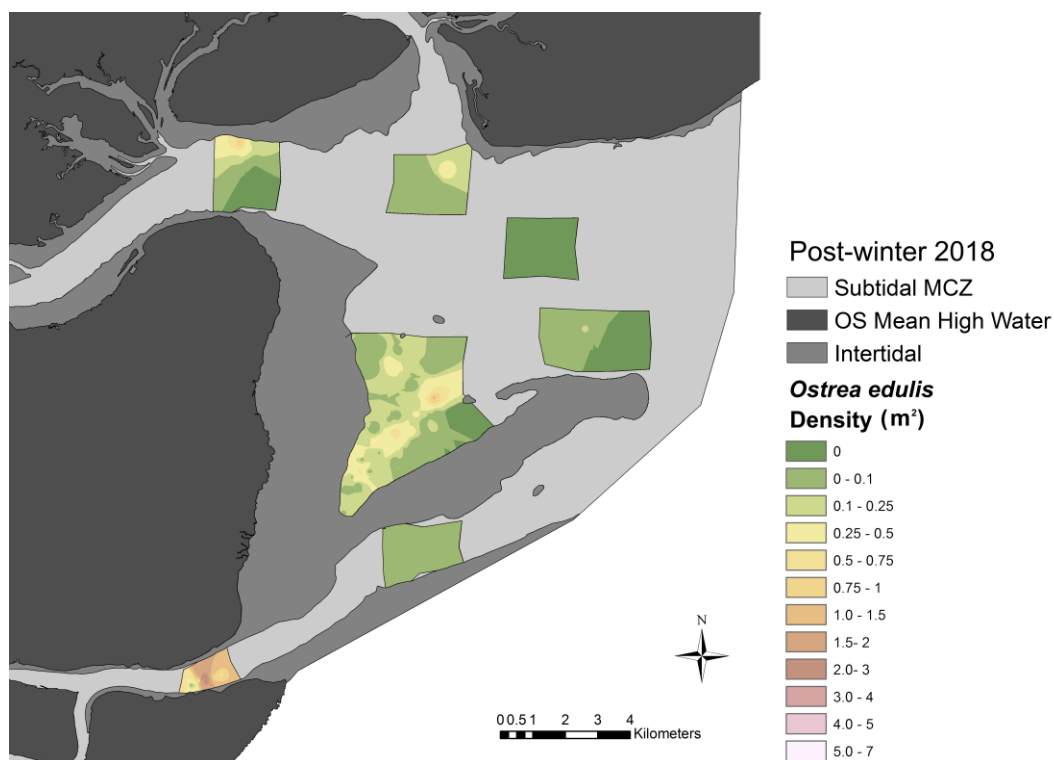


Figure 2.19. Interpolated distribution of *Ostrea edulis* counts (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-winter 2018 dredge surveys within resampled specified oyster bed areas only.

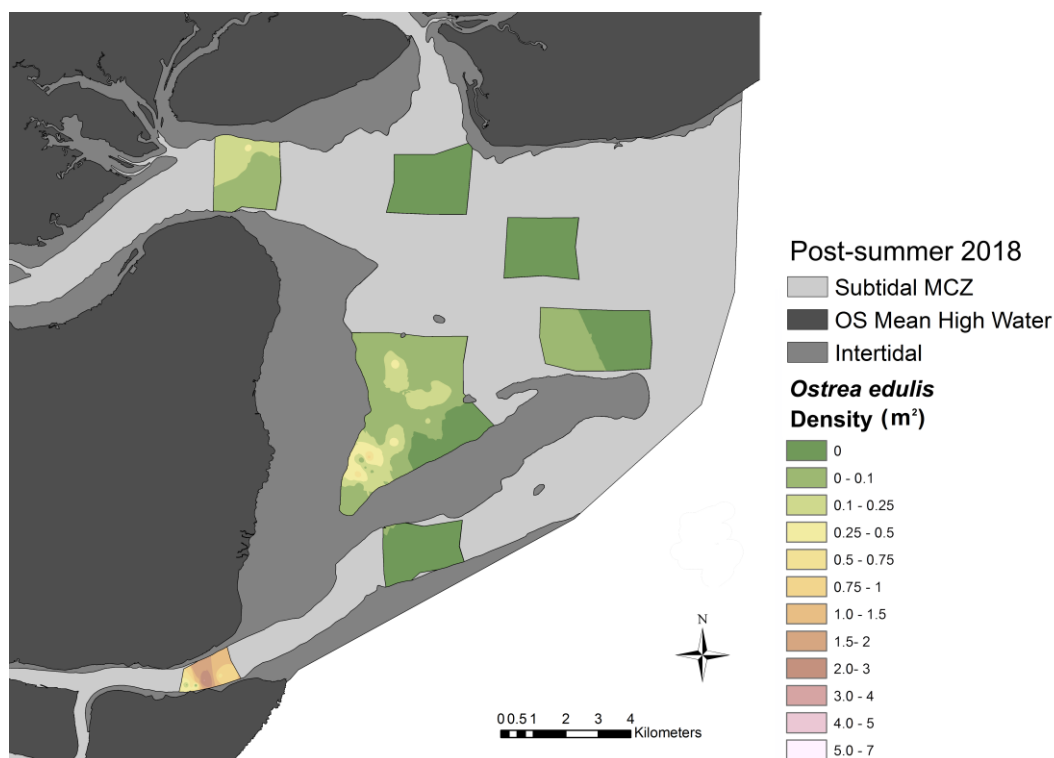


Figure 2.20. Interpolated distribution of *Ostrea edulis* counts (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-summer 2018 dredge surveys within resampled specified oyster bed areas only.

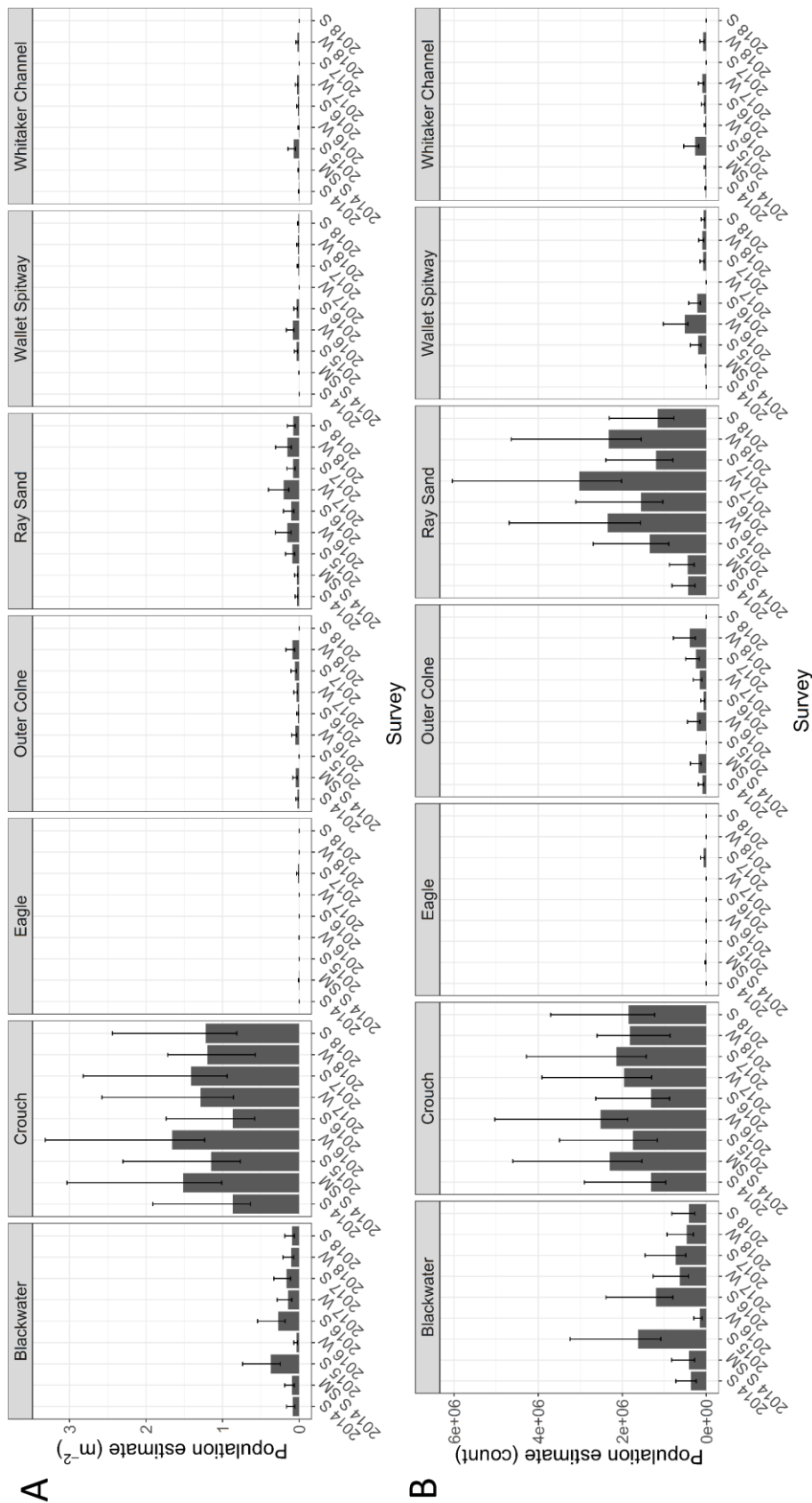


Figure 2.21. A. Average estimated density of native oysters for individual beds between post-summer 2014 (2014 S) and post-summer 2018 (2018 S) with standard deviation extracted from interpolation Inverse Distance Weighting calculations. And **B.** multiplied by bed area to give estimated population number. **S** specifies post-summer surveys and **W** specifies post-winter surveys. Bed areas have been specified in Figure 2.1. 1 2014 S SM indicates IDW analysis for 2014 S removing dredges which were not resampled in 2016 onwards with beds therefore not constrained by dredges where no oysters were found. Error bars show the effect of using 10% and 30% dredge efficiency with 20% shown in bars.

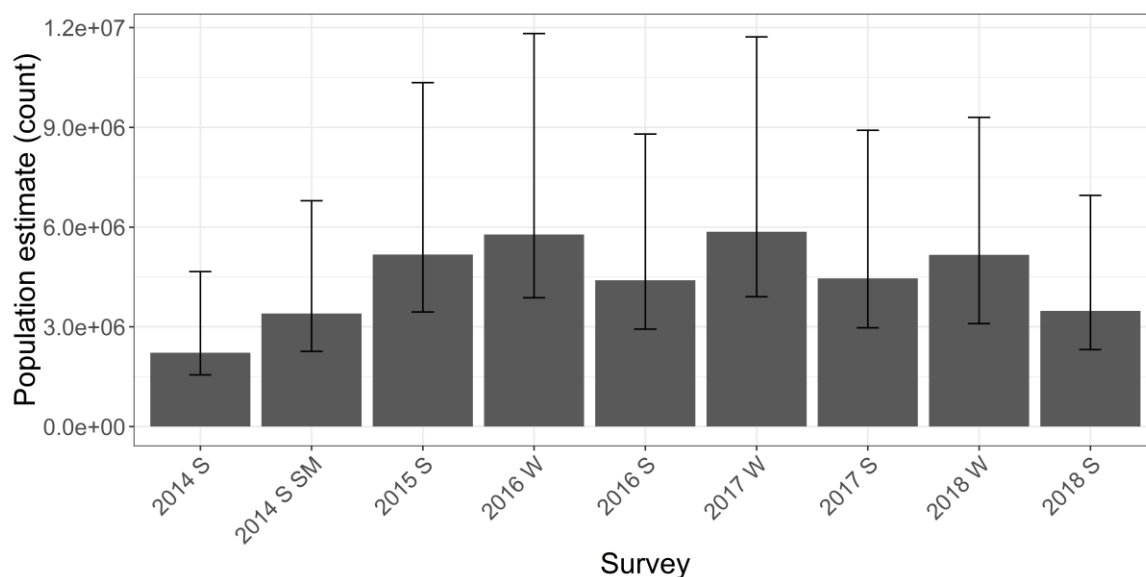


Figure 2.22. Estimated native oyster population abundance (counts) incorporating a 20% dredge efficiency for all beds combined between post-summer 2014 (2014 S) and post-summer 2018 (2018 S) with standard deviation extracted from interpolation Inverse Distance Weighting calculations. **S** specifies post-summer surveys and **W** specifies post-winter surveys. Bed areas have been specified in Figure 2.1 2014 S SM indicates IDW analysis for 2014 S removing dredges which were not resampled in 2016 onwards with beds therefore not constrained by dredges where no oysters were found. Error bars show the effect of using 10% and 30% dredge efficiency.

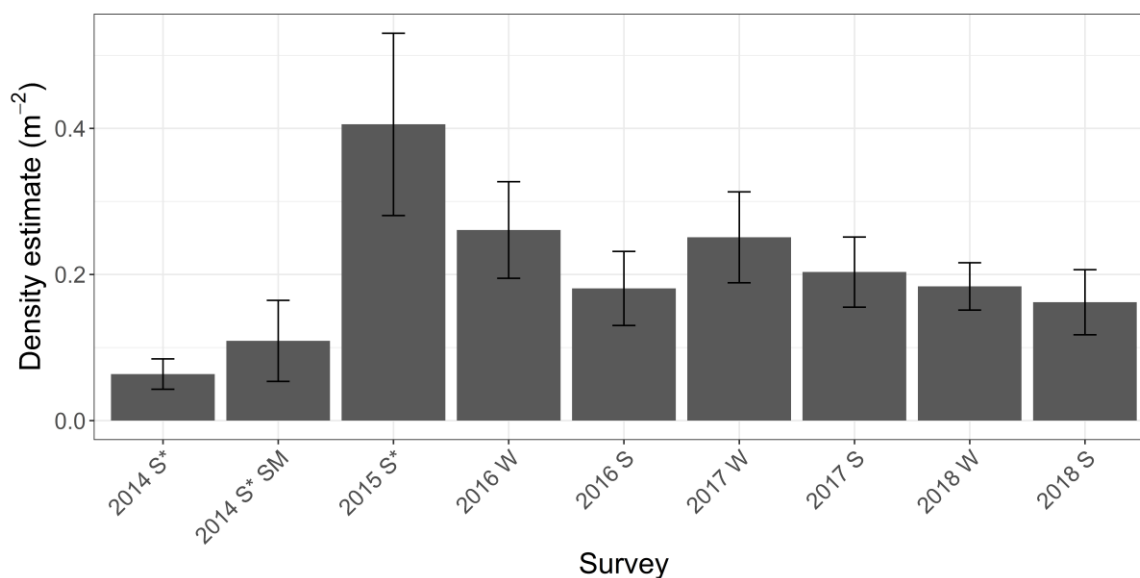


Figure 2.23. Average density of native oysters abundance (counts) for all dredges between post-summer 2014 (2014 S) and post-summer 2018 (2018 S) with standard error calculated through Jackknife resampling. **S** specifies post-summer surveys and **W** specifies post-winter surveys. *indicates different sampling protocol, with different areas sampled, e.g. 2014 includes full MCZ where survey was highly zero-inflated. 2014 S SM indicates removal of sites not sampled in subsequent years.

Length frequency distributions of Ostrea edulis

Length frequency distribution of all *O. edulis* caught in all surveys between 2014-2018 can be found in Figure 2.24. Confidence intervals estimated using 999 bootstrap permutations show significantly higher proportions of smaller oysters in 2015 and post-summer 2016 surveys with a significantly lower proportion of oysters at approximately 50mm in length in post-summer 2018. It appears cohorts merge as growth rates slow with increasing age of oyster.

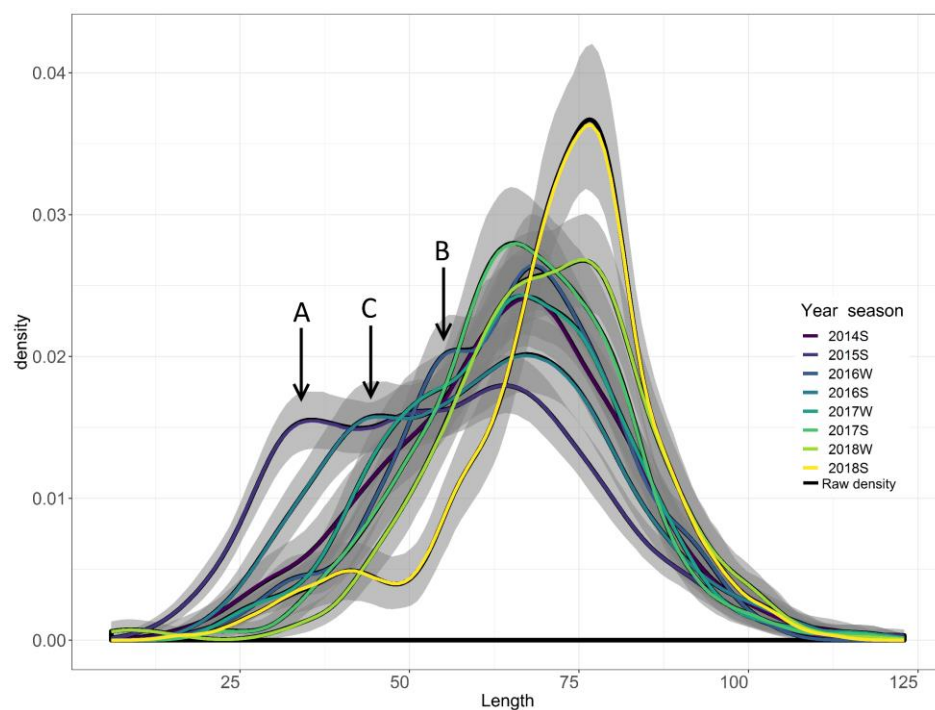


Figure 2.24. Distribution of length frequency density of all *Ostrea edulis* caught in dredge surveys within the Blackwater Roach Crouch and Colne surveys between 2014 and 2018 with bootstrapped 95% confidence intervals. Legend specified year of survey with S representing post-summer surveys and W post-winter. Points A, B and C represent a potential new cohort from 2014 (A) with growth of that cohort (B) and a secondary new cohort from 2015 (C)

Crepidula fornicata population and distribution

Interpolated maps highlight key areas of high- and low-density *C. fornicata* biomass within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone (Figure 2.25-2.30 for animated gif please visit <https://j.gifs.com/Mw5P3Q.gif>). The highest population densities are found in the Blackwater and Ray Sand (Figure 2.31). The estimated population in the Blackwater ranges between 1944 tonnes in the post-summer 2016 survey to 1618 tonnes in the post-summer 2017 and on the Ray Sand the population ranges between 3030.5 tonnes in the post-summer 2016 with the highest estimated population of 4891.5 tonnes also in the post-summer 2017 survey. There was a statistically significant difference in *C. fornicata* biomass between sites (Anova, $F_{6,586} = 75.016$, $P < 0.001$) however, no difference was observed between individual surveys, with this predictor subsequently removed. Full breakdown of *Post-hoc* analysis can be found in Appendix I: Supplementary Information Table 2.4.

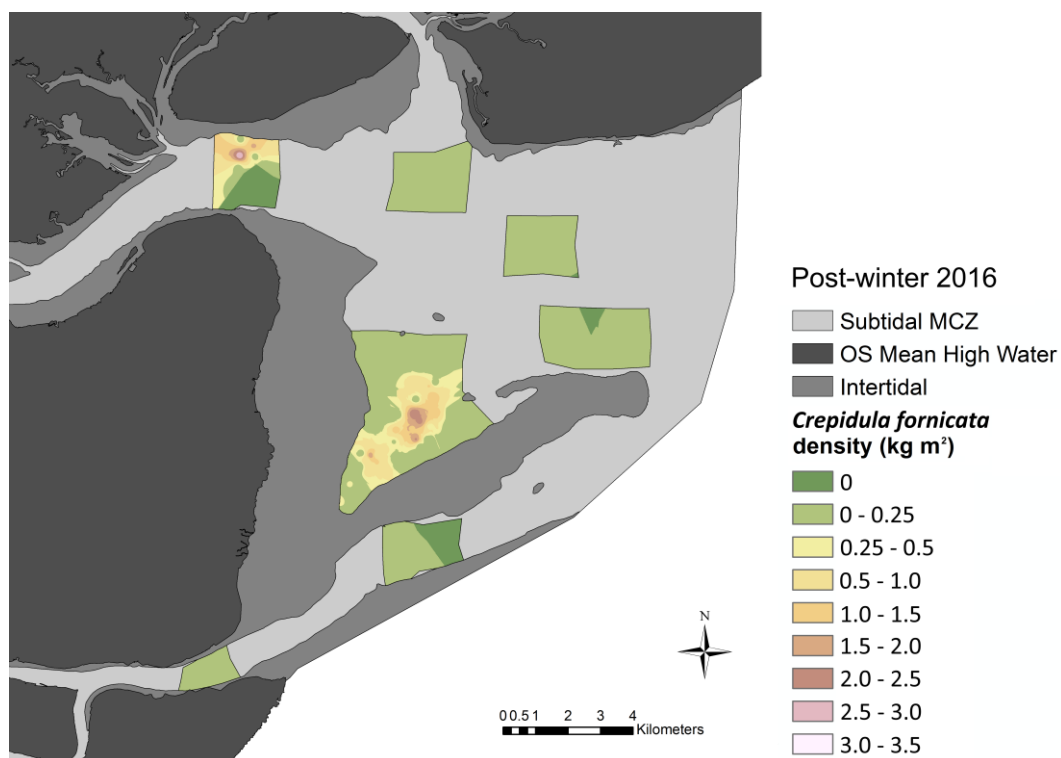


Figure 2.25. Interpolated distribution of *Crepidula fornicata* biomass (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-winter 2016 dredge surveys within resampled specified oyster bed areas only.

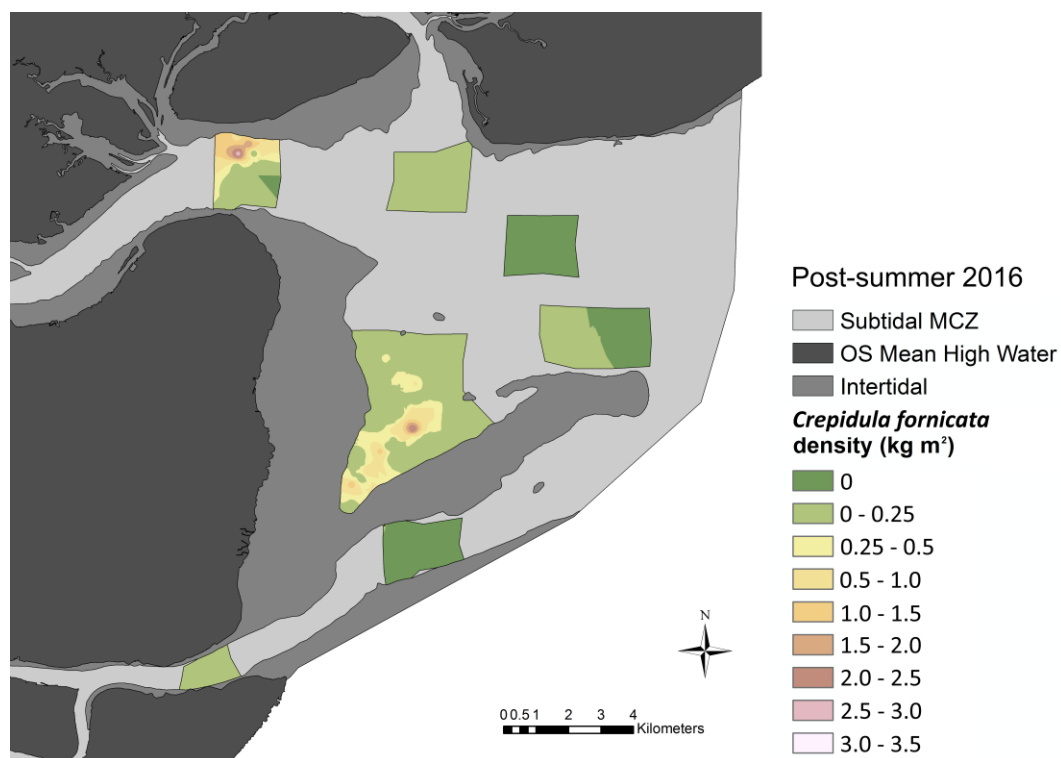


Figure 2.26. Interpolated distribution of *Crepidula fornicata* biomass (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-summer 2016 dredge surveys within resampled specified oyster bed areas only.

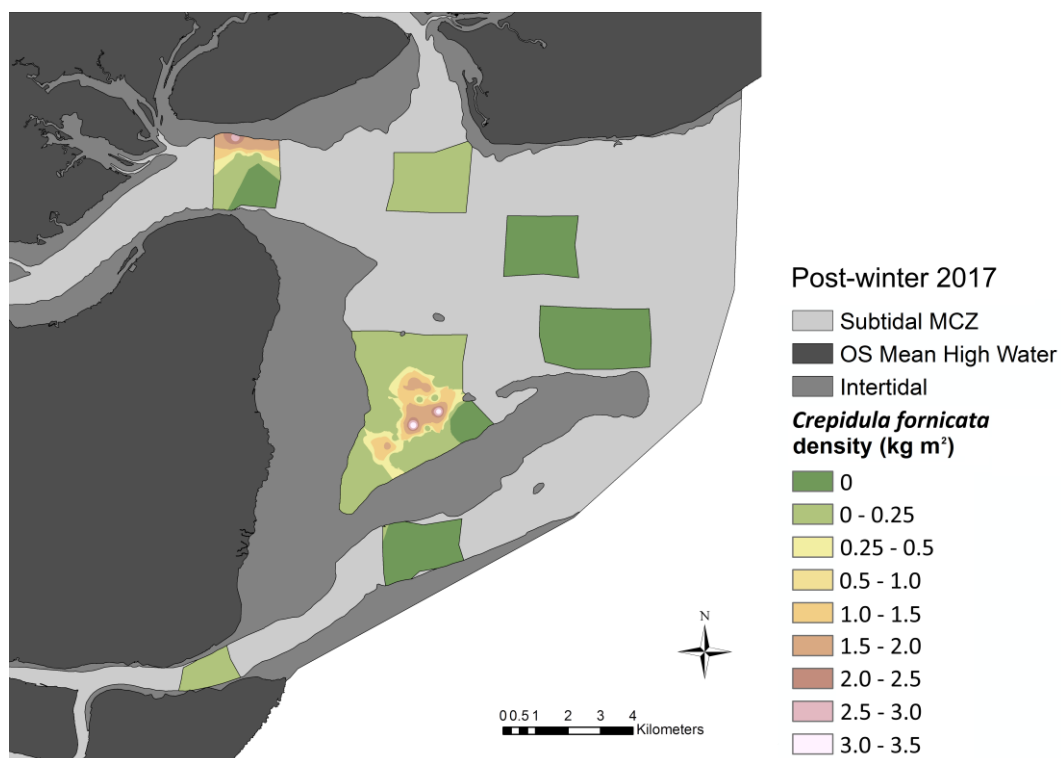


Figure 2.27. Interpolated distribution of *Crepidula fornicata* biomass (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-winter 2017 dredge surveys within resampled specified oyster bed areas only.

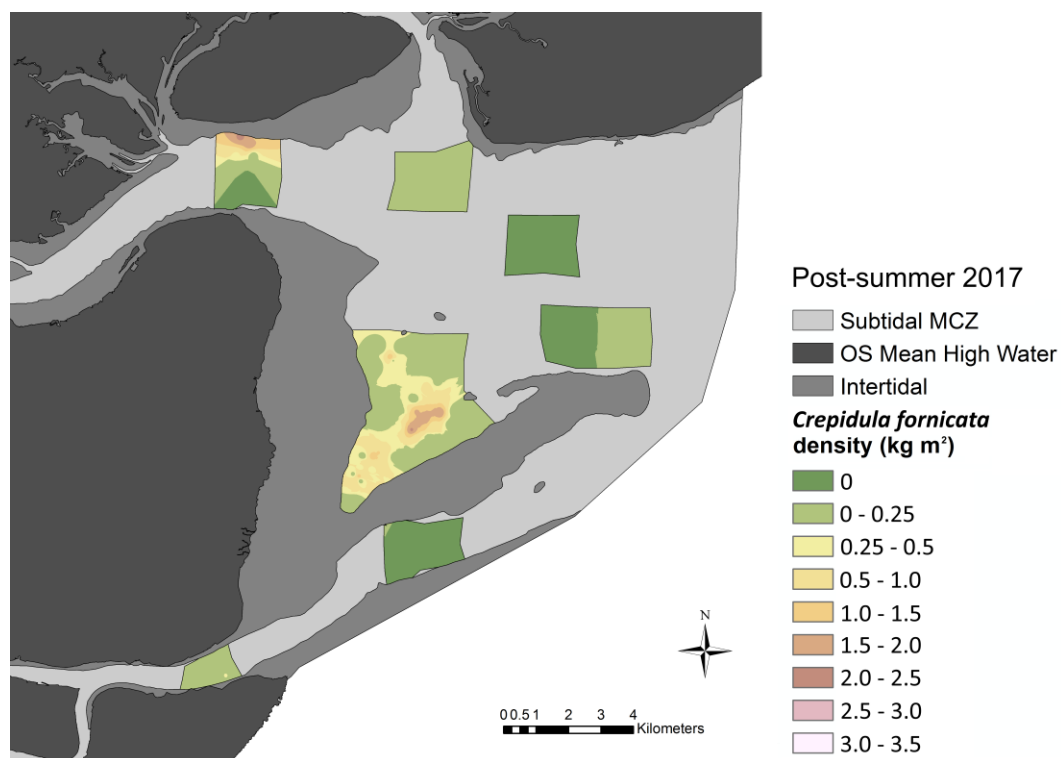


Figure 2.28. Interpolated distribution of *Crepidula fornicata* biomass (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-summer 2017 dredge surveys within resampled specified oyster bed areas only.

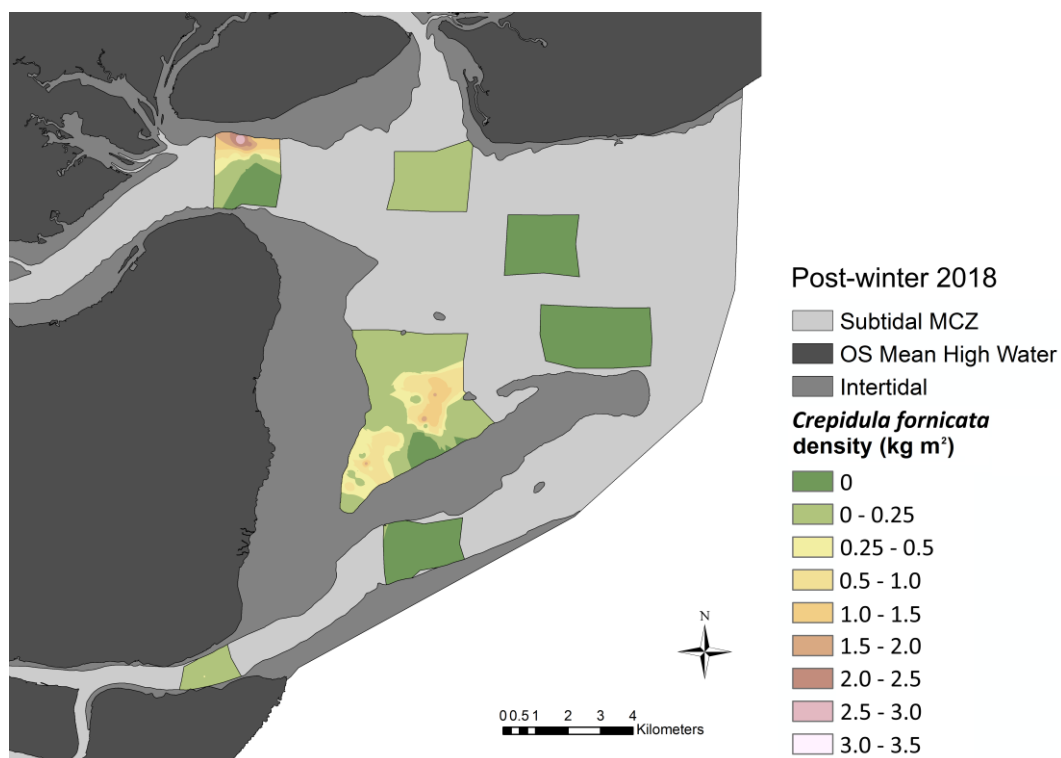


Figure 2.29. Interpolated distribution of *Crepidula fornicata* biomass (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-winter 2018 dredge surveys within resampled specified oyster bed areas only.

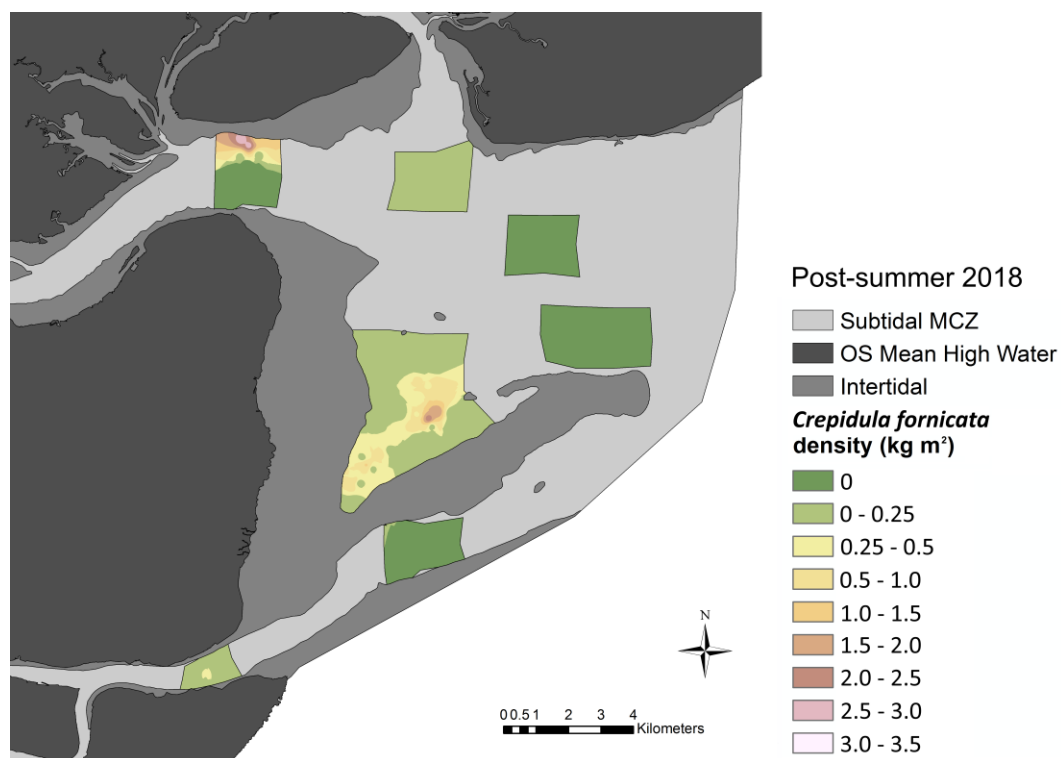


Figure 2.30. Interpolated distribution of *Crepidula fornicata* biomass (adjusted to 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-summer 2018 dredge surveys within resampled specified oyster bed areas only.

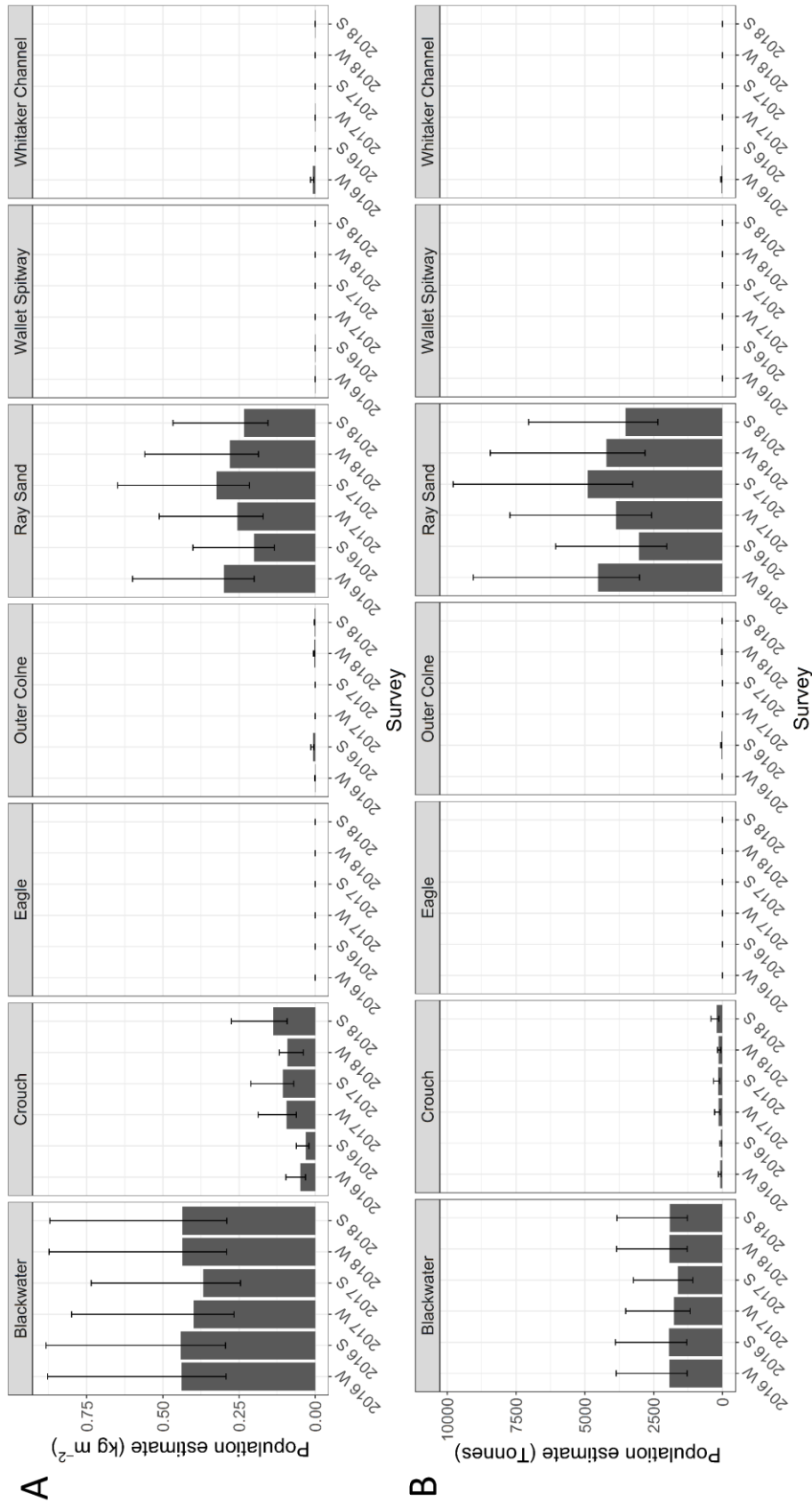


Figure 2.31. A. Average estimated density of *Crepidula fornicata* for individual beds between Post-winter 2016 (2016 W) and post-summer 2018 (2018 S) with standard deviation extracted from interpolation Inverse Distance Weighting calculations. And **B.** multiplied by bed area to give estimated biomass. **S** specifies post-summer surveys and **W** specifies post-winter surveys. Bed areas have been specified in Figure 2.1. Error bars show the effect of using 10% and 30% dredge efficiency with mean of 20% shown in bars.

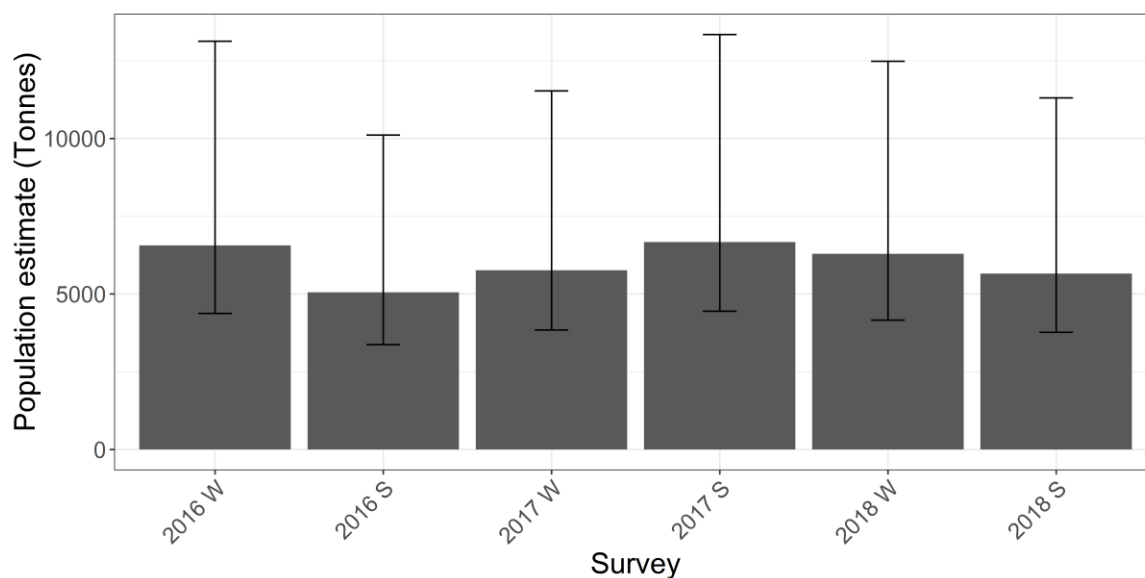


Figure 2.32. Estimated *Crepidula fornicata* biomass incorporating a 20% dredge efficiency for all beds combined between post-winter 2016 (2016 W) and post-summer 2018 (2018 S) with standard deviation extracted from interpolation Inverse Distance Weighting calculations. **S** specifies post-summer surveys and **W** specifies winter surveys. Bed areas have been specified in Figure 2.1. Error bars show the effect of using 10% and 30% dredge efficiency with mean as 20%.

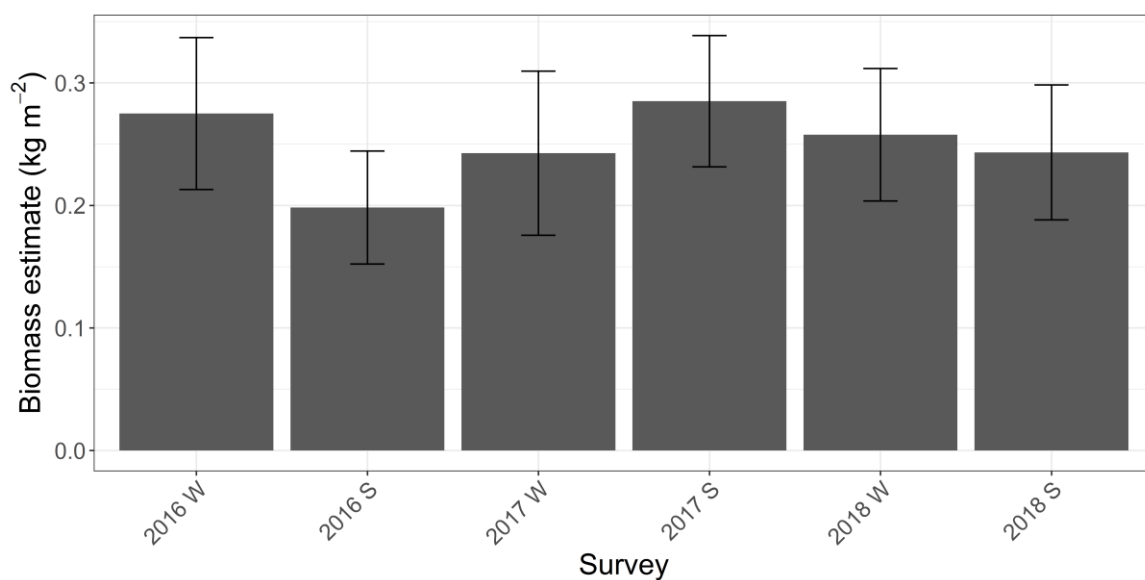


Figure 2.33. Average biomass density of *Crepidula fornicata* for all dredges between post-winter 2016 (2016 W) and post-summer 2018 (2018 S) with standard error calculated through Jackknife resampling. **S** specifies post-summer surveys and **W** specifies winter surveys.

Asterias Rubens population and distribution

Interpolated maps of estimated counts of *A. rubens* are shown in the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone (Figure 2.34-2.39, for animated gif please go to <https://gifs.com/gif/Q0ZwBL>). The densities surveyed are highest offshore in the Eagle and Wallet Spitway sites with estimated populations ranging from 882,132 in post-winter 2017 surveys down to 215,780 in post-summer 2017 on the Eagle bed. On the Wallet Spitway, estimated populations ranged between 91,244 post-summer 2018 up to 636,058 post-winter 2016. Lowest populations were found in the Crouch with estimated populations ranging from 698 in post-summer 2016 surveys to 12,882 in post-winter 2016 surveys (Figure 2.40.). Total populations range from 515,702 in post-summer 2018 to 1,458,099 in post-winter 2017. Statistical differences in population were observed between different surveys (Anova, $F_{5,581} = 5.8455$, $P < 0.001$) with Tukey's HSD *post hoc* analysis consistently highlighting differences between post-summer and post-winter surveys, with no differences observed between different post-winter surveys or between different post-summer surveys (Appendix I: Supplementary Information Table 2.5). There was also a statistical difference in the number of *A. rubens* observed between sites (Anova, $F_{6,581} = 25.7162$, $P < 0.001$) with the full *post-hoc* breakdown available in Appendix I: Supplementary Information Table 2.6.

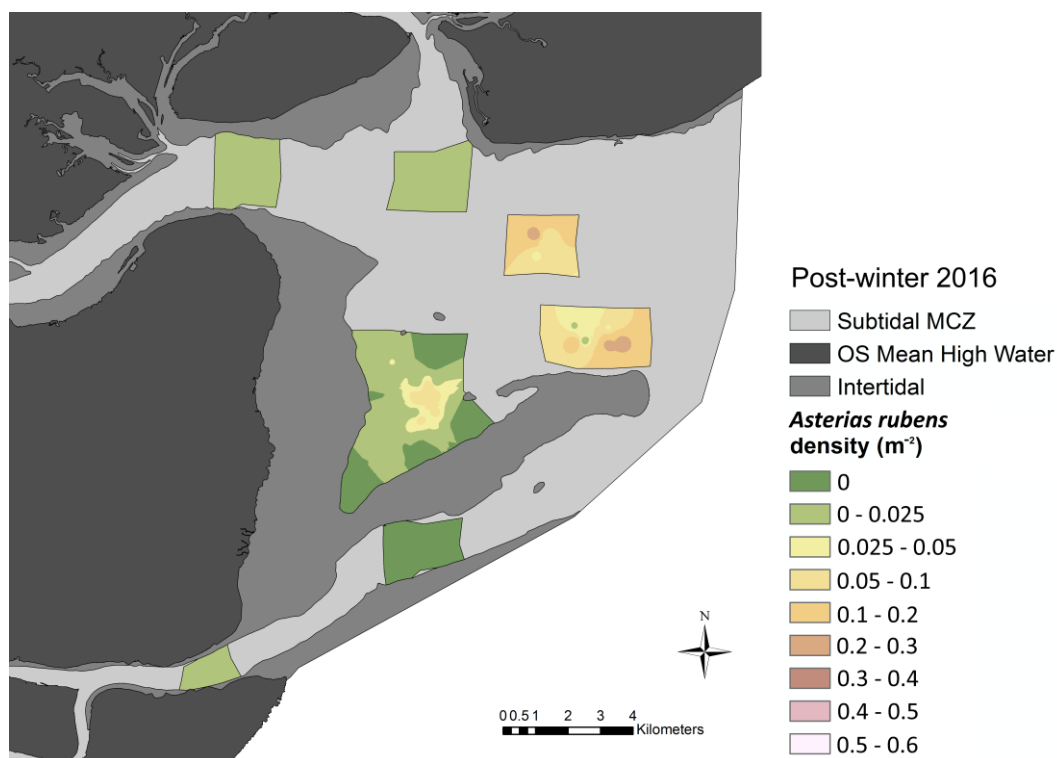


Figure 2.34. Interpolated distribution of *Asterias rubens* abundance within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-winter 2016 dredge surveys within resampled specified oyster bed areas only.

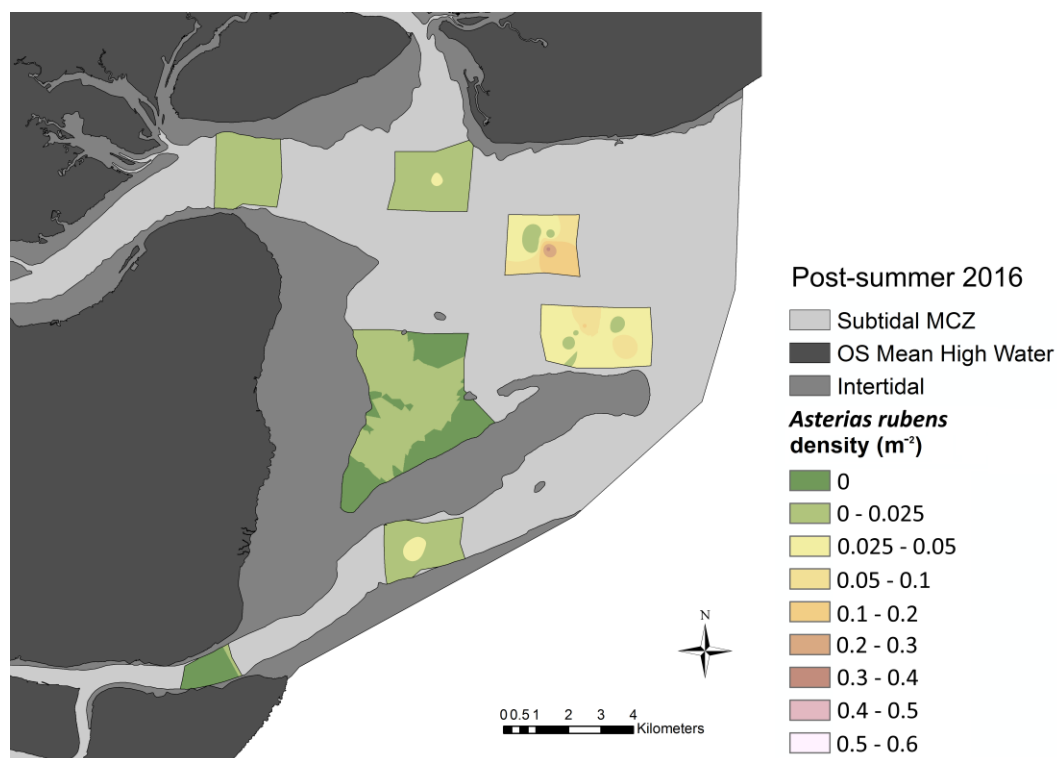


Figure 2.35. Interpolated distribution of *Asterias rubens* abundance within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-summer 2016 dredge surveys within resampled specified oyster bed areas only.

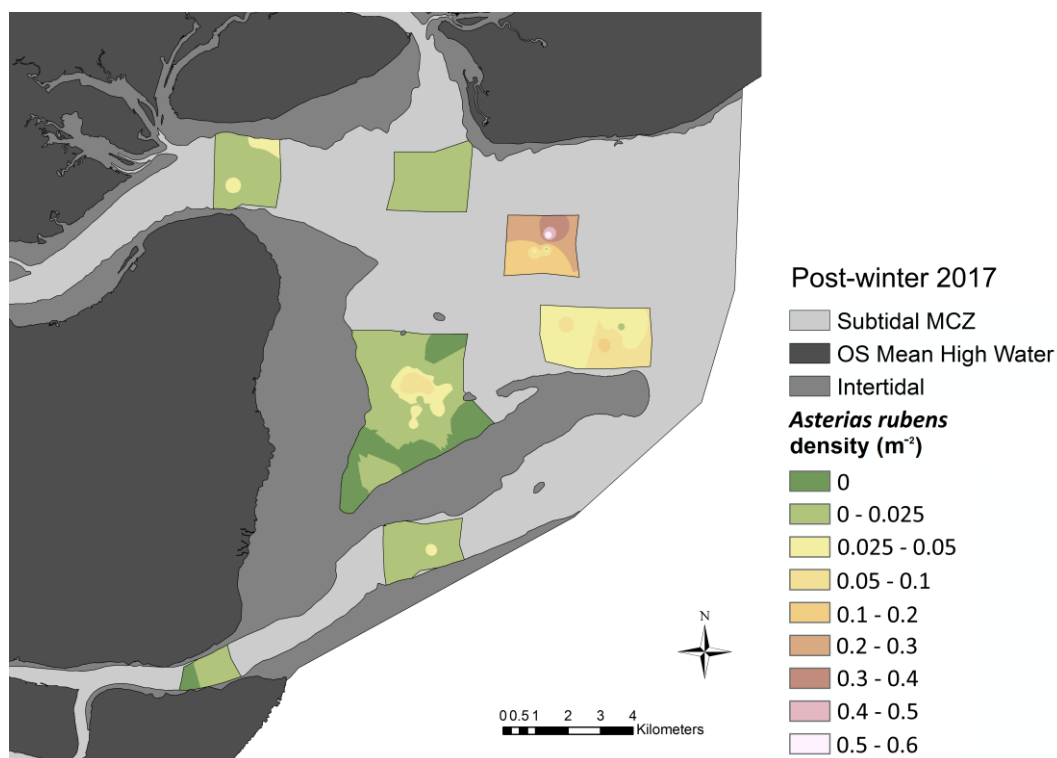


Figure 2.36. Interpolated distribution of *Asterias rubens* abundance within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-winter 2017 dredge surveys within resampled specified oyster bed areas only.

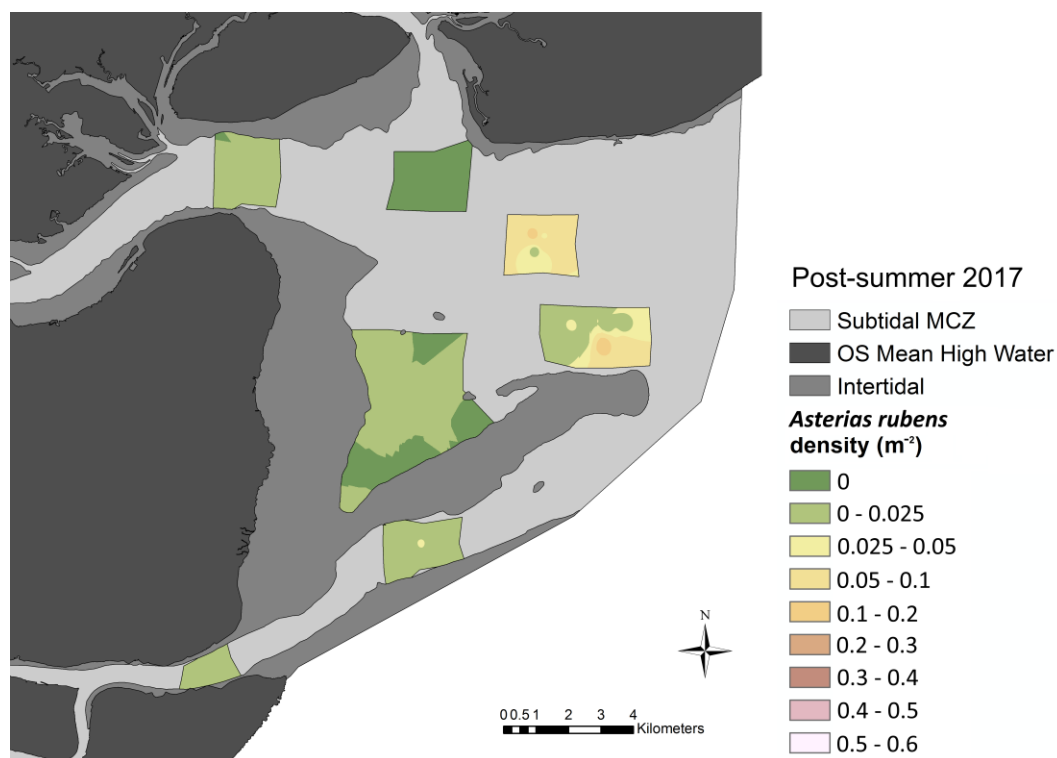


Figure 2.37. Interpolated distribution of *Asterias rubens* abundance within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-summer 2017 dredge surveys within resampled specified oyster bed areas only.

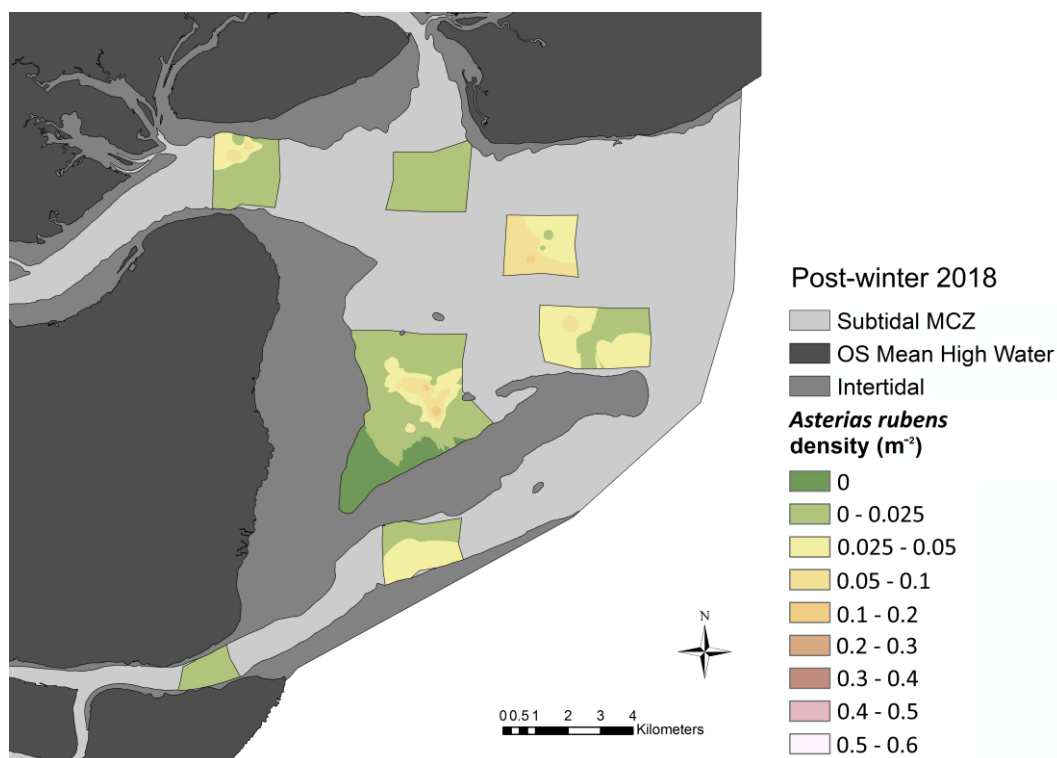


Figure 2.38. Interpolated distribution of *Asterias rubens* abundance within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-winter 2018 dredge surveys within resampled specified oyster bed areas only.

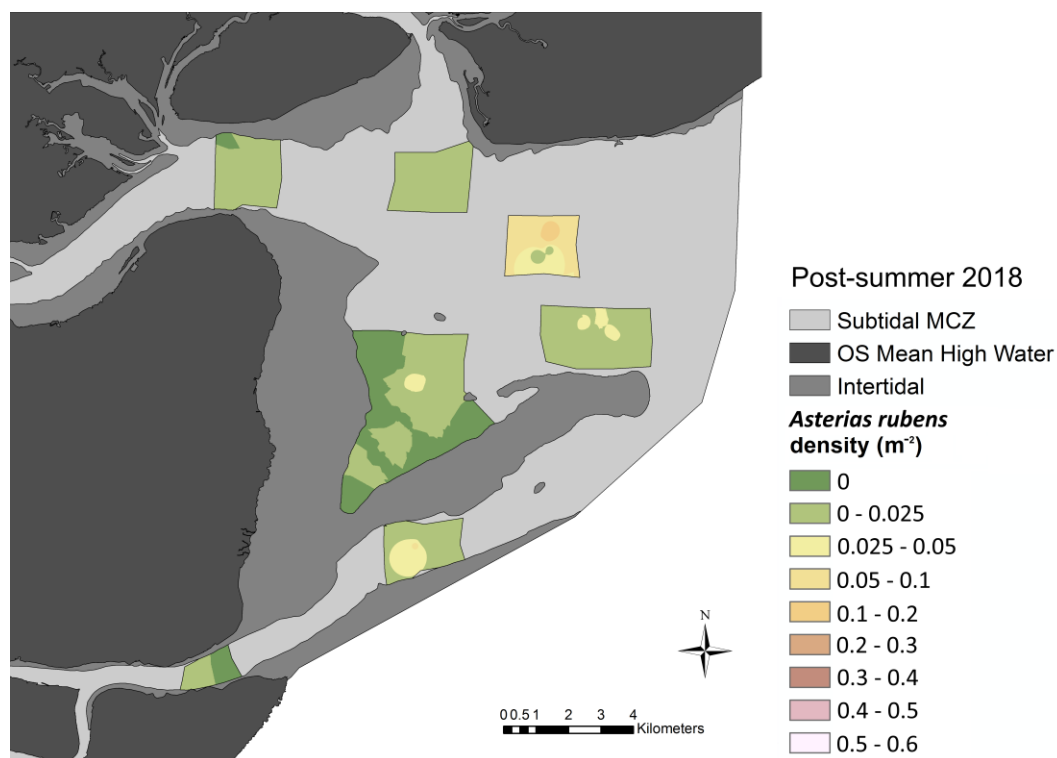


Figure 2.39. Interpolated distribution of *Asterias rubens* abundance within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-summer 2018 dredge surveys within resampled specified oyster bed areas only.

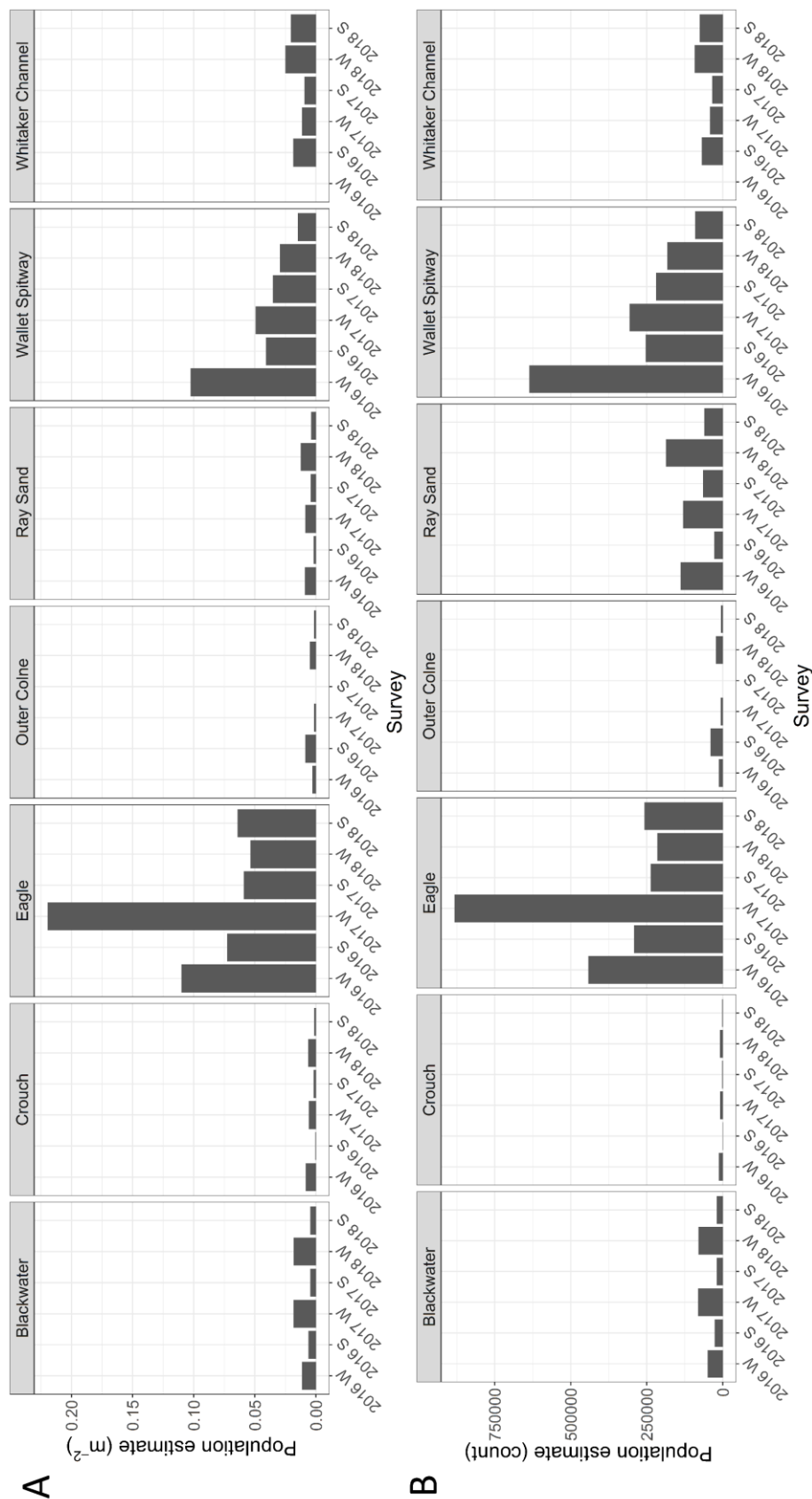


Figure 2.40. A. Average estimated density of *Asterias rubens* for individual beds between post-winter 2016 (2016 W) and post-summer 2018 (2018 S) with standard deviation extracted from interpolation Inverse Distance Weighting calculations. And **B.** multiplied by bed area to give estimated populations. **S** specifies post-summer surveys and **W** specifies winter surveys. Bed areas have been specified in Figure 2.1. 100% dredge efficiency has been assumed.

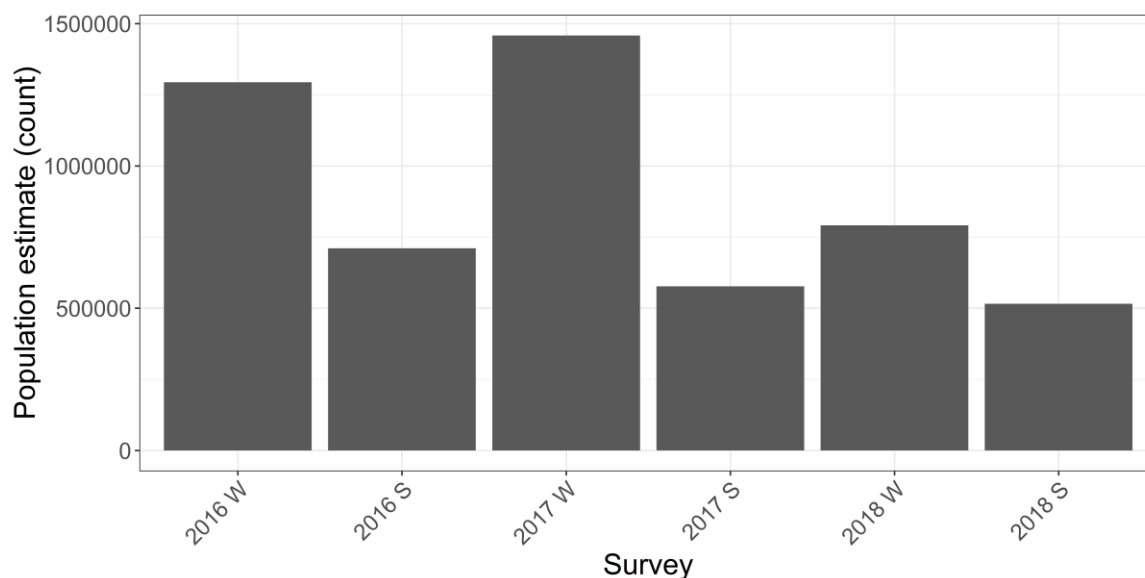


Figure 2.41. Estimated *Asterias rubens* populations for all beds combined between post-winter 2016 (2016 W) and post-summer 2018 (2018 S) with standard deviation extracted from interpolation Inverse Distance Weighting calculations. **S** specifies post-summer surveys and **W** specifies winter surveys. Bed areas have been specified in Figure 2.1. 100% dredge efficiency has been assumed.

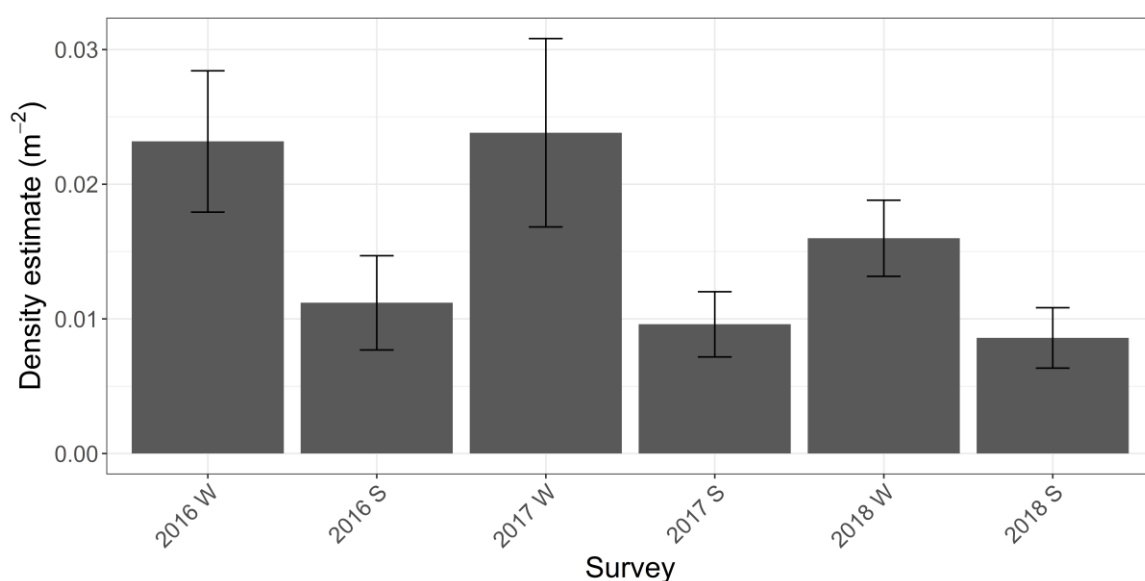


Figure 2.42. Average density of *Asterias rubens* for all dredges between post-winter 2016 (2016 W) and post-summer 2018 (2018 S) with standard error calculated through Jackknife resampling. **S** specifies post-summer surveys and **W** specifies winter surveys.

Crassostrea gigas population and distribution

Interpolated maps of estimated counts of *C. gigas* are shown in the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone (Figure 2.43-2.48 for animated gif go to <https://gifs.com/gif/E9r5gk>). As with populations of *O. edulis*, densities surveyed are consistently highest in the Crouch where populations range from 2,410,849 in post-summer 2017 down to 817,554 in post-winter 2018 (Figure 2.49). Here, live oysters of both species were regularly found attached growing to each other (Figure 2.52). Total subtidal estimated populations range from 2,264,794 in post-winter 2018 surveys up to 5,828,274 in post-summer 2016 (Figure 2.50). No statistical difference was observed in the abundance of *gigas* between surveys however there was a statistically significant difference between different sites (Anova, $F_{6,583} = 34.098$, $P < 0.001$) with full *post-hoc* breakdown of the analysis found in Appendix I: Supplementary Information Table 2.7.

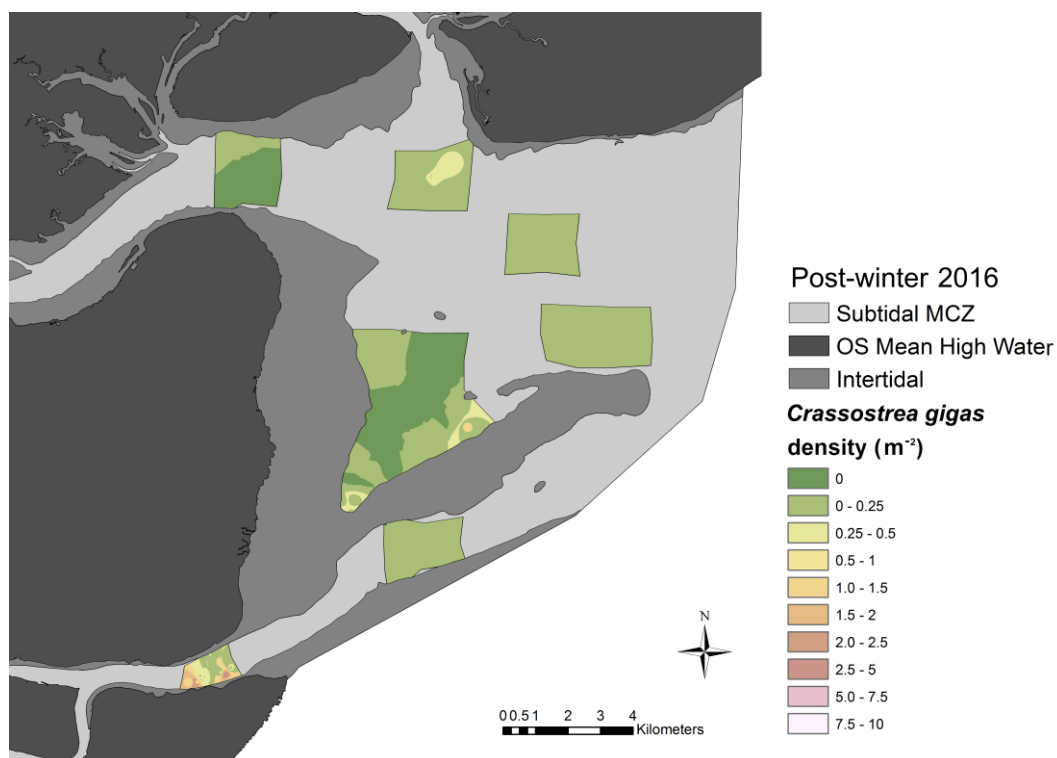


Figure 2.43. Interpolated distribution of *Crassostrea gigas* abundance (incorporating 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-winter 2016 dredge surveys within resampled specified oyster bed areas only.

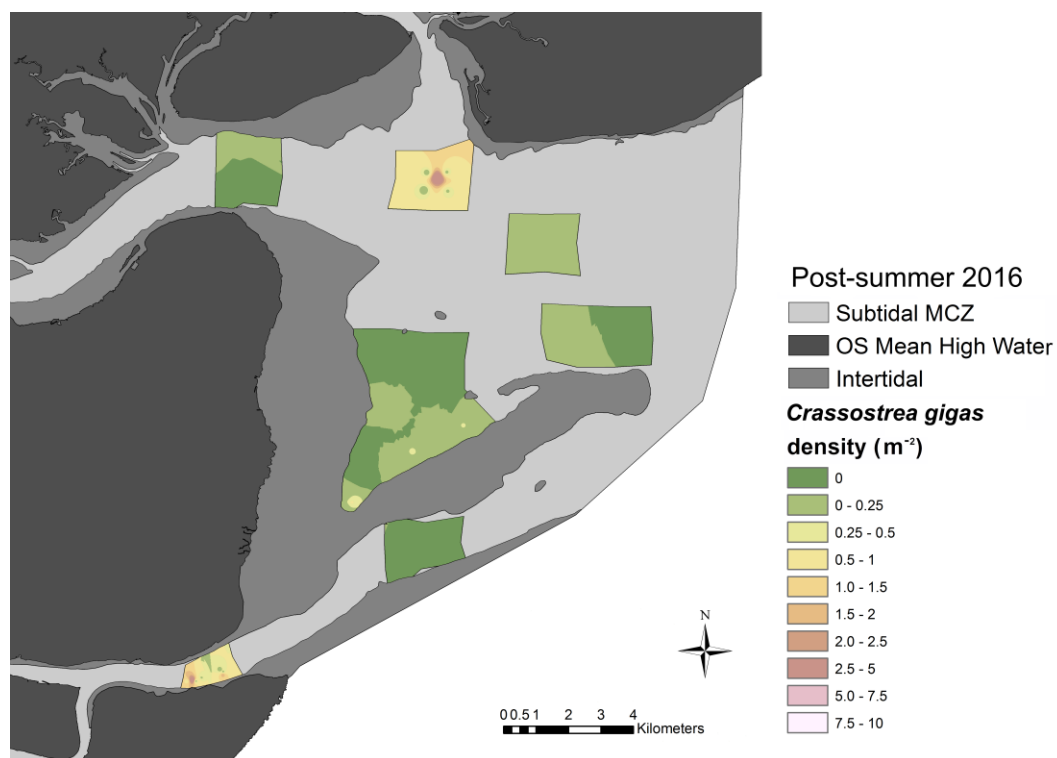


Figure 2.44. Interpolated distribution of *Crassostrea gigas* abundance (incorporating 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-summer 2016 dredge surveys within resampled specified oyster bed areas only.

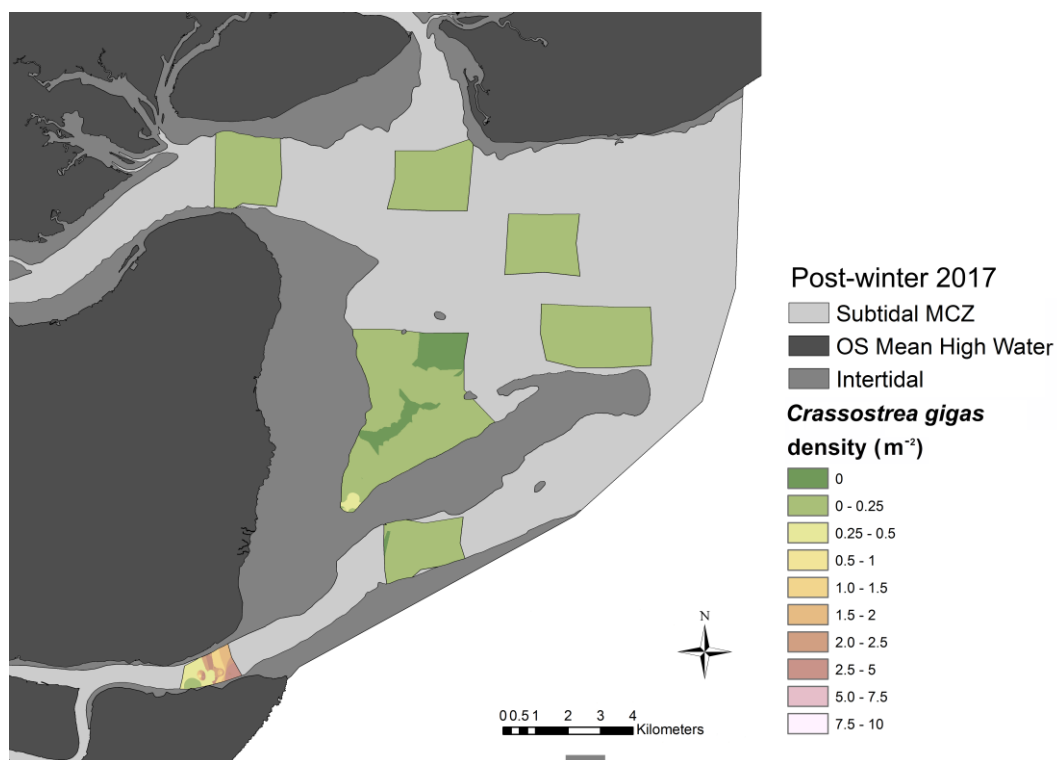


Figure 2.45. Interpolated distribution of *Crassostrea gigas* abundance (incorporating 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-winter 2017 dredge surveys within resampled specified oyster bed areas only.

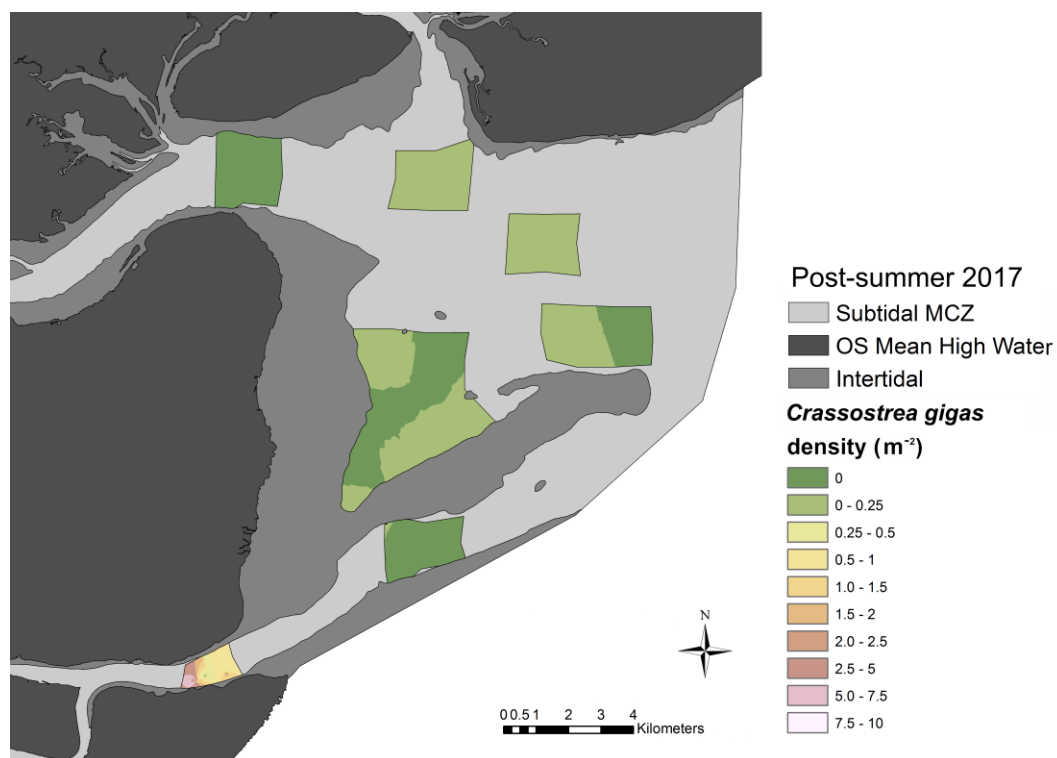


Figure 2.46. Interpolated distribution of *Crassostrea gigas* abundance (incorporating 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-summer 2017 dredge surveys within resampled specified oyster bed areas only.

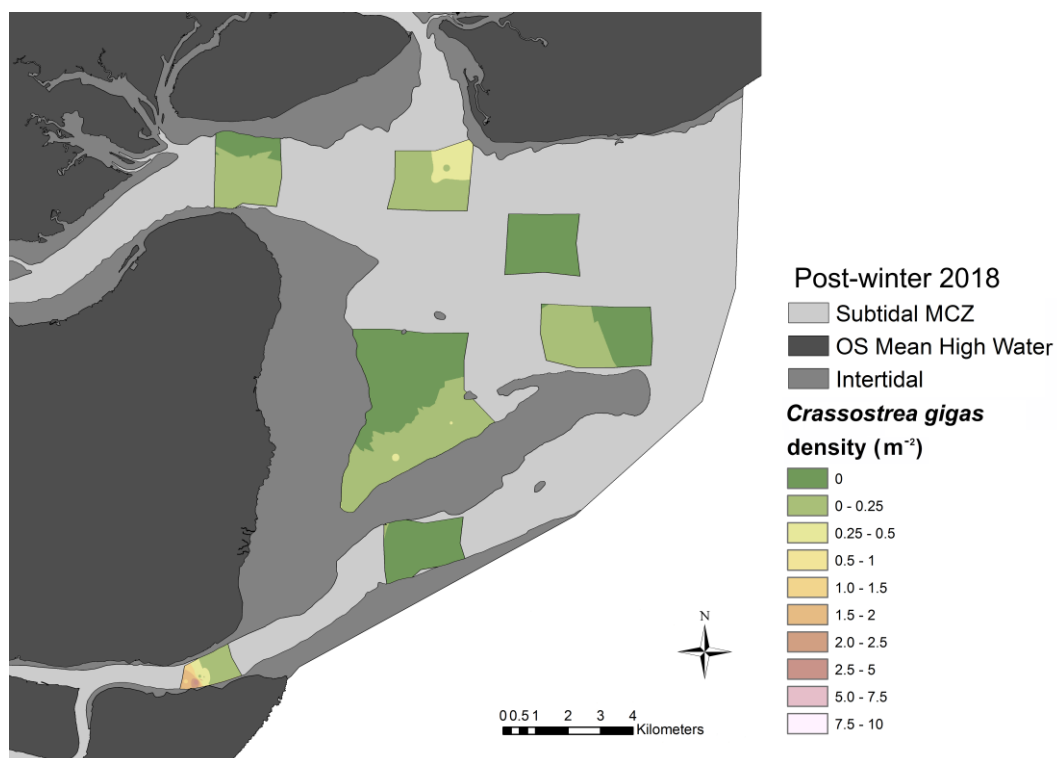


Figure 2.47. Interpolated distribution of *Crassostrea gigas* abundance (incorporating 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-winter 2018 dredge surveys within resampled specified oyster bed areas only.

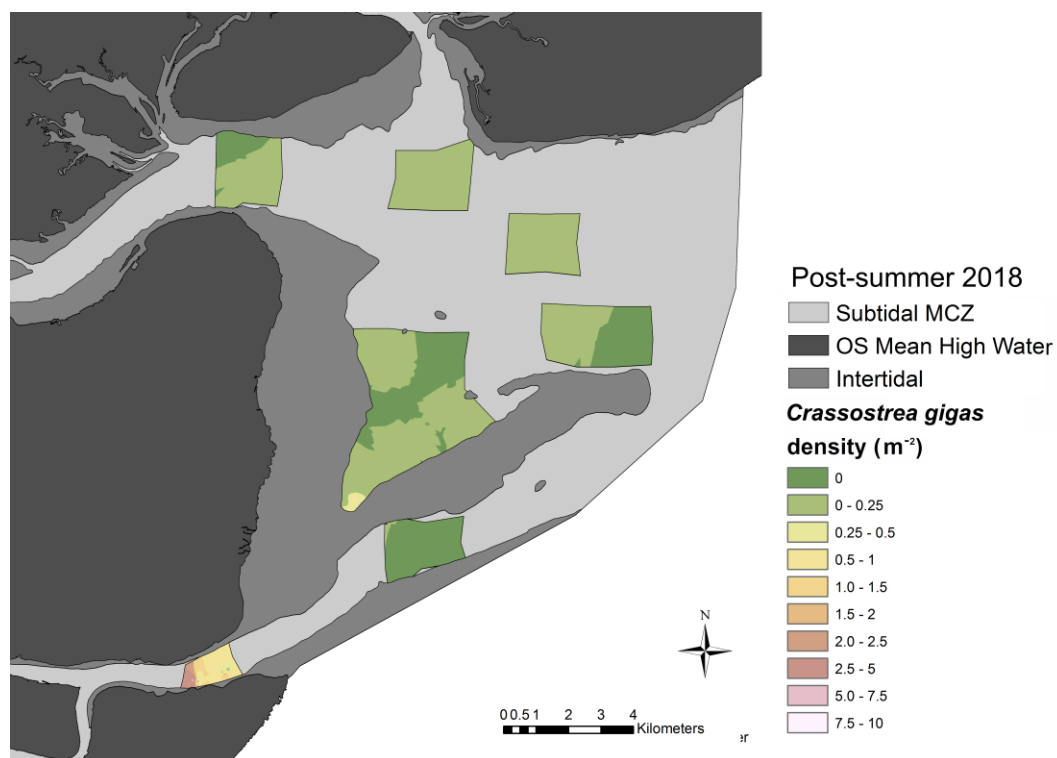


Figure 2.48. Interpolated distribution of *Crassostrea gigas* abundance (incorporating 20% dredge efficiency) within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone from post-summer 2018 dredge surveys within resampled specified oyster bed areas only.

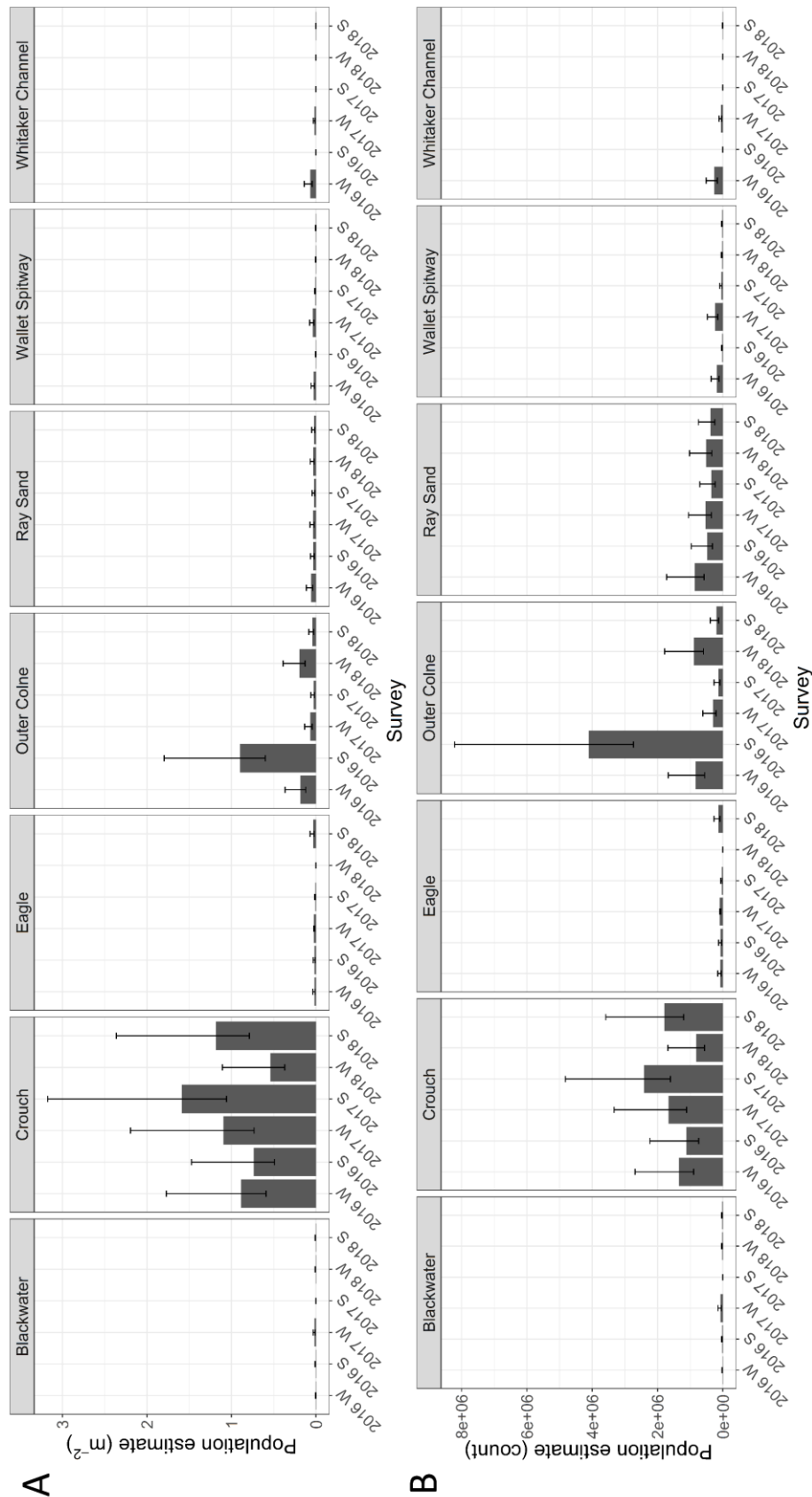


Figure 2.49. A. Average estimated density of *Crassostrea gigas* for individual beds between post-winter 2016 (2016 W) and post-summer 2018 (2018 S) with standard deviation extracted from interpolation Inverse Distance Weighting calculations. And **B.** multiplied by bed area to give estimated populations. **S** specifies post-summer surveys and **W** specifies winter surveys. Bed areas have been specified in Figure 2.1. Error bars show the effect of using 10% and 30% dredge efficiency.

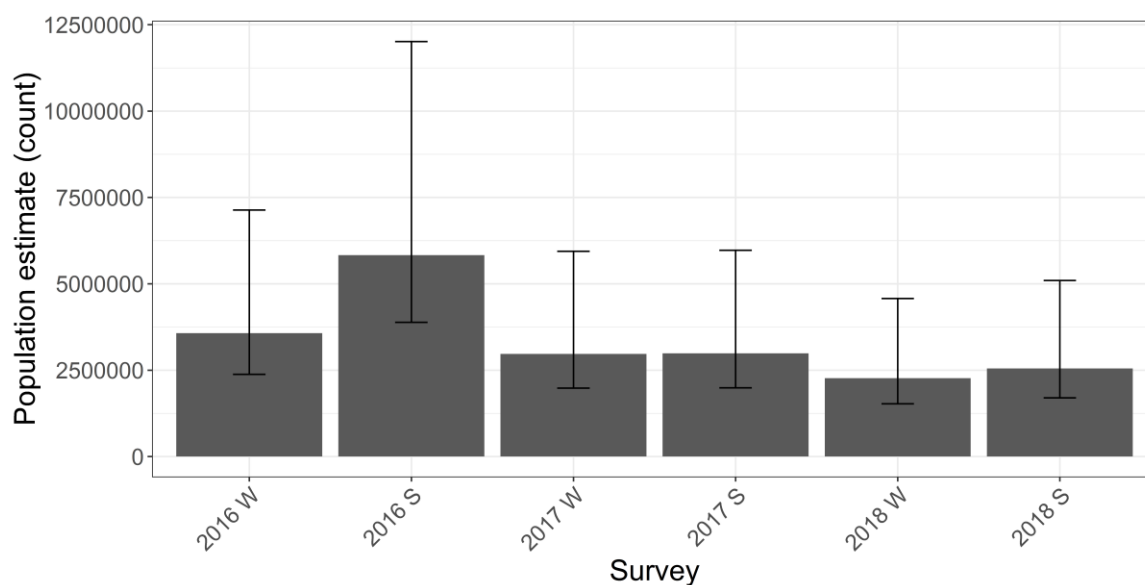


Figure 2.50. Estimated *Crassostrea gigas* populations for all beds combined between post-winter 2016 (2016W) and post-summer 2018 (2018 S) with standard deviation extracted from interpolation Inverse Distance Weighting calculations. **S** specifies post-summer surveys and **W** specifies winter surveys. Bed areas have been specified in Figure 2.1. Error bars show the effect of using 10% and 30% dredge efficiency and mean of 20%.

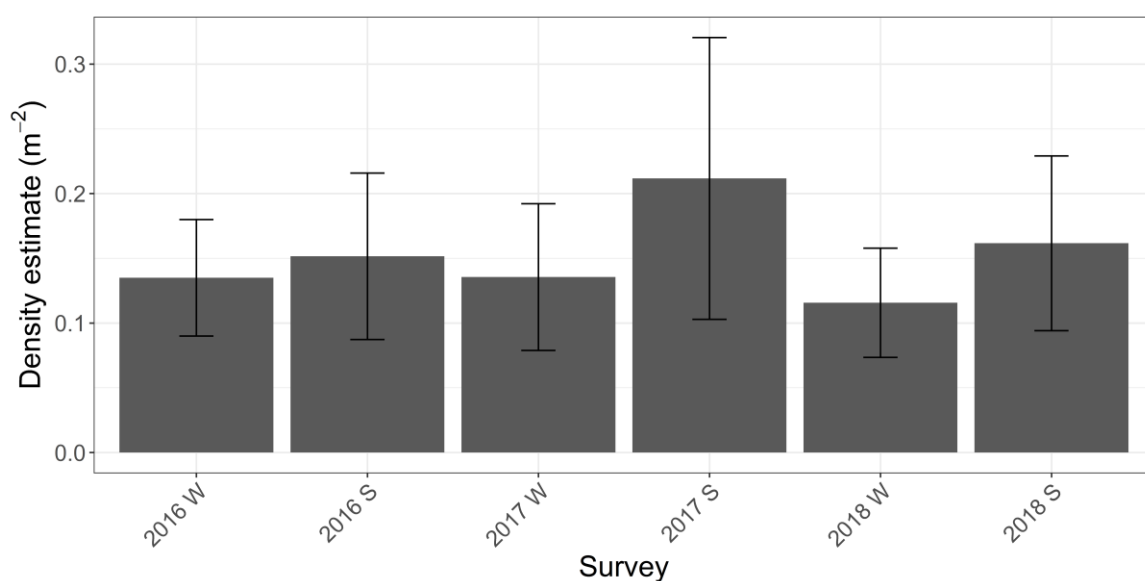


Figure 2.51. Average density of *Crassostrea gigas* for all dredges between post-winter 2016 (2016 W) and post-summer 2018 (2018 S) with standard error calculated through Jackknife resampling. **S** specifies post-summer surveys and **W** specifies winter surveys.



Figure 2.52. Co-occurrence of *Crassostrea gigas* and *Ostrea edulis* in Essex, UK highlighting the presence of mixed reefs of live oysters as found at the Crouch site. Red indicated *O. edulis* and blue *C. gigas*.

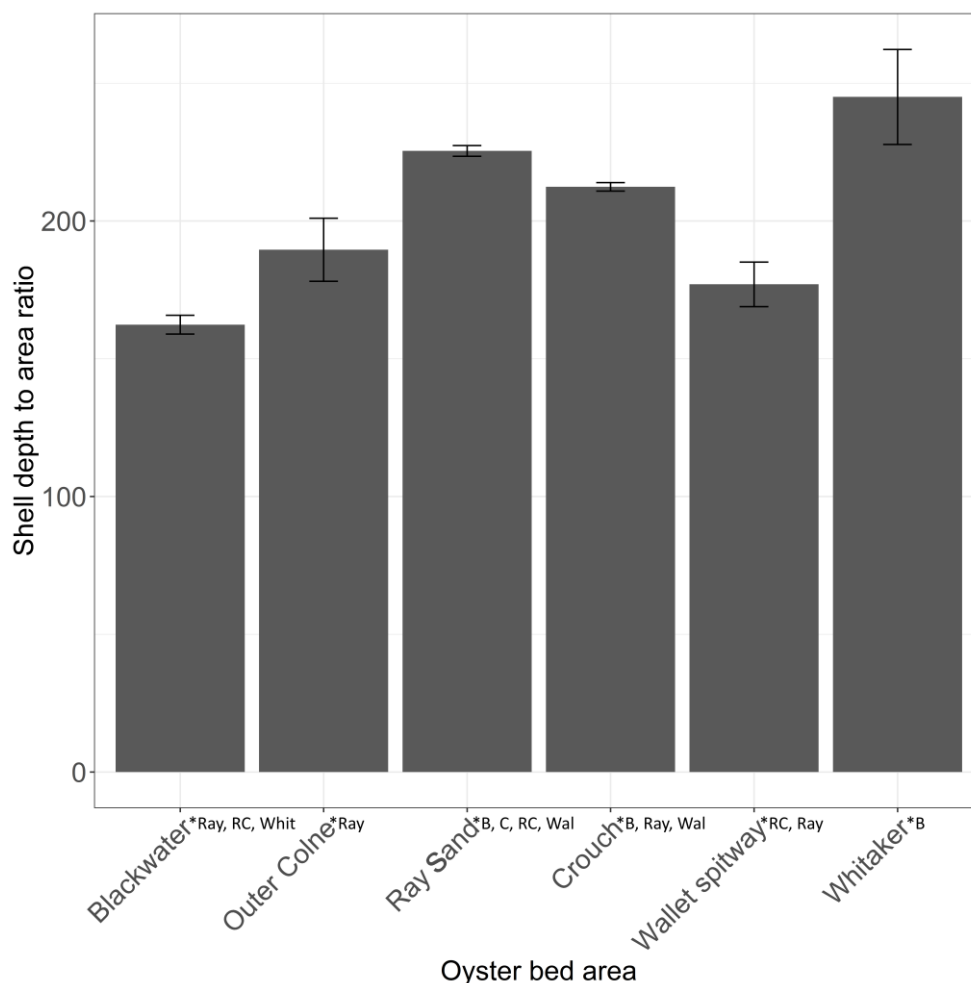


Figure 2.53. Oyster shell depth to area of shell ratio differences by oyster bed (Blackwater, Outer Colne, Ray Sand, Crouch, Wallet Spitway and Whitaker channel) with standard error bars. Lower ratios indicate deeper shelled oysters. Significant differences from Tukey's HSD *post hoc* analysis are highlighted with * and site names with B = Blackwater, Ray= Ray Sand, C=Colne, RC = Crouch (Roach Crouch mouth), Wal = Wallet Spitway and Whit = Whitaker Channel.

Variation in shell shape of Ostrea edulis

All oysters measured for length, width and depth had an mean length to width ratio of 1.0236:1 and a mean area to depth ratio of 212.17:1. Oyster bed area was found to be a significant predictor of oyster shell depth to shell area ratio (Dev=283.547, $P<0.001$) however, oyster shell depth to area ratio was not affected by the depth of dredge tow location (Dev=1.650, $P=0.80$). Variation in shell depth ratios and significant site differences calculated using Tukey's HSD *post hoc* analysis can be found in Figure 2.23 with oysters from Wallet Spitway and Blackwater sites significantly deeper than oysters found on Ray Sand and Whitaker sites.

2.4 Discussion

This chapter provides information on current estimated population trends of the European native oyster in Essex over 5 years (2014-2018) with seasonal trends in European native oyster populations and key associated species provided over 3 years (2016-2018). This gives an understanding of the baseline and subsequent status of the native oyster population following the designation of the BCRC.MCZ in 2013 and is essential for developing a suitable and sustainable management plan for the restoration of this species. These figures will be used in ongoing assessments to understand what tools should be used for oyster species and oyster habitat restoration, and to determine when restoration has been achieved.

Ostrea edulis counts and biomass population and trends

In the most recent survey (in post-summer 2018), current estimated populations of *O. edulis* stand at 3,479,060 individuals or 221.45 tonnes within the constrained areas of IDW. Between 2014 and 2015 there appears to have been an uplift in the population biomass and abundance, mirrored with an increase in the size frequencies of smaller size classes in 2015. This highlights a likely successful spatfall in 2014 with 1-year old recruits observed in the population. It is highly important to note that population estimates of biomass in the 2014 surveys increased by approximately 60 tonnes when removing survey points which were not resampled in subsequent surveys. This is a clear caveat with using this method of population estimation and is the primary reason bed boundaries were specified as opposed to calculating densities for the entire BCRC.MCZ to prevent extrapolation of densities outside of survey areas. A further source of error when estimating these populations is through the difficulties in sampling patchy distributions of oysters. Whilst every effort has been made to put oysters back in the same area and sample the same places every year, there is some variation in this and this likely resulted in some level of survey-based variation in population estimate. In addition, a large source of error in accurately estimating this population of native oysters derives from dredge efficiency being unclear. Whilst the use of a 20% dredge efficiency has been advised, following discussions and research between Natural England and KEIFCA, this value has not been experimentally tested and can result in populations

estimated at approximately 300 tonnes or over 800 tonnes for the same year either using 10 or 30% dredge efficiency (or between 0.006 and 0.021 kg m⁻² if using average density), within the same survey (2016 W). This level of variation is larger than that resulting from Jackknife resampling which ranges from 0.013 to 0.021 kg m⁻² for the same survey, highlighting that dredge efficiency is a large source of error for estimating *O. edulis* populations.

Whilst it is recognised that there are large sources of error in these estimates, this has been unavoidable to create a baseline of the distribution and trends of *O. edulis* populations in the BCRC.MCZ over a large geographic area. Consistent seasonal trends are being observed in *O. edulis* populations with current estimates showing increases in native oyster biomass and population abundance in post-winter surveys in comparison to post-summer surveys. Whilst this is not observed on all beds, it is an overall trend for all beds combined across the BCRC.MCZ with this fluctuation most apparent on the Ray Sand (Figure 2.10 and 2.22). Consistent reductions in estimated biomass in winter surveys may be due to sampling patchy oyster beds with highly variable densities and or seasonal impacts in sampling. Increased algal growth throughout the summer results in increased catches of dead algae in the dredge (personal observation). This may influence oyster catch rates, however, as this trend is not observed in other species such as *C. gigas* or *C. fornicata*, seasonal environmental factors influencing dredge and or detection efficiency may be unlikely.

Reductions in population following the post-summer breeding season have, however, been observed in other species of oyster, blue mussel and scallops, occurring worldwide (Renault, Ford and Samain, 2005). This post-summer population reduction is known as “Post-summer Mortality Syndrome” and is most commonly associated with *C. gigas* when it was first described in the 1940s, however remains to be fully understood. Post-summer mortalities in *C. gigas* have been primarily associated with increased disease prevalence and susceptibility, thermal stress, reduced energy reserves following reproduction and smothering by increased macroalgal growth resulting in hypoxia (Renault, Ford and Samain, 2005; Moullac *et al.*, 2007; Wendling and Wegner, 2013). In *C. gigas*, post-summer disease mortalities are primarily attributed to increased transference of the OsHV-1 (Osteris herpesvirus 1) virus and multiple strains of *Vibrios*, primarily *V. splendidus* and *V. estuarianus* (Renault, Ford and Samain, 2005). Both

OsHV-1 and *Vibrios* are known to be more prevalent and cause increased mortality in oysters during warmer months with OsHV-1 particularly more virulent once water temperatures reach 16°C (Renault *et al.*, 2014). Whilst it was previously thought that OsHV-1 does not infect *O. edulis*, recent studies have found *in-situ* hybridization of a micro variant of this virus can infect European native oysters (López Sanmartín *et al.*, 2016). *Vibrio* spp. are also documented to negatively impact *O. edulis* (Jeffries, 1982). In addition to these two diseases, which have been associated to *C. gigas* post-summer mortalities, *O. edulis* also suffer from a fungal shell disease (*Ostracoblabe implexa*) and the haplosporidian parasite *Bonamia*, both of which are found to be more prevalent and spread during warmer weather. *O. implexa*, otherwise known as Dutch shell disease, is thought to only be found in areas which have water temperatures exceeding 22°C for at least 2 weeks of the year and initially causes shell malformations which subsequently penetrates the mantle/shell interface and can lead to shell weakness. This fungus may then infect the adductor muscle causing difficulties in closing the shell resulting in mortality of *O. edulis*, along with other species of oyster (McGladdery, 2011; Laing *et al.*, 2005). *Bonamia* is a genus of protozoan parasite, *Bonamia ostreae*, and is thought to be of particular concern to *O. edulis*. It is known to be present in Essex since it was first detected in 1983 (Laing *et al.*, 2014). It is the cause of bonamiosis which has been known to cause widescale mortalities of *O. edulis* across Europe. This parasite remains to be fully understood, with disease prevalence thought to be lower in warmer water however mortality may be higher (Engelsma *et al.*, 2010; Laing *et al.*, 2014).

Other areas, particularly the Blackwater, show a consistent decline in *O. edulis* abundance. The Blackwater area is of particular concern due to the close proximity to the largest native oyster Several Order area in Essex, the Tollesbury and Mersea Several Order, where oyster cultivation and harvest is permitted. Oyster growers reported particularly high levels of sedimentation resulting in smothering of oysters on the several order beds upstream, to the west of the Blackwater bed, following storm Hartmut and the cold front storm Emma in March 2018; this is discussed further in Chapter 4.

Crepidula fornicata biomass population and trends

Biomass estimates for *C. fornicata* have remained relatively stable between 2016 and 2018 with estimates ranging from 5000-6500 tonnes, with little seasonal variation (Figure 2.32.). Highest densities are found in the Blackwater and the Ray Sand with no *C. fornicata* present on the Eagle bed since March 2016 when populations were estimated to be approximately 350kg for the entire bed. Density of *C. fornicata* on the Crouch bed, however, appears to be increasing and despite densities remaining low at this site, regular monitoring of *C. fornicata* populations here are recommended, particularly due to the co-occurrence of high *O. edulis* and *C. gigas* densities (Figure 2.2-2.10 and 2.25-2.30).

Asterias rubens population and trends

Populations of *A. rubens* are primarily found in offshore areas such as the Eagle and Wallet Spitway (Fig. 2.34-2.39). Densities are generally found to be highest at these sites in post-winter surveys and lowest in post-summer surveys however, the seasonal increase in population of *A. rubens* in winter 2018 appears suppressed in comparison to previous years. This lack of offshore post-winter increase in population density is likely due to the widely reported anti-cyclone (Emma) and combined anticyclonic storm (Hartmut) that resulted in the mass mortality of nearshore invertebrates along UK coastlines, including on the Frinton and Walton shores (personal observation).

Areas which have highest populations of *A. rubens* in post-winter surveys are known herring spawning grounds, with spawning occurring in late March, following the post-winter surveys. In British Columbia, other species of starfish are known predators of herring spawn. In this area starfish are not thought to be major predators of Pacific herring due to their low densities (Haegerle, 1993), with starfish listed as “probable” predators of herring eggs (Stevenson & Scott, 2005). However, quantification of predation rates of herring spawn by *A. rubens* has not yet been undertaken.

Crassostrea gigas population and trends

Populations of the non-native *C. gigas* appear highly variable between sites with the highest densities observed in the Crouch (Figure 2.2.43-2.49). Here, there was a large population decline between post-summer 2017 and post-winter 2018. This large population decline may be due to variation in sampling sites and patchiness of *C. gigas* beds or due to widescale mortalities during the anti-cyclone (Emma) and combined anticyclonic storm (Hartmut). *C. gigas* are thought to be more susceptible to mortality at low temperatures than *O. edulis* with approximately 95% mortality observed in juvenile *C. gigas* when maintained at 3°C for 7-11 weeks in comparison to only 5% *O. edulis* (Child & Laing, 1998). *C. gigas* is also found to be more susceptible to stress from increased temperatures and ocean acidification than *O. edulis*, showing reduced condition and reduced clearing rates respectively at increased temperature and acidification levels, with *O. edulis* unaffected in the same conditions (Lemasson *et al.*, 2018). *C. gigas* may, therefore, be more vulnerable to global climate change with climatic models predicting increased frequency of both summer heatwaves and winter storms (Beniston *et al.*, 2007). *In-situ* monitoring of the survival of individual *C. gigas* populations may be required to further assess population dynamics of this species and make predictions on changes in their populations in future years.

Reports of wild populations of *C. gigas* within Europe are not new, with reports of *C. gigas* spatfalls in Europe dating back to 1975 (Drinkwaard, 1999). Subtidal populations are becoming more prevalent with both oyster species now recognised to co-occur in some Irish populations (Zwerschke *et al.*, 2017). This is the first report, to our knowledge, of both *O. edulis* and *C. gigas* not only co-occurring within the same area, but growing attached to live, adult oyster heterospecifics. Studies of the expansion of Irish mixed species beds indicate the potential for out-competition of *O. edulis* by *C. gigas* under certain conditions, therefore regular monitoring of these populations should be of high priority (Zwerschke *et al.*, 2017). Harvesting of these populations may be of use in some areas however oysters should be carefully assessed to ensure *O. edulis* spat are not removed in the process and available hard substrate for *O. edulis* spat is not depleted with *C. gigas* shells often seen as valuable cultch (Haelters & Kerckhof, 2009).

Length frequency distribution

Understanding the length frequency distribution of populations helps to highlight when successful spawning events may have occurred. Figure 2.24 shows how the length frequency distribution of *O. edulis* has changed over time between 2014 and 2018. Years 2015 and post-summer 2016 show increased juvenile proportion of populations, potentially showing juvenile oysters which have settled in the previous post-summer recruiting into the size classes which are able to be captured by the dredge in this survey. Point A highlights a likely single cohort of juvenile *O. edulis* in September 2015 with point B highlighting potential growth of this cohort to the post-winter 2016 survey. Point C then shows a potential additional cohort of new juveniles entering the survey in the post-summer 2016 survey (Figure 2.24). Size-frequency distributions show low juvenile to adult ratios in 2018 surveys following storm Hartmut and cold front Emma. It has been noted that high levels of turbidity during the storms resulted in the smothering of some oyster beds in the Several Order and subsequently lead to high levels of mortality in the Blackwater. In the Tollesbury and Mersea Several Order, oyster growers dredged and harrowed these grounds to clear this sediment. This did not occur within the MCZ area (personal observation). However, as this plot (Figure 2.24) is a size-frequency plot it is important to note that it is possible that these apparent increases in juvenile abundances in 2015 and 2016 may also be due to high adult mortality, increasing the relative proportion of small and juvenile individuals within a population. To assess which life stage is most at risk of mortality, individual size-specific assessments of survival are required. This is discussed further in Chapter 4.

Variation in shell morphology

Wide variation in shell depth to shell area ratio has been observed in different areas across the BCRC.MCZ with oysters from the Ray Sand and Whitaker Channel significantly shallower than oysters in the Blackwater or the Wallet Spitway (Fig. 2.53). It is currently unclear from this data if this variation in oyster morphology translates to any variation in meat availability or if this variation is primarily due to differences in shell thickness, however, clear differences are present between small geographic areas.

Large variation in relative shell dimensions have been recognised in multiple geographic regions. Oysters of various shapes have previously been described as 1. “Dumpy” – where oysters have smaller yet deeper shells, 2. “Normal” – where oysters are large with narrower depth to their shells and 3. “Semi-dumpy” – where oyster shells are deeper than normal but not to the extent of a “dumpy” oyster (Orton, 1927). Dumpy oysters known to be common on Essex grounds, however the exact causes of this variation in shell shape, whilst hypothesised, has not been confirmed (Cole & Waugh, 1959). This variation in relative *O. edulis* shell dimensions has been hypothesised to be due to two primary environmental factors: water depth, temperature and speed of water flow, with oysters in deeper and faster flowing water more likely to be either “dumpy” or “semi-dumpy” oysters (Orton 1938; Cole & Waugh, 1959). This has been attributed to the increased encounter rate of food as it flows past in faster flowing water and bombardment of silt particles. This study did not find any influence of water depth on the shell depth to area ratio however it should be noted that the BCRC.MCZ is shallow with maximum depths of approximately 19m below chart datum, whereas *O. edulis* are known to be found in depths up to 200m. Current data suggests that shell depth to area ratios are influenced by other differences rather than a general trend of deeper-shelled oysters in deeper water. This may be through genetic differences caused by physical separation of populations or differences in water quality between different areas. To clarify this, further physiological and genetic analysis is required.

In addition to the potential influence of water flow rate and depth, presence of predators, specifically oyster drills, has also been suggested to increase shell thickness of *O. edulis* and result in slower growing oysters. This is due to oysters needing to increase protection against potential attack from predation (Carriker, 1955). The non-native oyster drill *Urosalpinx cinerea* has been observed to preferentially feed on young, thinner shelled oysters, however once shells were observed to be thicker than that of mussels *U. cinerea* will favour mussel species (Carriker, 1955).

Conclusions

With the distribution of the native oyster in the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone now clear, and population estimates and trends becoming apparent, management of the native oyster and any intervention work will be targeted to the correct geographic areas. Variation in populations of the native oyster have been observed both between years and between seasons and this seasonal variation in predicted population number will need to be considered when sampling this population for census data in future years. Ray Sand populations of *O. edulis* appear highly variable between seasons yet stable overall however Blackwater populations appear to be declining.

Populations of *C. fornicata*, and *C. gigas*, whilst currently appear stable, need to be monitored to ensure proliferation of these species do not occur into native oyster strongholds, with negative impacts of both species recorded for the native oyster in previous studies (Decottignies *et al.*, 2007; Blanchard *et al.*, 2008; Zwerschke *et al.*, 2018).

Whilst it is clear that oyster distribution is highly patchy throughout the BCRC.MCZ, a primary management task should be to accurately calculate dredge efficiency for a range of habitats and oceanic conditions. Large error bars calculated through varying dredge efficiency on the predicted populations of native oysters, *C. gigas* and *C. fornicata* highlight the effect even small changes in dredge efficiency can have on estimated population number.

Chapter 3:

Density and seasonally dependent associations of biodiversity with the European flat oyster (*Ostrea edulis*)

3.1. Introduction aims and hypotheses

Conservation and restoration historically focuses on single species or habitats, with policy largely directed at achieving specific population targets of individual protected species or through the protection of a known area of habitat (Brooks, Fonseca and Rodrigues, 2004). There is now an increasing trend to recognise the importance of biodiversity and ecosystem function as a whole when directing conservation activities. It then becomes important to make predictions on how a community may change through increasing or decreasing populations of what remain single species restoration targets (Nicholson *et al.*, 2012).

With coastal and estuarine areas becoming progressively more populated, human impacts are putting increasing strain on estuarine ecosystems, this is having detrimental impacts on biodiversity in many estuarine areas and said to have resulted in the depletion of 90% of important species worldwide (Lotze *et al.*, 2006). Oyster reefs are estimated to have declined by as much as 85% globally (Beck *et al.*, 2011). This recognised decline has resulted in an increase in shellfish reef restoration activities with multimillion dollar grants now awarded for some projects in the USA (Penhirin, 2014; Mcleod *et al.*, 2019).

The European native oyster (*Ostrea edulis*) is primarily subtidal and, as an ecosystem engineer, creates hard substrate through the production of shell. The resulting increased habitat complexity is documented as being important to provide nursery habitats for small fish species, support a wide range of epifaunal species (Korringa 1951; Mistakidis 1951; Barnes 1973; Haelters & Kerckhof 2009), with a flat oyster “bed” now a recognised habitat type (Temple and Cox, 2009). In addition, flat oysters could be important for filtering sediment from the water column, excreting indigestible particles as pseudofaeces, and contributing to nutrient cycling, as has been found with rock oysters in other geographic regions

(Newell *et al.*, 2006). The native oyster is an economically important species which has supported multiple fisheries across the UK and Europe since Roman times (1st century AD) (Tyler, 2009). Production has declined throughout the last 60 years, from a maximum recorded global production of 29,595 tonnes in 1961 to 2809 tonnes in 2014 (FAO, 2017). Conversely, global production of the Pacific oyster, (*Crassostrea gigas*; sometimes *Magallena*), which is now also widely present as a non-native species around the European coast, has risen from 281,193 tonnes to 625,925 (FAO, 2016). With such a potential range of ecosystem services offered from the presence of oysters there has been a large increase in efforts to restore rock oysters in the USA, Canada and New Zealand and subtidal flat oysters across Europe (Lipcius *et al.*, 2015; Roberts 2017).

In the UK, *O. edulis* was listed as a Priority Species in the Post-2010 biodiversity framework, now with its own Native Oyster Species Action Plan for increasing the abundance and geographic range where possible (Syvret and Woolmer, 2015). Due to the presence of native oysters as a species and native oyster beds as a habitat, the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone (hereafter BCRC.MCZ) was established in 2013 in Essex, UK. There is now, therefore, a legal obligation to “recover these two features to a favourable condition” within the BCRC.MCZ and a requirement to understand how these two features differ, and how to classify and manage each (UK Government, 2013; Natural England, 2015). When termed “native oysters”, individuals could be harvested within sustainable fishery guidelines. However, once determined as an “oyster bed” extraction could be prohibited, instead protecting the bed as a habitat. This is a highly contentious issue due to the nature of oyster conservation and restoration which often requires the collaborative efforts of oyster growers, fishers and conservation scientists together (Allison 2018). Of particular interest to environmental regulators is whether there is a relationship between native oyster density and associated species diversity.

Current definitions of oyster beds require oysters to be at or higher than 5 oysters m⁻² (Haelters & Kerckhof 2009), however this fails to recognise size variation of oysters, and may bring concerns due to density dependent disease risks from *Bonamia ostreae* (Doonan *et al.*, 1999).

Previous studies on native oyster communities identify potential predators and competitors (Table 3.1), focus on smaller epifaunal species, or were completed decades ago when disease prevalence, habitat

and environmental conditions were different to that of today (Mistakidis 1951; Hancock 1969; Barnes *et al.*, 1973; Smyth & Roberts 2010; Laing 2014). Specifically, recent quantitative studies of the response of biodiversity to variation in the density of European native oysters in the wild are lacking (McLeod *et al.*, 2019). Those experimental studies that have considered the association between native oysters and biodiversity have not done so across a gradient of natural density or body sizes as would be found in their natural habitat (e.g. experiments in high density cages with either large adults or small juveniles, e.g. Zwerschke *et al.*, 2016).

In order to provide the evidence required to assist policy makers in defining native oyster bed density thresholds, to understand estuarine seasonal community dynamics and the association native oysters have with biodiversity and make predictions on how more mobile species may respond to marine habitat restoration, I undertook a seasonally and spatially extensive study on European flat oysters in the BCRC.MCZ. My aim was to examine the relationship between remaining and recovering shallow subtidal estuarine mud, sand and mixed sediment oyster habitats and biodiversity in an area where native oysters can be found over a large spatial scale and, uniquely, over a range of natural densities (average 0-4.2 oysters m⁻² as estimated from a 120m² dredge tow). I formally explore the hypothesis that aggregations of native oysters are associated with estuarine biodiversity above that provided by hard shell habitats alone. The results of this study will help inform national and international efforts towards native oyster restoration, but also the development of policy, specifically oyster density thresholds for management decisions in the management plan associated with the Native Oyster Fishery Flexible Permit Byelaw (KEIFCA, 2018).

3.2. Method

Methods for dredge surveys have been described in Chapter 2 and were based on Wiggins (2014). This was to enable comparisons between subsequent years and to establish baseline oyster distribution and abundances for future management. Following the initial surveys in 2014 and 2015, data was collected by dredge within the BCRC.MCZ biannually between 2016 and 2017 in post-winter (March/April) and post-summer (September/October; Chapter 2, Figure 2.1). Further site information can be found in Chapter 1.2 and Chapter 2. Data from 2016-17 only are included in this study as measurements taken on 2014-2015 dredge surveys differed, with a more detailed species survey completed from 2016 onwards (Chapter 2, Table 2.1). Surveys from 2018 were not included in the current analysis due to the widely reported cyclone (Emma) and combined anticyclonic storm (Hartmut) that resulted in mass mortality of nearshore invertebrates.

All epibenthic macrofaunal species were recorded, however, due to low efficiencies of catch of fish in the dredge these species were removed from the analysis. A full species list can be found in Table 3.2.

Scaling of biodiversity predictors

In all models, total dead shell weight, live *C. fornicata* weight and native oyster counts were used as predictors. Collinearity was assessed using a pairs plot and Variance Inflation Factors (VIF) (Zuur *et al.*, 2013). All VIF statistics were below 1.5 and so all predictors were used in regression analyses. (Figure 3.1). Initial analysis included *C. gigas* however, as the number of dredges containing *C. gigas* were relatively rare in comparison to other predictors (n=126/397) and, following non-significance in preliminary analysis, this species was removed and included in the species number defined below. As it was not possible to quantify the patchy and variable densities of native of oysters over small distances, predictors were all converted to average densities per m² across the 120 m² dredge. It is also recognised that dredges and trawls only sample a fraction of the fauna on the seabed (Eleftheriou & Moore 2013). Following discussions between Natural England and Kent & Essex Inshore Fisheries Conservation Authority (KEIFCA), average native oyster abundance was adjusted to a 20% dredge efficiency, where it is assumed 20% of the native oysters are caught in the first pass. This 20% efficiency was also used to calculate scaled-up estimates of live *C. fornicata* (kg m⁻²) with stacks being a similar weight and size

to *O. edulis*. Dredge efficiency has previously been calculated to be approximately twice as efficient for capturing live eastern oysters as it is for cultch (Taylor *et al.*, 2014), therefore a dredge efficiency of 10% was used for dead shell average density (kg m^{-2}). All future abundances for predictors are stated as scaled up average densities incorporating these dredge

efficiencies. Uncentred counts of native oysters were used in all analysis, however, analysis was repeated using all predictors standardised to kg m^{-2} and for centred predictors with comparative analysis found in Appendix II: Supplementary Information 3.1. This did not alter our conclusions.

Rare species such as worms and shrimp were grouped in terms of presence/absence and termed “other species” and species such as *Sabellaria* spp. and barnacles were only able to be measured in terms of presence/absence too due to inaccuracies in being able to count individuals for these species over large dredge scales (Table 3.2). Species number was determined by total number of observed species within a dredge (presence/absence).

Table 3.1. A list of known predators and competitors of *Ostrea edulis* in the UK.

*indicates low level competitor and only competes when in particularly high densities.

Oyster "pest"	Predator/competitor	native/non-	
		native	reference
<i>Carcinus maenas</i>	Predator	Native	Hancock, 1969
<i>Urosalpinx cinerea</i>	Predator	non-native	Hancock, 1969
<i>Ocenebra erinaceus</i>	Predator	Native	Hancock, 1969
<i>Asterias rubens</i>	Predator	Native	Hancock, 1969
<i>Marthasterias glacialis</i>	Predator	Native	Hancock, 1969
<i>Psammechinus miliaris</i>	Predator	Native	Hancock, 1969
<i>Crepidula fornicata</i>	Competitor/Predator of larvae	non-native	Hancock, 1969
<i>Crassostrea gigas</i>	Competitor/Predator of larvae	non-native	Tully & Clarke, 2012
<i>Elminius modestus</i> *	Competitor	non-native	Hancock, 1969
<i>Pomatoceros</i> *	Competitor	Native	Hancock, 1969
<i>Dendrodoa</i> *	Competitor	Native	Hancock, 1969
<i>Ciona and Ascidiella</i> *	Competitor	Native	Hancock, 1969
<i>Sabella</i> *	Competitor	Native	Hancock, 1969

Table 3.2. Full species list from 2016 and 2017 dredge surveys.

Species	Common name	Measure ment	Reliably quantified within dredge	Reliably caught within dredge	Prevalent (i.e. total count >10/dredge)
<i>Ostrea edulis</i>	Native oyster	count & weight	Yes	Yes	Yes
<i>Magallena gigas</i>	Rock oyster	count & weight	Yes	Yes	Yes
<i>Asterias rubens</i>	common starfish	count	Yes	Yes	Yes
<i>Crossaster papposus</i>	sunstar	count	Yes	Yes	Yes
<i>Crepidula fornicata</i>	slipper limpet	weight	Yes	Yes	Yes
<i>Ophuroidea spp.</i>	brittlestar	count	Yes	Yes	Yes
<i>Oceanebra erinacea</i>	European sting winkle/oyster drill	count	Yes	Yes	Yes
<i>Carcinus maenas</i>	European green shore crab	count	Yes	Yes	Yes
<i>Cancer pagarus</i>	Brown/edible crab	count	Yes	Yes	Yes
<i>Majoidea spp.</i>	spidercrab species	count	Yes	Yes	Yes
<i>Paguroidea spp.</i>	hermit crab species	count	Yes	Yes	Yes
<i>Actiniaria spp.</i>	Anemone species	count	Yes	Yes	Yes
<i>Necora puber</i>	Velvet swimming crab	count	Yes	Yes	No
<i>Aphrodita aculeata</i>	sea mouse	count	Yes	Yes	Yes
<i>Liocarcinus holsatus</i>	flying crab	count	Yes	Yes	Yes
<i>Thyone fusus</i>	[pink sea cucumber]	count	Yes	Yes	Yes
<i>Psammechinus miliaris</i>	Green sea urchin	count	Yes	Yes	Yes
<i>Mytillus edulis</i>	blue mussel	count	Yes	Yes	No
<i>Cerastoderma edule</i>	common cockle	count	Yes	Yes	No
<i>Pectinidae</i>	scallop species	count	Yes	Yes	No
<i>Soleidae</i>	sole species	count	Yes	No	No
<i>Callionymidae</i>	dragonet species	count	Yes	No	No
<i>Ensis arcuatus</i>	razorclam	count	Yes	Yes	No
<i>Raja clavata</i>	Thornback ray	count	Yes	No	No
<i>Pleuronectes platessa</i>	European plaice	count	Yes	No	No
<i>Mercenaria mercenaria</i>	hard shell clam	count	Yes	Yes	No
<i>Polyplacophora</i>	chiton species.	count	No	No	No
<i>Dendrodoa grossularia</i>	baked bean ascidian	presence	No	No	Yes
<i>Urochordata spp.</i>	tunicate species	presence	No	No	No
<i>Hadromerida spp</i>	Orange encrusting sponge	presence	No	No	No

<i>Calliostoma</i>						
<i>zizyphinum</i>	painted topshell	count	Yes	Yes	No	
<i>Cirripedia</i>	barnacle sp.	presence	No	No	Yes	
	encrusting worm					
<i>Serpulidae spp.</i>	species	presence	No	No	Yes	
<i>Nudibranchia</i>						
<i>spp.</i>	Nuudibranch species	count	No	No	No	
<i>Tritia reticulata</i>	netted dogwhelk	count	Yes	Maybe	No	
Decapoda						
(shrimp) species		count	No	No	No	
<i>Medusozoa</i>	jellyfish species	count	Yes	No	No	
	Porcelain crab					
<i>Pisidia spp.</i>	species	count	No	No	No	
Goby species		count	Yes	No	No	
<i>Buccinum</i>						
<i>undatum</i>	Common whelk	count	Yes	Yes	Yes	
			No - Brittle and easily break	No - Brittle and easily break		
<i>Echinocardium</i>						
<i>cordatum</i>	sea potato	count			No	
<i>Merlangius</i>						
<i>merlangus</i>	whiting	count	Yes	No	No	
<i>Sabellaria</i>		presence	No	No	Yes	
<i>Limanda</i>						
<i>limanda</i>	dab	count	Yes	No	No	
	polychaete worm					
Nereidae	species	count	No	No	No	
<i>Corystes</i>						
<i>cassivelaunus</i>	masked crab	count	Yes	Yes	No	
<i>Syngnathidae</i>	pipefish	count	Yes	Yes	No	

Model selection

To visualise the effects of oyster density on biodiversity, number of species observed vs average *O. edulis* density (m^{-2}) were plotted with a loess smoother with 95% confidence interval to aid interpretation. This was repeated for the other variables deemed to be sources of hard substrate- total shell budget (kg m^{-2}) and total weight of live *Crepidula fornicata* (kg m^{-2}).

Variation in both i) number of species and ii) species presence/absence composition were tested using multivariate generalized linear models in R (R Core Team 2017) to give a community level metric not constrained by the distribution of the data (ManyGLM, i) negative binomial response and ii) binomial response respectively, Wang *et al.*, 2012). Plotted Dunn-Smyth residuals were used to check model distribution fit. All models used 999 bootstrap permutations. Due to logistical problems in post-winter 2016 surveys, some sites were not able to be sampled every time, subsequently “case” resampling was used to incorporate the repeated measures design of multiple surveys.

All predictors were added to the model incorporating a fully factorial design to identify potential interaction effects which may be occurring between different sources of hard substrate. Stepwise AIC selection was used to select the best model. Coefficients were extracted from best models to determine the impact of predictors on individual species (see Appendix II: Supplementary Information part 3.6 for full R code).

A single multispecies Species Distribution Model (SDM) was fitted for all species counts at all sites using a LASSO-GLM in the mvabund package using the fourth corner traitglm function. This was used to estimate how each species responds to environmental variables (weight of shell, live *C. fornicata* and average density of live *O. edulis*) in the two different seasons (Brown *et al.*, 2014).

3.3. Results

A total of 396 dredges were completed between February 2016 and October 2017 identifying a total of 47 species, of which 8 were fish species and subsequently removed (Table 3.2). Individual oyster weights ranged from 0.0015 to 0.4 kg with an average of 0.06kg each.

Does oyster density influence number of observed species?

Increases in average abundance of each predictor was associated with an initial increase in the number of species observed, from an average of 3 species in the absence of each environmental variable, to reaching an asymptote at 5-7 species (Figure 3.2).

Increasing total shell weight, live *C. fornicata* weight (kg m⁻²) and *O. edulis* density (number of oysters m⁻²) all had significant effects on increasing the number of species recorded in the dredge with the most deviance explained by total shell weight (LR=75.87, $P<0.001$, LR=20.44, $P<0.001$ and LR=12.03, $P<0.001$ respectively). Significant interactions between average total shell and *C. fornicata* weight (LR=10.91, $P=0.003$), total shell and *O. edulis* average density (LR=15.24, $P<0.001$) and *C. fornicata* weight and *O. edulis* density (LR=5.14, $P<0.001$) were also observed. Coefficients extracted from this model were plotted to identify the impact of 1 kg of total shell, live *C. fornicata* or one *O. edulis* m⁻² on species diversity. Increases in associated species number observed were driven by main predictors in isolation while interactions between predictors resulted in negative impacts on the number of associated

species (Figure 3.3; Appendix II: Supplementary Information part 3.1 and 3.2 for analysis using weights of oysters as predictors as opposed to counts and also, a comparative analysis using mixed models. These alternative models made no qualitative difference to our conclusions).

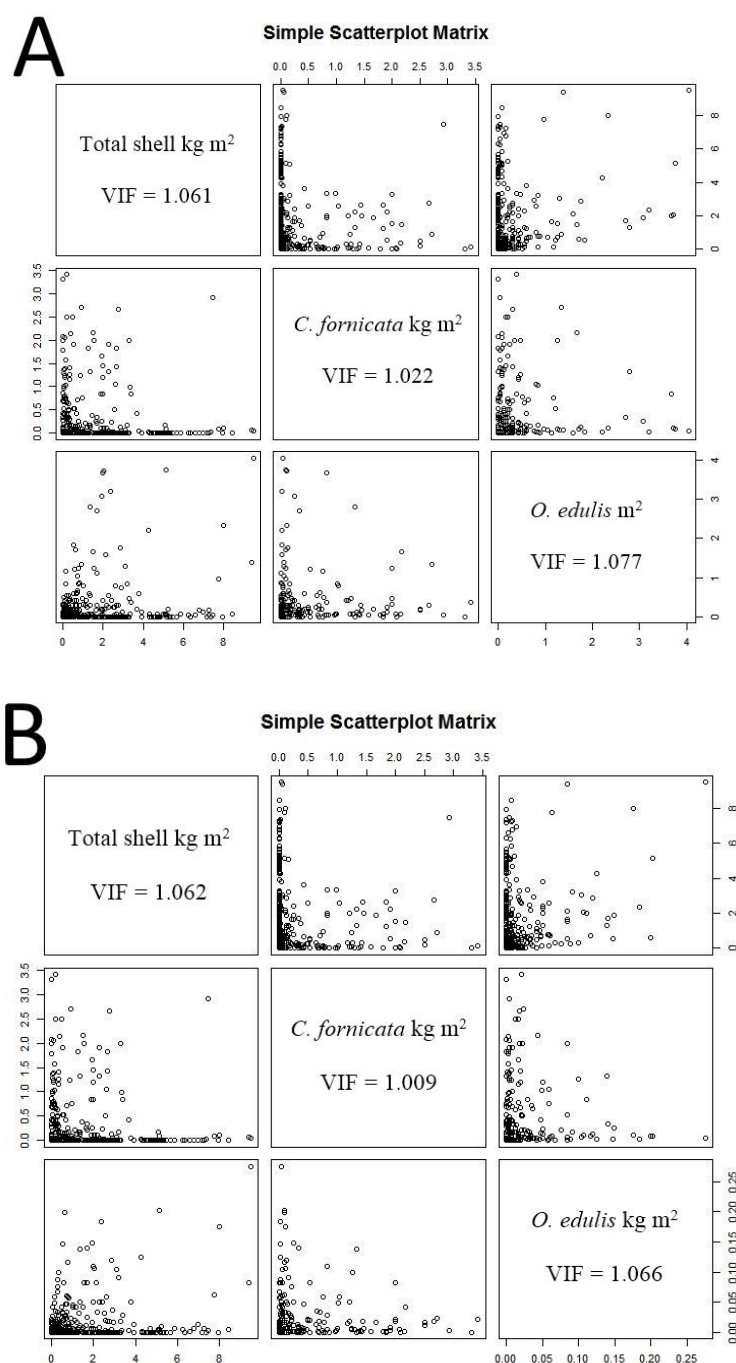


Figure 3.1. Pairs plots with Variance Inflation Factors (VIF) for predictors used in regression analyses.

Plot A shows correlations and VIF when counts of *Ostrea edulis* are used as predictors and plot B when

O. edulis weight is used as a predictor.

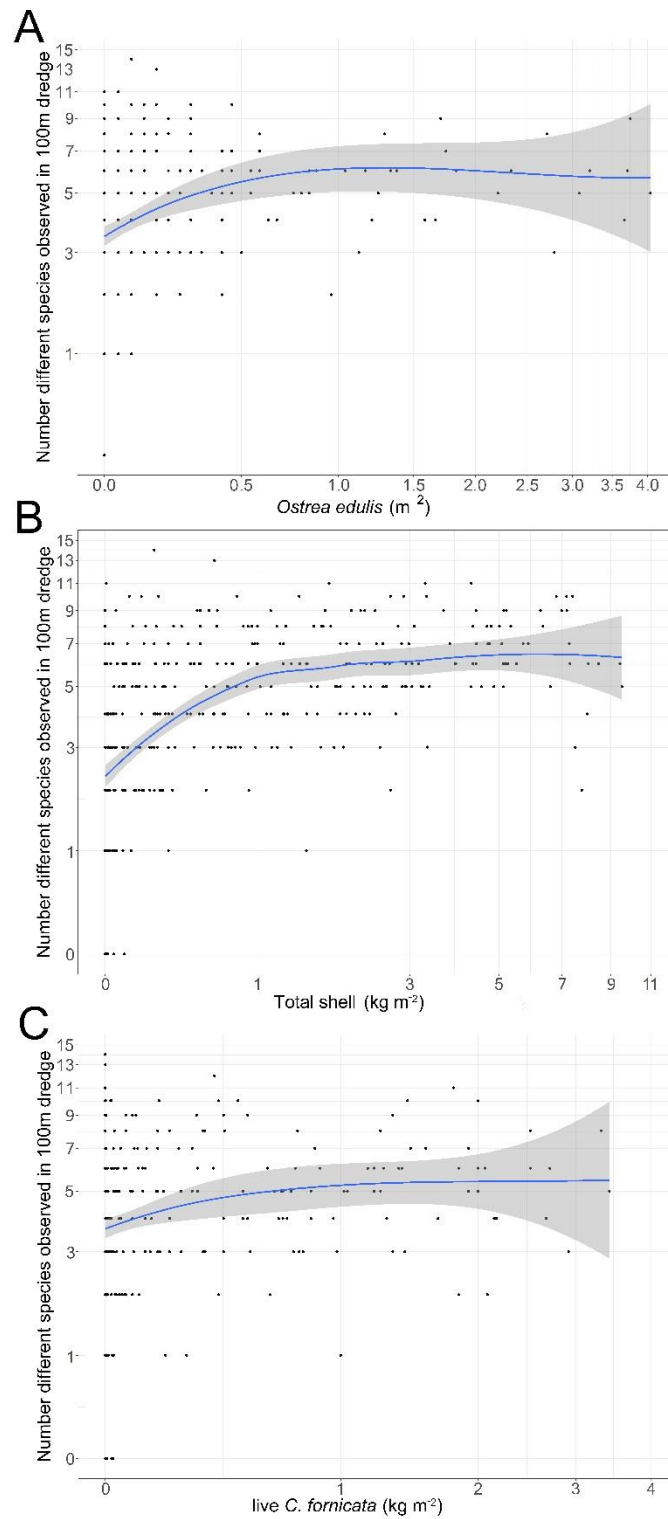


Figure 3.2. A: Average number of native oysters (m^{-2}) (adjusted to 20% dredge efficiency) vs number of different species within the dredge. B: Total shell (kg m^{-2}) (adjusted to 10% dredge efficiency) vs number of different species within the dredge. C: Weight of live *C. fornicata* (kg m^{-2}) (adjusted to 20% dredge efficiency) vs number of different species within the dredge.

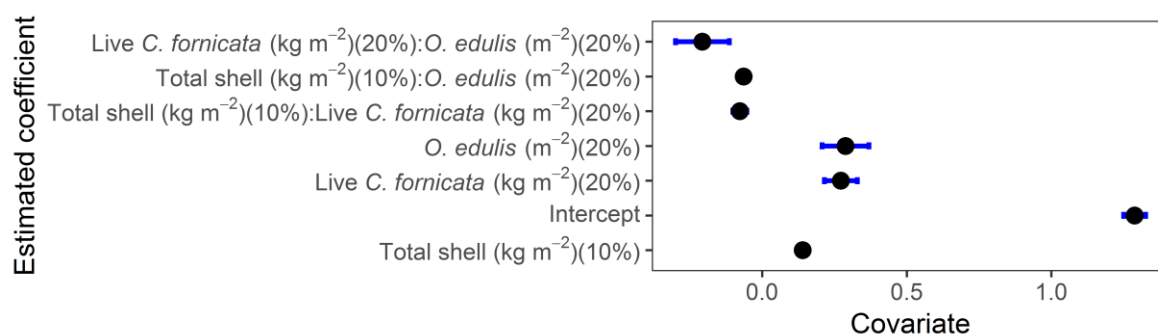


Figure 3.3. Coefficients with standard errors extracted from negative binomial manyglm identifying effect of total shell weight (kg m⁻²), live *C. fornicata* weight (kg m⁻²), *O. edulis* density (m⁻²) and interactions between total shell: live *C. fornicata* weight, total shell:*O. edulis* density and also *C. fornicata* weight:*O. edulis* density with intercept value on the total number of species observed in a dredge. Respective dredge efficiency percentages used are shown in brackets with densities calculated over an average of 120m².

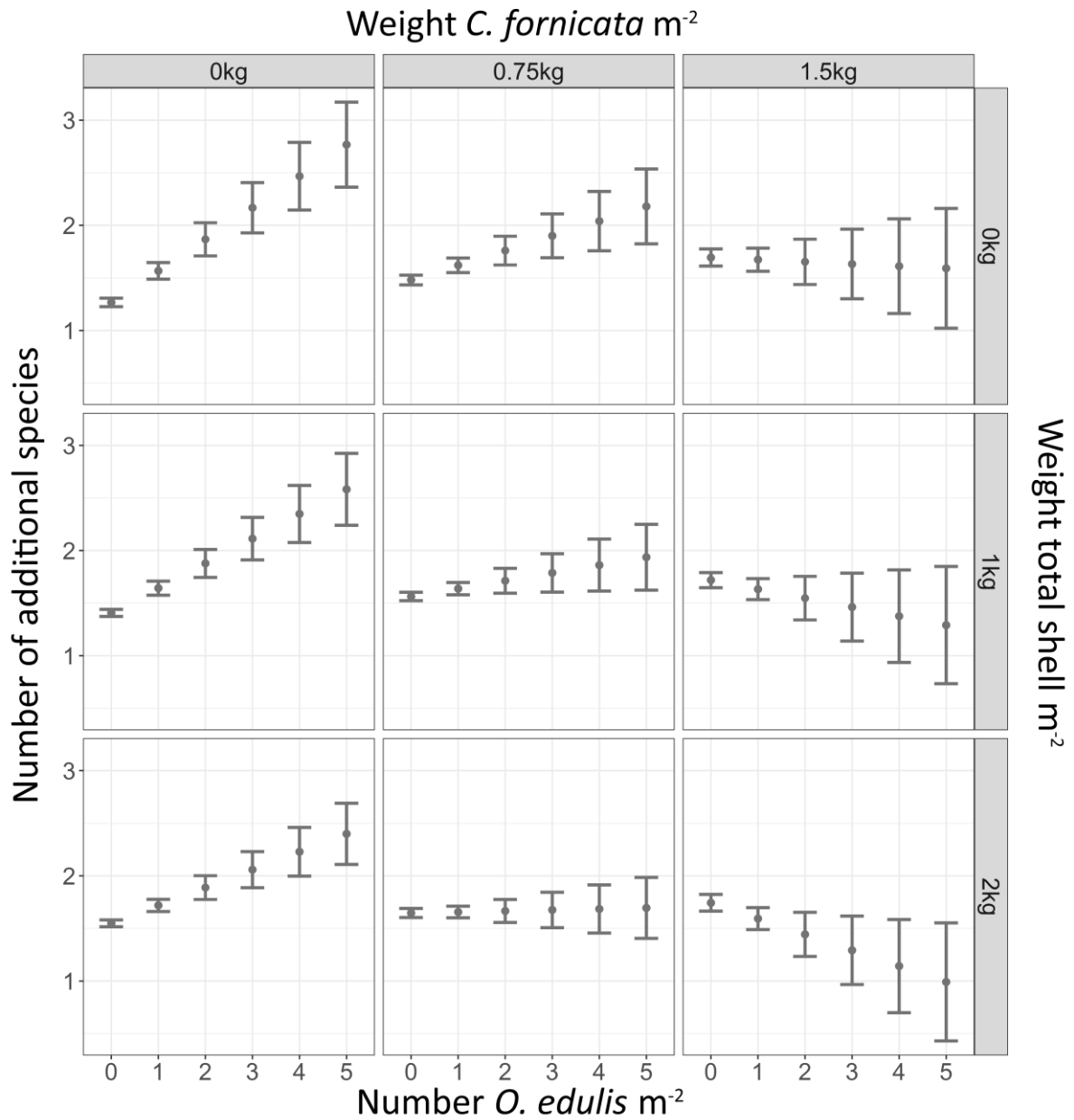
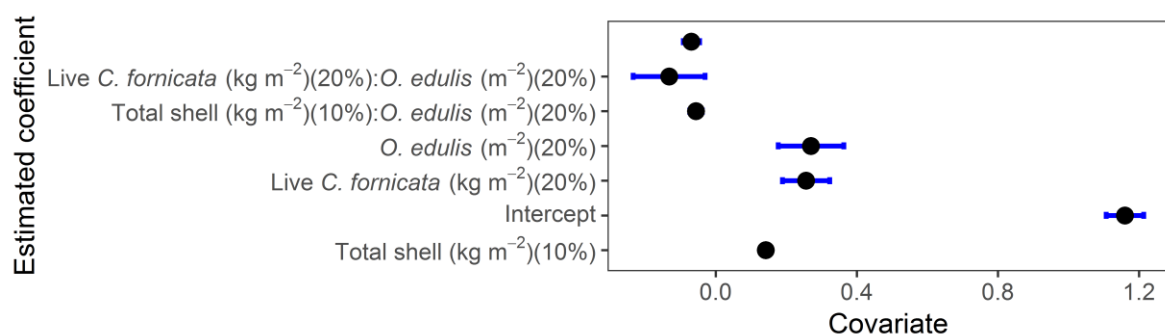


Figure 3.4. Predictions in changes in number of species per m^2 with standard errors extracted from the negative binomial ManyGLM model. Predictors are number of native oyster m^{-2} , weight of *Crepidula fornicata* kg m^{-2} and weight of total shell kg m^{-2} . Columns of plots are split by incremental weight of *C. fornicata* (0-1.5 kg m^{-2}) with rows split by incremental total shell weight (0-2 kg m^{-2}).

To investigate the interactions better, incremental increases in *O. edulis* density, *C. fornicata* weight and shell weight were used to make model predictions of changes in number of associated species m^{-2} (Figure 3.4). The predictions allow us to tease apart the relative effects of the constituent habitat on associated species richness, for example, the number of associated species observed with increasing average shell or *C. fornicata* abundance in the absence of native oyster. A key result is increased associated species observed with increasing average *O. edulis* abundance in the absence of *C. fornicata* (e.g. +1.6 additional species at 1 oyster m^{-2} or + 2.8 species at 5 oysters m^{-2}). However, in the presence of *C. fornicata*, the positive effects of oysters on species richness is eroded such that associated species diversity is maximised at approximately 1 native oyster m^2 (Figure 3.4).

Analysis was repeated using the same model for each post-summer and post-winter survey separately to identify if one season was driving these results. In post-winter surveys, significant effects of total shell weight, *C. fornicata* weight and *O. edulis* weight were observed (LR=60.707, $P<0.001$, LR=12.701, $P<0.001$ and LR=7.628, $P=0.002$ respectively) with significant interactions observed between total shell and *O. edulis* weight (LR=10.487, $P=0.011$) between live *C. fornicata* weight and *O. edulis* weight (LR=1.740, $P=0.009$) and between total shell and live *C. fornicata* weight (LR=1.740, $P=0.009$). In post-summer surveys, significant effects of total shell weight, *C. fornicata* weight and *O. edulis* weight were again observed (LR=30.953, $P<0.001$, LR=6.859, $P=0.004$ and LR=7.067, $P=0.004$ respectively) with significant interactions observed between total shell and *O. edulis* weight (LR=6.840, $P=0.005$) and between live *C. fornicata* weight and *O. edulis* weight (LR=4.484, $P=0.002$) however here significant interactions were not observed between total shell and live *C. fornicata* weight and so this interaction was removed for post-summer analysis. Coefficients for each model were extracted to show the effect sizes of changes in shellfish and shell abundance on the number of observed species between seasons (Figure 3.5). However, changes in the number of species observed indicate increased overall diversity in post-summer surveys (LR=5.303, $P=0.021$). A simplified analysis that discretises oyster density into bins and treats it as a factor, to help clarify where the greatest changes in diversity occur are found in Appendix II: Supplementary Information part 3.4, this did not qualitatively affect the results.

Post-winter



Post-summer

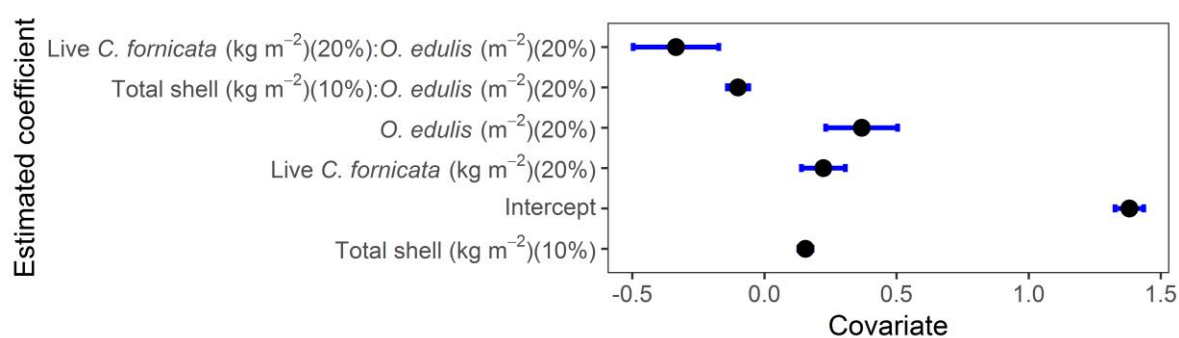


Figure 3.5. Coefficients with standard errors extracted from negative binomial manyglms separated by season identifying effect of total shell weight (kg m⁻² adjusted to 10% dredge efficiency), live *C. fornicata* weight (kg m⁻² adjusted to 20% dredge efficiency), *O. edulis* density (m⁻² adjusted to 20% dredge efficiency) and interactions between total shell: live *C. fornicata* weight, total shell:*O. edulis* density and also *C. fornicata* weight:*O. edulis* weight density with intercept value on the total number of species observed in a dredge.

Does oyster density influence species composition?

Using a full matrix of all species presence/absence highlighted significant effects of average total shell, live *C. fornicata* weight and average *O. edulis* density, on the compositional presence/absence of all individual species (Total shell (kg m^{-2}) LR=196.0 $P=0.045$, Live *C. fornicata* (kg m^{-2}) LR=106.5, $P=0.039$ and *O. edulis* density (m^{-2}) LR=105.7, $P=0.021$) showing each type of hard substrate is significant in determining community structure. There were no significant interactions and so these were removed from the model. Unstandardized coefficients for the model for individual species presence/absence response are plotted in Figure S5 highlighting increases in *Actinatia spp.*, *C. gigas*, *Aequipecten opercularis*, *Cancer pagarus*, *Necora puber* and *Veneroidea* species with increasing native oyster abundance.

Regression coefficients of the interaction variables between predictors and species (abundance) response using a single multispecies SDM are found in Figure 3.6. A strong negative association across all sites was found between the presence of live *C. fornicata* and *C. gigas*, conversely, strong positive associations between *O. edulis* and *C. gigas* were indicated. Figure 3.6 also illustrates changes in these trends between seasons. When analysis is split by season, to show differences in specific species associations with environmental factors, there is a clear movement of *A. rubens* away from areas of native oyster during post-winter surveys. In post-summer surveys, *Psammechinus milliaris* show decreased association with increased density of *O. edulis*. In both seasons *Carcinus maenas* show a positive association with *O. edulis*.

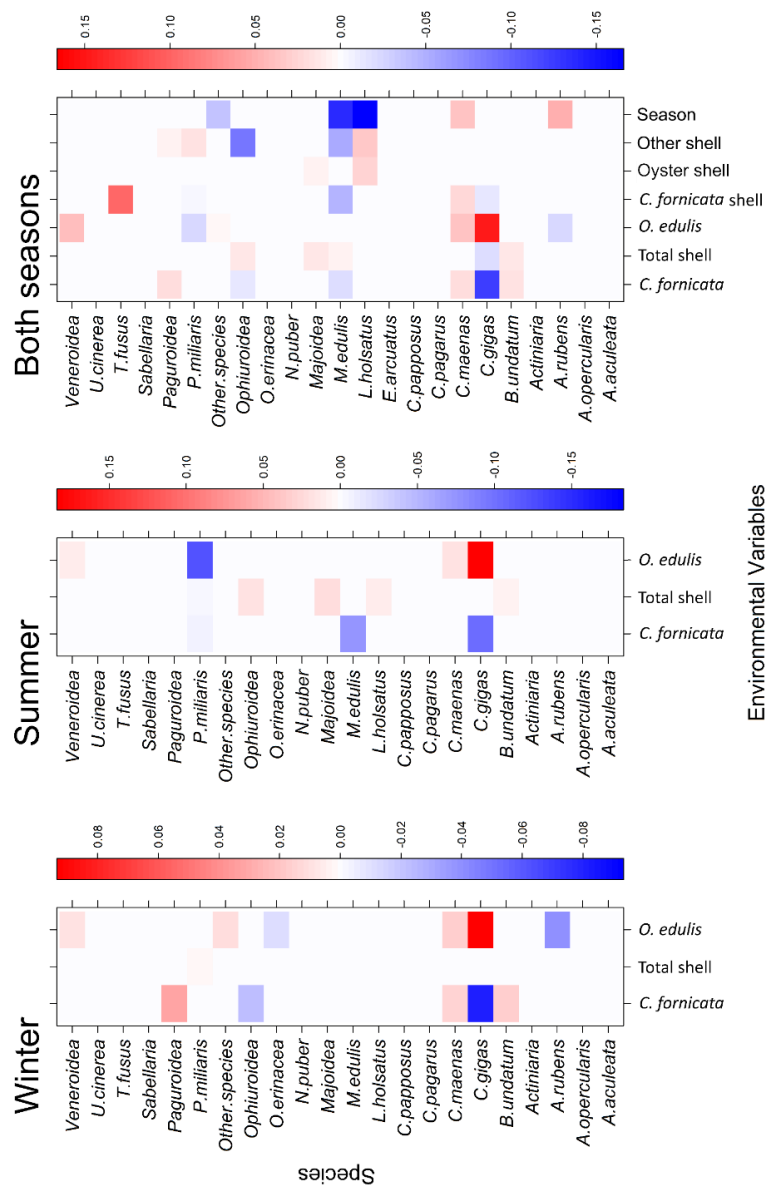


Figure 3.6. Differences in standardised regression coefficients of the interaction variables between environmental variables and count species response between seasons and overall (both seasons) using fourth corner Species Distribution Models individually modelled for *C. fornicata* (live kg m⁻² at 20% dredge efficiency), Total shell (All types kg m⁻² at 10% dredge efficiency), *O. edulis* (live m⁻² at 20% dredge efficiency), *C. fornicata* shell (kg m⁻² at 10% dredge efficiency), Oyster shell (mixed species of oyster kg m⁻² at 10% dredge efficiency), other mixed shell not oyster or *C. fornicata* (kg m⁻² at 10% dredge efficiency) and season (red indicates increased abundance in post-winter in plot representing both seasons). Due to smaller sample sizes associated with splitting analysis between seasons separate shell types were not included within seasonal analysis due to non-convergence of the model.

3.4. Discussion

Previous literature has highlighted the importance or potential of *Ostrea edulis* as a species which could boost biodiversity through acting as a preferable substrate to epifaunal species and engineering habitat for a range of species (Korringa 1951; Mistakidis 1951; Haelters & Kerckhof 2009; Smyth & Roberts, 2010). This study has shown that whilst *O. edulis* are associated with increases in species richness up to an observed average density of approximately 1 native oyster m^{-2} (24 oysters per experimental dredge, Figure 3.2), no further increases are observed across the range of habitats sampled in the current conditions of the BCRC.MCZ (with average densities up to 4.2 oysters m^{-2} in a 120 m^2 dredge). With greatest increases in species diversity *in-situ* found between oyster-free areas and areas of low average density (0.5-1 oyster m^{-2}), this indicates moderate increases (+approximately 2.5 species per dredge) in associated species richness are found at average animal densities far below those required to classify an oyster bed by the OSPAR definition of 5 oysters m^{-2} (Haelters and Kerckhof, 2009). Whilst our model only predicts an additional 1.5 species at this density (1 oyster m^{-2} or an additional 2.8 species at 5 oysters m^{-2}), this is due to limitations in predictions further than the maximum observed species richness in this study. In addition to us including more taxonomic groups to improve on future predictions, e.g. algae or fishes, it is possible a low regional species pool or dredge selectivity affects the maximum associated species richness in our study.

Potential for density dependent associations with species richness

Whilst it is notable no further increases in associated species was observed above 1 oyster m^{-2} / dredge, this may be due to a variety of reasons: firstly, increases in associated species with increasing average native oyster density are not being observed due to the negative effects caused by interactions between *O. edulis* and *C. fornicata*. Predicted estimates of the number of additional species under varying scenarios show a linear increase with increasing native oyster density, however, these increases are suppressed and even reversed when *C. fornicata* are also at high density. Management of *C. fornicata* abundance in areas designated for the protection of *O. edulis* may be necessary to promote increased biodiversity associated with native oysters. Previous studies have found the colonisation of *C. fornicata* results in a homogeneous permanent seafloor which alters the community

type (Blanchard, 2009). In addition, there are several studies that conclude high-density oyster and mussel beds can result in decreased biodiversity, with lower biodiversity observed in all but very low density mussel beds compared to control areas in the Menai Strait (Beadman *et al.*, 2004). Decreased species richness was also observed in areas of particularly high *C. gigas* density (100% cover) when compared to low or medium density (5 or 50% cover; (Green and Crowe, 2014). In this study *C. gigas* were relatively rare in comparison to other sources of hard substrate and were not found to influence species richness within our models however, surveys were focused on subtidal areas where *C. gigas* are less abundant. Reductions in diversity in the aforementioned study were attributed to competition for space, the physical covering of the sea floor with hard shell resulting in a reduction of predator species which feed on soft bodied worms, and also due to the potential variation in hydrodynamics caused by the presence of oyster shell substrate. Conversely, Blanchard (2009) found increases in carnivorous microfaunal populations with high *C. fornicata* density due to the use of empty shells as refuge. This may be occurring in the Essex estuaries as there are areas of high *C. fornicata* abundance, however percentage cover and the presence of microfauna were not evaluated in this present study (Moulin *et al.*, 2007; Green and Crowe, 2014). In a soft mud dominant estuary system there may, however, still be biodiversity benefits, however small, of the hard surface mosaic that slipper limpets can create (de Montaudouin & Sauriau 1999). This was most notable in the absence of *O. edulis* when seen in our plots of model predicted species richness (Fig. 4). As with many benthic species, slipper limpets are able to re-emerge after being buried by light sedimentation (Beadman *et al.*, 2004). With fairly high levels of suspended sediment in the BCRC.MCZ, with concentrations of over 50mg l⁻¹ suspended sediment observed around the East Anglian coast (Moffat, 1995), it is likely live *C. fornicata* are able to provide a constant influx of hard substrate thus providing clean shell for settlement of new species year-round, including flat oysters. The site or habitat specific context of where *C. fornicata* is a needed pioneer for biodiversity, including for the recovery of native oyster, or where they prevent maximum biodiversity gains from oyster restoration requires further research. Such management is possible as Essex native oyster producers regularly state that slipper limpets can be useful to establish suitable oyster settlement habitat, if that habitat is *managed* and prevented from becoming homogeneous.

Secondly, areas of higher oyster density are relatively rare in this study system, and while they are common in comparison to other sites in the UK (Clark, 2017), this may be limiting patterns of higher associated species emerging in our study. To validate the conclusions taken from our regression analyses at those higher oyster densities, I repeated the dredge survey methodology in managed private oyster cultivation areas outside the BCRC.MCZ. However, in these areas I obtained similar oyster density ranges and associated species numbers (3-12 species with a range of 0.54-4.6 oysters m^{-2} within the private areas and 0-15 species with a range of 0-4.1 oysters m^{-2} within the BCRC.MCZ). This suggests that high biodiversity~high oyster density relationships can exist, but something is limiting their occurrence.

Finally, *O. edulis* may only be found in habitats already supporting increased biodiversity (e.g. due to high food availability or some unmeasured variable) and is not directly impacting diversity of species. While increases in associated species diversity were observed with the presence of live *C. fornicata*, one native oyster was found to be associated with a similar level of species richness to that of 1 kg *C. fornicata*. I have not directly measured total hard substrate surface availability in this study, however, one native oyster has a vastly smaller volume and mass than that of 1 kg *C. fornicata*. Therefore, the observed increases in species richness associated with oyster presence is not due to increased hard substrate availability alone and subsequently due to some other benefit from the presence of live *O. edulis* or due to *O. edulis* being found in habitats also suitable for a range of other species. Previous studies have recognised that live native oysters provide improved biogenic engineering qualities than that of non-living hard substrate (Smyth and Roberts, 2010). Despite not quantifying all epibiotic species in this study, where associated species are not direct predators of *O. edulis*, they may be benefiting from the association of their prey with *O. edulis* i.e. the reef/bed effect (Langhamer, 2012).

While these three ideas for limited biodiversity effects remain hypotheses that need to be tested, our statistical model clearly predicts species richness gains of restored oysters when *C. fornicata* are at low abundance. Therefore, reductions in *C. fornicata* may be an appropriate management tool to explore in order to maximise biodiversity associations from flat oyster restoration. I can recommend a restoration and fishery management objective should be to restore and maintain native flat oysters of at least an average density of 1 m^{-2} across a 120 m^2 dredge. This is well

below the oyster bed definition of 5 oysters m⁻², however should improve associated species diversity while taking into account the cautions raised above. If site specific, funding or broodstock availability were to be an issue – based on our results it may be more strategic for restoration projects to maximise the areas at low to moderate oyster density instead of smaller areas with higher density – on order to maximise recovery of marine biodiversity.

Interspecific competition between shellfish

Both *C. fornicata* and *C. gigas* are generally found to inhabit the same habitats as *O. edulis*, however *C. gigas* is generally intertidal to shallow subtidal whereas *C. fornicata* is found from the sublittoral fringe and deeper (Blanchard, 1997). There is a significant dietary overlap between the two species (Decottignies *et al.*, 2007) which may result in competitive exclusion of *C. gigas* by *C. fornicata* at increased depth. In addition to physical grazing when in the mobile phase, *C. fornicata* have also been found to suspension filter feed a wider range of plankton sizes at higher rates than *C. gigas*, which in turn have been found to consume a wider range of particle sizes than that of *O. edulis* (Blanchard *et al.*, 2008; Nielsen, Winding and Bent, 2017). This ultimately means *C. fornicata* may be able to out-compete both *O. edulis* and *C. gigas* in food-limited areas. Therefore, there is potential for native oysters to experience competition with invasive species from above and below the sublittoral fringe. These potential mechanisms lead to the often proposed statement that *C. fornicata*, a non-native species, can have negative effects on ecosystems – including oysters (Hancock, 1960). I would note that in a soft mud dominant estuary system there may still be biodiversity benefits, however small, of the hard surface mosaic that slipper limpets create. As with many benthic species, slipper limpets are able to re-emerge after being buried by light sedimentation (Beadman *et al.*, 2004). With fairly high levels of sediment within the water in the BCRC.MCZ it is likely live *C. fornicata* are able to provide a constant influx of hard substrate which is able to retain itself above a (low level) constant sediment load, thus providing clean shell for settlement of new species year-round, including oysters. This resurfacing from sedimentation is not possible with dead shell. The site or habitat specific context of where *C. fornicata* is a needed pioneer for biodiversity, including for the native oyster, therefore requires further research. Essex native oyster producers regularly state that slipper limpets can be useful to establish suitable

oyster settlement habitat, if that habitat is *managed*. Distribution, abundance and potential propagation of *C. fornicata* should be closely monitored to identify if out-competition is occurring with *O. edulis* over time.

Density thresholds for native oyster beds

Previous studies highlight epifaunal variation due to *O. edulis* size, age and density, and this subsequently appears to drive a reef effect and promote further biodiversity (Smyth and Roberts, 2010). Population structure will therefore impact oyster bed community diversity and further impact on how an oyster bed should be defined. Larger, older oysters may have a greater impact on species diversity allowing for a more developed community, however a population lacking smaller, younger generations is unlikely to sustain itself due to a lack of new recruits (Munkittrick and Dixon, 1989). Population structure has been used for many years as an indicator of health of commercial finfish stocks (Pope, Lochmann and Young, 2010). With the definition of an oyster bed set to 5 oysters m⁻² this is unlikely to be a suitable measure for determining oyster bed health and resilience as it does not incorporate population structure. While size of oysters has not been analysed in terms of varying impact on species diversity within this study, it is clear that 1 kg *O. edulis* provides a greater increase in species diversity than when oysters are quantified by count alone (Appendix II: Supplementary Information parts 3.1-3.3). A more general measure of biomass m⁻² and the inclusion of a population size structure may be beneficial in further defining an “oyster bed” as a habitat. It is important to consider the size and other ontogenetic factors that affect oysters when planning restoration.

Marine sampling challenges and observing real density dependent biodiversity relationships

While I observed average native oyster densities in 120m² dredge areas up to 4.2 oysters m⁻², like all dredge sampling it is not possible to measure absolute density in every meter². Likewise, grab sampling in these same areas fails to capture density without very high sampling effort (Eleftheriou & Moore, 2013). However, it is likely that native oysters are not uniformly distributed and have a patchy distribution. Subsequently, there will be native oysters which surpass the OSPAR definition of 5 oysters m⁻² in the BCRC.MCZ.

Despite these challenges, I emphasise a focus on both optimism and caution when presenting the case of *O. edulis* restoration. I suggest optimism in promoting current observed levels of species richness as an achievable goal for restoration, as a doubling of species richness can occur at relatively low oyster seabed densities that are within safe disease-risk limits (up to 1.26 oyster m⁻², Doonan *et al.*, 1999). But I also suggest caution in promising that restored natural densities of European flat oyster will deliver large increases in subtidal benthic diversity. Restoring and protecting higher density aggregations, while good precautionary practice, may lead to increased disease risk (Doonan, Cranfield and Michael, 1999). Whereas protection of high-density oysters as “beds” could create unnecessary conflict due to policy implications of native oysters as a species vs a habitat in regards to fishery management. This conflict can likely be minimised through adaptive and spatial management, and consideration that ecological restoration and fishery “recovery” are two different objectives. This study does not yet take account of other ecosystem services (e.g. denitrification or fish nursery or foraging habitat potential) or minimum densities for successful reproduction, which may also affect the oyster density thresholds that benefits to society accrue.

Does oyster biomass and seasonality influence species composition?

All types of hard substrate were found to significantly affect presence/absence of species composition with SDMs further highlighting *O. edulis* associations with *C. gigas* and *C. maenas*. Species most associated with live *O. edulis* include *C. maenas*, *C. gigas*, and *Veneroidea* species, indicating these species are likely to benefit from expansion of native oyster habitat. Post-winter surveys show a negative association between *O. edulis* and *A. rubens* (n=801 *A. rubens* individuals in 148 dredges), a reported predator of *O. edulis* (Figure 3.6), with a weak negative association observed overall. This indicates *A. rubens* may be preferentially feeding on other species within Essex or potentially suppressing native oyster expansion in other areas due to high predation levels on oyster spat in the late winter following spatfall. *A. rubens* accumulations were most notably high in post-winter surveys at sites further offshore where herring are known to spawn, it is therefore likely that starfish are accumulating here to feed on herring eggs (Dempsey and Bamber, 1983). Previous studies indicate *A. rubens* may be able to consume large amounts of adult oysters in the absence of other food, however,

preferentially select other species such as *C. fornicata*, *M. edulis* or barnacle species (Hancock 1955; Hancock, 1958). *A. rubens* populations have been said to increase intertidally in number on intertidal mussel beds in the Menai Strait between April and July and subsequently decline between September and April (Gallagher *et al.*, 2008). In this study I observed increased populations subtidally during winter surveys.

Other seasonal changes highlight many known reproductive migrations and trends with increased diversity noted in post-summer surveys. Many species reproduce over the summer months, and this has likely been reflected in increased detection of many species (Strathmann 1987). *C. maenas* subtidal abundance increases in winter months when females are known to move into deeper water to protect themselves when berried (Crothers 1968). This species is found to be associated with native oysters throughout the year, however increased association with *C. fornicata*, which is more commonly found in deeper water during winter further supports this movement. A similar migration is observed in *L. holsatus*, moving from estuarine areas during summer months to deeper waters of the North Sea in winter resulting in summer increases in populations in the coastal areas surveyed in this study (Venema & Creutzberg 1973).

Conclusions

This study has quantified the importance of the presence and density of *O. edulis* in the diversity of coastal benthic communities. This has implications for the management of this species, both in terms of restoration and in any future harvesting. Whether the positive association is due to autocorrelation in habitat suitabilities or due to increased epifaunal colonisation of live oysters over other sources of hard substrate remains unclear. No additional biodiversity benefits associated with higher density oyster areas were observed in current conditions of the BCRC.MCZ, however, I have shown this to be driven by high *C. fornicata* abundances in many sites, predicting steeper biodiversity gains from oyster density in low *C. fornicata* density areas. Under current conditions (high *C. fornicata* abundance) of the BCRC.MCZ, increasing the area over which oysters inhabit is therefore likely to be more beneficial to coastal macrofaunal biodiversity than attempting to boost existing densities specifically to meet the oyster bed definition of 5 oysters m⁻². In addition, exploring the outcomes of oyster restoration in areas

varying in *C. fornicata* density will help to inform management and practice – and determine whether density reductions of *C. fornicata* prior to restoration efforts is worthwhile.

Chapter 4:

A novel method for tracking growth rate and survival of individual native oysters at low density

4.1. Introduction aims and hypotheses

When undertaking restoration activities of any species, understanding expected survival and growth rates prior to restoration allows for a more accurate estimation of success and enables informed decisions for the direction of best practice (Wortley *et al.*, 2013). In addition, appropriately monitoring the growth and survival of a restoration species throughout a restoration project increases efficacy of restoration and will enable potential intervention work to occur if any mortality is observed.

With many factors known to influence oyster growth rates (See Chapter 1 section 1.4 for more information), measuring all factors likely to influence the success of oyster restoration projects may not be feasible, particularly if the area designated for restoration is geographically large (such as the BCRC.MCZ). Instead, assessing spatial differences in growth and survival of target restoration species *in-situ* is a cost-effective way to guide restoration. Success may then be driven by concentrating on areas where survival is low, attempting to improve the lowest quality habitat, or by expanding areas where growth and survival is high. Alternatively, success can be driven by a combination of the two, using sub-site-specific management based on the requirements of individual areas.

In addition to estimating the success of restoration processes, assessing growth and survival rates of target fishery species can help to achieve sustainability of fisheries, particularly in species where assessing age is difficult and growth rates can greatly vary (Moore *et al.*, 2016). Knowing how long a target species takes to reach harvestable size is key for fisheries management with many demographic processes such as reproduction based on both age and size (Laing *et al.*, 2005; Moore *et al.*, 2016). Assessing size at age is therefore essential in ensuring landing sizes are set large enough to allow all individuals to reproduce as female within a population before they are harvested. *Ostrea edulis* are

notoriously difficult to age (Richardson *et al.*, 1993). Traditionally bivalves are aged through the counting of nacreous layer “rings”. However, these are not always clear in the European native oyster, and when they are, oysters commonly lay down multiple rings in a single year, therefore counting rings to assess age-based sizes would not be accurate (Orton & Amirthalingam, 1927; Richardson *et al.*, 1993). A previous study has attempted to assess size at age in native oysters using acetate peels to age individuals at a single point in time, referring age back to size. Multiple independent groups were then used to count the umbonal growth lines of acetate peels taken from cross sections of native oyster, these groups commonly found interpretation of the first umbonal line difficult in younger oysters. In addition, instances were reported where shell abrasion resulted in growth lines being lost meaning lines had to be traced back into the outer prismatic shell layer and were not visible from the outside (Richardson *et al.*, 1993). Studies like this are highly laborious, requiring multiple independent groups to verify numbers of umbonal growth lines and require oysters to be sacrificed, this means survival of individuals cannot be assessed, and also may not be an option within protected areas such as the BCRC.MCZ where extraction and sacrifice of animals may be highly regulated or prohibited.

Previous studies in oyster survival have been largely directed towards *Crassostrea* (sometimes *Magallana*) species or are focused on oysters cultivated in boxes, estimated from photographic surveys of farmed populations, focus on intertidal oysters disregarding any subtidal oysters, or from laboratory-based studies (Orton, 1938; Walne 1958; Goulletquer *et al.*, 1998; Ford *et al.*, 2006). These studies, therefore, do not take into account the natural conditions in which oysters live, or may result in large levels of error. In addition, the entire area of the BCRC.MCZ has been designated as positive for the oyster parasite *Bonamia ostreae*, dating back to 1982 (Dunn *et al.*, 2014). Positive results have been recorded in the Blackwater, Colne and most recently detected on the Ray Sand (Cefas, 2017, ENORI meeting minutes, 2018). Any historical assessments of survival or growth of native oysters such as Orton, 1938 or Walne, 1958 will therefore not include the effect that this protozoan parasite has on growth and survival and will only be suitable for use in historical conditions. No study has to date assessed individual based growth and survival simultaneously in naturally occurring low densities of the European native oyster. Similar studies have assessed *O. edulis*, or other oyster species, growth or

survival using frames or tiles where oysters are maintained above the sea bed or in densities above those measured in *O. edulis* in Essex (Garland & Kimbro, 2015; Zwerschke *et al.*, 2018).

This study aims to quantify growth and survival rates of individual native oysters of varying size and age across different areas of the Essex estuaries (Figure 4.2). Sites have been selected both in the public grounds of the Blackwater, Crouch Roach and Colne Estuaries Marine Conservation Zone (BCRC.MCZ) and in some sheltered areas of private layings where active culture of *Ostrea edulis* and *C. gigas* occurs. Tracking the growth and survival of individual oysters around the Essex estuaries *in-situ* will provide information on where optimal areas may be for oyster restoration. This information can then be linked to variation in temperature, data obtained in the widescale MCZ dredge survey (Chapter 2), and other previous studies on what is known about potential predator effects. This individual based approach has never been undertaken for native oysters and is generally a rare approach for subtidal shellfish. These methods will enable stakeholders involved in restoration of native oysters in this area to make informed decisions about where best to target initial and future restoration efforts, particularly if any relaying of oysters is to occur. The findings of this study are designed to feed into a demographic population model presented in a later chapter (Chapter 5).

Oyster cultivation has a history of moving oysters into shallow, more sheltered areas for “fattening” (Benham *et al.* 1993). Therefore, it is hypothesised that oysters in more sheltered areas will have highest growth rates with offshore oysters expected to encounter higher levels of shell erosion from wave action, a process more common in winter months (Walne 1958). Growth rates are also expected to be lower in areas of high predator abundance due to the increased investment required to produce thicker shell to combat predation attempts (Robinson *et al.* 2014), in addition to this, frequent attacks by predators can break off thin new shell growth resulting in slower growth rates (Hancock 1892) and reduce the amount of gaping time available when oysters filter feed.

4.2. Method 1 - Pilot

This method was devised to track growth and survival of individual known oysters over time in different areas of the Essex estuaries under natural conditions, in order to highlight potential optimum areas and conditions for oyster growth.

Following the initial failure of a similar experiment in Florida due to problems with adhesives, a pilot trial was completed in summer 2016 in order to develop the best experimental set-up (Kimbrow, pers. com. 2016; Garland & Kimbro, 2015). Four types of concrete block (Hollow dense concrete block, Marshalls Richmond paving Natural flag utility, Solid Dense Concrete Block 7.3N 140mm and Solid Medium Density Concrete Block 7.3N 100mm, Travis Perkins, UK) and 4 types of non-toxic epoxy adhesives (PC-11 two-part marine grade epoxy, Aquascape epoxy, Milliput and JB Quickweld 2-part quick setting epoxy), chosen due to their non-toxic nature in water once dry (Table 4.1). This tested the most reliable block-adhesive combination with lowest levels of movement and most reliable adhesive. A total of 8 blocks were tested in the pilot trial (2 of each block type) with 8 oysters of *C. gigas* (for pilot) between 71-116 mm attached to each block (2 oysters per adhesive type per block) resulting in 4 oysters per glue-block combination (n=64). Blocks were marked with a buoy and placed in the Colne river, Essex next to Colne Island in the Pyefleet Channel on the 27th June 2016. Blocks were checked and returned to the same area every 3 weeks at which point the presence of and length of each oyster was recorded. Blocks were retrieved after 9 weeks.

4.3. Pilot results

Pilot results indicate some major flaws with the use of larger, flat paving slabs (Marshalls Richmond paving) as opposed to smaller, heavier blocks with the paving types prone to drifting downstream. Other block types remained stationary (Table 4.1).

JB quickweld adhesive shows poor long-term retention. Milliput resulted in the highest number of retained oysters at the end of the pilot trial (Figure 4.1).

Table 4.1. Success of each types of concrete block in the pilot trial. Numbers in brackets show the number of blocks affected.

Slab type			Move?	Smothered?	Lost?	Other
Hollow	dense	concrete				
block			No	No	No	
Marshalls Richmond paving			Yes			
Natural flag utility			(n=1)	Yes (n=1)	No	
Solid Dense Concrete Block						
7.3N 140mm			No	No	No	
						*due to buoys being too small and
Solid	Medium	Density			Yes	sinking. These were recovered
Concrete Block 7.3N 100m			No	No	(n=2)*	later.

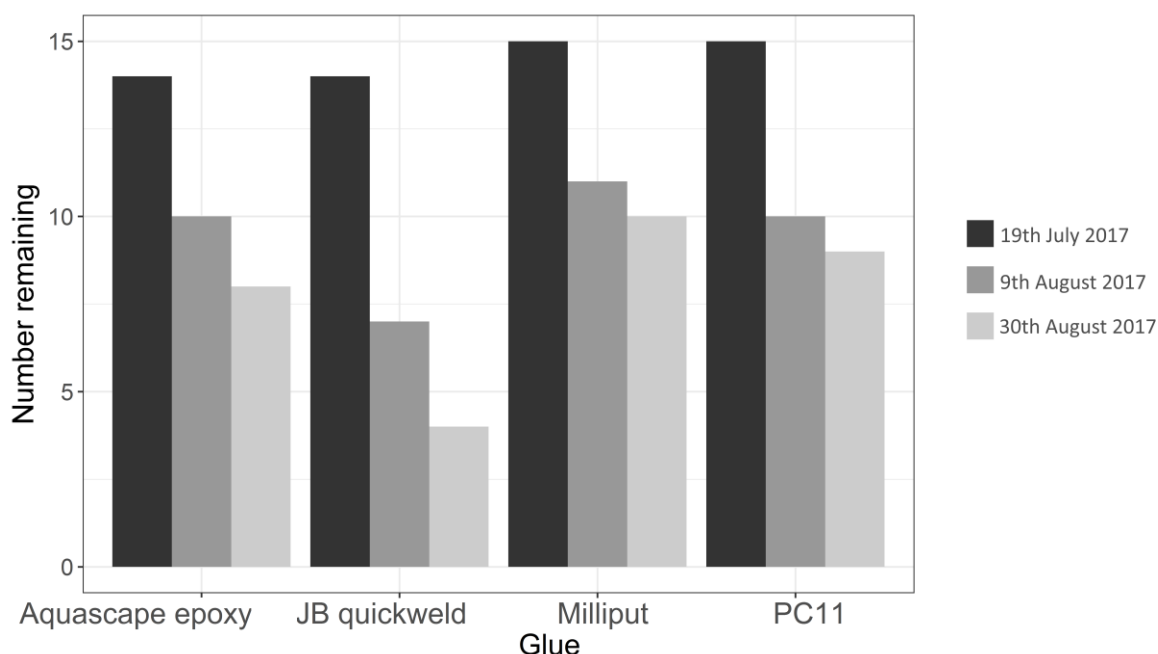


Figure 4.1. Number of oysters attached to concrete slabs after 3-week time intervals across all slabs, attached using different non-toxic epoxy adhesive types. All glue types started off with 16 oysters and have been tested on the same selection of slab types.

4.4. Pilot Discussion

Pilot trials have highlighted a possible experimental set up in terms of most effective adhesive to use, with Milliput providing the most durable attachment method (Figure 4.1). This adhesive was also the most inexpensive and therefore, the preferable choice economically. This pilot has also highlighted issues in terms of concrete block choice. Throughout this trial, it became clear that one slab type (Richmond paving slab) was not suitable, this is due to one sinking easily into the soft mud and the other drifting downstream. It is also recognised that attaching 8 oysters to a small breeze-block will result in oysters being kept at unusually high densities. These high densities are known to result in increased disease transference, particularly increasing the risk of *Bonamia ostreae* (Doonan *et al.*, 1999). In addition to this, by attaching oysters onto the top of concrete blocks this slightly lifts oysters out of any sediment areas and results in unnatural conditions which will therefore affect predicted growth and survival. This was clearly noticed in the increased fouling of barnacles experienced by oysters in this pilot trial in comparison to oysters dredged from the area.

This new experimental design incorporates the best adhesive (Milliput) and concrete block (Solid Dense Concrete Block 7.3N 140mm) as an anchor weight and knowledge about the presence of *B. ostrae* resulting in a design highlighted in Figure 4.2.

4.5. Method 2 – Main experiment

Following a short 2-month pilot trial (previously discussed) in the Colne estuary using *C. gigas* attached to concrete blocks, an updated experimental design was developed to reduce the density at which oysters are laid, and to ensure oysters are laid directly onto the ground sediment. This is in-part due to the prevalence of the parasite *B. ostrae* in the area which is more quickly spread in areas of high oyster density (Doonan *et al.*, 1999). In addition to this, by laying oysters on the sea floor, thus placing oysters in more natural conditions, it may be possible to record mortalities from sedimentation and burying which would otherwise not be possible if the oysters are raised slightly above the seabed on concrete blocks, this resulted in an experimental set up which mirrored natural conditions as closely as possible.

To create this new experimental design, 2.5mm braided polyester rope (Rope a, Figure 4.2) was run between two concrete blocks. Between the concrete blocks paired oysters were attached using Milliput at 1m intervals using 15cm “offbranches” from the original line resulting in pairs for a total of 10m (n=20). A knot was added to the end of the line which sat inside the Milliput to prevent the “offbranch” line from slipping out. The exact distance of line between the two concrete blocks varied slightly and depended on the depth the lines were to be deployed with excess lengths of 2.5mm rope accounted for maintenance (Figure 4.2).

Chapter 1 of this thesis has highlighted the primary areas where *O. edulis* are found within the public grounds of the MCZ with subsequent bi-annual dredge surveys monitoring the overall population and associated species. Seven sites around the Blackwater, Crouch, Roach and Colne Marine Conservation Zone (MCZ) were selected: 4 on “private grounds or “Several Order” areas and 3 within the MCZ (Figure 4.3). MCZ sites were selected based on the areas in the Blackwater, Ray Sand and Crouch measured to have the highest densities in the post-summer 2016 dredge surveys (Chapter 2). Private

ground sites were selected by local fishers to both incorporate areas where oysters are known to be present but experimental set up will not conflict with commercial fishing activities.

For this experiment, it was essential that oysters used were relayed to the proximity in which they were caught in order to assess growth and survival rates of naturally occurring oysters. For sites within the BCRC.MCZ, oysters caught from the closest 10 dredge points during the surveys described in Chapter 1 were used, with a maximum 60 oysters across 3 strings at each site per season. If more than 60 oysters were caught, oysters from closer sites were selected first. For sites within the BCRC.MCZ two sets of strings were laid out: one set in March (post-winter) and one in September (post-summer) 2017. This resulted in a total of 6 strings or 120 oysters originally placed at each MCZ site except for Site 5 (Blackwater MCZ) where a total of 48 oysters were found in post-winter 2017 therefore only 16 oysters per string were used at this site, however 59 were used for this site in post-summer 2017. If juvenile oysters were found settled on adult oysters within the experiment they were subsequently followed and measured along with the other oysters on the string throughout the experiment or until death, whichever came sooner. A total of 14 oysters were added to the study in this way throughout the experiment.

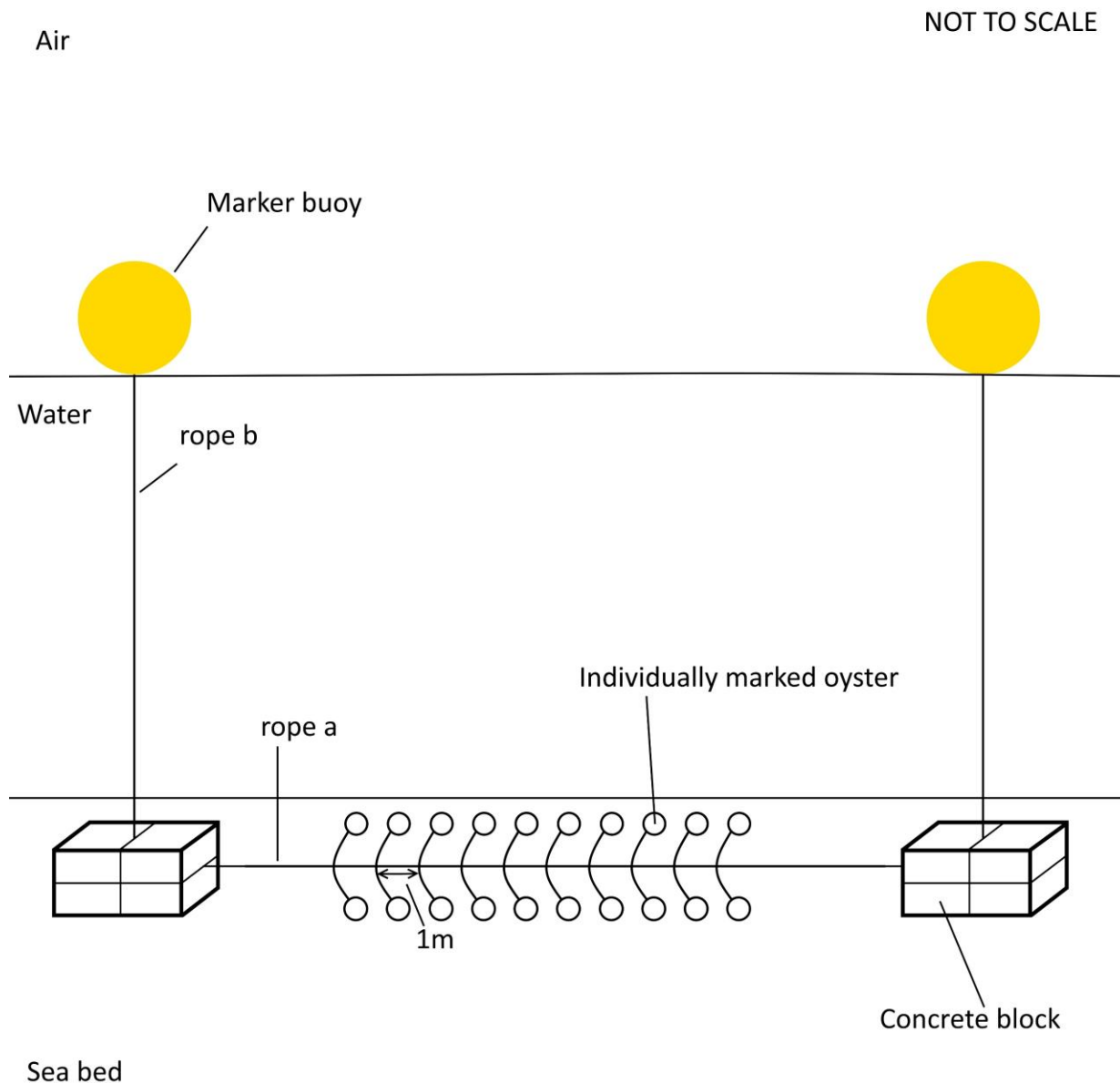


Figure 4.2. New experimental design set up following initial pilot trial to track individual growth rates of oysters around the Essex estuaries. The concrete blocks act as anchors, marked using buoys/fenders and oysters are directly attached to rope using Milliput adhesive. Two types of rope were used: a 5mm polypropylene rope (rope b) was used to attach marker buoys and a 2.5mm polyester rope used to tether the oysters (rope a).

For private ground sites, oysters were provided from the local area by Colchester Oyster Fishery for Sites 1 and 2 (Colne Pyefleet, now referred to just as the Colne, and Colne Raft), the Blackwater oystermen for site 4 (Tollesbury and Mersea Several Order, hereafter the Several Order) and Richard Haward's Oysters for site 3 (Brightlingsea Creek; Figure 4.3). Oysters were not brought in from other areas in order to remove the potential spread of disease from other areas and to ensure oysters used in

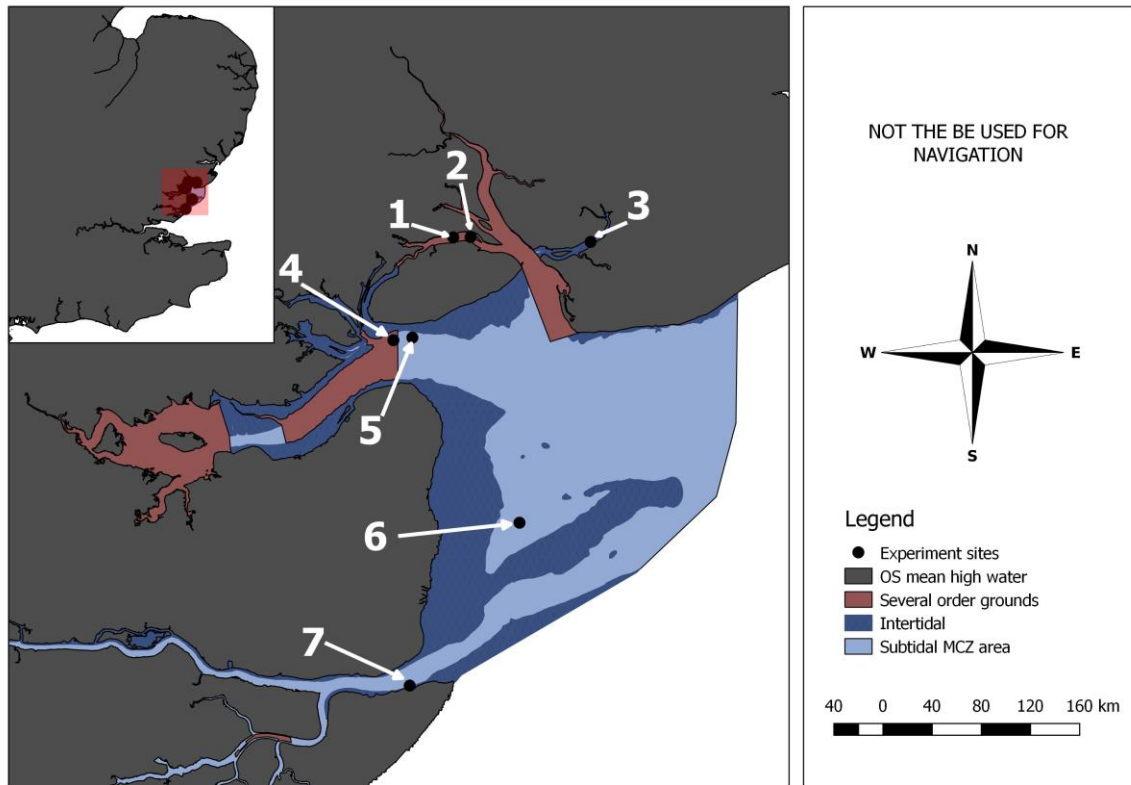


Figure 4.3. Map of sites where string experiment has been deployed: 1 – Colne Raft, 2 – Colne Pyefleet, 3 – Brightlingsea Creek, 4 – Blackwater Several Order, 5 – Blackwater MCZ, 6 – Ray Sand MCZ and 7 – Crouch MCZ.

each area were already acclimatised to the specific conditions to that site (for a full breakdown of the number of oysters per site and dates deployed see Table 4.2). Due to boat constraints it was not possible to collect all oysters and prepare all strings within a short time frame therefore a staggered start was required primarily focused around the post-winter 2017 surveys and the post-summer 2017 surveys.

Strings were checked four times a year (September/October, December/January, March/April and June/July) depending on boat availability and weather conditions. Each time strings were checked, water temperature, salinity, mortality of individual oysters and length and width of individual oysters were recorded. Any algae was removed from marker buoy lines in order to reduce the likelihood of markers becoming too heavily weighted by algal growth, a problem which occurred in the pilot trial. Where possible, potential cause of death was also recorded, however it was not possible to confirm with 100% certainty cause of mortality for most oysters. If the shell was blackened by anoxic mud, this implied death from burying in soft sediment or being engulfed by sediment load and or decomposing

algae. If small holes (<1mm diameter) were seen, this implied mortality from predation by drill species (either *Ocenebra erinaceus* or *Urosalpinx cinerea*). Finally, if shell malformations such as those seen in Figure 4.4 were observed, it was likely this was caused by the fungus shell disease *Ostracoblabe implexa* (Figure 4.4).

Table 4.2. Dates of string deployment and number of oysters used at each site

Site Number	Site Name	MCZ or Private grounds	Number of string sets (3 strings)	Date 1st string set deployed	Number of oysters	Date 2nd string set deployed	Number of oysters
1	Colne Raft	Private	1	01/08/2017	60	-	-
2	Colne Pyefleet	Private	1	15/02/2017	60	-	-
3	Flag Creek	Private	1	12/04/2017	60	-	-
4	Blackwater Several Order	Private	1	16/08/2017	60	-	-
5	Blackwater MCZ	MCZ	2	06/03/2017	48	07/09/2017	59
6	Ray Sand MCZ	MCZ	2	08/03/2017	60	14/09/2017	60
7	Crouch MCZ	MCZ	2	09/03/2017	60	13/10/2017	60



Figure 4.4. Small native oyster found during September 2017 Essex oyster dredge surveys showing clear predation attempt from an oyster drill species above and oyster showing signs on shell disease *Ostracoblabe implexa* (taken from Azov Scientific Research Institute of Fisheries, 2018)

Oysters were initially measured as described in Chapter 2 (i.e. length, width and depth). However, once attached to the strings it was not possible to measure the depth of the oysters due to the presence of Milliput, increasing the thickness of the oyster close to the hinge. This resulted in only the “area” of the oyster being used for analysis.

Reproduction

To estimate reproduction rates of *O. edulis* in Essex, 149 oysters were sacrificed between 22nd June and 2nd July 2018 to assess size-based likelihood of reproduction (mean=73.26mm length \pm 0.99 standard error). Here, oysters were measured, weighed and opened to check for the presence of white, grey and black “sick” indicating the oyster is a ripe female through the presence of unfertilised, fertilised and developing and ripe eggs within the female mantle cavity (Younge, 1960). Fecundity was estimated using size-based fecundity rates from (Cole, 1941). As it was not possible to perform a widescale dredge survey throughout the summer reproduction season or sacrifice large numbers of *O. edulis* within this protected area, these estimates are currently the most accurate methods of quantifying reproduction available.

4.5. Data analysis

Size and weight

It is recognised that length measurements are not ideal for quantifying oyster growth due to mismatch between “meat” biomass growth and shell growth however larger oysters are generally known to have larger meat mass (Walne 1958). Logistically it was not possible to weigh each oyster in the field due to the difficulties in obtaining accurate weight measurements on a small vessel and due to each oyster being attached to Milliput and the string. Approximate “area” of oyster was calculated as described in Chapter 2, assuming an elliptical shape for each oyster (i.e. $Area = (length/2)(width/2)\pi$), with area to weight conversions completed calculated using a linear model following the use of AIC scores.

All oysters were measured to the nearest mm and weighed to the nearest 0.1g. A total of 743 oysters were accurately measured in the laboratory of which 587 were used directly within the experiment. All analysis has been completed using R studio (R Development Core Team, 2007).

Analysis of Variance with Tukey's HSD *post hoc* analysis was used to identify differences in starting size of oysters between sites.

Growth rates

To assess seasonal variation in growth, relative growth rate in terms of increase in shell size was calculated for each measurement as:

$$\frac{\ln(\text{measurement2}) - \ln(\text{measurement1})}{\text{time2} - \text{time1}} \quad [\text{Equation 4.1}]$$

where measurement 1 and 2 were approximated area of oyster and time2-time1 is the time in days between measurements (Liu *et al.* 2015). Differences in growth rates between sites were calculated using linear mixed effects models, incorporating oyster area (mm²), season of growth occurrence and site as covariates, and individual oyster ID as a random factor to incorporate the repeated measure design. *Post hoc* analysis to compare differences in growth rate between sites was completed using Tukey's HSD.

Previous studies have highlighted the known growth trajectory of *Ostrea edulis* over time with *O. edulis* following a Von Bertalanffy (VB) Growth curve (Richardson *et al.* 1993). VB models were therefore used to assess lifetime growth of *O. edulis* from measured data. Due to known seasonal variation in growth potentially impacting the growth coefficients of the model, only oysters which had been monitored for and survived a full year were used to analyse lifetime growth rate, (n=177).

The original parameterisation of the VB growth function is written as follows:

$$L_t = L_\infty - (L_\infty - L_o)e^{-Kt} \quad [\text{Equation 4.2}]$$

Where L_t is length at age t , L_∞ is the asymptotic growth length, K is the Brody growth rate coefficient, and t_0 represents the time when average length equals zero.

As previously discussed, aging *O. edulis* is notoriously difficult and results in extremely high error rates (Orton 1938; Richardson *et al.* 1993). Absolute age of oysters, therefore, is not known and so the VB growth function method required adaptation for the use of capture-recapture data where size at first capture, size at second capture (1 year later) and time between are known but age is not. For this study, the methods used by Fabens (1965) have been followed so that L and K can be estimated without the requirement for t_0 . The VB model used is:

$$L_r = L_m + (L_\infty - L_m)(1 - e^{K\delta t}) \quad [\text{Equation 4.3}]$$

Where L_r is the length recapture, L_m is the length at time of first capture (and marking), $t - t_r = \delta t$ and is the time between first capture and recapture. This therefore allows for K to be estimated.

This equation can then be adapted to give

$$L_t = L_r + (L_\infty - L_r)[1 - e^{-Kt - t_r}] \quad [\text{Equation 4.4}]$$

Where L_t is the length at time t . When $t = t_r$ then $L_t = L_r$. In addition, when $t_r = 0$ then $L_r = t_0$ (Ogle & Isermann 2017).

VB growth curves were calculated from measurements of oyster area as calculated in Chapter 2 where each oyster was assumed to have an elliptical shape and for length (mm) measurements alone. This is due to the differences in growth observed between individual oysters with some oysters growing wider over time, with little increase in overall length but also due to length being a more commonly used metric for oyster size.

Parameters were estimated using the Fabens model within the FishR package using a non-linear least squares approach in R studio (Ogle, 2015; R Software development team, 2018) using the measured size at first capture and measured size 1 year later. Starting parameters were estimated from the maximum measured size of oyster and mean daily growth rate for all oysters which survived over 1 year. Starting size (size at birth) was set to the published size of pediveliger larvae of 254 μm length

and 233 μm width (Acarli & Lok, 2009). To assess accuracy of K (growth rate) and Linf (maximum size) coefficients, nls models were bootstrap resampled 999 times in order to obtain 95% confidence intervals of the coefficients. Coefficients were then varied between these maximum and minimum values to observe the effect of changing these covariates to make predictions on size at age.

Survival

A time dependent Cox proportional hazards model using last measured oyster “area” as a covariate, stratified by site and clustered by individual oyster ID, was used to assess survival probability in native oysters between sites over the course of the experiment. Survival rates over the course of the experiment were plotted using ggadjusted curves using a “conditional” method to balance sub-populations between sites (Therneau *et al.*, 2015). Size, in area, was divided by 100 in survival analysis to model survival of oysters for every 1cm^2 increase in size due to 1mm^2 increase in size deemed too small a size increase to assess changes in survival. Cox proportional hazards were chosen over logistic regression at this stage to assess survival over the course of the experiment due to the need to incorporate staggered entry and right censoring into the model without removing large quantities of data. This is because different strings were deployed on different days (and sometimes seasons) and due to various strings being lost at sea throughout the trial.

Population dynamics are commonly assessed using parameters obtained from survival regressions, therefore, an equivalent model using generalised linear mixed models using mortality status after approximately 12 months as the response variable was used for all individuals that were not lost at sea to assess probability of an oyster surviving 1 year based on individual starting size, this method was required to assess age dependent survival due to insufficient numbers of replicates available for each age group to complete a cox regression split by age. Each oyster was assigned an approximate age, calculated from the closest size at age from the previously calculated VB growth curves. Approximate starting age was used as the predictor and individual site as the random variable within this model, with survival probability calculated from summary model coefficients as follows:

$$EP = \frac{e^{(\beta_0 + \beta_1 * x)}}{1 + e^{(\beta_0 + \beta_1 * x)}} \quad [\text{Equation 4.5}]$$

Where β_0 is the estimated intercept coefficient of the model and β_1 is the coefficient for age extracted from the model and x is the size at the start of the year of observation in cm^2 . This second model did not incorporate variation in survival over the course of the year and is only able to predict size dependent mortality.

Reproduction and cause of death

A Chi-squared test was used to compare frequency of oysters showing various causes of death between sites. The natural log of oyster length was plotted against the natural log of the number of embryos estimated within the mantle cavity by Cole (1941). AIC scores were used to find the best fit with a straight linear model resulting in the best fit. A binomial Generalised Linear Model was used to identify the effect of weight and oyster size on likelihood of ripeness (i.e. carrying a brood of eggs or embryos).

4.6 Results

Size and weight

A simple linear regression was used to predict the weight of *O. edulis* from an area measurement ($t=75.39$, $P<0.001$ with adjusted $R^2=0.8847$) (Figure 4.5). Predicted weight from oyster area is calculated to be:

$$y = 1.505x - 8.751 \quad [\text{Equation 4.6}]$$

where $y = \ln$ weight of native oyster (g) and $x = \ln$ area of native oyster (mm^2). An oyster of average length to width dimensions of 1.0236:1 would have an area of 3759.94mm^2 (Chapter 2) would therefore have an estimated weight of 38.03g.

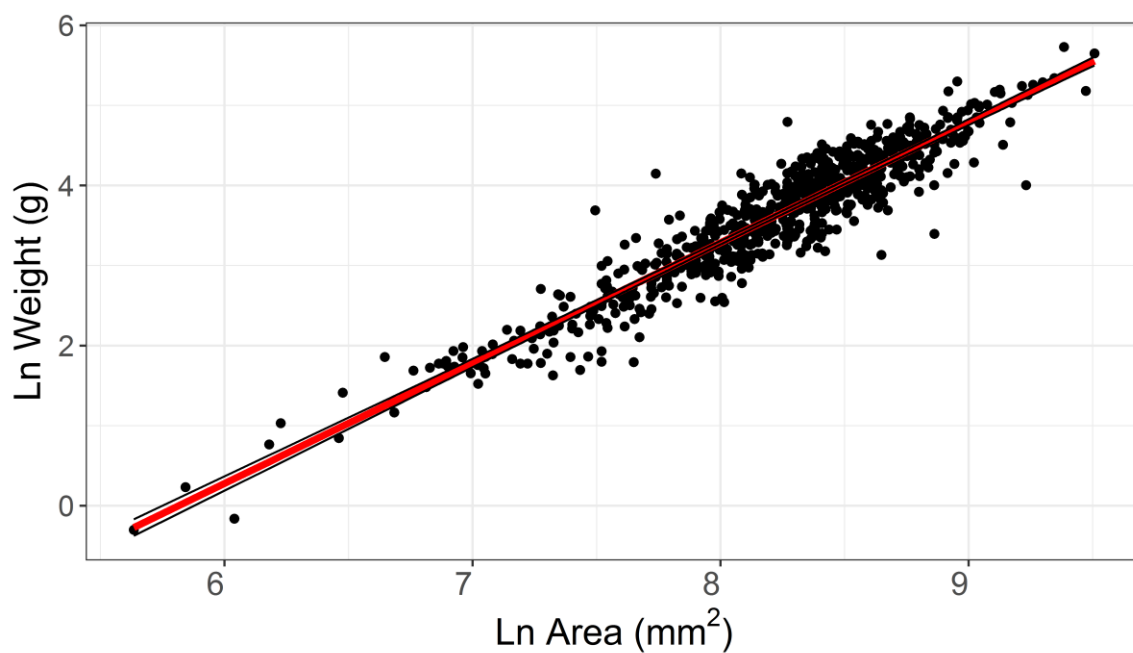


Figure 4.5. All native oyster ln area measurements vs ln accurate weights with best fit line calculated using a linear model following $y = 1.505x - 8.751$. Best fit line is shown in red with 95% confidence intervals

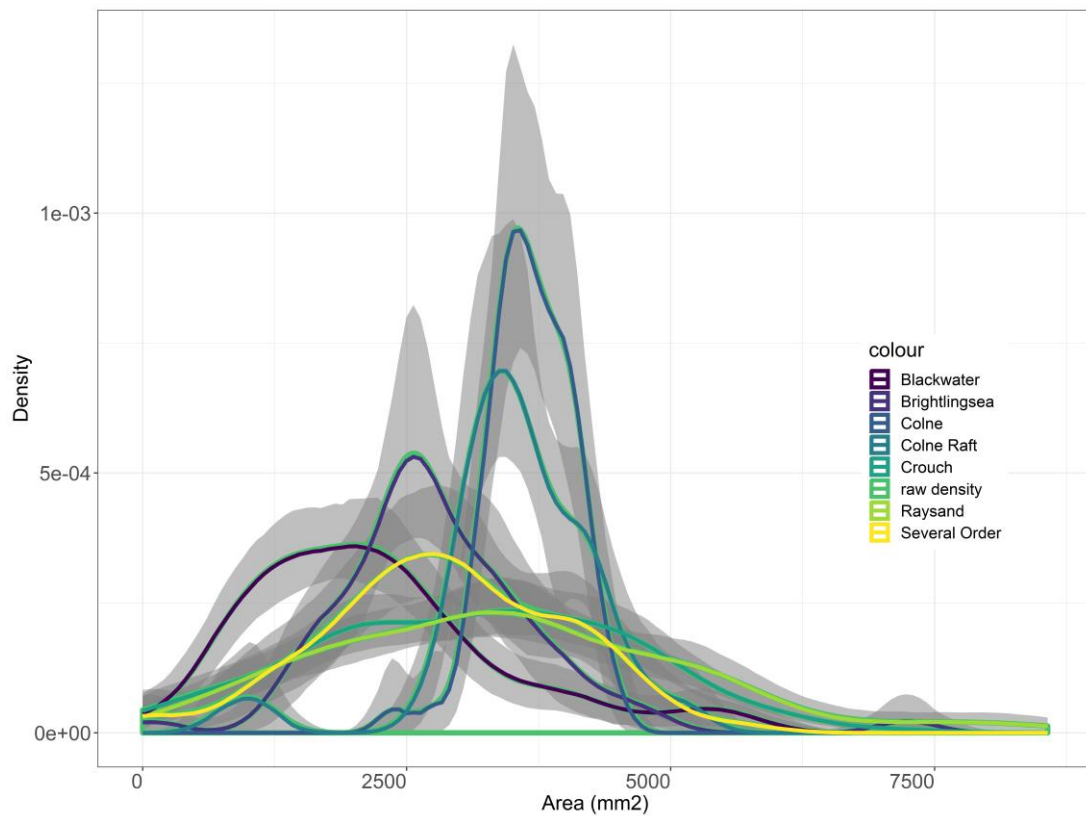


Figure 4.6. Density curve of starting size of oysters in mm² between sites. Blackwater, Crouch and Ray Sand sites used oysters collected during full surveys of the Marine Conservation Zone with the Kent and Essex Inshore Fisheries Conservation Authority with other sites using oysters collected locally by local oystermen. 95% confidence intervals are shown from 999 bootstrapped permutations.

The mean starting area for all oysters in this study was calculated to be $3065.783\text{mm}^2 \pm \text{standard error } 55.0917$ ($n=598$). Distribution of initial starting area of oysters by site can be found in Figure 4.6. A statistically significant difference in starting area between sites was observed (Anova, $F_{6,591}=13.18$, $P<0.001$). Significant differences in starting area were found between Blackwater MCZ-Colne raft, Blackwater MCZ-Crouch, Blackwater MCZ-Colne, Blackwater MCZ-Rayand, Blackwater MCZ-Several Order, Brightlingsea Creek-Colne raft, Brightlingsea Creek-Colne Island, Brightlingsea Creek-Ray Sand and Colne Island-Several Order (Figure 4.6).

Temperature

Temperature ranged from a low of 1.42°C in the Blackwater MCZ site in March 2018 up to a maximum of 28.25°C. Temperatures in 2017 reached highs of 19.7°C in the Crouch in June 2017 with 19.2°C recorded 1 year later with highs in 2018 of 24.5°C on 27th July 2018, no temperatures were able to be taken on this date in 2017. Highest Blackwater MCZ temperatures in 2017 of 19.55°C were recorded on 23rd June 2017 with temperatures of 19°C recorded 1 year later and a maximum temperature of 25.1°C recorded on 27th July 2018. Colne raft temperatures were recorded as 17.26°C on 1st August 2017 with temperatures of 22.9°C recorded 1 year later and Several Order temperatures recorded as 18.18°C on the 18th August 2017 with high temperatures of 19.8°C recorded 1 year later (Figure 4.7).

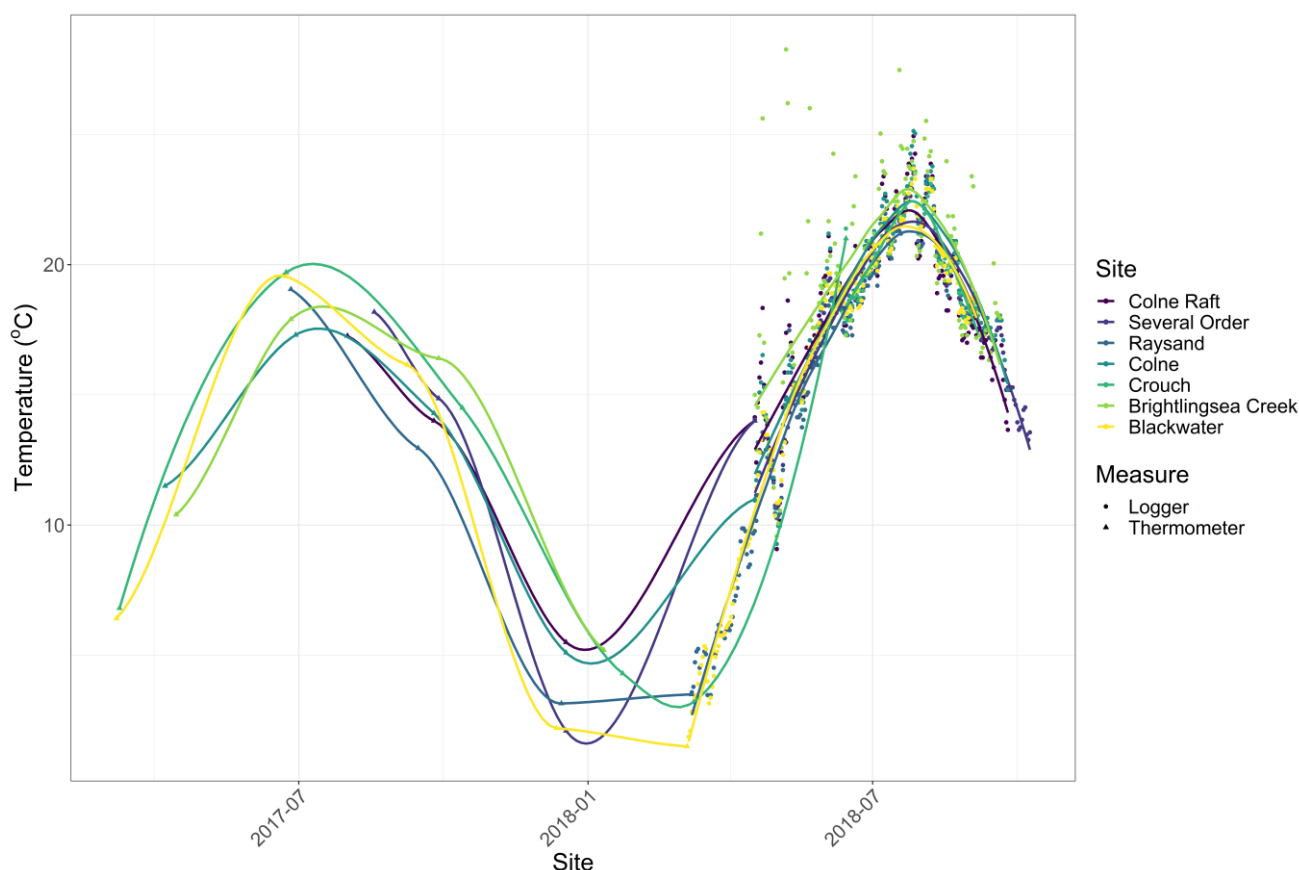


Figure 4.7. Individual site temperature throughout 2017 and 2018. Thermometer measurements are taken from surface samples with logger temperatures recorded at the sea bed daily at midday.

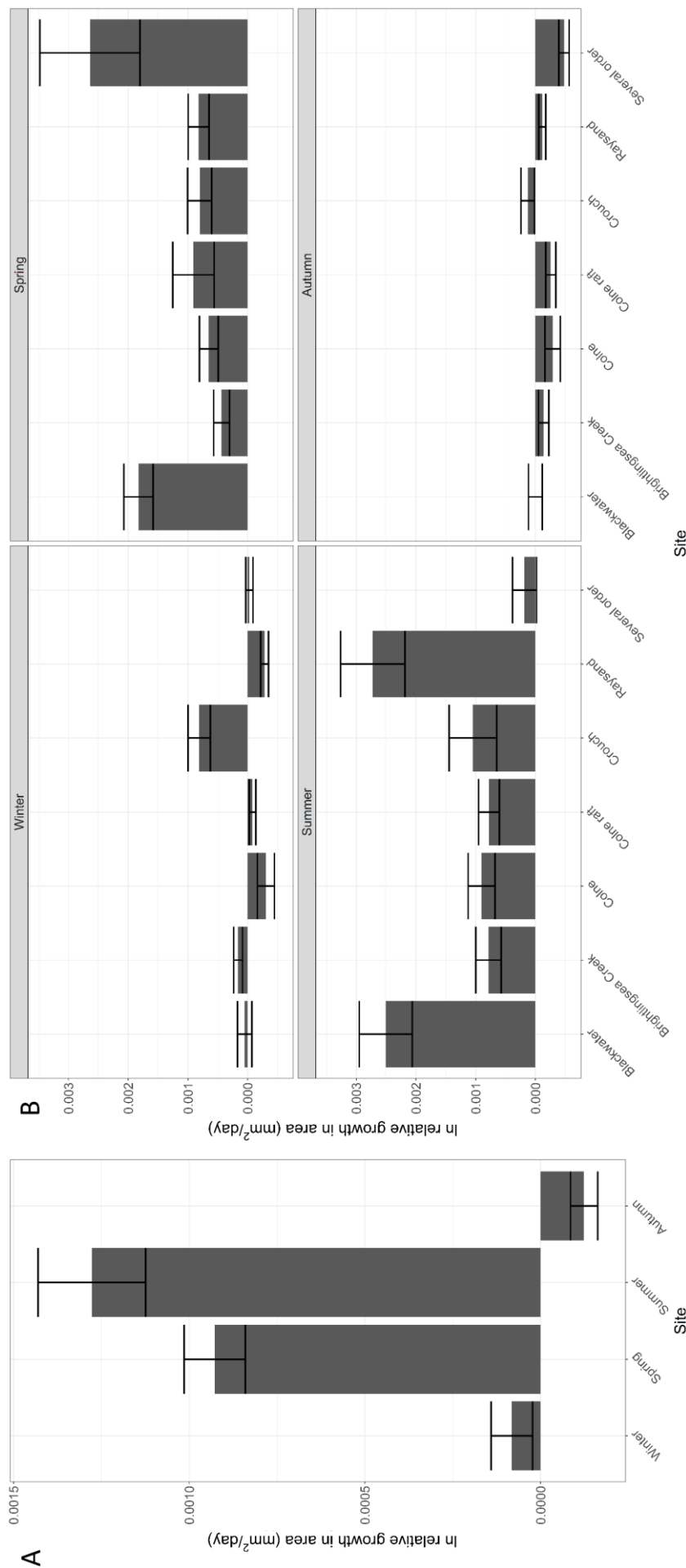


Figure 4.8. Average relative log daily growth rate in area of native oysters in Essex +/- standard error. A) All sites combined, between seasons B) Spring only, split by individual sites C) Summer only, split by site D) Autumn only, split by site E) Winter only, split by site.

Growth rate

A linear model highlighted a statistically significant interaction between site and season ($F_{18,1501}=7.73$, $P<0.001$) with both a difference in growth rates between sites ($F_{6,1501}=6.36$, $P<0.001$) and between seasons ($F_{3,1501}=71.29$, $P<0.001$) also observed. Highest relative growth rate occurred during spring and summer months with a small reduction in size occurring in Autumn between October and December (Figure 4.8). There was a statistically significant difference in daily growth rates between sites, when starting size (area mm^2) and season (to account for different numbers of individuals included in different seasons) are included as random factors (Anova, $F_{6,1501}=9.12$, $P<0.001$). Significant site differences are highlighted in Table 4.3 with sites in the MCZ found to have consistently higher growth rates than in the Several Order and Creek sites.

The highest growth rates overall are observed in Blackwater MCZ site for both spring and summer months however it is important to note that this site also had the smallest starting size oysters. Increases in growth are first notable in the Blackwater and Several Order sites in spring while other sites appear suppressed in comparison. By summer the Ray Sand site also shows high growth rates. Brightlingsea creek has the lowest growth rates for both spring and summer growing seasons (Figure 4.8).

VB Growth curves highlight the variation in growth when using area measurements or length measurements alone. When using oyster area as a proxy for size, minimum growth rates (lowest K) and minimum Linf (maximum size) predict oysters to be of landable size after approximately 10-11 years with maximum growth rates and maximum Linf values predicting oysters to be harvestable between 1-2 years. Mean growth rates and Linf values predict oysters to be harvestable after 4-5 years (Figure 4.9). Here, landable oysters are specified to have both length of 70mm and width of 68.39mm assuming mean a length to width ratio of 1.0236:1 (Chapter 2). An oyster with these dimensions would have an area of 3759.94mm^2 .

Using length measurements alone shows lower variation between predicted time to harvestable size when varying coefficients for maximum size (Linf) and growth rate (k). Here, oysters with the lowest 2.5% growth rate and smallest Linf are likely to be harvestable between 6-7 years with the fastest

growing oysters with highest Linf, harvestable within 3-4 years with mean growth rates and Linf values predicting oysters to be harvestable after 4-5 years using length only as a proxy for measurement (Figure 4.10).

Table 4.3. Results from Tukey's Post-hoc analysis of a linear mixed model identifying differences in growth rate between sites, with starting size (area mm²) and season included as random factors. * Indicates area of oyster mariculture and MCZ indicating sites in the Blackwater, Crouch, Roach and Colne Marine Conservation Zone. A positive coefficient estimates indicates the first site listed has higher growth rates with a negative coefficient indicating the second site listed has higher growth rates.

Sites compared	Coefficient	Std. Error	z value	Pr(> z)
Brightlingsea Creek * - Blackwater MCZ	-7.78E-04	1.66E-04	-4.686	< 0.001
Colne * - Blackwater MCZ	-7.86E-04	1.72E-04	-4.58	< 0.001
Colne raft * - Blackwater MCZ	-7.25E-04	1.74E-04	-4.173	< 0.001
Crouch MCZ - Blackwater MCZ	-1.86E-04	1.55E-04	-1.203	0.89209
Ray Sand MCZ - Blackwater MCZ	-2.81E-04	1.48E-04	-1.905	0.47349
Several Order * - Blackwater MCZ	-8.76E-04	1.74E-04	-5.047	< 0.001
Colne * - Brightlingsea Creek *	-7.90E-06	1.73E-04	-0.046	1
Colne raft * - Brightlingsea Creek *	5.31E-05	1.76E-04	0.302	0.99994
Crouch MCZ - Brightlingsea Creek *	5.92E-04	1.58E-04	3.752	0.00331
Ray Sand MCZ - Brightlingsea Creek *	4.96E-04	1.49E-04	3.335	0.01456
Several Order * - Brightlingsea Creek *	-9.83E-05	1.76E-04	-0.56	0.99781
Colne raft * - Colne *	6.10E-05	1.82E-04	0.336	0.99988
Crouch MCZ - Colne *	5.99E-04	1.63E-04	3.688	0.00422
Ray Sand MCZ - Colne *	5.04E-04	1.56E-04	3.234	0.02047
Several Order * - Colne *	-9.04E-05	1.82E-04	-0.497	0.99888
Crouch MCZ - Colne raft *	5.38E-04	1.66E-04	3.241	0.02018
Ray Sand MCZ - Colne raft *	4.43E-04	1.58E-04	2.806	0.07358
Several Order * - Colne raft *	-1.51E-04	1.80E-04	-0.843	0.98005
Ray Sand MCZ - Crouch MCZ	-9.50E-05	1.38E-04	-0.689	0.99313
Several Order * - Crouch MCZ	-6.90E-04	1.66E-04	-4.145	< 0.001
Several Order * - Ray Sand MCZ	-5.95E-04	1.58E-04	-3.771	0.00309

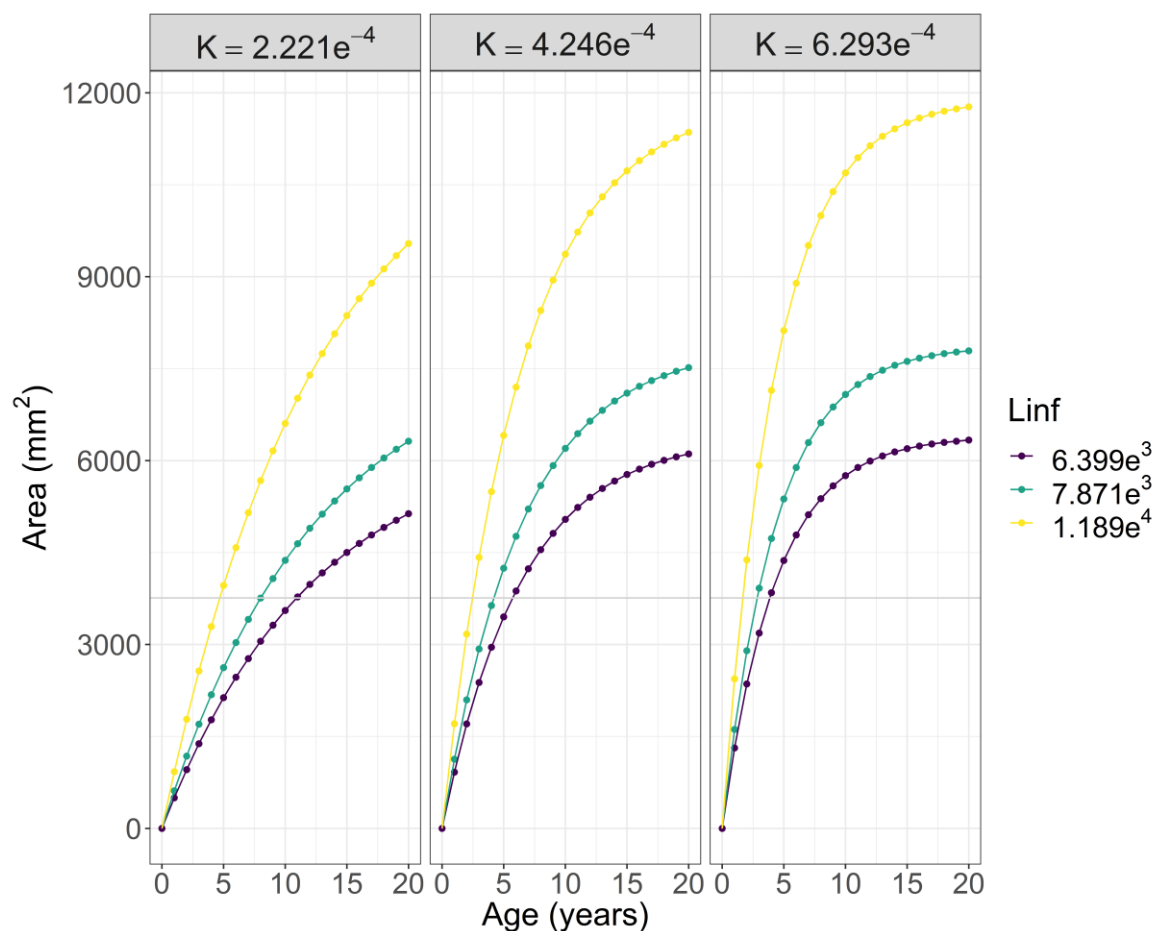


Figure 4.9. Von Bertalanffy Growth Curves calculated from area of oyster at first capture and area of the same oyster 1 year later. Models were bootstrap resampled to provide 95% Confidence Intervals for coefficients K (growth rate) and L_{inf} (maximum growth size). The light grey horizontal line denotes the size of an oyster when Length = 70mm and width = 68.39mm assuming mean a length to width ratio of 1.0236:1 (Chapter 2). An oyster with these dimensions would have an area of 3759.94mm² and is therefore an approximate area for landable size.

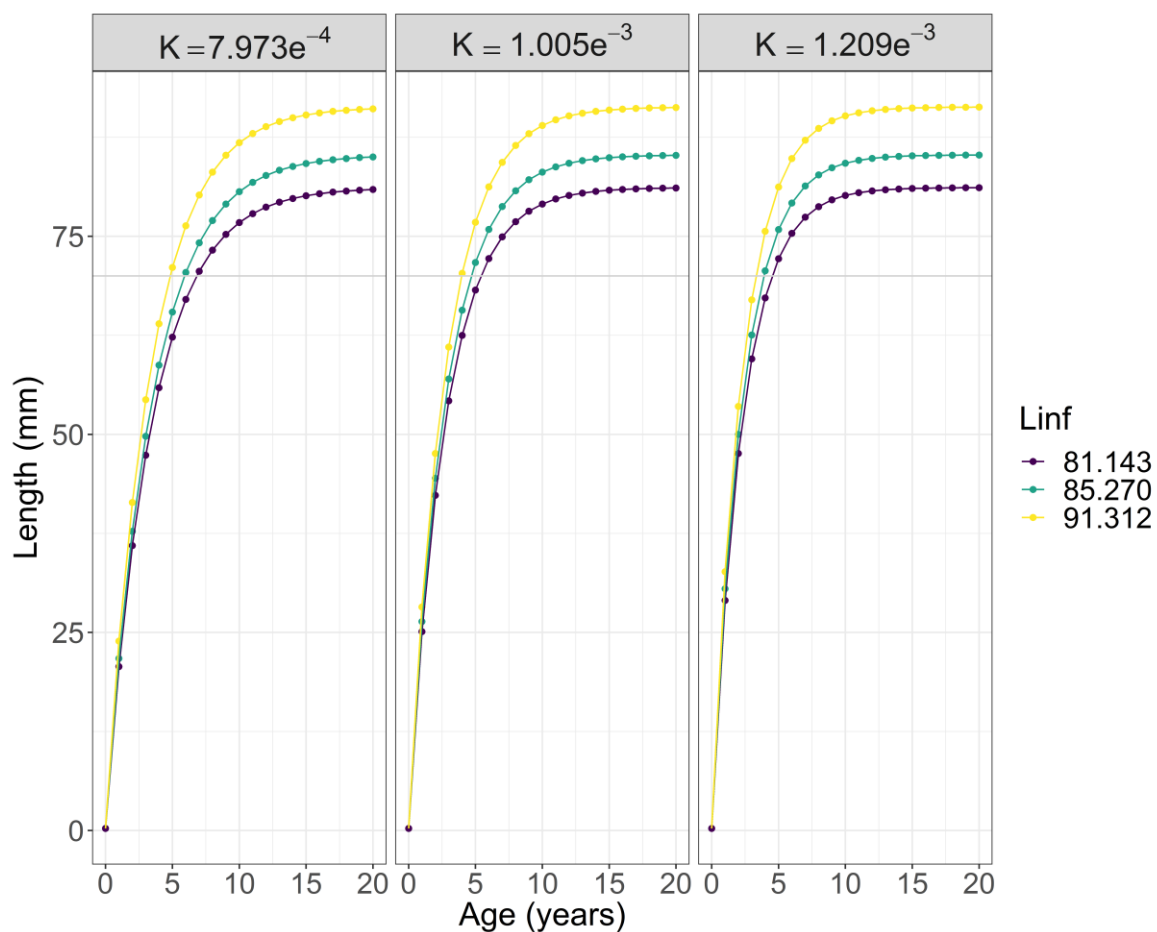


Figure 4.10. Von Bertalanffy Growth Curves calculated from length (mm) of oyster at first capture and length of the same oyster 1 year later. Models were bootstrap resampled to provide 95% Confidence Intervals for coefficients K (growth rate) and Linf (maximum growth size). The light grey horizontal line denotes the size of an oyster when length = 70mm and is therefore a landable size of oyster within the Essex estuaries.

Survival

After 1 year, a total of 240 oysters had been recorded dead, 178 surviving and 183 lost with a total of 282 oysters recorded dead, 251 lost and 68 surviving over the course of the experiment, including any oysters which were found growing attached to strings throughout the study. When using a cox regression survival model using site as a stratified term, the main effect of initial size as measured by area was found to be statistically significant in determining mortality between sites with the risk of mortality higher in smaller oysters ($\exp(\text{coef})=0.987$, $z=-2.28$, $P=0.022$) meaning oysters on average have a hazard ratio of dying of 0.987 for every 1cm^2 increase in area over the course of the experiment. However, when using site as a predicting factor rather than stratifying the data by site, size was not significant ($\exp(\text{coef})=0.990$, $z=-1.75$, $P=0.079$), but individual sites were found to have significantly different survival to each other. Hazard ratios and significance values can be found in Table 4.3. All sites, other than the Colne site had significantly better survival of native oysters than the mouth of the Blackwater with the Ray Sand found to have the highest survival with hazard ratio the lowest at 0.181 in comparison to oysters in the Blackwater mouth ($z=-5.82$, $P<0.001$). Figure 5.11 shows variation in survival over time between sites whilst controlling for size, indicating highest survival rates on the Ray Sand and lowest in the Blackwater and Colne.

Using the more commonly used generalised linear mixed models with a logit binomial distribution to estimate probability of survival after 1 year, using site as a random variable, there was no statistically significant effect of age on survival probability ($P=0.196$) however extracted coefficients showed a small decrease in the odds of dying with increasing age. Annual, age-based survival coefficients were calculated to be $\beta_0 = -0.72849$ and $\beta_1 = 0.011521$ for full year survival and $x = \text{size in cm}^2$ with annual survival probability calculated to be 0.3281 at size = 1cm^2 increasing by 0.0115 with every $+1\text{cm}^2$ increase in area.

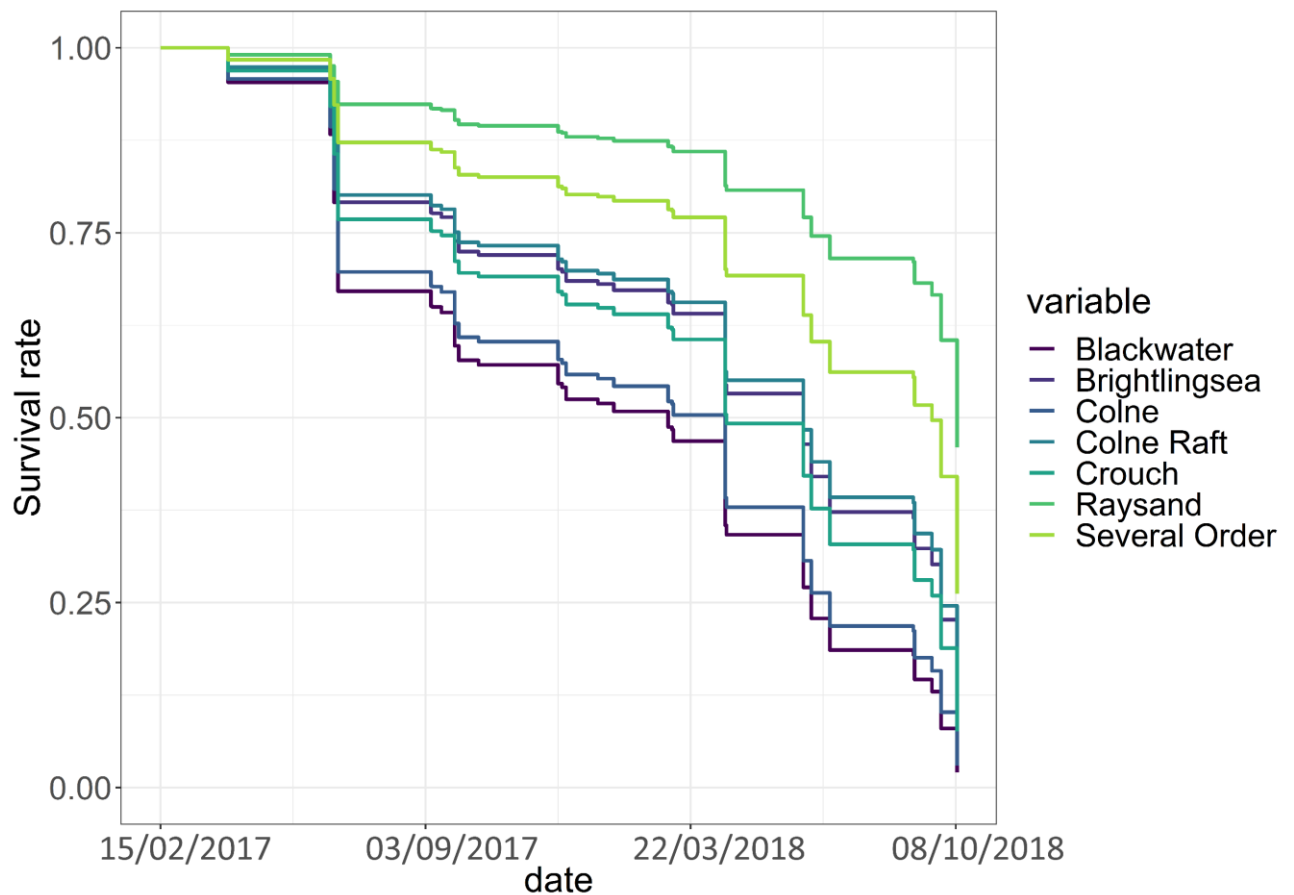


Figure 4.11. Survival curve of native oysters at various sites around the Essex estuaries throughout 2017 and 2018 using cox proportional hazards regression. A “conditional” method has been used to balance sub-populations between sites. Area of oyster (cm²) is used as a covariate.

Cause of death

It was not always possible to determine a cause of death for many oysters and no signs of drilling by oyster drills were observed throughout this study. It was possible to identify a likely cause of death in 94/282 of mortalities. Many of these were shells found to be highly blackened, implying burying by anoxic sediment (n=60). Recorded potential causes of death are shown in Figure 4.12. There was a statistically significant difference in the number of mortalities attributed to each cause of death between sites ($\chi^2 = 59.349$, $df=4$, $P < 0.001$) with anoxic mud attributed to a high mortality rate at the Blackwater MCZ, Colne raft and Colne sites.

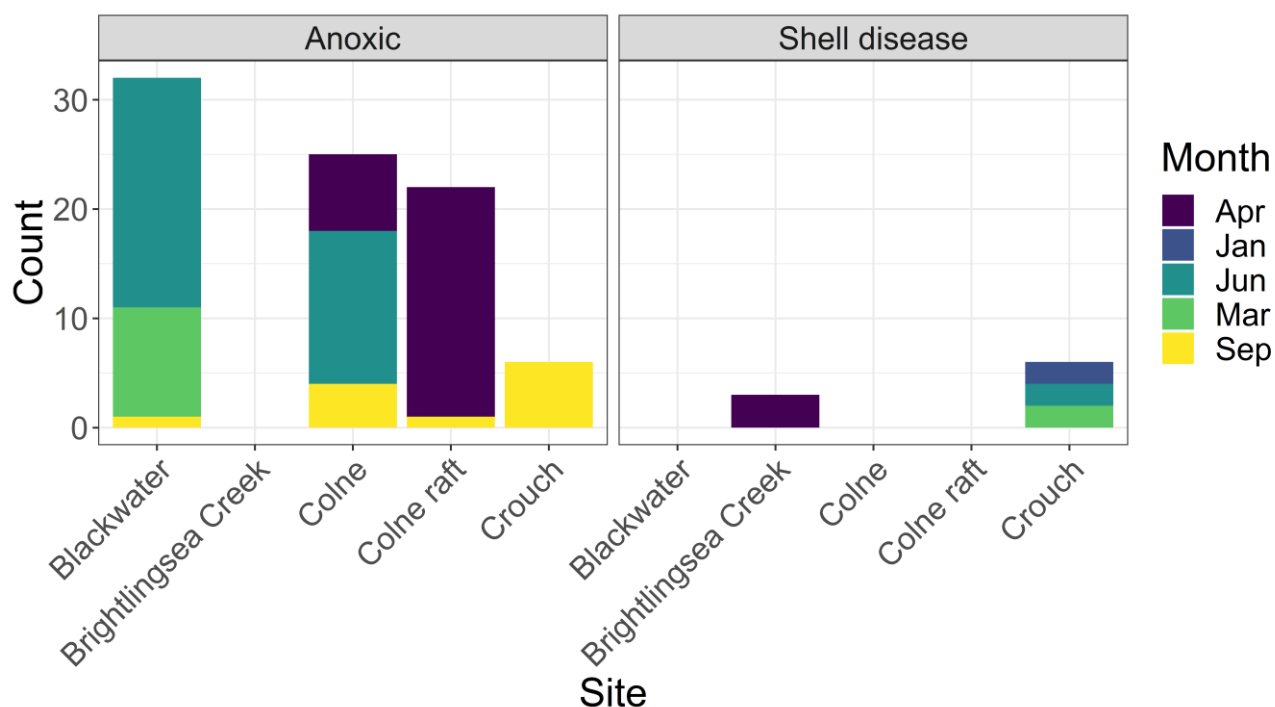


Figure 4.12. Number of individual oysters appearing to have died from smothering by anoxic mud (“anoxic”) or from lesions typical of shell disease (“shell disease”). Note not all sites are listed due cause of death not possible for all oysters.

Reproduction

A simple linear regression was used to predict the number of embryos of a ripe female *O. edulis* ($t=6.207$, $P<0.001$ with adjusted $R^2=0.490$) (Figure 4.13). Predicted fecundity rate from oyster length is calculated to be:

$$y = 3.3587x - 0.8067 \quad [\text{Equation 4.7}]$$

where y = ln number of embryos and x = ln length of ripe female oyster (mm). 15.7% of oysters opened by Cole between June and July were found to carry broods of embryos from the Menai Straits, Wales whereas 0 oysters were found at this site containing embryos from August to September. Details regarding the number of oysters required to obtain brood estimates are not available for other sites in this study.

In 2018 samples from the Blackwater and the Ray Sand a total of 13.4% (20/149) oysters appeared as ripe females, of these 2 contained black sick, 4 were grey and 14 were white. Generalised linear models with a binomial distribution found both length or weight were not statistically significant predictors of ripeness ($z=0.792$ $P=0.429$ and $z=-1.605$, $P=0.128$ respectively).

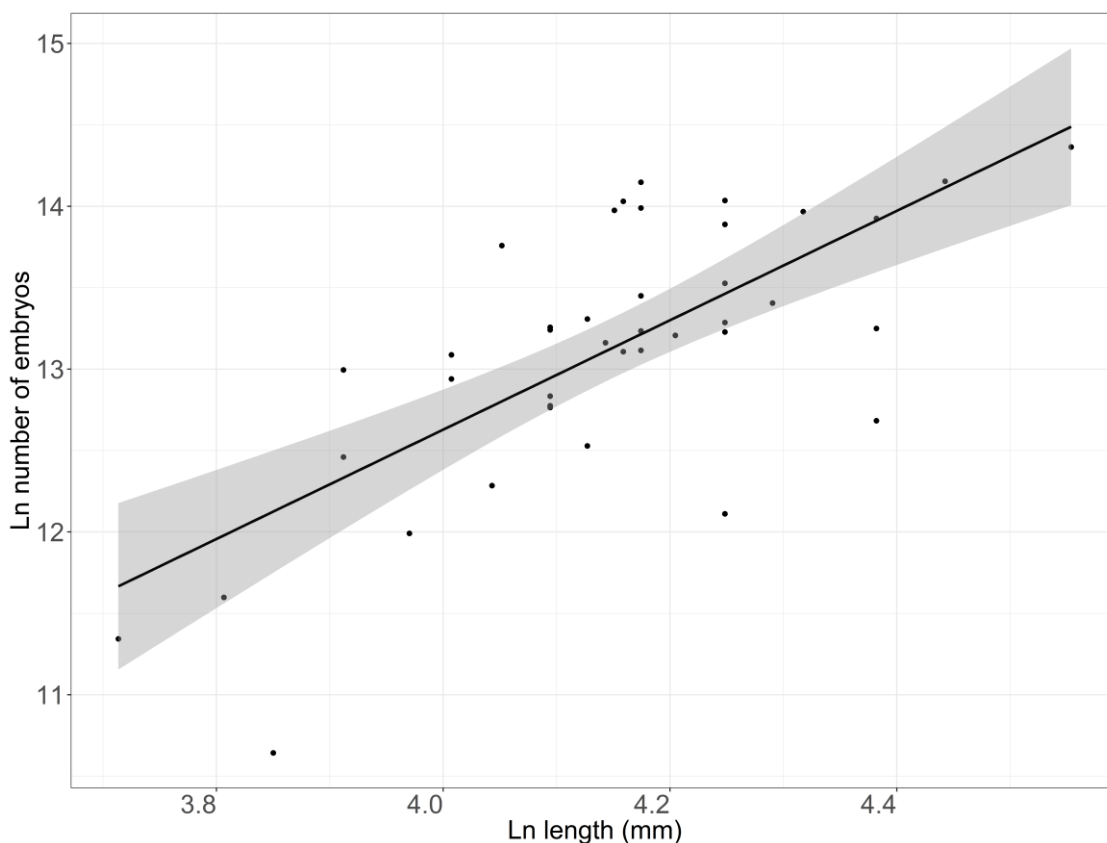


Figure 4.13. Estimated size-based fecundity from data extracted from Cole (1941) with 95% Confidence Intervals. Oysters were collected from Helford, Cornwall, Menai Straight, Wales and breeding tanks at Conway, Wales.

4.7. Discussion

This chapter has highlighted variation in growth rates and survival in different areas of the BCRC.MCZ. It is the first study to assess growth and survival rates of *O. edulis* at low, naturally occurring, local densities in the subtidal zone. These results have been designed to feed into models exploring population dynamics of the European native oyster (Chapter 5).

Growth rates

Changes in growth rate between seasons highlight the well-studied “growing season” for *O. edulis* throughout spring and summer. It is common for native oysters to stop growing altogether over winter months (Renault *et al.*, 2005). The observed reduction in size during autumn months has been commonly observed and has previously been attributed to the newly formed spring and summer shell being more fragile than that of old shell, resulting in this new growth being broken off during the first storm and rough weather winter events (Orton 1938).

High variation in growth rate was found between sites. Highest growth rates were observed in the Blackwater and the Several Order during spring months and the Blackwater and the Ray Sand during summer months. Whilst the Blackwater MCZ site shows some of the highest growth rates, this site also shows some of the highest mortality rates with all MCZ sites showing significantly higher growth rates than mariculture sites (i.e. Several Order, Brightlingsea Creek and Colne sites) when incorporating starting size and season (due to the different starting times between sites) as random factors. This difference in growth rate between managed and “wild” areas may be due to multiple reasons: firstly, in areas where oysters are actively relayed and moved to maintain high densities, or if *C. gigas* are cultured in close proximity (as occurs at the Colne Raft site), oysters may suffer competition for food resources (Zwerschke *et al.*, 2018). Secondly, differences in growth rate may be due to differences in food availability or water conditions between creek sites and the more open MCZ sites. In this case, high growth rates would also be expected in the more open Several Order site. Apparent growth rates in the Several Order during the summer may be suppressed due to the small sample size available by that time

(n=8), 1 year after deployment of the strings. This was likely due to loss of strings in this area resulting in small sample sizes. High growth rates were however, observed during spring measurements. Alternatively, the exact location of the placement of the strings within the mariculture sites may have been sub-optimal. This is due to the requirement for the strings to be situated slightly away from key working areas so that strings are not dredged by working boats. This was unavoidable at this time. Finally, growth rates of oysters within private grounds may be lower due to oysters not being as adapted to local condition as wild-type oysters. Whilst every effort was made to ensure oysters used in the MCZ sites originated from local site vicinities, oysters in private oyster grounds in the Colne area may have been imported from other areas of the country such as the Fal where conditions are different to Essex. Previous studies have shown a high degree of local adaptation of *O. edulis* with growth rates differing between oysters grown at a single site, imported from various places around Europe (da Silva *et al.*, 2005).

Whilst growth rate is extremely important to maximise profits and turnover in a sustainable fishery, there are some potential benefits to slower growing oysters: firstly, previous studies have shown fast growing oysters are likely to test positive for *Bonamia* sooner with size rather than age a more important factor in determining bomaniosis (Cáceres-Martínez *et al.*, 1995). Secondly, fast growing oysters may have reduced thermal tolerance to that of slow growing oysters as shown with the primarily intertidal, Sydney rock oyster (*Saccostrea glomerata*), here faster growing oysters also suffered higher mortality (McAfee *et al.*, 2017). Furthermore, oysters may be growing slower in length and in width but growing more in depth, indicating oysters are potentially experiencing higher “fattening” rates, increasing meat content and quality for condition indexes for sale at market (Acarli *et al.*, 2011). Finally, oysters may be increasing shell thickness in order to prevent increased mortality rates from higher abundances of predators (Carriker, 1955). It was not possible to assess deepening of oyster shell or shell thickness due to the Milliput placement on the oyster in this study. (For more information on oyster area to depth ratio see Chapter 2). Alternatively, potential adverse causes of slow growth may be due to limited food resources, potential pollutants or genetic differences between wild and cultivated populations (Utting, 1988; Laing *et al.* 2005). Whilst this study is not currently able to determine which primary factor of

these (if any) may be influencing *O. edulis* growth across Essex, identifying where differences in growth rate are occurring helps to guide the direction of future research and restoration and increase the sustainability and efficiency of *O. edulis* mariculture in Essex by pinpointing areas already best suitable for oyster growth and survival.

It is notoriously difficult to age European native oysters due to many oysters not exhibiting clear surface nacreous rings on the exterior of the shell (Richardson *et al.*, 1993). Previous studies of oyster growth calculated native oysters in the Blackwater to reach 70mm length within 2-3 years (Richardson *et al.*, 1993). This may indicate a potential decrease in growth rate of *O. edulis* over the past 2 decades as, by using these methods, this study calculates that oysters will, on average, take between 4-5 years to reach 70mm in size. Whilst using individual growth rate data may not provide the most accurate method of assessing age of oysters, methods tracing back umbonal lines using acetate peels are highly laborious and require oysters to be sacrificed, meaning aging oysters prior to inclusion in this experiment was not feasible. To increase accuracy of aging oysters, collection of spat during a known breeding season would be required with individual oysters followed throughout their lives. As European native oysters are slow growing, long lived species living up to 20 years, this method would be highly time consuming and require huge numbers of 1-year old oysters to fully assess the full lifespan of *O. edulis* in different areas (FAO, 2004). Another previous study has also measured growth rates of *O. edulis* collected from various beds around the river Blackwater. Here growth shoots between 3-10mm in length in the summer of 1923 (Orton, 1938). This study, however, fails to state the initial starting size of the oysters studied and so were likely a range of oysters of all ages and is therefore not suitable to be used to assess differences in growth rate over the life span of an oyster (Orton, 1938).

Survival

This study has found survival of oysters is primarily dependent on different site characteristics rather than size or age-based survival. Whilst size was initially found to be a statistically significant predictor of survival when using site to strata the cox regression, this may have been driven by differences in

initial starting size between different sites. When incorporating site as a predictor, size becomes borderline significant ($P=0.07$) with site highly significant ($P<0.001$). Whilst it is possible that there could be genetic causes for differences in survival, the movement of oysters from one area to another by oyster growers has been common practice for many decades meaning populations are likely to be well mixed, however sites within private grounds may have imported oysters from other areas more recently which may impact results for the Colne raft and Colne sites (Personal Correspondence). Oyster growers would regularly move oysters from sea beds to creeks and oyster pits during winter months and import oysters from elsewhere in the country for fattening in Essex waters (Benham *et al.*, 1993). These methods, combined with the method of spawning in oysters and low genetic differentiation in oysters across Europe, likely results in a well-mixed gene exchange, however further research would be required to confirm this in the Essex populations (Saavedra *et al.*, 1995).

Lowest survival rates were observed in the Blackwater MCZ site. This site had low temperatures of 1.4°C recorded in March 2018 and local oyster growers reported a large quantity of sediment washed into the Blackwater estuary in that time due to the Storm “Emma” (Personal Correspondence). This sediment was “worked” by the oyster growers using both dredges and harrow chains to remove the soft sediment from their oyster layings within the Tollesbury and Mersea Several Order grounds. These activities to clear the sediment were not completed at any MCZ sites. This likely resulted in the large quantities of deceased oysters being discovered in the Blackwater MCZ site in 2018 showing signs of blackened shell, implying these shells were buried under anoxic sediment. Whilst it was not possible to fully determine whether sediment loading was the cause of death or if burying occurred after mortality, possibly following stress caused by the low temperatures, it is clear that the Blackwater MCZ and the Colne sites both experienced high levels of evidence of anoxia in comparison to other areas (Figure 4.11).

The Ray Sand was found to have highest survival rates with 13 oysters recorded dead and 51 living after 1 year (58 missing), with 1 string of 20 oysters experiencing 0 mortalities over the course of a year. Growth rates were high at this site during summer months and while *Crepidula fornicata* are present at this site, densities are lower than those found in the Blackwater (Chapter 2). These high

survival and high growth rates indicate that the Ray Sand may be a suitable area to focus on for further restoration work. Natural densities in this area are, however, low in comparison to other sites with maximum densities regularly observed at approximately 1 oyster m⁻² with average densities are generally much lower, generally between 0.1 and 0.5 oyster m⁻² (Chapter 2). Oysters have been observed to have lower fertilisation success when spaced more than 1.5m apart and as the Ray Sand has otherwise high growth and survival rates, lack of propagation by this population may be influenced by an allee effect (Guy *et al.* 2018). Further research into the potential impacts on increasing oyster density in the Ray Sand area on survival vs fertilisation success may help to clarify these mechanisms, as has been performed recently in the Solent with oysters collected from surrounding low-density areas and re-laid at higher density with the aim of creating a broodstock (Sawusdee, 2015).

The results in this chapter have shown that survival rates between sites even within close proximity (e.g. Several Order to Blackwater MCZ) are highly variable. Due to the close proximity between sites in the Blackwater, variation in suitable water quality between areas is unlikely to be a significant influence in determining these differences in survival. Benthic habitat type (e.g. shell vs sand or mud) or influence of human intervention (managed vs wild) may therefore be more influential in determining mortality rates. If water quality issues are a primary cause of mortality for *O. edulis* in Essex, similar mortality rates between sites of close proximity would be expected. However, with survival of oysters at the Several Order site 2nd to highest, and the Blackwater MCZ site the lowest, this does not appear to be the case (Figure 4.11). To confirm differences in survival due to benthic habitat effects rather than water quality problems, a repeat of this experiment may be useful using oysters laid on the sea floor, as is performed here, and also an additional line of oysters suspended within the water column using additional sub-surface floating buoys to maintain a set height of a second line within the water column, similar to that of a gill net without a mesh net in the middle (Appendix III, Figure S4.1). This will help to identify if site-dependent mortality is driven by water quality issues or due to high levels of sedimentation. Repeating this experiment using this updated design in the current sites will need to be carefully considered due to the potential for suspended lines to interrupt boat traffic.

Cause of death

With 188/282 mortalities unaccounted for, a large number of oysters were not attributed a cause of death. Nine oysters were found to have black lesions typical of shell disease, a disease which has thought to have been present in Essex since 1950 (Cole & Hancock, 1954). Levels of this disease, thought to be caused by the fungus *O. implexa*, have usually remained at a low level, however, with transference thought to increase when water temperatures rise above 22°C for at least 2 weeks, as has clearly occurred in all sites in Essex in 2018, it is likely that this disease may become more prevalent under future climate scenarios with warmer summers predicted to increase in frequency (Alderman, 1985; Beniston *et al.*, 2007).

In addition to shell disease, which leaves clear scars on the shells of deceased oysters, *Bonamia ostreae* is also known to be present within the Essex estuaries (DEFRA, 2016). This parasite causes widescale mortalities of oysters, generally after the first two years of life, however does not leave any clear shell scarring or malformations (Culloty & Mulcahy, 1996). Testing for the presence of *Bonamia* occurs semi-regularly in Essex with the last test completed in May 2018. Oysters positive for this parasite are regularly found in the Blackwater and on Mersea shore, however in 2018 records indicate oysters now positive for *Bonamia* are present for the first time in the Ray Sand (ENORI, 2018). Due to small sample sizes taken to assess *Bonamia* in this area (n=30) confirming disease absence was not possible therefore it may be likely that this parasite has been present on the Ray Sand since it was first detected in Essex in 1982 and not a recent occurrence (Gubbins – personal communication, 2019). Survival rates are highest at the Ray Sand site therefore it is possible that disease prevalence is low, or this population is generally healthy with good conditions meaning increased mortality rates observed in other areas are due to factors largely unrelated to the presence of *Bonamia*. If survival rates do suddenly start to decrease on the Ray Sand, this may imply that a new contamination has occurred in the Essex area and careful consideration should be taken to monitor this. If no mortality is observed in the next 2-3 years this may imply oysters are somewhat immune to *Bonamia*, or experience a latency to infection in this area, and the Ray Sand site may provide an excellent habitat on which to expand restoration efforts (Ronza *et al.*, 2018).

No oysters were found to have clearly suffered predation from drills in this experiment. While oyster shells were found during full census surveys with clear drill evidence, this did not occur in oysters selected for this growth and survival experiment (Personal observation). Previous studies highlight the potential impact various predators may have on oyster populations with starfish and oyster drills previously described as “voracious” predators of the European native oyster (Hancock, 1955; Hancock, 1958; Buhle & Ruesink, 2009). More recent laboratory-based studies investigating functional responses of *Asterias rubens*, *Urosalpinx cinerea* and *Ocenebra erinaceus* have highlighted the readiness of oyster drills of both species to feed on adult oysters (both *C. gigas* and *O. edulis* above 35mm length) with *A. rubens* complexly failing to successfully feed on any oyster. It should be noted that *A. rubens* did feed successfully on barnacles, mussels, cockles and clams (Lown, unpublished data).

Fecundity and proportion of mature females.

While exact fecundity rates were not measured within this study, it is clear that older and larger oysters have higher fecundity rates (Cole, 1941). While this study found a slightly lower percentage of mature brooding females (13.4% vs approximately 15.4%), this was not substantially different, however statistical analysis was not possible due to compare due to lack of clear sample sizes in previous studies. Other studies investigating fecundity rates of healthy populations in the Fal (Cornwall) were found to have at least 50% ripe and functional females at the start of the breeding season (Orton, 1933). While proportion of ripe females observed in June and July 2018 in Essex is well below this, further monitoring of the prevalence of reproductively ripe females is required due to the well documented nature of *O. edulis* tending to have highly variable rates of reproduction between years (Laing *et al.*, 2005). It is not clear if prevalence of ripe females observed in this study is high or low level for this geographic area or how this may change with future climate models and continuation of monitoring prevalence of brooding females may help to predict the level of recruitment expected.

Conclusion

This study has provided a unique data set of highly detailed individual based growth and survival of native oysters in near-natural conditions. This has highlighted the large variation in growth and survival between different areas of the Essex estuaries with survival highest at the Ray Sand and lowest in the Blackwater MCZ site. Large numbers of oyster shells have been attributed to mortality from anoxic smothering, however it remains unclear if sediment load was the cause of death, or if shells were more likely to become buried after oysters have deceased. Proportion of population found to be mature, ripe female were found to be low compared to some historical reports, however further monitoring of how this may change between years with high and low reported spatfalls needs to be assessed. While 2017 was reported to have provided conditions suitable for a large spatfall, the cold winter of March 2018 and hot summer of July 2018 is likely to have impacted recruitment rates with no large spatfall reported from local oyster growers in this time. While higher growth rates have been observed in all public grounds (MCZ) sites with lower growth rates observed in creeks and private layings, consistent differences in survival between MCZ sites and private layings have not been observed. Using these methods, it is now possible to monitor the growth and survival of *O. edulis* in near-natural conditions without the use of dredges, grabs or dives following an initial collection at low cost with this method useful to assess survival of existing oysters *in-situ* or to be used to monitor survival rates in relayed oysters as part of a stock restoration project.

Chapter 5:

Demographic modelling of *Ostrea edulis* in Essex, UK.

5.1. Introduction aims and hypotheses

While challenging it is imperative to be able to make predictions on how animal populations may respond to interventions such as restoration, harvesting or environmental change. The European native oyster, *Ostrea edulis*, has been an important fishery target species in Essex since the Roman times with reported catch rates declining since the industrial revolution (Utting & Spencer, 1992). Occasional monitoring of populations has been undertaken in small areas of the Essex estuaries (Hardy, 2014), and a wide scale survey was completed in 2012 prior to the designation of the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone (BCRC.MCZ) (Allison, 2018). Like many other areas in the UK and Europe there has been much more focus on determining the abundance and extent of remnant populations in more recent years (Chapter 2). However, as yet no attempt has been made to model the population dynamics of this species, with records maintained as counts, CPUE or maps of general distribution. In addition to this, no regular monitoring prior to the designation of the BCRC.MCZ has occurred, meaning any available population data is sparse and only provides a snapshot of evidence to describe the population distribution. Following the designation of the MCZ, it was determined that regular monitoring would be essential to not only assess the native oyster population but also to capture how it is responding to anthropogenic and environmental changes. While such survey-based assessments are necessary – they do not on their own allow prediction of potential changes to a population under any given set of scenarios. For prediction, some form of model or decision analysis tool is required. The overall objective of this chapter is to develop such a model to predict native oyster population dynamics.

When assessing populations of any species, abundance or size structure alone rarely provides enough information to accurately predict any future population change or assess population health, fishery stock

size or resilience to natural or anthropogenic disturbances (de Roos, 2014). It is therefore necessary to include individual life history traits and population structure into any population model in order to provide the most accurate assessment of any population change (Pope *et al.* 2010). Body size, stage and age are all highly influential traits through which to assess the impact an organism may have on its environment, conspecifics or heterospecifics. All three may determine predation risk, food choice, behaviour, reproduction, individual somatic and developmental growth rate, foraging capacity and therefore competition and mortality (de Roos *et al.*, 2003). However, whilst intrinsically linked, it is important to understand body size, stage and age are not the same. Many factors influence each, including but not limited to, genetic differences, resource availability, density, temperature, predation risk and pollutants (For more information see Chapter 1.4).

Types of population model

There is a broad array of population models, each with limitations and data requirements. Initial differences in model types are based on which aspects of the population are being modelled. Individual based models (IBMs) follow every individual within a population to assess individual outcomes and consider individual-specific characteristics and dynamics, with population dynamics a sum of these outcomes. Conversely, distribution based models follow populations and their dynamics via population-level distributional changes (Picard & Liang, 2014). Subsequent differences between model types are largely due to the continuous or discrete nature in which age or size, time and/or reproduction are treated. Examples of these range from matrix based models whereby both time and size or age is discretised, to Ordinary Differential Equations (ODEs) or Physiologically Structured Population Models (PSPMs) where both time, reproduction and size are usually treated as continuous variables (de Roos & Persson, 2013; Picard & Liang, 2014).

Matrix models are a branch of population modelling which use both discrete age or size structuring and discrete time intervals (Caswell, 2000). Models are based on life history variables calculated either from monitoring of individuals within a subset of a population or through population-level distributional

changes (Cassini, 2013). Environmental variation in these models can be easily implemented through the use of multiple matrices which incorporate various scenarios such as switching between years of low or high environment quality (Smallegange & Coulson, 2013), high mortality or variable levels of reproduction. Matrices can be cycled through a set order (periodic i.e. seasons) or drawn randomly from a set of potential scenarios (stochastic environment) (Caswell & Trevisan, 1994).

Similar to matrix models, Integral Projection Models (IPMs) also use discretised time, calculating dynamics of, and changes in, populations via time segments. Time segments can be set to any unit, e.g. 1 day, 1 month or 1 year, depending on the lifespan of the species under investigation. However, IPMs use a series of regression equations to parameterise growth, survival and reproduction rates to incorporate individual based variation within the growth transition part of the model, a pitfall of matrix models which assume no variation between individuals within the same size and time category (Picard & Liang, 2014). IPMs are centred around an integral equation called a kernel. This kernel describes changes of state of individuals from one timestep to another. IPMs can be age or size based or both, or based on another level of discrete or continuous categorisation that changes throughout an individual's life, such as the number of methyl groups in DNA in whales (Merow *et al.*, 2014; Polanowski *et al.*, 2014). This means IPMs are useful in situations where abundance estimates from census data are based on discrete timesteps whereas data has been collected as a continuous distribution of life history information such as annual growth rates and survival.

Stochasticity

Simple size structured population models will only account for juvenile growth and mortality and adult reproduction and mortality assuming constant rates for all of these processes. These types of model are limited in use and do not allow for fluctuations in environmental variables and subsequent effects on populations. Without stochastic variation, populations may appear to be able to maintain themselves or even grow, however once fluctuations in growth, reproduction and survival are included within the model, populations may appear to go extinct (Lande *et al.*, 2003). Drivers in oyster population dynamics

such as spatfall are well known to be highly variable and unpredictable in many areas, with recruitment success seemingly driven by a wide range of environmental factors (Cano *et al.*, 1997). Incorporation of processes such as variation in spatfall is therefore essential to understand the long-term status of oyster populations.

Oyster population management

Populations undergo three main processes influencing demographic change. These are growth, survival and reproduction. In laboratory-based studies it is possible to easily manipulate growth rates of oysters through the use of different food types, water quality and temperature regimes, however this is not easily implemented at scale in the field environment (Laing & Millican, 1986). This results in two main directions of restoring native oysters without physically introducing more oysters from an external population. These are through boosting recruitment and increasing adult survival. Boosting recruitment can be achieved through cultch management such as laying down shell during the breeding season to ensure suitable settlement substrate is available for spat (Smyth *et al.*, 2018). Alternatively, spat survival can be boosted by using collectors suspended in the water column, such as coupelles or Chinese hats coated in lime (Syvret & Woolmer, 2015). Once spat have been collected they can then be grown on to a larger size, raised off the sea floor, either kept on the collectors or transferred to mesh bags until they spatting ponds oyster restoration can be distributed onto the sea bed (Buestel *et al.*, 2009). If recruitment appears to be limited by successful fertilisation of brooding female oysters, populations may be undergoing an Allee effect whereby the density of oysters is too low for successful fertilisation. It may therefore be possible to move oysters closer together to promote successful fertilisation with oysters found to brood significantly more larvae when their nearest neighbour is less than 1.5 m away (Guy *et al.*, 2018). Alternatively, successful recruitment may be boosted through the use of spatting ponds where a broodstock of adults are maintained in controlled ponds where spat may be recovered from collectors with oysters redistributed to sites once they reach 1 year old. This technique may result in population bottle necks and so it is necessary to maintain these cultures at large scales (Lallias *et al.*, 2010). In addition, the use of spatting ponds may be more costly than laying cultch or setting spat collectors and

so may only be suitable for localised restoration projects, with success also reported to be highly variable (Laing *et al.*, 2006).

Secondly, oyster populations can be restored through boosting adult survival to maintain a large broodstock. Whilst the majority of research has been completed in assessing how to maximise spat success, a limited number of studies have looked at maximising adult oyster survival. A primary cause of adult mortality (outside of fishing induced mortality in harvested populations) is that of disease (Laing *et al.*, 2005). The current primary disease of concern for *O. edulis* is thought to be the haplosporidian parasite, *Bonamia* subsequently causing the disease bonamiosis. This said to be “the greatest biological factor limiting stock restoration” (Laing *et al.*, 2005). Mortalities from bonamiosis generally occur after the first 2 years of age with the spread of the disease thought to be linked to reproductive processes and stress (Robert *et al.*, 1991; Lallias *et al.*, 2008; Martín-Gómez *et al.*, 2012). Whilst no clear cure has yet been developed, there are a number of potential management interventions possible to limit the impact and spread. The impacts of bonamiosis is thought to be reduced by ensuring native oyster density remains at relatively low levels (Doonan *et al.* 1999) or through cultivating oysters at the surface (in 1-2m depth) rather than in slightly deeper waters (8-9m) (Lama & Montre, 1993). In addition to disease management, adult physiological performance may be boosted through the creation of a caged broodstock where oysters are suspended above the sea floor, away from the impacts of smothering from sedimentation and other predators, this in turn may increase growth and survival rates in adult oysters (Sawusdee *et al.* 2015). Attempts at this are currently being trailed in the Solent where adult oysters are maintained in cages suspended below pontoons (Harding *et al.*, 2016).

Aims and hypothesis

The aim of this chapter is to make long term population projections for a population of which we now have age and size-based life-history traits (survival, growth and reproduction: Chapter 4 and full census data: Chapter 1). I therefore develop a model that can use this life history information and make predictions in population change. As this model is currently designed to be a work in progress, with

new and developing data planned to be added over time, simple models such as matrix models, IPMs or biomass-based fishery models are deemed most appropriate for flexible use by stakeholders. In addition, this chapter aims to identify which life stages are most influential to population growth and recovery and assess predicted effects of restoration management techniques.

As with many, particularly long-lived, invertebrate species, it is hypothesised that *O. edulis* populations in Essex will be highly sensitive to reproduction success and juvenile survival with the potential for the population to be sustained through infrequent, large pulses of spatfall.

5.2. Method

Due to available data being based on discrete steps between abundance estimates and a continuous distribution of life history information, size (area of oyster mm²) based IPMs were used to describe the population of native oysters in Essex, UK. Models were used to make projections of this population in future years and to assess sensitivity of population growth to variation in abundance of life history stages transitions of *O. edulis* (e.g. survival, recruitment etc.) in order to guide management and restoration. For the first, most basic model, all oysters which were not lost within a year of the string-based growth and survival experiment (Chapter 4) were used to estimate survival and growth coefficients, with all oysters alive for June measurements used to estimate reproduction coefficients. Information regarding the size distribution of oysters on the growth and survival strings and string site placements can be found in Chapter 4 with information regarding the census populations in Chapter 2.

Whilst it is recognised that some strings of oysters were deployed prior to the oyster breeding season in March 2017 and others in September 2017, due access to oysters only being available during census surveys and due to difficulties in incorporating a staggered design within the model or reducing the sample size of the data available to use, it was assumed that all oysters were first measured in September. This was classified as a post-reproductive census with oysters deemed able to reproduce if they were alive for the following June measurement. It is understood that this is not ideal, however, access to oysters within the MCZ restricted deployment times. Growth and survival rates were measured

through the use of the novel *in-situ* experiment described in Chapter 4, to parameterize models based at 7 sites throughout the Essex Estuaries: Colne (Pyefleet), Colne Raft, Brightlingsea Creek, Blackwater Several Order (hereafter Several Order), Blackwater MCZ (hereafter Blackwater), Ray Sand and Crouch. Sites within the Blackwater, Crouch, Roach and Colne Marine conservation Zone are the Ray Sand, Blackwater and Crouch sites with all other sites on Several Order grounds.

The integral kernel used to create the IPM and map the size distribution at time t to the distribution at $t+1$ (1 year later) was taken from (Merow *et al.*, 2014) where:

$$N_{t+1}(z') = \int_{\Omega} K(z', z)n(z)dz \quad [\text{Equation 5.1}]$$

$$N_{t+1}(z') = \int_{\Omega} [P(z'z) + F(z'z)]n(z)dz \quad [\text{Equation 5.2}]$$

Where z is the area in mm^2 of the oyster at time t , z' is the area in mm^2 at time $t+1$. $n(z)$ is the size distribution of the population at time t with Ω denoting the possible range of sizes of the population. K is the full kernel, comprised of P and F . P is the growth and survival kernel calculated to be:

$$P(z', z) = s(z)g(z'|z) \quad [\text{Equation 5.3}]$$

Where $s(z)$ is the area based annual survival of an oyster from time t to time $t+1$ and $g(z'|z)$ describes the probability density of size z' that an individual of area z can grow a single time step conditioned on it having survived and $F(z', z)$ is the fecundity kernel where:

$$F(z', z) = p_{rep}(z)f_{eggs}(z)P_{estab}R \quad [\text{Equation 5.4}]$$

With $p_{rep}(z)$ the size-based probability of reproducing, $f_{eggs}(z)$ is the size-based fecundity, P_{estab} the probability of an egg establishing and surviving 1 year and R the size distribution of 1-year old recruits (Merow *et al.*, 2014). Fecundity and recruitment have therefore been modelled as a single population with no external recruitment from outside of the MCZ. As with many plants and broadcast spawning invertebrates, mortality of oysters through loss of larvae and spat mortality is unknown therefore the use of an establishment probability was deemed necessary to bridge the gap between the measured size-based fecundity data and subsequent settled oyster survival. Establishment probability was calculated as:

$$p_{estab} = \frac{N_r}{N_e} \quad [\text{Equation 5.5}]$$

Where N_r is the number of new recruits alive in June on the string experiment and N_e is the sum number of eggs estimated through size-based fecundity data from oysters in the string experiment. An oyster was deemed able to reproduce if it was within the study and alive for the summer measurement (June/July) and measured over 40mm length. Length was used to estimate fecundity values due to egg estimates only being available based on length measurements with no area measurements available (Cole, 1941). All oysters of reproductive size were assumed to reproduce however individual fecundity estimated as 13.4% of the size-based fecundity shown in Equation 4.7 (Chapter 4). This was both to incorporate size-based variation in fecundity but also include the sex ratio of 13.4% ripe females. As no data is currently available regarding likelihood of an individual oyster reproducing twice in one year or measured speeds of transition between male and female and vice versa in Essex, all oysters were assumed to reproduce once per year. The simple incorporation of the sex ratio in this way also enables this to be easily changed in future stakeholder use of this model. In addition to recording newly settled recruits on to string experiment annual establishment probability was estimated from census data for 2015-2018 using:

$$P_{estab2} = \frac{J_{t+1}T_{t+1}}{A_tT_tf_{eggst}} * 2.95 \quad [\text{Equation 5.6}]$$

Where J_{t+1} is the ratio of the measured population under 30mm length at time $t+1$, T_{t+1} is the total estimated population calculated through inverse distance weighting (Chapter 2). A_t is the ratio of the measured population 40mm length or greater at time t with T_t the total estimated population at time t and f_{eggst} the size-based fecundity for the average length adult at time t , incorporating a 13.4% reduction to individual fecundity estimates due to sex ratios described previously. This figure was then multiplied by 2.95 to standardise the measured establishment probability from the dredge surveys to the measured establishment on the strings (i.e. the estimated dredge survey establishment appeared 2.95 times smaller than the string-based establishment). This difference was attributed to decreased detection of smaller oysters during the dredge surveys due to the influence of increased mud and shell within dredge samples, potential damage to smaller oysters from dredge impacts and impact of smaller oysters

being more likely to slip through the dredge ring size of 40mm if the oyster was not attached to a large enough shell.

To estimate growth, survival and fecundity coefficients, vital rates were first plotted, and regression lines fitted to assess trends in size-based growth, survival and fecundity. Oysters were deemed to have been recruited into the population when they reach 1 year old with all oysters assumed to have been born in June. As newly settled oysters were recorded during the growth and survival string experiment, the number alive for a June measurement was low ($n=9$), the size distribution of recruits was extracted from estimated size at age calculations and set to mean=1000 mm² with standard deviation=500 mm². Following the initial plotting of vital rates, survival coefficients were calculated through the use of GLM regressions with a binomial distribution (i.e. alive/dead), growth coefficients calculated using linear models and fecundity calculated using GLM regressions with a Poisson distribution. EP_{st} . Methods were all repeated for each site individual to gain insight into site-based variation of coefficients. Due to small recruitment sample sizes, the same mean and standard deviation for recruit size was required for all sites with P_{estab} maintained at site level measurements (i.e. if no recruits were observed at that site $P_{estab} = 0$). All site-based models were also re-run with P_{estab} =average establishment for all sites to assess how small, measured differences in P_{estab} might change population growth rate (λ).

All coefficients calculated from string data and sample sizes used to calculate each metric are shown in Table 5.1. The midpoint rule using 91 bins was used to approximate the integral operators between sizes 25.4469 mm² and 9981.939 mm² in area with lambda or population growth rate (λ) calculated from the first eigenvector of K (the full IPM kernel) (Merow *et al.*, 2014). Calculations first performed for, all sites combined and then repeated for each string site individually.

To calculate the parameters used to make predictions at the scale of the entire MCZ, it is recognised that not all sub- populations are split evenly across each oyster site and different sites had different numbers of lost oysters, therefore a weighted average of coefficients based on the proportions present at post-summer 2017 sub- populations, extracted from IDW calculations in Chapter 2, were used to assign a weighted average to the MCZ. This resulted in MCZ coefficients being calculated as:

$$MCZ_{coeff} = Blackwater * 0.179258 + Crouch * 0.526499 + Raysand * 0.294243$$

All further population analysis has been completed for the weighted analysis of MCZ sites only, unless otherwise stated, to maximise sample sizes and assess best practice for restoration for the BCRC.MCZ as a whole.

Population summary plots

The kernel of the model was graphically represented using a heat plot to show the primary transitions of the IPM in R studio (Figure 5.2; R studio team, 2018). In addition, the reproductive value (i.e. the left eigen vector corresponding to the dominant eigenvalue of the kernel and is the measure of how many offspring an individual will have overall was assessed and plotted as described by Easterling *et al.*, 2000) is shown. This was to assign a size-based value to an oyster depending on the number of decedents it has, incorporating size dependent survival of those dependents to age 1 (i.e. when they are recruited into the population). This allowed visualisation of the size dependent contribution to population growth through reproduction. Conversely the right eigen vector corresponding to the dominant eigenvalue of the kernel represents the stable size distribution of the population and was also plotted (Figure 5.2; Easterling *et al.*, 2000).

Sensitivity and elasticity of λ (proportional sensitivity) for the weighted average MCZ model was calculated to determine how small changes to the growth, survival and fecundity of individuals lead to proportional changes in λ . These show the relative importance of transitions between sizes and fecundity rates and highlight which vital rates contribute most to changes in λ (Ellner *et al.*, 2016).

Model Validation

To assess model accuracy for the use in management of the BCRC.MCZ, the MCZ weighted average model (Table 5.1), was run forward for 1 timestep (1 year) using the size distribution of all oysters measured in the 2017 post-summer (September) dredge survey across the BCRC.MCZ (n0). The estimated density distribution of oysters extracted from the model was then compared to the measured

density distribution of oysters observed 1 year later on the post-summer 2018 dredge survey incorporating 95% confidence intervals calculated through 999 bootstrap permutations. Bootstrap permutation bins were maintained at equal value to kernel bins to maintain consistency (i.e. the boundary points are the same as those specified in the kernel ranging from 14.137 mm² to 9981.939 mm²). Two-sample Kolmogorov-Smirnov tests were used to statistically compare fit of the mean bootstrapped post-summer 2018 distribution to the extracted estimated IPM distribution.

In addition, to check accuracy of λ , population abundance estimates for post-summer 2018 were estimated using the total population of *O. edulis* estimated using Inverse Distance Weighting and dredge survey data from post-summer 2017 (Chapter 1) and multiplying by λ . The new population estimate was then compared to the abundance estimated from dredge surveys in post-summer 2018 using IDW techniques. Model accuracy check using the post-summer 2017 population multiplied by λ was also repeated for each MCZ area individually to highlight variation in model accuracy by oyster bed.

Stochastic iterations and population dynamic projections

Once the model was established using vital rate functions, the IPM kernels were sampled and iterated through a stochastic environment for 50 years using different years of establishment probability selected at random from P_{estab2} (Equation 5.6) for the whole MCZ combined and each site within the MCZ individually. λ_s was then calculated as described in (Metcalf *et al.*, 2015) using:

$$\log \lambda_s = \lim_{t \rightarrow \infty} \frac{1}{t} \log ||K_t \dots K_0 n_0|| \text{ [Equation 5.7]}$$

With $||\bullet||$ denoting total population size calculated in each year, as also used in (Metcalf *et al.*, 2015) with the population size distribution measured in the September 2017 survey used as the starting population distribution from dredge survey data (n_0). Models were replicated for 400 runs over 50 years with the mean log population size plotted over time. Extracted populations were multiplied by 8982.528 for the weighted MCZ model, 12354.372 for the Blackwater, 8425.84 for the Ray Sand and 6384.31 for the Crouch (Estimated post-summer 2017 population size through IDW calculations at 20% dredge

efficiency divided by the real number of measured oysters in that area in the post-summer 2017 dredge survey: Chapter 2) to give the estimated scaled up total population estimates.

5.3 Results

Vital rates (growth, survival, fecundity and recruit size distribution) are shown in Figure 5.1. Larger oysters are more likely to survive, and smaller oysters tend to grow more than larger oysters over the course of 1 year.

Population summary plots including the kernel used in the IPM, stable size distribution for current parameters, reproductive values and elasticity and sensitivity plots are shown in Figure 5.2 for the MCZ only sites combined by a weighted average of the Blackwater, Crouch and Ray Sand parameters. Individual site-based population summary plots for MCZ sites can be found in Appendix IV, Figures S5.1-S5.3. Kernel plots highlight growth transitions along the $x=y$ axis and reproductive transitions in the lower right-hand segment of the kernel plot (Figure 5.2).

Lambda (λ) values for the model using a a weighted average of all sub-sites combined and for each sub-site individually are shown in Table 5.1. Overall $\lambda = 0.629$ for all sites combined and $\lambda = 0.777$ for all MCZ sites only using coefficients combined under a weighted average, predicting population declines.

Sub-site-based models predict the Ray Sand area to have the highest lambda values at with $\lambda = 0.842$ and the Blackwater MCZ site the lowest at with $\lambda = 0.263$. In addition, site-based models were all re run with $P_{estab} = 4.40E-07$ (the establishment probability value for all sites combined from the string experiment). This resulted in small changes in λ , particularly for Brightlingsea creek where λ rose from 0.507 to 0.534 when incorporating the MCZ wide establishment probability. Conversely λ for the Several Order site in the Blackwater decreased from 0.436 to 0.323 when incorporating the MCZ-wide P_{estab} value.

Table 5.1. Values of coefficients of vital rates used within IPM calculated from growth and survival data by oyster AREA in Essex 2017-2018 (Chapter 4) and size-based fecundity data from Cole (1941).

Coefficient		All sites	Black-water	Brightlin-gsea	Colne	Colne Raft	Crouch	Ray Sand	Several Order	MCZ weighted average
Survival intercept	SI	-8.86E-01	-1.35E+00	8.77E-01	-2.50E+00	-4.84E-01	-7.25E-01	-3.19E-01	5.65E-01	-7.18E-01
Survival slope	SS	1.79E-04	-1.63E-05	-2.70E-04	4.49E-04	-1.03E-04	1.75E-04	5.99E-04	-4.69E-04	2.66E-04
Growth intercept	GI	1.04E+03	1.28E+03	7.73E+02	2.83E+03	1.44E+03	1.14E+03	1.15E+03	2.11E+03	1.17E+03
Growth slope	GS	8.84E-01	8.17E-01	8.83E-01	4.32E-01	6.70E-01	7.91E-01	9.21E-01	5.80E-01	8.34E-01
Growth standard deviation	GSD	5.65E+02	4.16E+02	2.88E+02	4.24E+02	6.11E+02	4.95E+02	6.11E+02	6.05E+02	5.15E+02
Fecundity intercept	FI	9.99E+00	9.46E+00	1.01E+01	1.05E+01	1.03E+01	9.77E+00	1.01E+01	9.82E+00	9.80E+00
Fecundity slope	FS	3.83E-04	5.51E-04	3.70E-04	2.56E-04	3.04E-04	4.35E-04	3.62E-04	4.74E-04	4.34E-04
Recruit size mean	RM	1.00E+03	1.00E+03	1.00E+03	1.00E+03	1.00E+03	1.00E+03	1.00E+03	1.00E+03	1.00E+03
Recruit size standard deviation	RMSD	5.00E+02	5.00E+02	5.00E+02	5.00E+02	5.00E+02	5.00E+02	5.00E+02	5.00E+02	5.00E+02
Establishment (string)	P_{estab}	4.08E-07	8.45E-07	2.85E-07	0.00E+00	0.00E+00	4.78E-07	1.26E-07	1.31E-06	4.40E-07
2018 Establishment	$P_{estab2.18}$	1.27E-07	1.35E-06	NA	NA	NA	5.18E-07	2.49E-07	NA	4.40E-07
2017 Establishment	$P_{estab2.17}$	9.49E-08	0	NA	NA	NA	2.24E-07	4.00E-07	NA	3.78E-07
2016 Establishment	$P_{estab2.16}$	4.38E-07	7.80E-06	NA	NA	NA	4.50E-07	1.03E-06	NA	1.95E-06
2015 Establishment	$P_{estab2.15}$	1.94E-06	2.27E-05	NA	NA	NA	9.97E-07	9.034E-06	NA	8.91E-06
Lambda using individual site recruit size and establishment prob		6.28E-01	2.63E-01	5.07E-01	3.54E-01	2.84E-01	5.89E-01	8.42E-01	4.36E-01	7.77E-01
Lambda using Weighted MCZ Establishment prob		6.29E-01	2.35E-01	5.34E-01	3.54E-01	3.26E-01	5.77E-01	9.29E-01	3.23E-01	7.77E-01
Sample size	All sites		Black-water	Brightlin-gsea	Colne	Colne Raft	Crouch	Ray Sand	Several Order	MCZ only
Growth	166		16	31	16	7	33	50	13	94
Survival	378		82	59	55	37	41	64	40	163
Reproduction *	298		48	46	36	11	62	76	19	141
Recruit	9		2	1	0	0	2	1	1	3
Lost (not dead) before	187		40	13	5	23	45	39	22	74

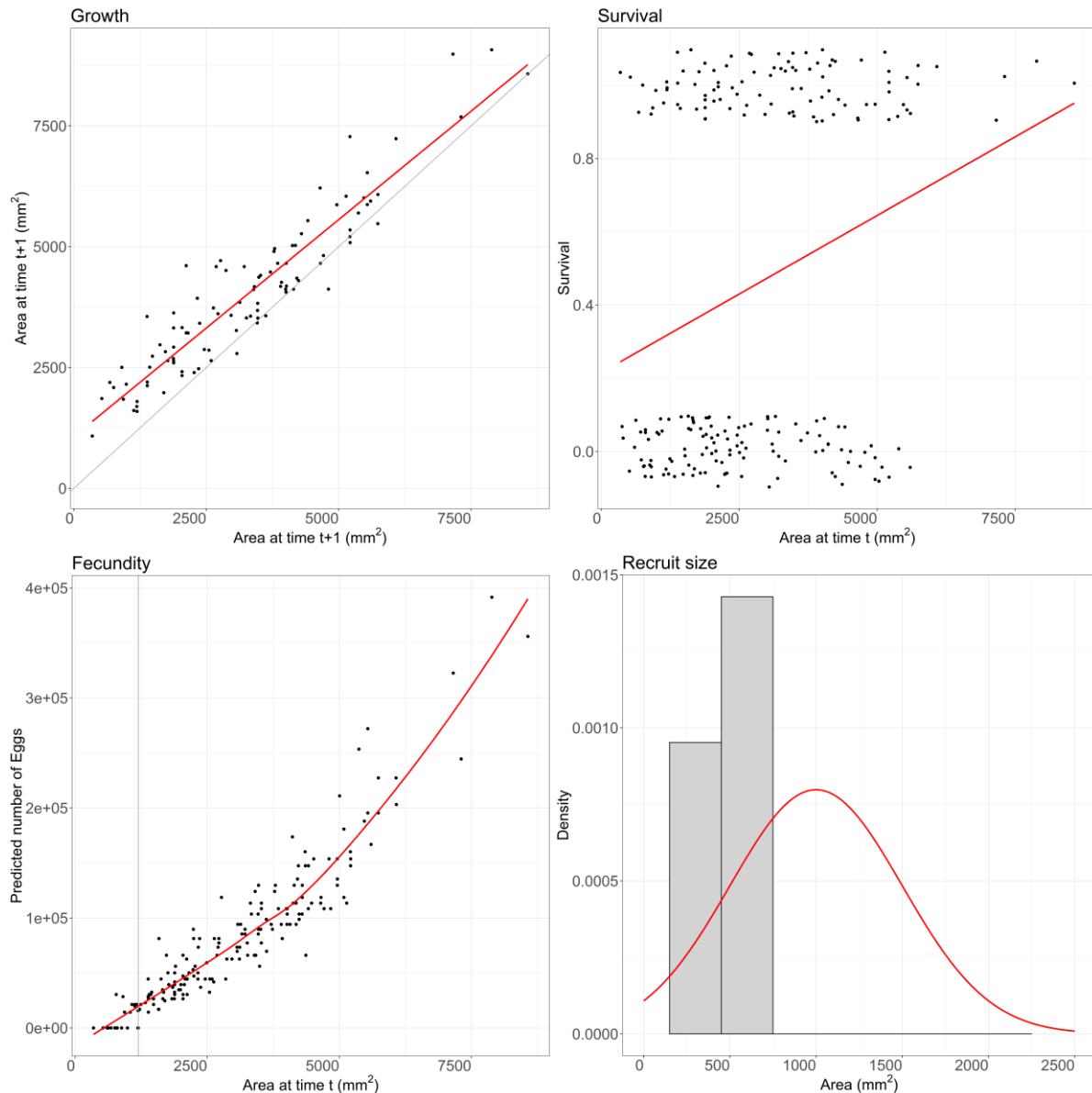


Figure 5.1. Vital rates plots for Integral Projection Model based on data collected from growth and survival experiment described in Chapter 4 from sites in the BCRC.MCZ area only. No sites within private oyster grounds have been included.

Growth shows area (mm²) of *O. edulis* oysters at time t and area (mm²) 1 year later. The light grey line denotes a $x=y$ line where no growth has occurred.

Survival shows size-based survival and binomial regression line. Data has been jittered for ease of interpretation.

Fecundity highlights the size-based fecundity estimated from summer length measurements after time t but before $t+1$ against size at time t with the vertical line highlighting 40mm length, below which oysters were deemed unable to reproduce within the IPM model.

Recruit size shows a density plot with fitted normal distribution mean=1000mm² sd=500mm².

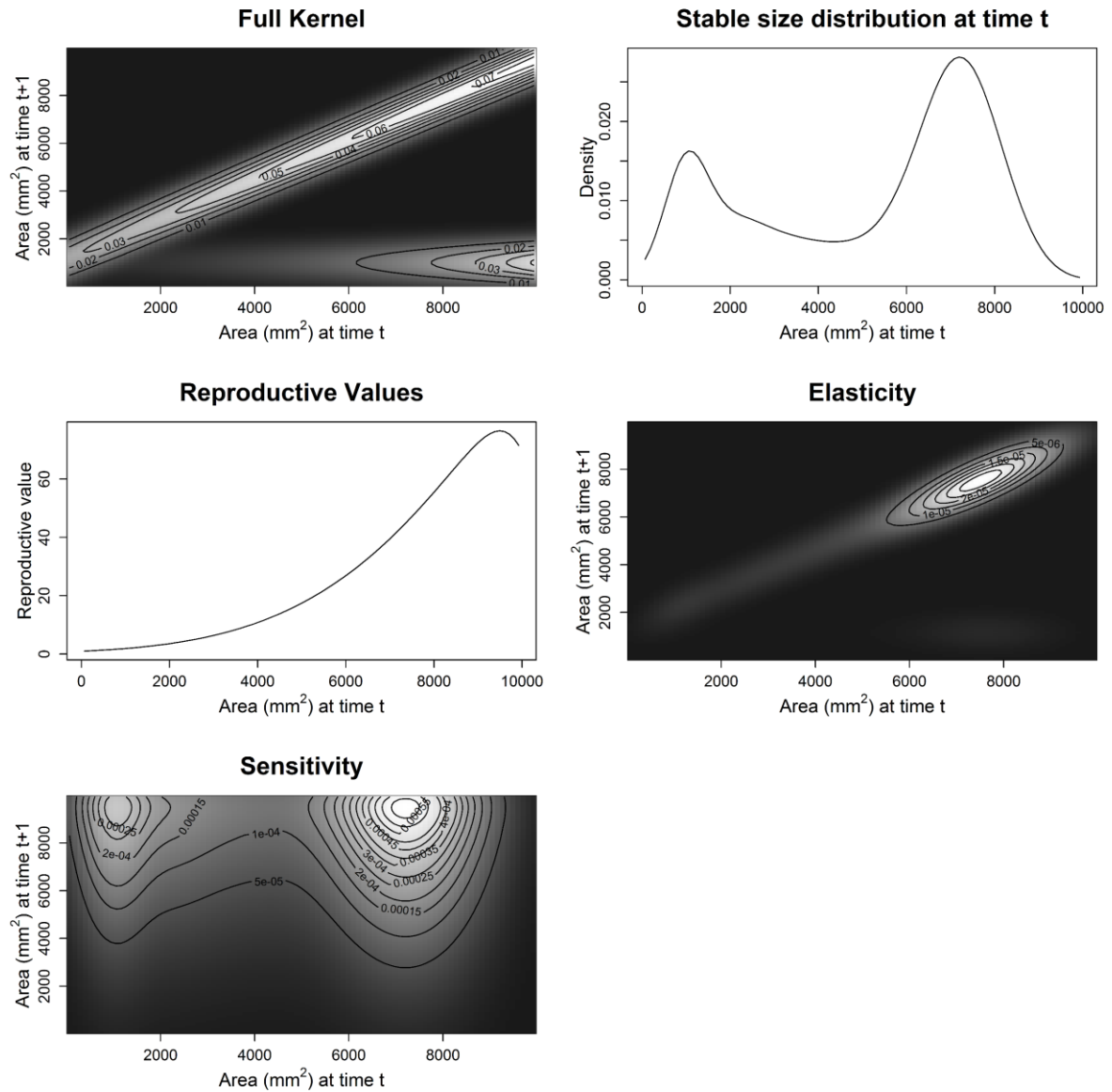


Figure 5.2. Population summary plots model output from a size structured IPM by area (mm²) for *Ostrea edulis* for all MCZ sites combined by weighted average of coefficient where $\lambda = 0.7772$.

Model validation

The estimated total population size for all beds in the BCRC.MCZ was estimated to be 4,455,334 oysters in the post-summer 2017 dredge survey (Chapter 1). Implementing the extracted MCZ IPM model population growth rate $\lambda = 0.7772$ would therefore result in an estimated population of 3,462,845 oysters in post-summer 2018, this is arguably close to the 3,479,060 estimated from measured dredge data at 20% dredge efficiency and IDW calculations in post-summer 2018 from dredge survey data (6,954,811-2,318,271 upper and lower bounds based on 10 and 30% dredge efficiency). The predicted distribution and measured distribution with 95% bootstrapped confidence intervals using 999 permutations for the measured data of oysters in the post-summer 2018 survey is shown in Figure 5.3. The predicted distribution extracted from the IPM model slightly underestimates the number of 1 year old spat however remains within the 95% confidence intervals of the data, and accurately estimates the mid to small size oysters however over estimates the proportion of larger oysters in the population with the model density curve lying outside of the 95% confidence intervals for oysters above oysters approximately 5000mm².

As dredge surveys are potentially damaging to oysters by knocking off new shell growth, with the string-based experiment not encountering this variable, it was found a 2.5% increase in oyster width and length could be added to the measured population of the post-summer 2018 survey with 95% bootstrapped confidence intervals with this density distribution also plotted and compared to the IPM model output distribution. This 2.5% increase in size is the equivalent of a 50mm length oyster losing 1.25mm on the length of the shell due to dredge damage. The adjusted distribution of “undamaged” dredged oysters fits the model prediction well, only falling outside of 95% confidence intervals between approximately 8000 and 8500mm² (Figure 5.3). There was no statistical difference in the density distribution extracted from the IPM prediction and the measured density distribution from the post-summer 2018 survey (KS-test, $D=0.15385$, $P= 0.2325$) with no difference also between the adjusted distribution and the IPM prediction (KS-test, $D = 0.14286$, $P = 0.3123$).

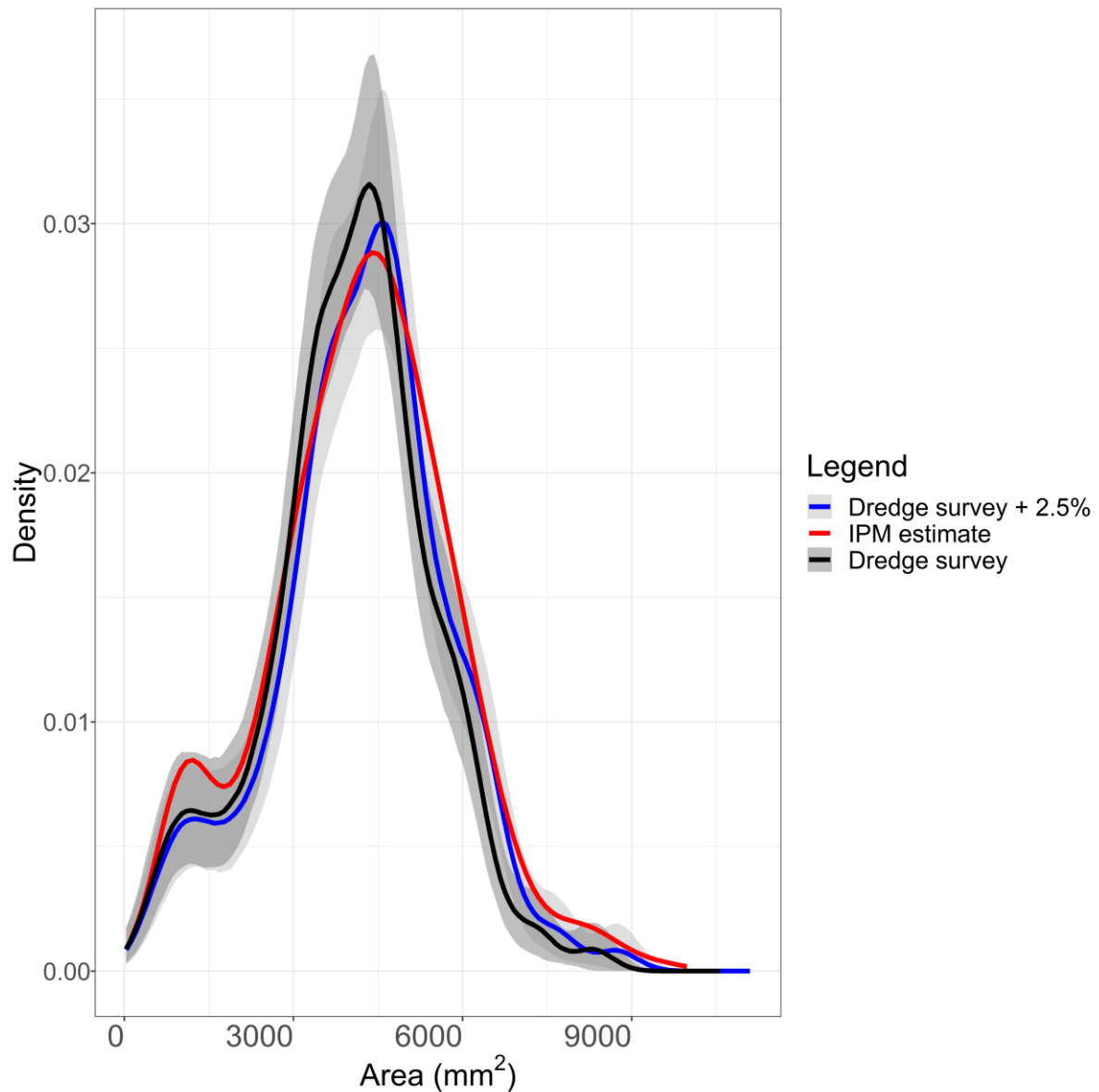


Figure 5.3. Density plot for *Ostrea edulis* for all MCZ sites combined from the measured size distribution of oysters from dredge survey data in the post-summer 2018 survey (black), this same measured distribution of oysters in the post-summer 2018 with a 2.5% increase in length and width to replicate dredge damage (blue), and the predicted size distribution of oysters extracted from the MCZ site only IPM where $\lambda = 0.7772$ (red). Shaded regions represent 95% confidence intervals of the measured dredge survey data.

Understanding the consequences of increased oyster recruitment: establishment probability

P_{estab} was changed incrementally for each MCZ site and all MCZ sites combined under a weighted average to identify what level of establishment of new recruits is required to improve population growth to $\lambda = 1$. In the current conditions between 2017 and 2018 for $\lambda = 1$ on the Ray Sand, P_{estab} needs increase by 400% compared to settlement observed in 2018 to $= 1.00E-06$ (i.e. the current establishment is 25% of the required level to maintain the population). The Crouch establishment probability is currently $5.18E-07$ which is 3.5% of the required establishment for $\lambda = 1$ with the required P_{estab} for the Crouch $= 1.65E-05$. The current measured establishment on the Blackwater is $1.35E-06$ which is 4.4% of the required establishment to maintain the population. For $\lambda = 1$, the Blackwater requires an establishment probability of $3.05E-05$. The highest observed establishment in the Blackwater occurred in 2015 which was 74.5% of the level of establishment required to sustain the population in the Blackwater area given current rates of growth and survival (or $2.27E-05$). For all MCZ sites combined under a weighted average, currently the establishment probability is measured at $4.40E-07$ or 4% of the required level to maintain the population. To maintain the whole MCZ the required P_{estab} needs to be $1.07E-05$ given current measured levels of growth and survival. The highest level of establishment observed for the whole MCZ combined was $8.91E-06$ in 2015 which is 83.3% of the required establishment to maintain $\lambda = 1$.

Curves to show impact of changing P_{estab} on λ for each of these sites can be found in Figure 5.4 with very low P_{estab} values resulting in lower than linearly predicted λ . Based on estimated egg abundances in the post-summer 2017 survey (Appendix IV, Table S5.1), this equated to a required spatfall of 3.89 million oysters surviving 1 year throughout the whole of the MCZ to sustain the population. Site based recruitment to sustain sub-sites are calculated to be 115,487 on the Ray Sand, 974,298 in the Blackwater and 2.9 million oysters in the Crouch. The sum of these numbers result in approximately 4 million recruits required due to rounding during inverse distance weighting calculations resulting in slight differences in total population calculations. The estimated number of spat at each site and the required number of additional recruits required to reach these levels of recruitment are summarised in Table 5.2. The approximate number of coupelles required to collect this number of spat was calculated and shown

in Table 5.2. Spat were assumed to be collected at approximately 326 spat m⁻² (Buitrago & Alvarado 2005) with the area of a single stack of 45 coupelle discs (approximately 1.5m² assuming both sides of the coupelle are available for settlement, with a diameter of 14.6cm²) (Go Deep Shellfish Aqua, 2019). Whilst it is recognised that previous spat settlement density trials to assess spat settlement density of collectors were performed with *Crassostrea rhizophorae*, these calculations are deemed to be approximations only.

Population summary plots highlighting the kernel used in the IPM, stable size distribution for current parameters, reproductive values and elasticity and sensitivity plots when $\lambda=1$ are shown in Figure 5.5 for the MCZ weighted average after changing P_{estab} to 1.07E-05. With high levels of recruitment to maintain $\lambda=1$, the population is proportionally sensitive (elastic) to changes in the survival and growth of smaller oysters with sensitivity analysis highlighting the sensitivity of lambda to rare transitions of fast-growing recruits (Ellner *et al.* 2016). The kernel itself is largely driven by recruitment and this is highlighted in the lower right area of the kernel plot.

Table 5.2. Required numbers of additional 1-year old oysters required in each year to sustain each population within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone. Calculations are based on site dependent establishment probability requirements and the estimated number of eggs produced by oysters in that area 2017 based on 13.4% of the population reproducing with length-based estimates of fecundity extracted from Cole (1949). Bold indicates natural spatfall was high enough to maintain population.

Site	Required settlement	Year	Observed settlement (estimated)	Additional number required	Approximate number of 45-disc coupelles
All (weighted)	3890000	2018	160262	3729738	7627.276
		2017	105927	3784073	7738.391
		2016	525636	3364364	6880.089
		2015	1475350	2414650	4937.935
Blackwater	924298	2018	43271	881027	1801.691
		2017	0	924298	1890.18
		2016	351806	572492	1170.741
		2015	536213	388085	793.6289
Ray Sand	115487	2018	28851	86636	177.1695
		2017	49681	65806	134.5723
		2016	152535	-37048	-75.7632
		2015	396299	-280812	-574.257
Crouch	2900000	2018	92514	2807486	5741.28
		2017	21764	2878236	5885.963
		2016	64301	2835699	5798.976
		2015	89333	2810667	5747.786

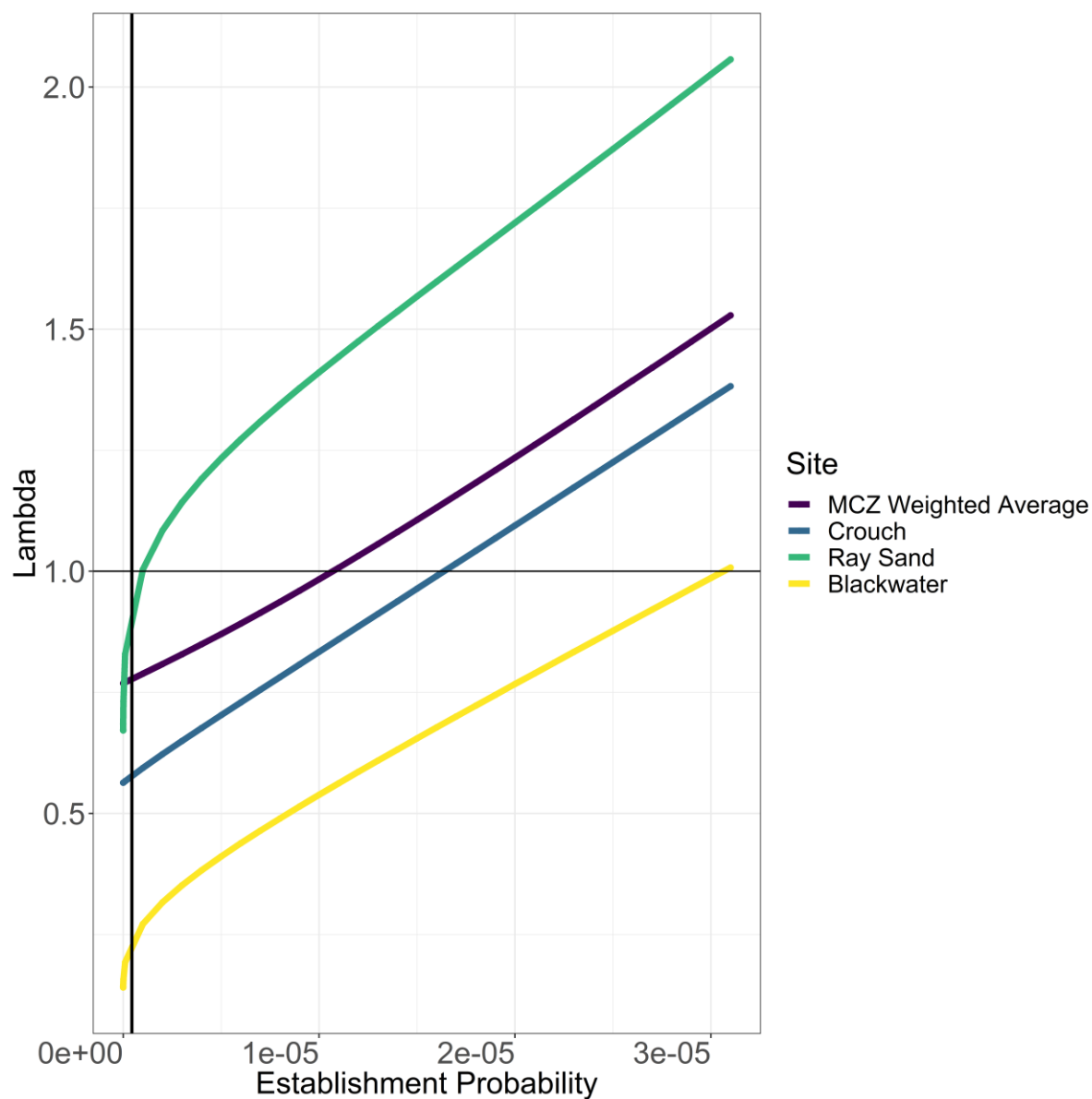


Figure 5.4. Levels of establishment probability required for λ to equal 1 (i.e. the population is not growing or shrinking) using measured conditions between 2017 and 2018 for MCZ sites combined as a weighted average and each MCZ site individually. The vertical grey line represents the current measured establishment probability for the whole of the MCZ area of 4.40E-7.

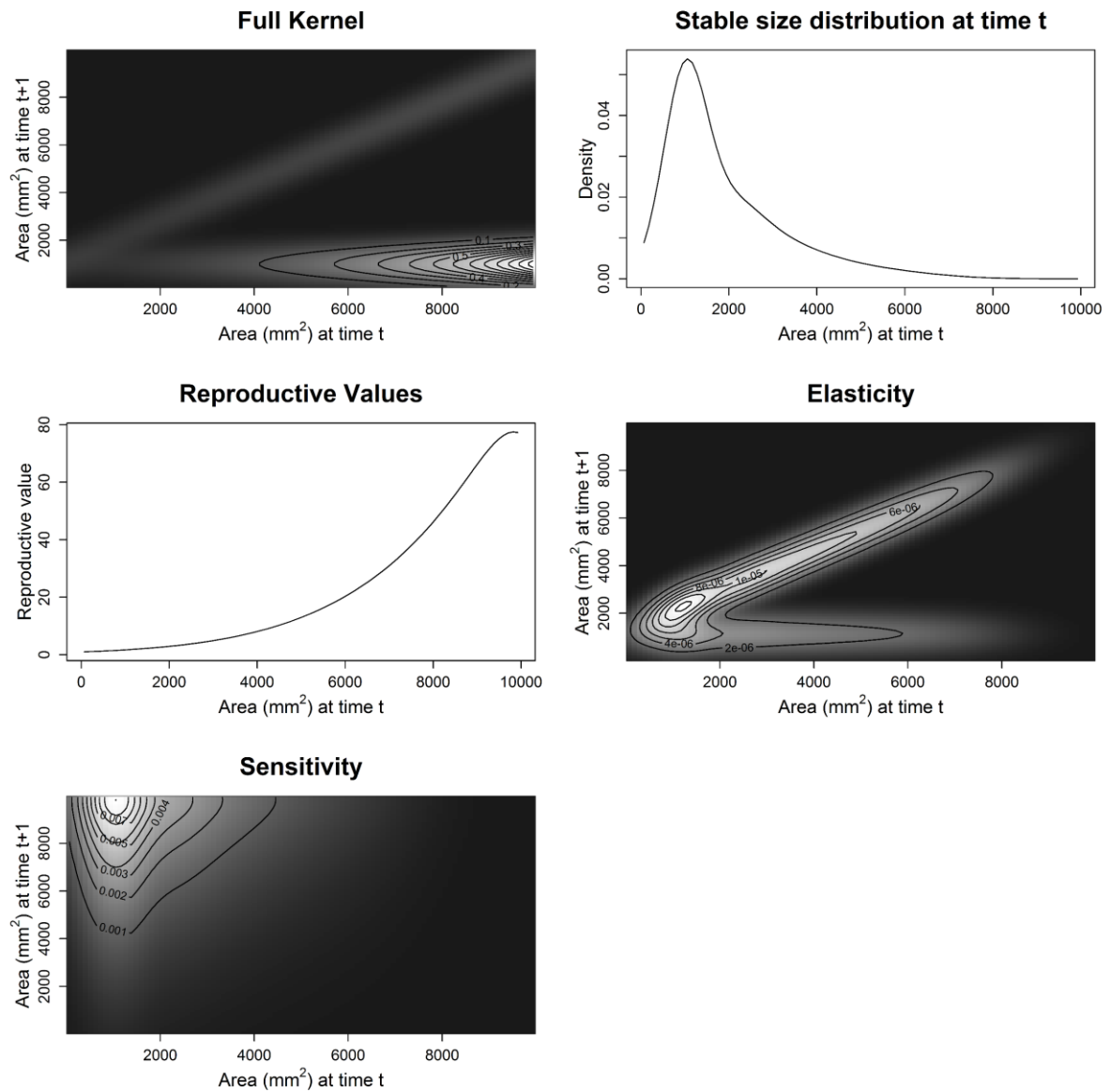


Figure 5.5. Population summary plots model output from a size structured IPM by area (mm²) for *Ostrea edulis* for all MCZ sites combined where $\lambda = 1$ through altering P_{estab} to 1.07E-05.

Understanding the consequences of restoration to improve oyster survival: changing survival slope

The survival slope coefficient was changed incrementally for each site within the MCZ and for all MCZ sites combine to identify what level of adult survival is required for $\lambda = 1$ given current levels of site-specific juvenile survival (survival intercept) and site-specific levels of P_{estab} (Table 5.1; Figure 5.6). Changes in survival result in a sigmoidal change in lambda. For the Crouch, current coefficient values for the survival slope are 0.000175, however for $\lambda = 1$ the required survival slope gradient is $ss = 0.0009$ (Figure 5.6). Therefore, small increases in adult survival will result in large increases in λ . This is the same for the Blackwater, where current survival slope conditions lie at the bottom of the steepest part of the sigmoidal curve, therefore small increases in adult survival will also have large effects on population rate of change for this area. For the Ray Sand, current survival slope estimates sit near to the curve asymptote, therefore large changes in adult survival will only result in small changes of λ however if adult survival falls, this would result in large decreases in λ .

Population summary plots highlighting the population kernel used in the IPM show the stable size distribution, reproductive values and elasticity when $\lambda=1$ in Figure 5.7 for the MCZ weighted average after the survival slope only. Here P_{estab} is maintained at the measured 2018 value of 4.40E-07. With this establishment probability now recognised as a low recruitment year, elasticity plots highlight if adult survival is boosted in low recruitment years change in λ is highly sensitive to changes in adult growth and survival (Figure 5.7).

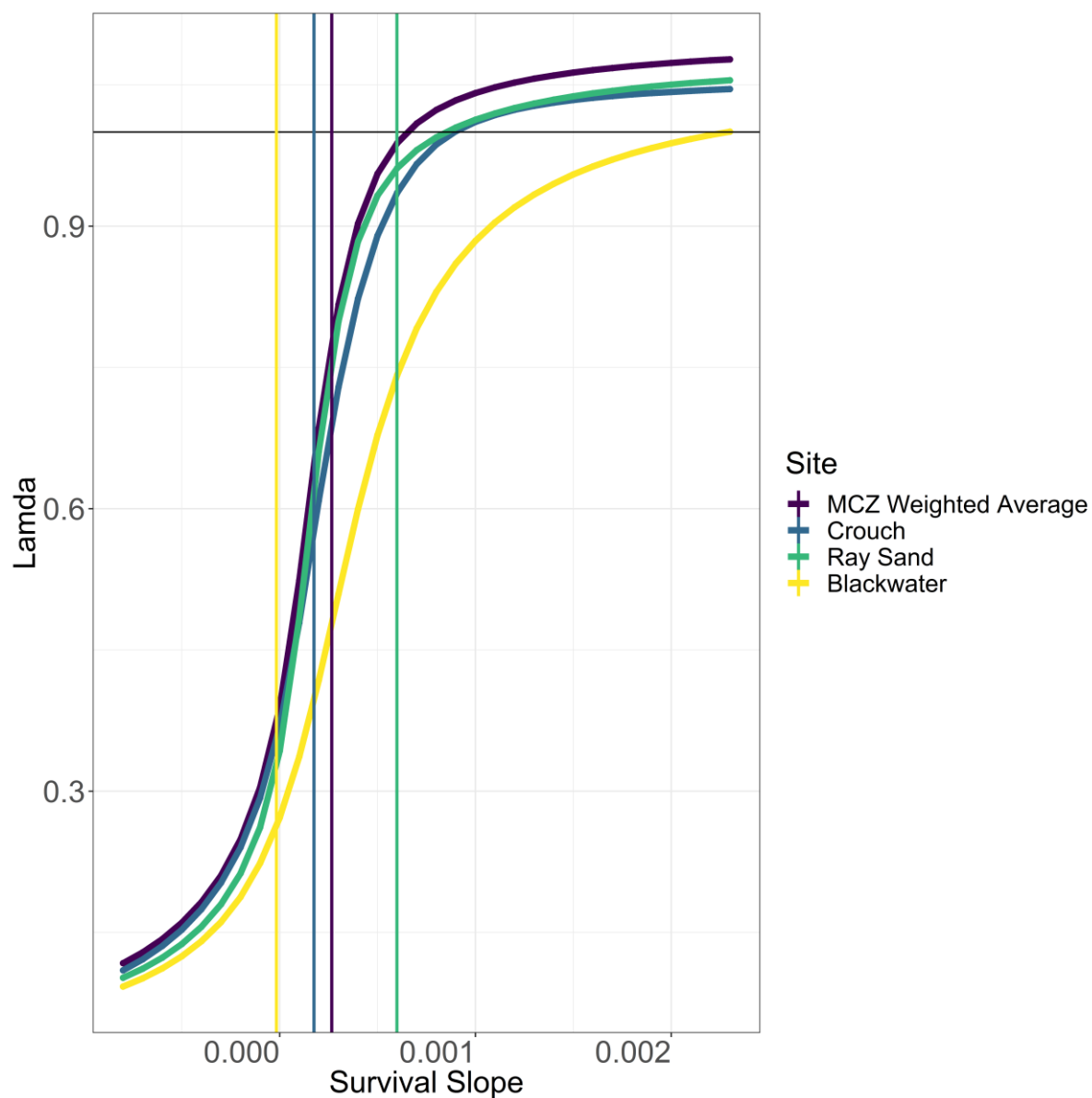


Figure 5.6. Levels of survival slope required for λ to equal 1 (i.e. the population is not growing or shrinking) using measured conditions between 2017 and 2018 for MCZ sites combined as a weighted average and each MCZ site individually.

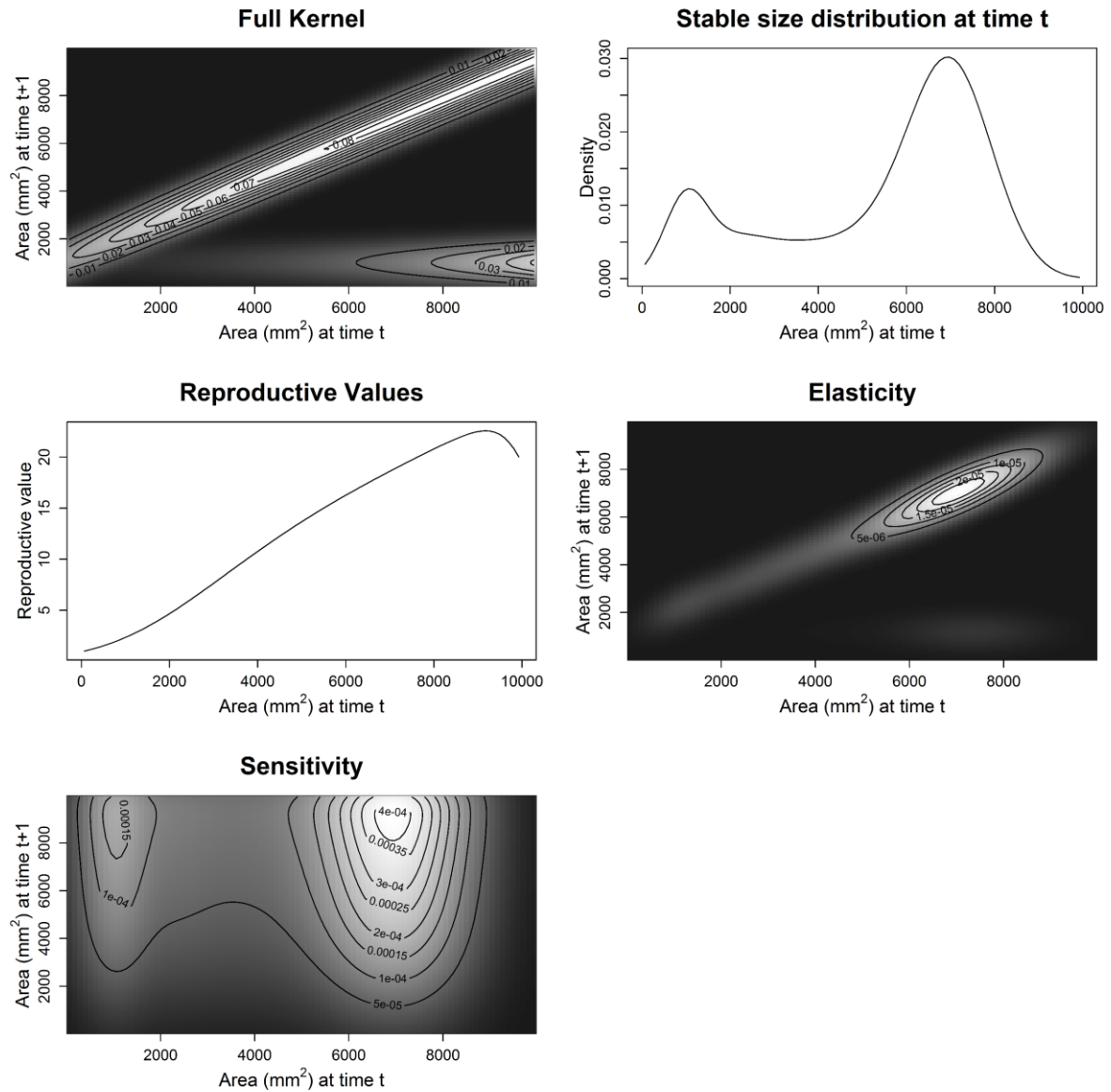


Figure 5.7. Population summary plots model output from a size structured IPM by area (mm²) for *Ostrea edulis* for all MCZ sites combined as a weighted average where $\lambda = 1$ through altering the survival slope coefficient (and therefore boosting adult survival) to 0.00066.

Understanding the consequences of restoration: finding the balance

With adult survival and establishment probability likely to vary over time, both the establishment probability and survival slope were changed incrementally to their combined effect on lambda. i.e. to visualise the likely value of lambda given current growth rates, recruit size and survival intercept if both were found to differ. This matrix was then plotted to highlight how these coefficients interact to result in a growing or shrinking population (Figure 5.8). This highlights that once the survival slope coefficient $=0.0006$, even low levels of recruitment (i.e. BCRC.MCZ site-wide average levels observed in 2016-2017) are able to sustain the population. If the recruitment probability falls below $1.0E-8$, large increases in adult survival are required to counteract the lack of recruits entering the population and below this value lambda was not observed to reach 1 within the survival slope parameters tested.

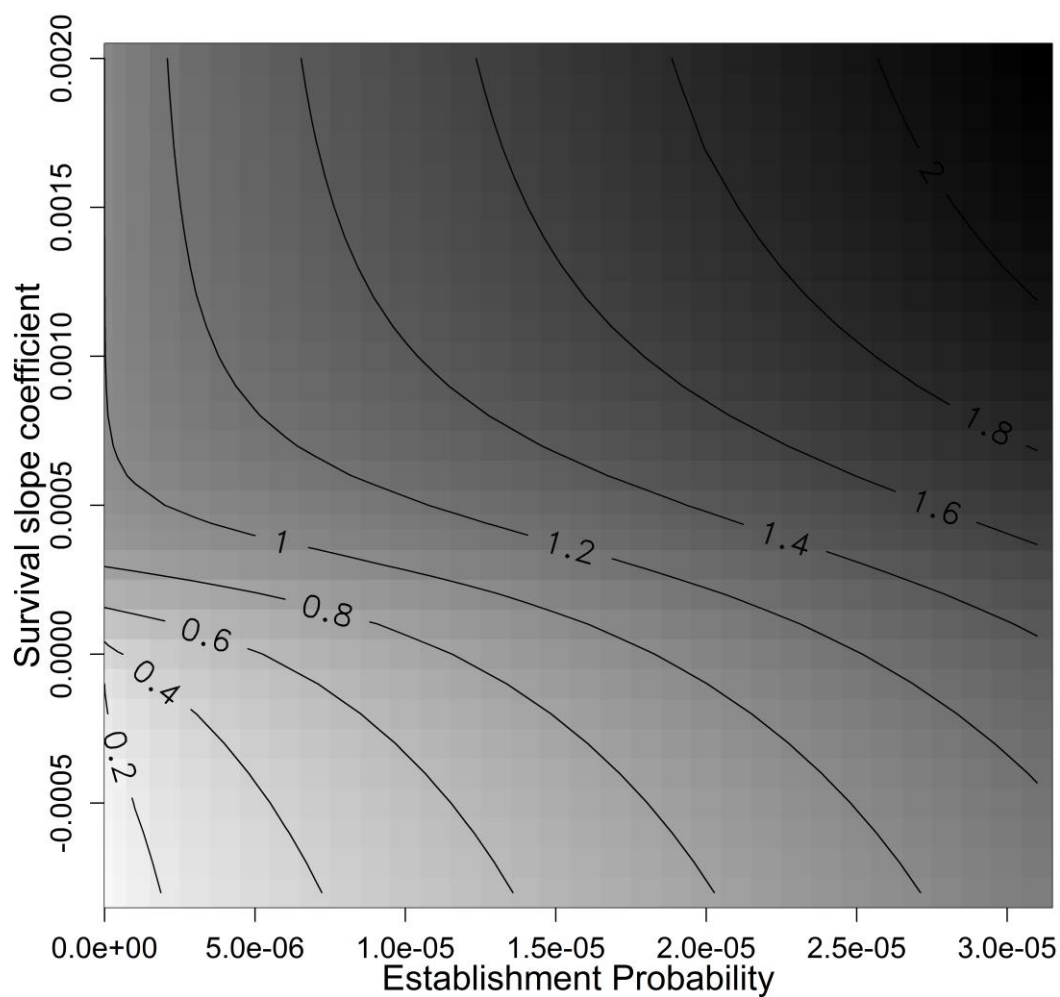


Figure 5.8. Effect on lambda by changing the establishment probability and survival slope incrementally given other values for the parameters for the Blackwater Crouch Roach and Colne Estuaries Marine Conservation Zone under a weighted average by post-summer 2017 population size. The highest spatfall calculated was 2.27E-05 in the Blackwater in 2015 and the highest survival slope within 2017-2018 was 0.000599 on the Ray Sand.

Future projections

With no stochasticity induced into future predictions (i.e. if the estimated establishment probability and site-based survival remain constant at measured levels) *O. edulis* populations within the BCRC.MCZ are expected to decrease over time with populations estimated to be near 0 after 10 years (Figure 5.9). Stochastic projections, only changing the establishment probability, at random selecting the estimated establishment from dredge survey data (i.e. using P_{estab2} Equation 5.6, Table 5.2) populations are also predicted to fall over time with mean $\lambda_s = 0.8262$ for the whole of the MCZ (Figure 5.10). Individual sites highlight that this is largely driven by trends at the Blackwater and Crouch where $\lambda_s = 0.4929$ and 0.5709 respectively using respective recorded establishment. Using locally recorded establishment, the Ray Sand site remains stable and increasing over time with $\lambda_s = 1.1112$. Differences in the effect of variation of establishment between sites are highlighted by the range of repeated runs with the Blackwater showing large variation in recruitment between years and the Crouch showing little variation in observed recruitment (Figure 5.10, Table 5.2).

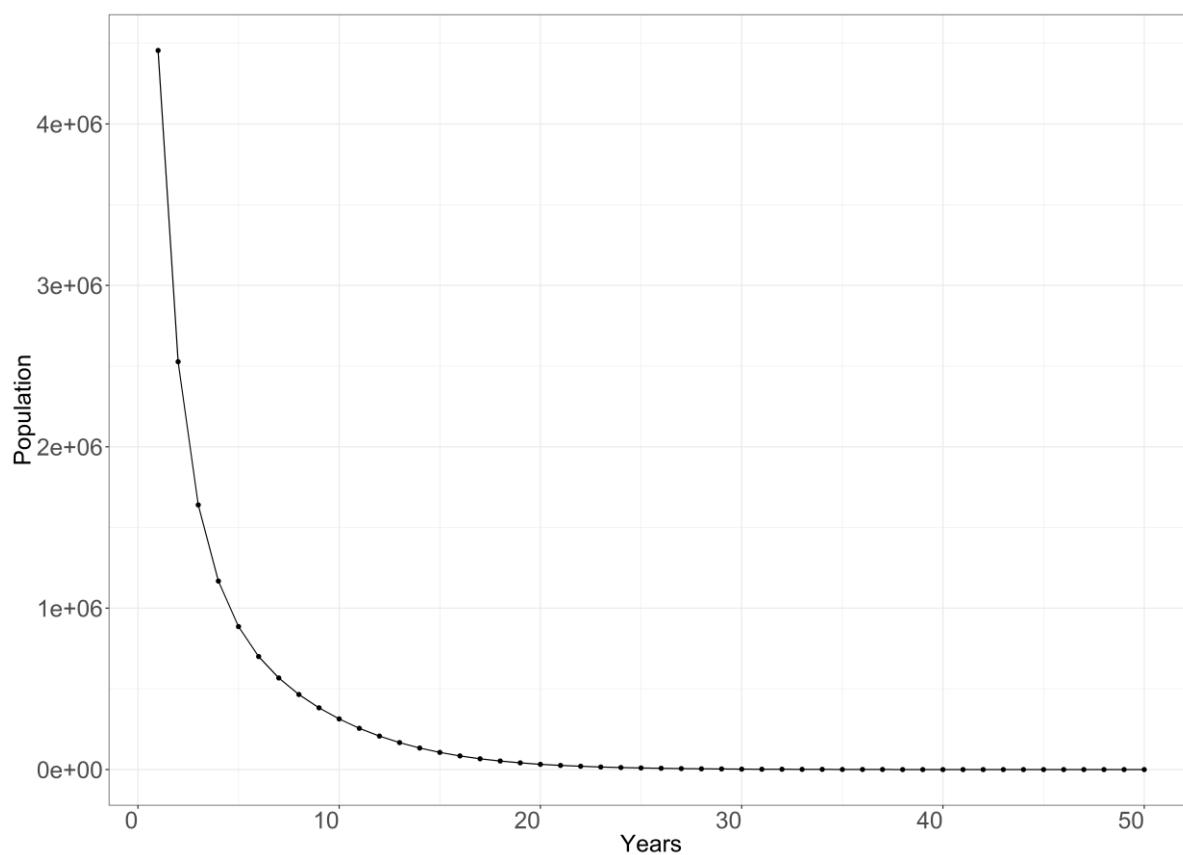


Figure 5.9. Future projection of *Ostrea edulis* population between 2017 (year 0) and 2067 (year 50) with no stochasticity where $\lambda = 0.7766$ for all MCZ sites combined.

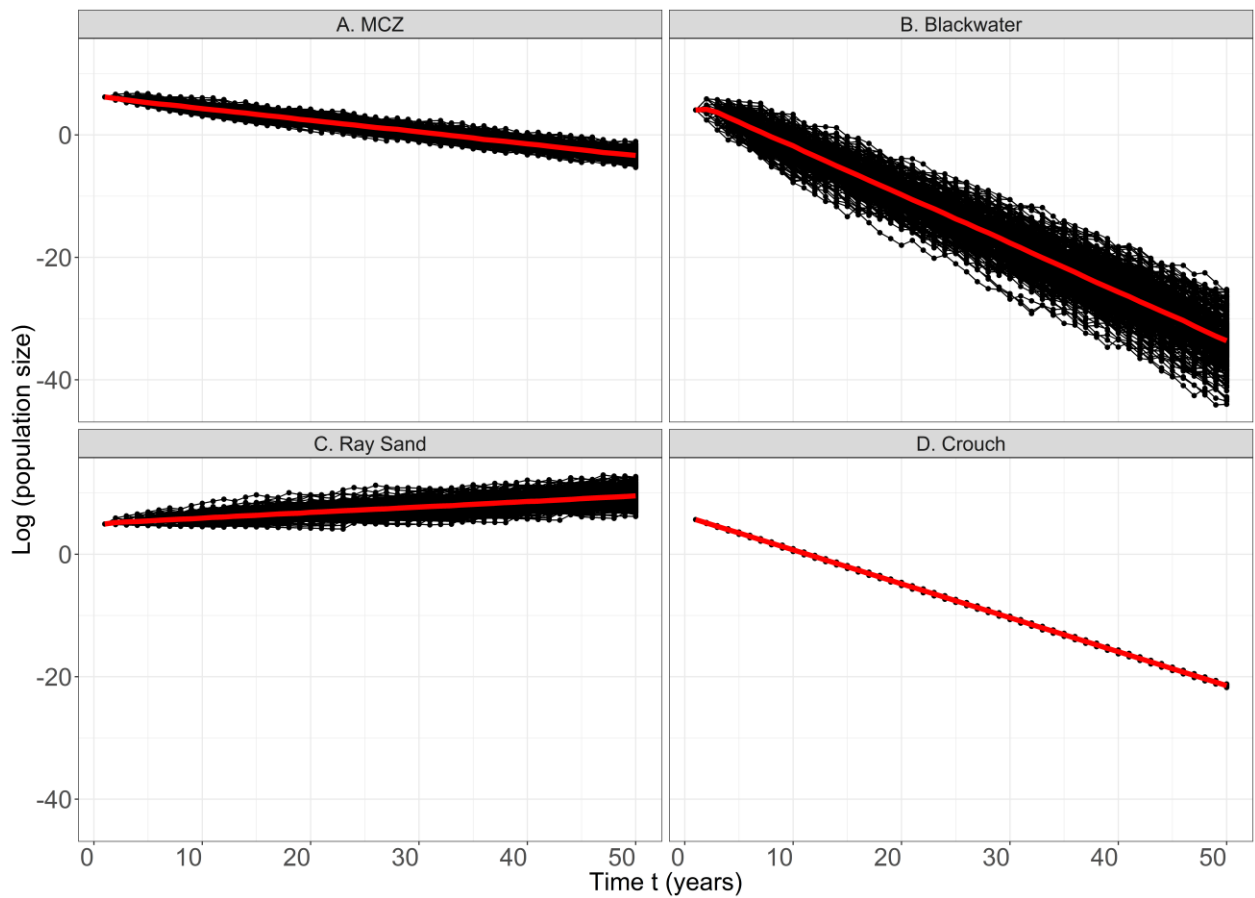


Figure 5.10. Population projection of *Ostrea edulis* populations in the BCRC.MCZ using the site-dependent starting size distribution measured in the post-summer 2017 surveys as n_0 (year 0) and stochastically iterating at random through **A.** varying establishment probabilities (P_{estab2} : Table 5.1) measured from dredge survey data between 2014 and 2018 for the full MCZ site ($\lambda_s = 0.8262$). **B.** varying establishment probabilities (P_{estab2} : Table 5.1) measured from dredge survey data between 2014 and 2018 for the Blackwater site only ($\lambda_s = 0.4929$). **C.** varying establishment probabilities (P_{estab2} : Table 5.1) measured from dredge survey data between 2014 and 2018 for the Ray Sand site only ($\lambda_s = 1.1112$). and **D.** varying establishment probabilities (P_{estab2} : Table 5.1) measured from dredge survey data between 2014 and 2018 for the Crouch site only ($\lambda_s = 0.5709$).

All models are iterated forwards 50 years with the mean log population size extracted from 400 repeated runs is highlighted in red with individual runs shown in black. All population estimates have been scaled up from dredge estimates to full population estimates to result in populations modelled as extracted IDW population number.

5.4 Discussion

Integral Projection Models provide the ideal framework for predicting *O. edulis* population change and assess the effects of different types of restoration management. Using data obtained from wide-scale dredge surveys (Chapter 2) and individual growth and survival experiments (Chapter 4), the resulting IPM is able to predict the measured population of native oysters in the post-summer 2018 survey accurately both in abundance, though implementing the measured lambda on the full population estimation, and in the measured size distribution.

Current assessments have found large variations in predicted population change between different areas of the BCRC.MCZ, requiring different management techniques. As a whole, *O. edulis* populations throughout the MCZ are declining with $\lambda_s = 0.8262$ when incorporating measured stochastic variation in recruitment (P_{estab2}). Without recognising the importance of stochastic variation in annual recruitment, the population of *O. edulis* in the BCRC.MCZ appear to go extinct (or near extinct) within 10 years with $\lambda = 0.7766$ (Figure 5.9). Recorded spat-fall was relatively low in 2018 comparison to that recorded in 2015 (approximately 50% lower). Given current conditions and spatfall, elasticity analysis highlights the sensitivity of population change to the growth and survival of existing adult oysters within the MCZ. Maintaining survival and growth rates, particularly prior to reproduction, are therefore essential in restoring native oysters throughout the MCZ, particularly in low recruitment years. If recruitment is boosted through recruitment management such as spat collection and or cultch management, this changes the elasticity of the system to be more sensitive to changes the growth and survival of juvenile oysters (Figure 5.5).

For the whole MCZ area, spatfall is observed to naturally fluctuate between 4 and 85% of the required level to maintain the population, if growth and survival rates do not change over time. This high level of natural variation highlights the well described dynamics of other oyster populations, with populations largely relying on large, relatively infrequent pulses of recruitment (Bromley *et al.*, 2016). Trials with *Crassostrea virginica* spat have shown that spat settlement can be increased approximately 8 times using limestone as opposed to sandstone settlement materials highlighting the influence suitable available substrate has on settlement success and recruitment in oyster larvae (Soniat & Burton 2005).

With other cultch settlement trials indicating treatment sites with the correct cultch type may boost recruitment by 125 times (Frederick *et al.* 2016). Assessments with *O. edulis* have shown a positive correlation between shell availability and settlement, with no preference over type of shell used (Smyth *et al.* 2018). Whilst this highlights the ability for suitable substrate to greatly boost recruitment of oysters achieving this at scale to influence the population dynamics is a different question. Spat settlement and recruitment trials are therefore required to assess the required scale of spat collection to ensure recruitment is maintained at a high level across a large area.

Varying both the survival slope and establishment highlights that once levels of survival result in a slope of 0.0006, given other measured coefficients, then even low levels of recruitment are able to sustain the population. With the measured survival slope for the Ray Sand being 0.000599, these levels of adult survival are occurring within the BCRC.MCZ and are therefore achievable for native oysters in Essex.

Individual areas

The Blackwater MCZ bed was found to have particularly low adult survival, with a negative survival slope coefficient, implying larger oysters are more likely to die (Table 5.1). This follows predicted impacts caused by the known presence of *Bonamia*, where oysters usually become more susceptible to mortality from the disease after 2-3 years of age (Culloty & Mulcahy 1996). The Blackwater shows the lowest levels of lambda, with sensitivity and elasticity analysis of this site (supplementary information) indicating lambda is highly sensitive to any change of all life stages. This area is therefore likely to benefit from a range of different management techniques with both recruitment and survival limiting population growth. Both the highest level of spatfall and the highest variation in spatfall between years has been observed in the Blackwater with P_{estab2} found to vary between 0 and 2.27E-05. The highest observed natural spatfall in the Blackwater occurred in 2015 and was 74.5% of the required spatfall to maintain the population size given measured levels of growth and survival in 2017-2018. To boost the recruitment to maintain the Blackwater population in 2015 an approximate 793 lengths of 45 coupelle

collectors were needed to have been put out in the area in the breeding season of 2014. With up to 50 lengths of couplet traditionally placed on a single A-frame, this would have required approximately 16 A frames of collectors to be placed out in years of high spatfall. This level of labour investment may be unachievable given current infrastructure, therefore further assessments of recruitment obtained through laying cultch of various types should be considered in this area to boost the probability of establishment. Raising adult oysters off the sea bed or managing the habitat to reduce smothering of adults by sediment during storm events and may also be beneficial given observed causes of mortality described in Chapter 4.

With the close proximity of this site to the Tollesbury and Mersea Several Order, it may be likely much of the recruitment observed in the Blackwater is originating from oysters within private grounds, therefore further research is recommended to assess the breeding status of oysters within the MCZ area and make direct comparisons with those inside of the managed area. Assessing spat fall and recruitment in managed areas within the Blackwater Several Order and making direct comparisons with data from the MCZ area may give insight into what realistic levels of recruitment and adult survival may be if certain management techniques such as “cleaning of grounds” through dredging or harrowing or laying cultch are applied. This was not possible with current data from the growth and survival study outlined in Chapter 4 due to string placement needing to be outside of directly managed areas due to the nature of the working fishery.

The Ray Sand has the highest adult survival and population growth rate, with $\lambda = 0.923$ when using the site-wide establishment probability, dropping to 0.842 when using the establishment measured on the Ray Sand. Long term lambda rates and locally calculated establishment indicate this population is stable and increasing in number (Figure 5.10). With clear evidence of previous recruitment in this population, along with the relatively small increase in establishment required to boost lambda to 1 (and above), this highlights the potential for this area to benefit from restoration techniques designed specifically to boost recruitment with no management likely required to boost adult survival in this area. Elasticity analysis show this area is sensitive to changes in growth, survival and reproduction/recruitment, therefore if

recruitment is boosted through restorative techniques such as spat collection, careful monitoring of the survival of adult oysters is also recommended.

The Crouch bed has very little measured variation in recruitment between years. Elasticity analysis therefore indicates that this area is highly sensitive to growth and survival of adults of reproductive size. This area is currently a mixed bed with *C. gigas* and *O. edulis* mixed on the same plane. Previous research has highlighted potential negative impacts on adult growth and survival when *C. gigas* and *O. edulis* grow together within the same plane, particularly in subtidal areas, however may facilitate survival of each species when grown together on a vertical plane (Zwerschke *et al.* 2018). Initial restorative management of a structured vertical base for a breeding stock may be beneficial in this area with careful monitoring of the spread of subtidal *C. gigas* populations recommended to reduce potential competitive interactions resulting from mixed beds of these two oyster species, however caution should be taken to ensure continued use of the shipping channel (Zwerschke *et al.* 2018). With site-based establishment probability on the Crouch site very low, and little variability recorded, further research into reproductive status of adult oysters is recommended. If oysters are found to be brooding fertilised eggs, increasing recruitment through the use of spat collectors may be useful, however should be used with caution in these areas due to the coexistence of *C. gigas* and *O. edulis*. With similar breeding season timing and habitat settlement requirements for the two species it is likely that spat collectors will collect both species of oyster which may result in difficulties in relaying following the initial growth period.

The causes of low levels and low variation in establishment of juvenile *O. edulis* in the Crouch area may be due to a variety of reasons. Firstly, adult oysters simply are not reproducing and brooding eggs. This can be assessed by taking larger subsamples of oysters from this area and opening them during breeding season to assess reproductive status, secondly, oysters may be brooding eggs but fertilisation may be too low. This is unlikely due to the highest densities of *O. edulis* observed in the MCZ present in the Crouch area. As oysters have been found to successfully fertilize in lower densities this is unlikely to be a factor (Guy *et al.* 2018). Alternatively, as currents are high in this area with the river mouth maintained as a narrow channel, constrained by a sea wall, larvae may be washed out of the area and

be settling in another, as yet undefined, area. Therefore, models assessing retention of spat may be beneficial for use in this area to assess where juvenile oysters may be most likely to be settling. Finally, *C. gigas* may be out-competing *O. edulis* and even consuming native oyster larvae. *C. gigas* have been previously observed to consume larvae of *Cerastoderma edule* and *Mytilus edulis* with both species larvae unable to swim against the inhalant jet strength of *C. gigas* (Troost 2009).

Pitfalls of the current dataset

It is recognised that data collected between 2017 and 2018 may be representative of more extreme years with unseasonably cold storms recorded during March 2018 preceding one of the driest and hottest summers on record for England (Met Office 2018), these growth and survival data may only represent particularly harsh years for *O. edulis* populations and therefore underestimate lambda and population growth rates. To assess if the adverse weather experienced between 2017 and 2018 reduced survival and growth, or potentially assisted oyster growth and survival repeating the growth and survival string-based experiment over multiple years is highly recommended to increase accuracy of this model. Due to time constraints associated with a PhD this was not possible for the model in this current form, however due to the low cost and simple design of the growth and survival experiment (Chapter 4), repeating this experiment to gain parameters for moderate and mild years will enable this model to be used for increasingly accurate predictions both of long and short term population change in the native oyster populations in the BCRC.MCZ.

In addition to the lack of repeated years data it is recognised that measured levels of establishment may be inaccurate due to the difficulty in detecting juvenile oysters both in dredge and string surveys, and the potential for oyster spat to be settling and growing outside of sampled areas. To assess recruitment more accurately, spat collection experiments specifically designed to assess recruitment are required across the primary areas of the MCZ. These should include the use of spat collectors within the water column, laying cultch designed to collect oysters and assessing natural substrates in local conditions.

Assessing settlement on artificially laid cultch is necessary so that any oysters observed on these substrates are of a known age and cohort.

Conclusion

This study has used an IPM parameterised using repeated dredge survey data to gain population size distribution and low-cost growth and survival experiments to assess current population dynamics of native oyster populations in the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone. This study has quantified the level of recruitment to maintain the current population given current site-dependent rates of adult growth and survival and identified the impact boosting adult survival may have on rate of population change within the three primary populations of *O. edulis* within the BCRC.MCZ.

Current conditions highlight the sensitivity of native oyster populations to change in the growth and survival of adult oysters in years where recruitment is low, with this changing to growth and survival of new recruits when establishment of spat is artificially boosted. Whilst this model appears to accurately predict the population change observed in dredge surveys between 2017 and 2018, further monitoring of individual oysters over milder years and linking growth and survival back to annual temperature variation is required to more accurately predict long-term population change and make predictions on how these populations of *O. edulis* may respond to future environmental change.

Current conditions indicate populations of *O. edulis* are declining within the BCRC.MCZ with these trends primarily due to reductions in abundance in the Blackwater MCZ and Crouch sites. Populations on the Ray Sand are increasing over time with natural fluctuations in observed recruitment able to sustain the population. Any boost to recruitment on the Ray Sand area will therefore have large benefits to growth over time with restoration here recommended to focus on spat settlement and recruitment.

This model can now be adapted for use in other areas where oyster restoration is being undertaken such as in the Solent where growth and survival stings, such as those used in Chapter 4, are already in situ.

By assessing growth rates and survival prior to restoration project occur and subsequently utilising this model, efficiency of restoration and likelihood of success can be greatly increased.

Chapter 6:

Conclusions, discussion and management recommendations for the restoration of *Ostrea edulis* in the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone.

6.1. Main findings

This thesis has described the distribution, abundance and status of native oyster populations and associated communities throughout the Blackwater, Crouch, Roach and Colne Marine Conservation Zone, creating a baseline for current and future management of this protected area.

*Distribution of *Ostrea edulis* and associated species*

This work has shown that *O. edulis* populations within the BCRC.MCZ, outside of Several Order areas, are primarily distributed around the Blackwater, Crouch and Ray Sand areas. Small, low density accumulations of native oysters are also dispersed in the Whitaker channel, Eagle, Wallet Spitway and outer Colne areas. Populations appear patchy with a maximum observed density of *O. edulis* observed of 7.25 oysters m⁻² across a 100m dredge tow in the Blackwater area, in the 2015 survey. This surpasses the OSPAR definition of an oyster bed of 5 oysters m⁻² (Haelters & Kerckhof 2009). However, following this, populations appear to have declined in this area. The Crouch has consistently high densities for the whole area, reaching averages of 1.10 oysters m⁻² for the whole area in the post-summer 2017 surveys and a maximum observed average density across a 100m dredge tow of 4.04 oysters m⁻² in the post-winter 2017 survey, incorporating a 20% dredge efficiency. Taking into account the nature of dredge surveys, only able to provide mean densities across a full 100m tow, and the patchy distribution of oyster beds, it is likely that real maximum densities are higher than the average densities specified in this study.

Seasonal movements of associated species have identified predictable movements of *Asterias rubens*, with increases in density observed in offshore areas following winter months. This moment coincides with known herring spawning events and it is therefore likely that starfish are actively moving to this area to feed on herring spawn (Fox 2001). Further research assessing stomach contents analysis and or in-lab feeding experiments of *A. rubens* on herring spawn may be required to fully confirm this.

Current densities on the non-native *C. fornicata* are stable throughout the course of this study, with little variation observed in the predicted total biomass or distribution. Despite this, continuation of monitoring of this species is essential for the success of the restoration of the European native oyster, with potential for this species to create homogeneous mats if densities become too high in unmanaged populations (Blanchard 2009). When managed, oyster growers report that this species can provide a much-needed settlement substrate, where hard substrate is limited.

Abundance of *C. gigas* also appears to remain stable between 2016 and 2018. Populations are focused in the mouth of the Crouch, where densities of both oyster species are high. *C. gigas* are primarily intertidal therefore this survey was not designed to accurately monitor the full proliferation of this species, however continuation of monitoring of *C. gigas* in subtidal areas is recommended with the potential for *C. gigas* to outcompete *O. edulis* in certain habitats (Zwerschke *et al.* 2018).

Associated species and macrofaunal epibenthic assemblages

This thesis provides policy makers with evidence of increasing species richness associated with increasing densities of the European native oyster. This association with macrofaunal epibenthic species, whilst recognised in other species of oyster such as *Crassostrea virginica* or *C. gigas*, had previously neglected to be verified in *Ostrea edulis* (Coen *et al.* 2007; Tolley & Volety 2005; Santiago *et al.* 2019). Whilst the cause and effect of this association remains unclear, this thesis provides evidence that improving habitat to be suitable for the proliferation of native oysters will likely to also result in increased biodiversity of other species, in the absence of the non-native slipper limpet *Crepidula fornicata*. When *C. fornicata* populations are left to proliferate and reach high densities, predicted

increases in the number of associated epibenthic faunal species with increasing native oyster density are suppressed and even reversed.

Population estimates and dynamics

Current population estimates for *O. edulis* for the whole of the BCRC.MCZ appear to be stable or declining, with downward trends primarily driven by Blackwater MCZ and Crouch MCZ areas. The population on the Ray Sand appears comparatively healthy with good survival and growth rates observed. Recruitment across the BCRC.MCZ has been highly variable between 2015 and 2018 with the largest observed recruitment event occurring in 2015 within the study period. Populations in the Blackwater appear to have declined since this study began with population rate of change (λ) currently sensitive to changes in adult growth rate and survival between 2017 and 2018. In this same year (2017-2018) recruitment was low, however, in 2015 estimated recruitment rates reached approximately 85% of the required level of settlement to maintain the population size for the Blackwater, given measured levels of growth and survival, with no intervention at this point.

It is therefore likely that that *O. edulis* populations throughout the BCRC.MCZ will respond well to widescale spat collection trials such as using spat collectors and or laying cultch, particularly on the Ray Sand where natural fluctuations in recruitment are able to sustain the population.

A widescale investigation to ensure the brooding of fertilised eggs is occurring in all areas is recommended, particularly in the Blackwater and Crouch. In the Blackwater, successful spatfall may be occurring due to the close proximity of the site to the Tollesbury and Mersea Several Order where successful spatfall was recorded in 2018 (Cameron, 2018).

6.2. Management recommendations and further research

Following this study, and the development of the demographic model, it is now possible to make clear management recommendations for individual areas of the BCRC.MCZ. These management

recommendations will clarify remaining gaps in knowledge, ensure appropriate restoration work is performed and achieved within the BCRC.MCZ and also improve accuracy of current models and predictions. Recommendations for progression of work within the BCRC.MCZ are summarised by site-wide recommendations with subsequent localised requirements also made.

Site-wide recommendations and further research

- Individual areas of the Blackwater, Ray Sand and Crouch should be treated as separate beds, requiring differing types of active management due to these areas showing different levels of recruitment and sensitivities.
- Offshore areas with extremely low *O. edulis* abundance should continue to be regularly monitored to assess potential spread of recovery. Previous spatfalls are known to have occurred in these areas however, due to the low populations in these areas this study has insufficient evidence to make specific recommendations for restoration.
- Repeat the string-based growth and survival experiment (Chapter 4) at the MCZ sites only over a range of years. This will enable more accurate ranges in growth and survival rates between storm years (2017-2018) and milder years where survival and growth rates may be higher. This will then increase accuracy of stochastic IPM model projections with site-based variation in growth and survival able to be accurately incorporated.
- Continue development and utilisation of IPM (Chapter 5) to guide restoration direction with site-based recommendations developed as more data is collected (i.e. as restoration progresses and sensitivity of the BCRC.MCZ system switches to be less sensitive to adult survival as recruitment increases to be able to sustain populations, this will indicate restoration is being achieved).
- Confirm movements of *A. rubens* in relation to feeding requirements on herring spawn through the use of stomach contents analysis and or lab-based feeding trials.

- With all areas indicating some level of sensitivity to reproduction further investigations into parameters surrounding reproduction should be investigated:
 - Assess oysters annually throughout the BCRC.MCZ for reproductive status during the breeding season. This will enable incorporation of varying the probability of reproduction within the IPM. Current estimates indicate 13.4% of the population are fecund females however if this proportion changes this may result in different abundances of larvae within the water.
 - Lay cultch of explicitly known shell type or spat collectors in the main areas. Using cultch and collectors of known deployment will allow for definitive classification of 1 year old oysters. Spat can then be assessed for abundance, size and survival 1 year later to assess recruitment probabilities and accurate recruitment sizes in natural conditions to increase accuracy of IPM (Chapter 5) for year class size range.

Ray Sand

- The IPM highlights that the Ray Sand is highly sensitive to increased spatfall, therefore a broodstock population should be considered in this area where survival rates are high. Location of this broodstock should be assessed in consideration to local hydrodynamics, spat behaviour and local shipping traffic.
- Assessment of presence of brooding females and or spat within the water column around the Ray Sand area will clarify if recruitment is limited through settlement of spat or through successful reproduction.
- If recruitment is limited through settlement (i.e. fertilised, brooding females are prevalent in breeding season and spat is present within the water column and not being carried to other areas), increased provision of settlement habitat through the laying of cultch will likely result in high levels of population growth.
- If recruitment is limited through reproduction (i.e. fertilised, brooding females are not found in breeding season and spat is not present within the water column), increased assessment of water

quality and or increasing densities of oysters during breeding season may assist with increasing successful fertilisation if Allee effects are occurring due to low densities (Guy *et al.* 2018).

Crouch

- The Crouch appears to be experiencing low levels of variation in spat-fall, this is highlighted through low levels of variation in stochastic projections of population abundance (Chapter 5).
- Assessment into competitive interactions between *C. gigas* and *O. edulis*, along with assessment of brooding status of oysters is required to disentangle potential causes for this lack of recruitment occurring in this area.
- It is recognised that current speeds are high in this area and if oysters are found to be brooding and no consumption of *O. edulis* larvae by *C. gigas* is likely to be occurring, further investigation in to the hydrodynamics of this area and assessment over where spat may be settling outside of this broodstock is required to understand larval dispersion.
- If *C. gigas* are found to interact with *O. edulis* larvae extracting *C. gigas* oysters from the area prior to breeding season and replacing with clean cultch may be useful to boost spatfall of *O. edulis*.

Blackwater

- The Blackwater MCZ area will benefit from a variety of management interventions with both adult survival and recruitment needing to be addressed.
- Habitat restoration, particularly following easterly storm events is recommended with high mortality from sedimentation observed (Chapter 4).
- Any relaying of adult oysters may benefit from the management of the proliferation of dense *Crepidula fornicata* beds of this area and trials into the impact various management techniques have on native oyster survival and biodiversity are recommended.

- Cultch relaying and spat collection are likely to boost populations in this area due to years of very high recruitment observed in previous years.

Other areas

- Populations in other areas such as the Whitaker channel, Eagle and outer Colne should be regularly assessed for any evidence of spatfall, particularly following any restoration work elsewhere surrounding the BCRC.MCZ.
- Hydrodynamics should be assessed to estimate where spat may originate from and or settle to, based on geographic location of oyster populations. This is due to the difficulties in assessing the whole of the BCRC.MCZ site and the likelihood of potentially missing small or newly forming populations.

6.3. Conclusion

The European native oyster provides well recognised cultural and economic benefits to Essex, having supported a fishery since the Roman times. This study highlights some of the environmental benefits associated with the native oyster, with increases in associated species observed with increasing oyster density. In areas where *C. fornicata* density are high, increases in species richness associated with increases in density of *O. edulis* appear to be suppressed and even reversed. Therefore, reductions in density of *C. fornicata* may be beneficial in areas where densities are particularly high.

This study has developed new methods to monitor the growth and survival of *O. edulis* at low densities, without the need to cage oysters or maintain oysters above the sea bed. These methods are already being used in the Solent area to assess growth and survival of oysters *in-situ*. Increasing the number of sites where string-based growth and survival experiments are being used will enable this IPM to be used, not only for other areas, but to potentially provide predictions on *O. edulis* populations across the UK and

Europe as a whole, pinpointing areas where growth and survival are high. This can then be linked to local conditions and genetic profiles of local oysters.

This list of management recommendations and further research should be used as a flexible active management approaches, with site-dependent restoration measures adapted as new research and data develops the now existing Integral Projection Model for this area. Whilst populations of this valuable species are recognised to be currently at a low level in comparison to historical records, directed long-term management of this species using stage-dependent management techniques and ongoing population assessments, will help to ensure the legal obligations to “protect and restore” the European native oyster within the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone, are met.

Appendix I

Supplementary Information for Chapter 2: Native Oysters and their community through space and time – a multi-annual study of the benthic community ecology in the Blackwater, Crouch, Roach and Colne Estuaries Marine Conservation Zone.

Supplementary Information Table 2.1: Mean density of native oysters and key associated species in the Blackwater, Crouch, Rouch and Colne Marine Conservation Zone between 2014 and 2018. Data is given by individual beds and all beds combined with estimates calculated using Inverse Distance Weighting. Weight populations are given in kg with all mean density data as per unit 1m2. 10%, 20% and 30% represent respective dredge efficiencies used in calculations of population estimate

Species	Year	Season	Bed	Mean	SD	Area m2	Population total 20%	Mean10	SD10	Population total 10%	Mean30	SD30	Population total 30%
O. edulis weight	2014	summer	ALL	0.003389	0.014893	39516808	133920.8	0.006735	0.029793	266141.633	0.002245	0.009931	88713.89
O. edulis weight	2014	summer	Blackwater	0.004814	0.005601	4397937	21169.55	0.009626	0.011202	42334.2937	0.003209	0.003734	14111.43
O. edulis weight	2014	summer	Crouch	0.056296	0.051694	1517307	85418.12	0.112569	0.103391	170801.471	0.037523	0.034464	56933.82
O. edulis weight	2014	summer	Eagle Outer	0	0	4018615	0	0	0	0	0	0	0
O. edulis weight	2014	summer	Colne	0.000758	0.001066	4569339	3464.785	0.001517	0.002132	6930.92926	0.000506	0.000711	2310.308
O. edulis weight	2014	summer	Raysand Wallet	0.00157	0.002186	15086912	23688.14	0.003027	0.004387	45665.4421	0.001009	0.001462	15221.82
O. edulis weight	2014	summer	Spitway Whitaker	0	0	6203952	0	0	0	0	0	0	0
O. edulis weight	2014	summer	Channel	5.29E-05	6.47E-05	3722746	197.0333	1.06E-04	1.29E-04	394.611094	3.53E-05	4.31E-05	131.4129
O. edulis weight	2014*	summer	ALL	0.004884	0.019739	39516808	192990.4	0.009721	0.039486	384162.572	0.00324	0.013162	128054.2
O. edulis weight	2014*	summer	Blackwater	0.005609	0.005132	4397937	24669.05	0.011217	0.010263	49332.1457	0.003739	0.003421	16444.05
O. edulis weight	2014*	summer	Crouch	0.088746	0.051343	1517307	134654.7	0.177479	0.102679	269289.524	0.05916	0.034226	89763.17
O. edulis weight	2014*	summer	Eagle Outer	0.0002	0.000136	4018615	805.0251	0.000401	0.000271	1610.03016	0.000134	9.04E-05	536.6781
O. edulis weight	2014*	summer	Colne	0.001361	0.001185	4569339	6219.268	0.002722	0.002369	12435.9688	0.000907	0.00079	4145.323
O. edulis weight	2014*	summer	Raysand Wallet	0.001674	0.002166	15086912	25256.35	0.003227	0.004355	48684.0314	0.001076	0.001452	16228.01
O. edulis weight	2014*	summer	Spitway Whitaker	0.000156	0.000141	6203952	968.0647	0.000312	0.000282	1936.37748	0.000104	9.40E-05	645.4592
O. edulis weight	2014*	summer	Channel	9.75E-05	5.88E-05	3722746	362.9678	1.95E-04	1.17E-04	725.935503	6.50E-05	3.91E-05	241.9785

Species	Year	Season	Bed	Mean	SD	Area m2	Population total 20%	Mean10	SD10	Population total 10%	Mean30	SD30	Population total 30%
O. edulis weight	2015	summer	ALL	0.008162	0.017095	39516808	322528.2	0.016327	0.034194	645171.327	0.005442	0.011398	215057.1
O. edulis weight	2015	summer	Blackwater	0.013693	0.025945	4397937	60220.45	0.027402	0.051908	120512.969	0.009134	0.017303	40170.99
O. edulis weight	2015	summer	Crouch	0.070631	0.026984	1517307	107168.8	0.141276	0.053949	214359.58	0.047092	0.017983	71453.19
O. edulis weight	2015	summer	Eagle	0	0	4018615	0	0	0	0	0	0	0
O. edulis weight	2015	summer	Outer Colne	3.07E-05	8.41E-05	4569339	140.2787	6.13E-05	1.68E-04	280.100501	2.04E-05	5.60E-05	93.21452
O. edulis weight	2015	summer	Raysand	0.008235	0.006108	15086912	124248.1	1.65E-02	1.22E-02	248934.046	0.005491	0.004072	82834.81
O. edulis weight	2015	summer	Wallet										
O. edulis weight	2015	summer	Spitway	0.00243	0.000474	6203952	15076.81	0.004861	0.000948	30157.7082	0.00162	0.000316	10052.57
O. edulis weight	2015	summer	Whitaker Channel	0.004214	0.003019	3722746	15688.12	0.008438	0.006043	31410.8719	0.002813	0.002014	10470.29
O. edulis weight	2016	summer	ALL	0.007258	0.013481	39516808	286824.7	0.014517	0.026962	573675.937	0.004838	0.008986	191176.6
O. edulis weight	2016	summer	Blackwater	0.010085	0.017956	4397937	44351.14	0.020166	0.035898	88687.8882	0.006723	0.011971	29568.94
O. edulis weight	2016	summer	Crouch	0.056795	0.018559	1517307	86175.82	0.113602	0.037108	172368.348	0.037865	0.012375	57452.63
O. edulis weight	2016	summer	Eagle	1.83E-05	0.000197	4018615	73.54066	3.69E-05	0.000396	148.286911	1.23E-05	0.000132	49.42897
O. edulis weight	2016	summer	Outer Colne	0.000367	0.000218	4569339	1676.029	0.000734	0.000436	3352.41918	0.000245	0.000145	1117.528
O. edulis weight	2016	summer	Raysand	0.008074	0.007238	15086912	121813.7	0.016149	0.014477	243636.775	0.005383	0.004825	81209.23
O. edulis weight	2016	summer	Wallet										
O. edulis weight	2016	summer	Spitway	0.004448	0.003415	6203952	27593.42	0.008897	0.006828	55193.6819	0.002966	0.002276	18398.55
O. edulis weight	2016	summer	Whitaker Channel	0.001374	0.000754	3722746	5115.865	0.002749	0.001509	10233.0958	0.000916	0.000503	3411.059
O. edulis weight	2016	winter	ALL	0.010586	0.020841	39516808	418339.1	0.021173	0.041686	836670.767	0.007058	0.013895	278890.3
O. edulis weight	2016	winter	Blackwater	0.002617	0.002798	4397937	11509.38	0.005234	0.005596	23018.4804	0.001745	0.001865	7672.825

Species	Year	Season	Bed	Mean	SD	Area m2	Population total 20%	Mean10	SD10	Population total 10%	Mean30	SD30	Population total 30%
O. edulis weight	2016	winter	Crouch	0.08702	0.032612	1517307	132036.6	0.174067	0.065212	264112.755	0.058022	0.021737	88037.59
O. edulis weight	2016	winter	Eagle	0.000124	0.000275	4018615	499.2929	0.000249	0.000551	999.586393	8.29E-05	0.000184	333.1432
O. edulis weight	2016	winter	Outer Colne	0.002586	0.00191	4569339	11816.24	0.005174	0.003824	23642.5979	0.001725	0.001275	7880.867
O. edulis weight	2016	winter	Raysand	0.014351	0.017671	15086912	216515.4	0.028692	0.035349	432866.193	0.009564	0.011783	144288.7
O. edulis weight	2016	winter	Wallet	0.007209	0.006117	6203952	44722.45	0.014424	0.012232	89485.3252	0.004808	0.004077	29828.44
O. edulis weight	2016	winter	Spitway Whitaker Channel	0.000329	0.000343	3722746	1225.018	0.000657	0.000685	2446.71536	0.000219	0.000228	815.5718
O. edulis weight	2017	summer	ALL	0.006508	0.019254	39516808	257194.8	0.013017	0.038509	514398.433	0.004339	0.012836	171466.2
O. edulis weight	2017	summer	Blackwater	0.00506	0.006693	4397937	22251.53	0.010119	0.013386	44504.6403	0.003373	0.004462	14834.88
O. edulis weight	2017	summer	Crouch	0.096232	0.026859	1517307	146014.1	0.192467	0.053716	292030.782	0.064156	0.017905	97343.6
O. edulis weight	2017	summer	Eagle	0.000634	0.00033	4018615	2547.678	0.001268	0.00066	5095.32311	0.000423	0.00022	1698.44
O. edulis weight	2017	summer	Outer Colne	0.00227	0.001523	4569339	10374.66	0.004541	0.003046	20747.8895	0.001514	0.001015	6915.965
O. edulis weight	2017	summer	Raysand	0.004954	0.005478	15086912	74743.71	0.009908	0.010955	149484.171	0.003303	0.003652	49828.06
O. edulis weight	2017	summer	Wallet	0.000189	0.00029	6203952	1170.524	0.000377	0.000579	2341.12331	0.000126	0.000193	780.3765
O. edulis weight	2017	summer	Spitway Whitaker Channel	6.48E-06	5.85E-05	3722746	24.1234	1.30E-05	1.17E-04	48.3957002	4.33E-06	3.90E-05	16.11949
O. edulis weight	2017	winter	ALL	0.010438	0.020191	39516808	412459.1	0.020875	0.040382	824920.484	0.006958	0.013461	274973.5
O. edulis weight	2017	winter	Blackwater	0.003483	0.005023	4397937	15317	0.006964	0.010046	30627.1046	0.002321	0.003349	10209.04
O. edulis weight	2017	winter	Crouch	0.080081	0.032794	1517307	121507.6	0.160158	0.065588	243009.057	0.053386	0.021863	81003.02
O. edulis weight	2017	winter	Eagle	0	0	4018615	0	0	0	0	0	0	0

Species	Year	Season	Bed	Mean	SD	Area m2	Population total 20%	Mean10	SD10	Population total 10%	Mean30	SD30	Population total 30%
O. edulis weight	2017	winter	Outer Colne	0.001506	0.001446	4569339	6881.069	0.003012	0.002893	13763.6589	0.001004	0.000964	4587.886
O. edulis weight	2017	winter	Raysand	0.016867	0.017287	15086912	254476.7	0.033735	0.034576	508964.229	0.011245	0.011525	169654.7
O. edulis weight	2017	winter	Willet										
O. edulis weight	2017	winter	Spitway	0	0	6203952	0	0	0	0	0	0	0
O. edulis weight	2017	winter	Whitaker Channel	0.003828	0.001592	3722746	14251.24	0.007657	0.003184	28504.5165	0.002552	0.001061	9501.505
O. edulis weight	2018	summer	ALL	0.005604	0.014196	39516808	221446.3	0.014112	0.039511	557664.951	0.004704	0.01317	185888.3
O. edulis weight	2018	summer	Blackwater	0.003954	0.002958	4397937	17390.88	0.012204	0.013503	53672.5399	0.004068	0.004501	17890.85
O. edulis weight	2018	summer	Crouch	0.06483	0.027351	1517307	98367.23	0.181457	0.079465	275325.547	0.060486	0.026488	91775.18
O. edulis weight	2018	summer	Eagle	0	0	4018615	0	0	0	0	0	0	0
O. edulis weight	2018	summer	Outer Colne	0	0	4569339	0	0	0	0	0	0	0
O. edulis weight	2018	summer	Raysand	0.006304	0.007852	15086912	95110.83	0.013758	0.019071	207564.859	0.004586	0.006357	69188.29
O. edulis weight	2018	summer	Willet										
O. edulis weight	2018	summer	Spitway	0.001698	0.002505	6203952	10536.94	0.003399	0.00501	21088.2936	0.001133	0.00167	7029.431
O. edulis weight	2018	summer	Whitaker Channel	8.70E-06	7.26E-05	3722746	32.38789	1.72E-05	1.44E-04	64.0312341	5.73E-06	4.80E-05	21.33134
O. edulis weight	2018	winter	ALL	0.008596	0.014096	39516808	339697.6	0.017194	0.028199	679457.493	0.005731	0.0094	226485.8
O. edulis weight	2018	winter	Blackwater	0.003921	0.007191	4397937	17243.4	0.007835	0.014372	34459.5371	0.002612	0.004791	11486.51
O. edulis weight	2018	winter	Crouch	0.059306	0.014129	1517307	89985.67	0.118622	0.028257	179986.526	0.039541	0.009419	59995.51
O. edulis weight	2018	winter	Eagle	3.14E-06	0.000107	4018615	12.61845	6.52E-06	0.000218	26.2013728	2.17E-06	7.27E-05	8.720396
O. edulis weight	2018	winter	Outer Colne	0.004397	0.00421	4569339	20091.31	0.008801	0.008432	40212.6079	0.002934	0.002811	13404.2
O. edulis weight	2018	winter	Raysand	0.012692	0.011918	15086912	191480.8	0.02538	0.023837	382899.623	0.00846	0.007946	127633.2

Species	Year	Season	Bed	Mean	SD	Area m2	Population total 20%	Mean10	SD10	Population total 10%	Mean30	SD30	Population total 30%
O. edulis weight	2018	winter	Wallet Spitway	0.001806	0.002433	6203952	11204.77	0.003613	0.004866	22416.4604	0.001204	0.001622	7472.151
O. edulis weight	2018	winter	Whitaker Channel	0.002599	0.001862	3722746	9674.364	0.005194	0.003721	19334.4434	0.001731	0.00124	6444.814
O. edulis count	2014	summer	ALL	0.056219	0.231958	39516808	2221607	0.118002	0.507648	4663066.87	0.039326	0.169184	1554041
O. edulis count	2014	summer	Blackwater	0.083695	0.095776	4397937	368084.2	0.164494	0.217886	723433.518	0.054847	0.072637	241215.5
O. edulis count	2014	summer	Crouch	0.867243	0.816624	1517307	1315873	1.912718	1.766913	2902179.43	0.637498	0.588909	967280.3
O. edulis count	2014	summer	Eagle	0	0	4018615	0	0	0	0	0	0	0
O. edulis count	2014	summer	Outer Colne	0.01989	0.020793	4569339	90882.32	0.041759	0.047141	190809.013	0.013915	0.015709	63581.2
O. edulis count	2014	summer	Raysand	0.028636	0.02676	15086912	432032.4	0.054022	0.060561	815019.119	0.018003	0.020185	271613
O. edulis count	2014	summer	Wallet Spitway	0	0	6203952	0	0	0	0	0	0	0
O. edulis count	2014	summer	Whitaker Channel	3.98E-03	4.67E-03	3722746	14816.53	0.008471	0.010352	31535.8184	0.002824	0.00345	10511.24
O. edulis count	2014*	summer	ALL	0.085977	0.337581	39516808	3397540	0.171954	0.675162	6795080.23	0.057318	0.225054	2265027
O. edulis count	2014*	summer	Blackwater	0.094206	0.102539	4397937	414312.2	0.188412	0.205079	828624.431	0.062804	0.06836	276208.1
O. edulis count	2014*	summer	Crouch	1.516215	0.884099	1517307	2300563	3.03243	1.768199	4601126.47	1.01081	0.5894	1533709
O. edulis count	2014*	summer	Eagle	0.004032	0.003042	4018615	16203.17	0.008064	0.006085	32406.3442	0.002688	0.002028	10802.11
O. edulis count	2014*	summer	Outer Colne	0.041092	0.026756	4569339	187762.2	0.082184	0.053513	375524.35	0.027395	0.017838	125174.8
O. edulis count	2014*	summer	Raysand	0.029107	0.029523	15086912	439138.1	0.058214	0.059046	878276.262	0.019405	0.019682	292758.7
O. edulis count	2014*	summer	Wallet Spitway	0.001859	0.001648	6203952	11535.81	0.003719	0.003296	23071.6288	0.00124	0.001099	7690.543
O. edulis count	2014*	summer	Whitaker Channel	0.007697	0.00444	3722746	28652.47	0.015393	0.008881	57304.9317	0.005131	0.00296	19101.65

Species	Year	Season	Bed	Mean	SD	Area m2	Population total 20%	Mean10	SD10	Population total 10%	Mean30	SD30	Population total 30%
O. edulis count	2015	summer	ALL	0.13086	0.370696	39516808	5171152	0.261778	0.741589	10344637.4	0.087259	0.247196	3448212
O. edulis count	2015	summer	Blackwater	0.368204	0.819752	4397937	1619336	0.736982	1.640265	3241198.54	0.245661	0.546755	1080400
O. edulis count	2015	summer	Crouch	1.151631	0.467664	1517307	1747377	2.303245	0.935467	3494728.54	0.767748	0.311822	1164910
O. edulis count	2015	summer	Eagle	0	0	4018615	0	0	0	0	0	0	0
O. edulis count	2015	summer	Outer Colne	4.04E-04	6.54E-04	4569339	1846.013	8.07E-04	1.31E-03	3687.45684	2.69E-04	4.36E-04	1229.152
O. edulis count	2015	summer	Raysand	0.089099	0.064942	15086912	1344232	0.178205	0.129879	2688566.26	0.059402	0.043293	896188.8
O. edulis count	2015	summer	Wallet	0.030779	0.004968	6203952	190948.5	0.061564	0.00994	381942.555	0.020521	0.003313	127314.2
O. edulis count	2015	summer	Spitway	0.071941	0.03055	3722746	267818.4	1.44E-01	6.11E-02	536075.449	0.047995	0.02038	178673.1
O. edulis count	2015	summer	Whitaker Channel	0.071941	0.03055	3722746	267818.4	1.44E-01	6.11E-02	536075.449	0.047995	0.02038	178673.1
O. edulis count	2016	summer	ALL	0.111292	0.274854	39516808	4397924	0.22267	0.549862	8799200.61	0.074223	0.183287	2933067
O. edulis count	2016	summer	Blackwater	0.271335	0.587152	4397937	1193314	0.542905	1.174543	2387661.65	0.180968	0.391514	795887.2
O. edulis count	2016	summer	Crouch	0.867265	0.337002	1517307	1315907	1.734805	0.673529	2632231.62	0.578268	0.22451	877410.5
O. edulis count	2016	summer	Eagle	1.30E-04	0.001432	4018615	522.42	2.61E-04	0.00286	1048.85864	8.69E-05	0.000953	349.2177
O. edulis count	2016	summer	Outer Colne	0.014671	0.008727	4569339	67037.4	0.029347	0.017455	134096.735	0.009782	0.005818	44698.91
O. edulis count	2016	summer	Raysand	0.102883	0.098815	15086912	1552184	0.205784	0.197682	3104651.86	0.068595	0.065894	1034884
O. edulis count	2016	summer	Wallet	0.033903	0.027105	6203952	210332.7	0.067841	0.054192	420882.639	0.022614	0.018064	140294.2
O. edulis count	2016	summer	Spitway	0.015779	0.008738	3722746	58740.12	0.03156	0.017479	117491.671	0.01052	0.005826	39163.89
O. edulis count	2016	summer	Whitaker Channel	0.015779	0.008738	3722746	58740.12	0.03156	0.017479	117491.671	0.01052	0.005826	39163.89
O. edulis count	2016	winter	ALL	0.146188	0.355233	39516808	5776871	0.299205	0.720923	11823623.2	0.098088	0.237594	3876125
O. edulis count	2016	winter	Blackwater	0.034158	0.033261	4397937	150226	0.06832	0.066522	300465.554	0.02196	0.02211	96580.64

Species	Year	Season	Bed	Mean	SD	Area m2	Population total 20%	Mean10	SD10	Population total 10%	Mean30	SD30	Population total 30%
O. edulis count	2016	winter	Crouch	1.658809	0.646379	1517307	2516921	3.317773	1.292786	5034078.8	1.233605	0.331375	1871757
O. edulis count	2016	winter	Eagle	0.001061	0.002448	4018615	4263.984	0.002121	0.004894	8525.30786	0.000707	0.001629	2840.651
O. edulis count	2016	winter	Outer Colne	0.049129	0.037182	4569339	224488.4	0.098255	0.074361	448961.775	0.032754	0.024789	149663.2
O. edulis count	2016	winter	Raysand	0.155476	0.192011	15086912	2345651	0.310949	0.384025	4691252.82	0.103635	0.128022	1563536
O. edulis count	2016	winter	Wallet	0.082424	0.072424	6203952	511354.7	0.164839	0.144847	1022652.16	0.069968	0.047377	434077.9
O. edulis count	2016	winter	Spitway Whitaker Channel	0.006584	0.006849	3722746	24511.85	0.013171	0.0137	49031.0613	0.004387	0.004564	16333.27
O. edulis count	2017	summer	ALL	0.112745	0.291256	39516808	4455334	0.225575	0.582685	8914015.47	0.075192	0.194228	2971338
O. edulis count	2017	summer	Blackwater	0.165739	0.266285	4397937	728908.6	0.331487	0.532586	1457857.97	0.110496	0.177529	485952.7
O. edulis count	2017	summer	Crouch	1.410974	0.303527	1517307	2140880	2.821997	0.607037	4281834.8	0.940666	0.202346	1427278
O. edulis count	2017	summer	Eagle	0.016898	0.008789	4018615	67905.54	0.033795	0.017578	135810.026	0.011265	0.005859	45270.01
O. edulis count	2017	summer	Outer Colne	0.054023	0.028905	4569339	246851.3	0.10803	0.057805	493624.458	0.03601	0.019268	164541.5
O. edulis count	2017	summer	Raysand	0.079305	0.098432	15086912	1196470	0.158638	0.196899	2393355.14	0.052879	0.065633	797785.1
O. edulis count	2017	summer	Wallet	0.011905	0.013475	6203952	73858.9	0.023818	0.026947	147765.616	0.007939	0.008982	49255.2
O. edulis count	2017	summer	Spitway Whitaker Channel	1.99E-04	1.78E-03	3722746	740.8265	3.93E-04	3.54E-03	1463.03925	1.31E-04	1.18E-03	487.6797
O. edulis count	2017	winter	ALL	0.14831	0.316475	39516808	5860738	0.296598	0.63292	11720614.4	0.098866	0.210973	3906871
O. edulis count	2017	winter	Blackwater	0.144049	0.258056	4397937	633517.2	0.288254	0.516407	1267721.95	0.096085	0.172136	422574
O. edulis count	2017	winter	Crouch	1.288218	0.576949	1517307	1954622	2.576385	1.153912	3909165.74	0.858795	0.384637	1303055
O. edulis count	2017	winter	Eagle	0	0	4018615	0	0	0	0	0	0	0

Species	Year	Season	Bed	Mean	SD	Area m2	Population total 20%	Mean10	SD10	Population total 10%	Mean30	SD30	Population total 30%
O. edulis count	2017	winter	Outer Colne	0.034412	0.033062	4569339	157241.7	0.06885	0.066117	314597.866	0.02295	0.022039	104866
O. edulis count	2017	winter	Raysand	0.200237	0.230213	15086912	3020953	0.400455	0.460461	6041635	0.133485	0.153487	2013878
O. edulis count	2017	winter	Willet										
O. edulis count	2017	winter	Spitway	0	0	6203952	0	0	0	0	0	0	0
O. edulis count	2017	winter	Whitaker Channel	0.025021	0.012683	3722746	93146.74	0.050044	0.02537	186302.226	0.016681	0.008457	62100.74
O. edulis count	2018	summer	ALL	0.08804	0.260976	39516808	3479060	0.175996	0.521881	6954811.67	0.058665	0.17396	2318271
O. edulis count	2018	summer	Blackwater	0.093446	0.071266	4397937	410967.7	0.186883	0.142522	821900.122	0.062294	0.047507	273966.7
O. edulis count	2018	summer	Crouch	1.220222	0.547834	1517307	1851451	2.440986	1.095805	3703723.65	0.813662	0.365268	1234575
O. edulis count	2018	summer	Eagle	0	0	4018615	0	0	0	0	0	0	0
O. edulis count	2018	summer	Outer Colne	0	0	4569339	0	0	0	0	0	0	0
O. edulis count	2018	summer	Raysand	0.076541	0.093023	15086912	1154772	0.153076	0.1861	2309437.38	0.051025	0.062033	769812.5
O. edulis count	2018	summer	Willet										
O. edulis count	2018	summer	Spitway	0.009699	0.014315	6203952	60170.5	0.019419	0.02863	120475.498	0.006473	0.009543	40158.5
O. edulis count	2018	summer	Whitaker Channel	7.86E-05	6.57E-04	3722746	292.6078	1.56E-04	1.31E-03	580.748403	5.20E-05	4.36E-04	193.5828
O. edulis count	2018	winter	ALL	0.130734	0.265174	39516808	5166172	0.235354	0.398898	9300419.39	0.078451	0.132966	3100140
O. edulis count	2018	winter	Blackwater	0.106025	0.165462	4397937	466290.2	0.212222	0.331011	933338.261	0.070741	0.110337	311112.8
O. edulis count	2018	winter	Crouch	1.196615	0.494099	1517307	1815632	1.715872	0.495484	2603503.18	0.571957	0.165161	867834.4
O. edulis count	2018	winter	Eagle	2.72E-05	0.000901	4018615	109.3063	5.16E-05	0.001759	207.360558	1.72E-05	0.000586	69.12019
O. edulis count	2018	winter	Outer Colne	0.086262	0.082657	4569339	394159.7	0.172436	0.165124	787916.953	0.057479	0.055041	262639
O. edulis count	2018	winter	Raysand	0.153853	0.143614	15086912	2321166	0.307549	0.287218	4639965.63	0.102516	0.095739	1546655

O. edulis count	2018	winter	Wallet Spitway	0.014763	0.019839	6203952	91591.06	0.029515	0.039674	183107.942	0.009838	0.013225	61035.98
O. edulis count	2018	winter	Whitaker Channel	0.020421	0.014944	3722746	76022.08	0.040842	0.02989	152045.888	0.013614	0.009963	50681.96
C. gigas count	2016	winter	Raysand	0.057028	0.163302	15086912	860377.1	0.114074	0.326604	1721017.14	0.038025	0.108868	573672.4
C. gigas count	2016	winter	Blackwater	0.002989	0.006787	4397937	13145.2	0.005979	0.013575	26293.5272	0.001993	0.004525	8764.508
C. gigas count	2016	winter	Crouch Whitaker	0.885973	0.380794	1517307	1344293	1.771921	0.76172	2688547.2	0.59064	0.253907	896182.4
C. gigas count	2016	winter	Channel	0.06921	0.058051	3722746	257649.9	0.13822	0.11598	514556.36	0.046073	0.03866	171518.8
C. gigas count	2016	winter	Wallet Spitway	0.029194	0.015337	6203952	181118.4	0.058384	0.030681	362211.245	0.019461	0.010227	120737.1
C. gigas count	2016	winter	Eagle Outer	0.019285	0.008055	4018615	77500.48	0.038573	0.016111	155009.705	0.012858	0.00537	51669.9
C. gigas count	2016	winter	Colne	0.182545	0.083448	4569339	834110.6	0.365157	0.167078	1668527.54	0.121719	0.055693	556175.8
C. gigas count	2016	winter	ALL	0.090304	0.21123	39516808	3568529	0.180578	0.42254	7135880.89	0.060193	0.140847	2378627
C. gigas count	2016	summer	Raysand	0.032036	0.060927	15086912	483321.9	0.064085	0.121885	966850.352	0.021362	0.040628	322283.5
C. gigas count	2016	summer	Blackwater	0.005147	0.00894	4397937	22635.82	0.010292	0.017875	45262.3116	0.003431	0.005958	15087.44
C. gigas count	2016	summer	Crouch Whitaker	0.7371	0.501226	1517307	1118407	1.474372	1.002609	2237074.66	0.491457	0.334203	745691.6
C. gigas count	2016	summer	Channel	0.000295	0.004487	3722746	1098.225	0.000553	0.008685	2058.33736	0.000184	0.002895	686.1125
C. gigas count	2016	summer	Wallet Spitway	0.00384	0.005509	6203952	23825.4	0.007679	0.011017	47638.0623	0.00256	0.003672	15879.35
C. gigas count	2016	summer	Eagle Outer	1.67E-02	0.006453	4018615	67110.88	0.033439	0.012906	134379.063	0.011146	0.004302	44793.02
C. gigas count	2016	summer	Colne	0.899142	0.470405	4569339	4108487	1.798203	0.940991	8216597.7	0.599401	0.313664	2738866
C. gigas count	2016	summer	ALL	0.147488	0.360254	39516808	5828274	0.303984	0.73026	12012477.8	0.098292	0.240142	3884202
C. gigas count	2017	winter	Raysand	0.03492	0.058438	15086912	526834.3	0.069765	0.116618	1052544.58	0.023255	0.038873	350848.2

C. gigas count	2017	winter	Blackwater	0.017125	0.013514	4397937	75314.17	0.034247	0.027024	150615.021	0.011416	0.009008	50205.01
C. gigas count	2017	winter	Crouch	1.096922	1.04601	1517307	1664367	2.195197	2.093084	3330786.76	0.731732	0.697695	1110262
C. gigas count	2017	winter	Whitaker										
C. gigas count	2017	winter	Channel	0.016543	0.017633	3722746	61585.01	0.032954	0.034786	122677.992	0.010985	0.011595	40892.66
C. gigas count	2017	winter	Wallet										
C. gigas count	2017	winter	Spitway	0.037992	0.024008	6203952	235700.2	0.075973	0.048015	471331.054	0.025324	0.016005	157110.4
C. gigas count	2017	winter	Eagle	0.024847	0.011838	4018615	99849.54	0.024847	0.01184	99852.2701	0.016565	0.007893	66568.18
C. gigas count	2017	winter	Outer										
C. gigas count	2017	winter	Colne	0.067117	0.03883	4569339	306680.3	0.134236	0.077711	613368.297	0.044745	0.025904	204456.1
C. gigas count	2017	winter	ALL	0.075162	0.292429	39516808	2970171	0.15037	0.585321	5942136.12	0.050123	0.195107	1980712
C. gigas count	2017	summer	Raysand	0.023463	0.040191	15086912	353988	0.046911	0.080374	707741.215	0.015636	0.026785	235904.1
C. gigas count	2017	summer	Blackwater	0	0	4397937	0	0	0	0	0	0	0
C. gigas count	2017	summer	Crouch	1.5889	1.653126	1517307	2410849	3.176622	3.30525	4819909.77	1.05912	1.101933	1607010
C. gigas count	2017	summer	Whitaker										
C. gigas count	2017	summer	Channel	2.56E-04	2.30E-03	3722746	953.023	0.00026	0.002321	966.147673	0.000175	0.001559	650.7794
C. gigas count	2017	summer	Wallet										
C. gigas count	2017	summer	Spitway	0.00816	0.011449	6203952	50622.2	0.01632	0.022897	101246.928	0.005431	0.007632	33691.33
C. gigas count	2017	summer	Eagle	0.008437	0.004385	4018615	33904.03	0.016873	0.008769	67807.1379	0.005624	0.002923	22600.71
C. gigas count	2017	summer	Outer										
C. gigas count	2017	summer	Colne	0.029446	0.017826	4569339	134549.2	0.058889	0.035662	269083.436	0.019628	0.011894	89688.11
C. gigas count	2017	summer	ALL	0.075536	0.444035	39516808	2984929	0.151086	0.888007	5970422.38	0.050352	0.295997	1989746
C. gigas count	2018	winter	Raysand	0.033776	0.057176	15086912	509580.5	0.067503	0.114324	1018411.73	0.022501	0.038108	339470.6
C. gigas count	2018	winter	Blackwater	0.004602	0.005114	4397937	20241.08	0.009209	0.010228	40499.0544	0.00307	0.003409	13499.68
C. gigas count	2018	winter	Crouch	0.538813	0.652629	1517307	817544.8	1.108397	1.337768	1681777.75	0.369466	0.445923	560592.6
C. gigas count	2018	winter	Whitaker										
C. gigas count	2018	winter	Channel	0.000211	0.003103	3722746	786.0951	0.000407	0.005747	1515.76358	0.000136	0.001916	505.2545

C. gigas count	2018	winter	Wallet Spitway	0.003966 0.00E+0	0.005509	6203952	24606.67	0.007933	0.011017	49216.1879	0.002644	0.003672	16405.4
C. gigas count	2018	winter	Eagle	0	0	4018615	0	0	0	0	0	0	0
C. gigas count	2018	winter	Outer Colne	0.195074	0.104685	4569339	891360.6	0.390163	0.209394	1782786.44	0.130054	0.069798	594262.1
C. gigas count	2018	winter	ALL	0.057312	0.177692	39516808	2264794	0.115778	0.363192	4575187.3	0.038593	0.121064	1525062
C. gigas count	2018	summer	Raysand	0.024731	0.057839	15086912	373121	0.04946	0.115639	746198.626	0.016487	0.038546	248732.9
C. gigas count	2018	summer	Blackwater	0.00488	0.005961	4397937	21463.9	0.009761	0.011924	42927.7376	0.003254	0.003975	14309.25
C. gigas count	2018	summer	Crouch	1.181882	0.888314	1517307	1793277	2.363131	1.776258	3585594.73	0.78771	0.592086	1195198
C. gigas count	2018	summer	Whitaker Channel	1.55E-03	1.06E-02	3722746	5770.257	0.003141	0.021601	11692.1586	0.001047	0.0072	3897.386
C. gigas count	2018	summer	Wallet Spitway	0.003789	0.003789	6203952	23506.63	0.007578	0.010706	47011.4489	0.002526	0.003569	15670.48
C. gigas count	2018	summer	Eagle	0.034308	0.024599	4018615	137871.6	0.068619	0.049203	275753.095	0.022873	0.016401	91917.7
C. gigas count	2018	summer	Outer Colne	0.042456	0.038719	4569339	193996.4	0.084883	0.077354	387859.214	0.028294	0.025785	129286.4
C. gigas count	2018	summer	ALL	0.064507	0.286146	39516808	2549101	0.128957	0.572005	5095950.14	0.042986	0.190668	1698650
C. forficata weight	2016	winter	Raysand	0.299928	0.424532	15086912	4524986	0.599854	0.849083	9049950.91	0.199951	0.283028	3016650
C. forficata weight	2016	winter	Blackwater	0.439237	0.533972	4397937	1931738	0.878501	1.068042	3863590.81	0.292834	0.356014	1287864
C. forficata weight	2016	winter	Crouch	0.048135	0.018354	1517307	73035.37	0.096254	0.036714	146046.46	0.032085	0.012238	48682.15
C. forficata weight	2016	winter	Whitaker Channel	0.007732	0.008409	3722746	28784.2	0.015448	0.016785	57509.7736	0.005149	0.005595	19169.92
C. forficata weight	2016	winter	Wallet Spitway	0.000198	0.000198	6203952	1227.731	0.000396	0.000396	2454.44381	0.000132	0.000132	818.1477

C. fornicata weight	2016	winter	Eagle	9.91E-05	6.25E-05	4018615	398.2448	0.000198	0.000125	796.188284	6.60E-05	4.16E-05	265.3961
C. fornicata weight	2016	winter	Outer Colne	0.000782	0.0003	4569339	3571.012	0.001564	0.0006	7145.83239	0.000521	0.0002	2381.944
C. fornicata weight	2016	winter	ALL	0.166073	0.358977	39516808	6562676	0.332227	0.71805	13128570	0.110742	0.23935	4376190
C. fornicata weight	2016	summer	Raysand	0.20087	0.271618	15086912	3030503	0.401808	0.543216	6062047.72	0.133936	0.181072	2020683
C. fornicata weight	2016	summer	Blackwater	0.441956	0.571321	4397937	1943693	0.883866	1.142638	3887188.21	0.294622	0.380879	1295729
C. fornicata weight	2016	summer	Crouch	0.03119	0.013175	1517307	47325.52	0.062378	0.026355	94646.0278	0.020793	0.008785	31548.68
C. fornicata weight	2016	summer	Whitaker Channel	5.84E-05	0.000927	3722746	217.4084	0.000122	0.001898	455.770824	4.08E-05	0.000633	151.9236
C. fornicata weight	2016	summer	Wallet Spitway	0.000192	0.000275	6203952	1189.409	0.000383	0.000551	2378.13654	0.000128	0.000184	792.7124
C. fornicata weight	2016	summer	Eagle	0.00E+0 0	0	4018615	0	0	0	0	0	0	0
C. fornicata weight	2016	summer	Outer Colne	0.007077	0.003046	4569339	32336.91	0.014155	0.006095	64681.2112	0.004718	0.002032	21560.4
C. fornicata weight	2016	summer	ALL	0.127923	0.292108	39516808	5055104	0.25591	0.584235	10112763.5	0.085303	0.194745	3370921
C. fornicata weight	2017	winter	Raysand	0.255964	0.474853	15086912	3861704	0.512065	0.949762	7725473.36	0.170747	0.316823	2576042
C. fornicata weight	2017	winter	Blackwater	0.39981	0.650823	4397937	1758337	0.799759	1.302091	3517287.83	0.266539	0.434107	1172221

C. fornicata weight	2017	winter	Crouch	0.093531	0.063019	1517307	141915.5	0.187218	0.126105	284067.033	0.062425	0.042077	94717.49
C. fornicata weight	2017	winter	Whitaker Channel	0.00024	0.002679	3722746	893.7681	0.000478	0.005353	1780.86539	0.00016	0.001788	594.6153
C. fornicata weight	2017	winter	Wallet Spitway	0	0	6203952	0	0	0	0	0	0	0
C. fornicata weight	2017	winter	Eagle	0	0	4018615	0	0	0	0	0	0	0
C. fornicata weight	2017	winter	Outer Colne	0.000103	8.20E-05	4569339	468.8188	0.000205	0.000164	937.935216	6.85E-05	5.48E-05	312.8991
C. fornicata weight	2017	winter	ALL	0.145849	0.394005	39516808	5763492	0.291772	0.788177	11529878.5	0.097275	0.262851	3843999
C. fornicata weight	2017	summer	Raysand	0.324219	0.361902	15086912	4891462	0.648457	0.723769	9783212.13	0.216152	0.241256	3261071
C. fornicata weight	2017	summer	Blackwater	0.367879	0.548122	4397937	1617907	0.735574	1.096096	3235007.55	0.245191	0.365365	1078336
C. fornicata weight	2017	summer	Crouch	0.105856	0.052336	1517307	160615.9	0.211738	0.104669	321271.552	0.070579	0.03489	107090.5
C. fornicata weight	2017	summer	Whitaker Channel	5.79E-05	5.24E-04	3722746	215.547	0.000117	0.001057	436.723859	3.91E-05	0.000352	145.5746
C. fornicata weight	2017	summer	Wallet Spitway	3.79E-05	4.85E-05	6203952	235.1298	7.59E-05	9.70E-05	470.941919	2.53E-05	3.23E-05	156.9806
C. fornicata weight	2017	summer	Eagle	0	0	4018615	0	0	0	0	0	0	0
C. fornicata weight	2017	summer	Outer Colne	8.08E-05	8.69E-05	4569339	369.2026	0.000162	0.000174	738.796935	5.39E-05	5.79E-05	246.2656

C. fornicata weight	2017	summer	ALL	0.168772	0.332658	39516808	6669321	0.337609	0.665311	13341215.6	0.112536	0.22177	4447072
C. fornicata weight	2018	winter	Raysand	0.27974	0.335002	15086912	4220412	0.559334	0.669956	8438615.69	0.186445	0.223319	2812872
C. fornicata weight	2018	winter	Blackwater	0.436586	0.67413	4397937	1920076	0.873233	1.348148	3840424.9	0.291078	0.449383	1280142
C. fornicata weight	2018	winter	Crouch	0.090666	0.048475	1517307	137567.6	0.117719	0.051049	178616.091	0.03924	0.017016	59538.7
C. fornicata weight	2018	winter	Whitaker Channel	0.000178	0.002066	3722746	664.1454	0.000353	0.004078	1312.99483	0.000118	0.001359	437.6649
C. fornicata weight	2018	winter	Wallet Spitway	0	0	0	0	0	0	0	0	0	0
C. fornicata weight	2018	winter	Eagle	0.00E+0 0	0	0	0	0	0	0	0	0	0
C. fornicata weight	2018	winter	Outer Colne	0.002932	0.002472	4569339	13396.96	0.005863	0.004945	26788.5703	0.001954	0.001648	8929.523
C. fornicata weight	2018	winter	ALL	0.159194	0.345767	39516808	6290841	0.315939	0.691901	12484891	0.105313	0.230634	4161630
C. fornicata weight	2018	summer	Raysand	0.233322	0.312911	15086912	3520109	0.466612	0.625693	7039728.39	0.155537	0.208564	2346576
C. fornicata weight	2018	summer	Blackwater	0.435817	0.711732	4397937	1916694	0.871493	1.423673	3832769.2	0.290498	0.474558	1277590
C. fornicata weight	2018	summer	Crouch	0.137519	0.07049	1517307	208657.9	0.274998	0.140976	417256.161	0.091666	0.046992	139085.4
C. fornicata weight	2018	summer	Whitaker Channel	2.55E-04	1.77E-03	3722746	949.3003	0.000507	0.003558	1888.43621	0.000169	0.001186	629.4787

C. fornicata weight	2018	summer	Wallet Spitway	0	0	0	0	0	0	0	0	0	0
C. fornicata weight	2018	summer	Eagle	0	0	0	0	0	0	0	0	0	0
C. fornicata weight	2018	summer	Outer Colne	0.001749	0.000677	4569339	7992.588	0.003497	0.001353	15980.704	0.001166	0.000451	5326.902
C. fornicata weight	2018	summer	ALL	0.143104	0.340799	39516808	5654998	0.286148	0.681487	11307668.4	0.095383	0.227162	3769223
A. rubens count	2016	winter	Raysand	0.00919	0.01465	15086912	138646.3						
A. rubens count	2016	winter	Blackwater	0.011289	0.004839	4397937	49647.84						
A. rubens count	2016	winter	Crouch	0.00849	0.003623	1517307	12882.08						
A. rubens count	2016	winter	Whitaker										
A. rubens count	2016	winter	Channel	0	0	3722746	0						
A. rubens count	2016	winter	Wallet										
A. rubens count	2016	winter	Spitway	0.102525	0.054754	6203952	636058.2						
A. rubens count	2016	winter	Eagle	1.10E-01	3.78E-02	4018615	442047.7						
A. rubens count	2016	winter	Outer Colne	0.002917	0.001653	4569339	13329.43						
A. rubens count	2016	winter	ALL	0.032737	0.050663	39516808	1293670						
A. rubens count	2016	summer	Raysand	0.001843	0.003315	15086912	27810.79						
A. rubens count	2016	summer	Blackwater	0.006159	0.003126	4397937	27087.68						
A. rubens count	2016	summer	Crouch	0.00046	0.001715	1517307	697.9307						
A. rubens count	2016	summer	Whitaker										
A. rubens count	2016	summer	Channel	1.85E-02	0.006193	3722746	68870.8						
A. rubens count	2016	summer	Wallet										
A. rubens count	2016	summer	Spitway	0.040889	0.012746	6203952	253670.5						

A. rubens count	2016	summer	Eagle	7.27E-02	0.053089	4018615	292153.3
A. rubens count	2016	summer	Outer Colne	0.008802	0.00524	4569339	40221.23
A. rubens count	2016	summer	ALL	0.017975	0.02911	39516808	710302.9
A. rubens count	2017	winter	Raysand	0.008652	0.014952	15086912	130526.1
A. rubens count	2017	winter	Blackwater	0.01841	0.006637	4397937	80965.34
A. rubens count	2017	winter	Crouch	0.005837	0.004681	1517307	8856.778
A. rubens count	2017	winter	Whitaker				
A. rubens count	2017	winter	Channel	0.01145	0.005802	3722746	42624.42
A. rubens count	2017	winter	Wallet				
A. rubens count	2017	winter	Spitway	0.049353	0.017677	6203952	306185
A. rubens count	2017	winter	Eagle	0.219527	0.084653	4018615	882193.2
A. rubens count	2017	winter	Outer Colne	0.001509	1.01E-03	4569339	6897.331
A. rubens count	2017	winter	ALL	0.036898	0.06981	39516808	1458099
A. rubens count	2017	summer	Raysand	0.004325	0.005619	15086912	65257.48
A. rubens count	2017	summer	Blackwater	0.004165	0.002489	4397937	18317.57
A. rubens count	2017	summer	Crouch	0.001861	0.001616	1517307	2824.084
A. rubens count	2017	summer	Whitaker				
A. rubens count	2017	summer	Channel	9.31E-03	4.36E-03	3722746	34659.45
A. rubens count	2017	summer	Wallet				
A. rubens count	2017	summer	Spitway	3.52E-02	2.85E-02	6203952	219170.9
A. rubens count	2017	summer	Eagle	0.058874	0.015531	4018615	236592.6
A. rubens count	2017	summer	Outer Colne	0	0	4569339	0
A. rubens count	2017	summer	ALL	0.014597	0.022795	39516808	576835.8

A. rubens count	2018	winter	Raysand	0.012414	0.018552	15086912	187287.2
A. rubens count	2018	winter	Blackwater	0.018258	0.014841	4397937	80298.94
A. rubens count	2018	winter	Crouch	0.006405	0.00457	1517307	9717.602
A. rubens count	2018	winter	Whitaker				
A. rubens count	2018	winter	Channel	0.02498	0.00762	3722746	92994.34
A. rubens count	2018	winter	Wallet				
A. rubens count	2018	winter	Spitway	0.02944	0.010554	6203952	182644.5
A. rubens count	2018	winter	Eagle	5.37E-02	0.017356	4018615	215799.7
A. rubens count	2018	winter	Outer				
A. rubens count	2018	winter	Colne	0.004919	0.001766	4569339	22476.04
A. rubens count	2018	winter	ALL	0.02002	0.019954	39516808	791136.8
A. rubens count	2018	summer	Raysand	0.004034	0.007339	15086912	60860.51
A. rubens count	2018	summer	Blackwater	0.004603	0.003359	4397937	20242.82
A. rubens count	2018	summer	Crouch	0.001439	0.001782	1517307	2183.714
A. rubens count	2018	summer	Whitaker				
A. rubens count	2018	summer	Channel	2.05E-02	9.03E-03	3722746	76316.3
A. rubens count	2018	summer	Wallet				
A. rubens count	2018	summer	Spitway	0.014707	0.006568	6203952	91244.08
A. rubens count	2018	summer	Eagle	0.064245	0.025807	4018615	258177.4
A. rubens count	2018	summer	Outer				
A. rubens count	2018	summer	Colne	0.00146	0.000838	4569339	6673.255
A. rubens count	2018	summer	ALL	0.01305	0.020883	39516808	515702.3
C. maenas count	2016	winter	Raysand	0.004837	0.006633	15086912	72976.84
C. maenas count	2016	winter	Blackwater	0.027858	0.02408	4397937	122517.2
C. maenas count	2016	winter	Crouch	0.02408	0.024131	1517307	36536.2

C. maenas count	2016	winter	Whitaker Channel	0.01795	0.00203	3722746	66821.79
C. maenas count	2016	winter	Wallet Spitway	0	0	6203952	0
C. maenas count	2016	winter	Eagle	0.00E+0	0.00E+0	4018615	0
C. maenas count	2016	winter	Outer Colne	0	0	4018615	0
C. maenas count	2016	winter	ALL	0.009001	0.015303	39516808	355691.3
C. maenas count	2016	summer	Raysand	0.002406	0.007104	15086912	36295.04
C. maenas count	2016	summer	Blackwater	0.010703	0.005454	4397937	47071.94
C. maenas count	2016	summer	Crouch	0.010194	0.005696	1517307	15467.55
C. maenas count	2016	summer	Whitaker Channel	1.19E-02	0.007854	3722746	44300.68
C. maenas count	2016	summer	Wallet Spitway	0.000856	0.001193	6203952	5308.858
C. maenas count	2016	summer	Eagle	1.82E-03	0.001415	4018615	7313.88
C. maenas count	2016	summer	Outer Colne	0.006145	0.002946	4569339	28077.32
C. maenas count	2016	summer	ALL	0.004653	0.006822	39516808	183860.1
C. maenas count	2017	winter	Raysand	0.010091	0.011493	15086912	152245.1
C. maenas count	2017	winter	Blackwater	0.048039	0.025871	4397937	211272.1
C. maenas count	2017	winter	Crouch	0.062075	0.013283	1517307	94187.24
C. maenas count	2017	winter	Whitaker Channel	0.041587	0.016122	3722746	154816.9
C. maenas count	2017	winter	Wallet Spitway	0.003449	0.004231	6203952	21396.7
C. maenas count	2017	winter	Eagle	2.76E-05	0.000318	4018615	110.9138

C. maenas count	2017	winter	Outer Colne	0.013064	7.08E-03	4569339	59693.36
C. maenas count	2017	winter	ALL	0.017552	0.022016	39516808	693616.9
C. maenas count	2017	summer	Raysand	0.006834	0.007434	15086912	103101.6
C. maenas count	2017	summer	Blackwater	0.023396	0.012682	4397937	102895
C. maenas count	2017	summer	Crouch	0.026493	0.011713	1517307	40197.76
C. maenas count	2017	summer	Whitaker				
C. maenas count	2017	summer	Channel	1.74E-02	5.45E-03	3722746	64775.78
C. maenas count	2017	summer	Wallet				
C. maenas count	2017	summer	Spitway	7.59E-04	9.70E-04	6203952	4708.8
C. maenas count	2017	summer	Eagle	0.006778	0.001605	4018615	27237.31
C. maenas count	2017	summer	Outer Colne	1.39E-02	5.91E-03	4569339	63513.82
C. maenas count	2017	summer	ALL	0.011073	0.011369	39516808	437580.9
C. maenas count	2018	winter	Raysand	0.019836	0.020741	15086912	299264.7
C. maenas count	2018	winter	Blackwater	0.025507	0.024519	4397937	112178.7
C. maenas count	2018	winter	Crouch	0.015859	0.015908	1517307	24062.36
C. maenas count	2018	winter	Whitaker				
C. maenas count	2018	winter	Channel	0.021543	0.007366	3722746	80198.8
C. maenas count	2018	winter	Wallet				
C. maenas count	2018	winter	Spitway	0.005879	0.002513	6203952	36474.6
C. maenas count	2018	winter	Eagle	1.63E-03	0.000945	4018615	6550.343
C. maenas count	2018	winter	Outer Colne	0.008037	0.003635	4569339	36726.01
C. maenas count	2018	winter	ALL	0.015068	0.017626	39516808	595436.2
C. maenas count	2018	summer	Raysand	0.003127	0.004375	15086912	47183.53

C. maenas count	2018	summer	Blackwater	0.007752	0.006991	4397937	34093.6
C. maenas count	2018	summer	Crouch	0.009738	0.006574	1517307	14774.89
C. maenas count	2018	summer	Whitaker Channel	8.81E-03	3.11E-03	3722746	32797.39
C. maenas count	2018	summer	Wallet Spitway	0	0	6203952	0
C. maenas count	2018	summer	Eagle	0.006461	0.002687	4018615	25964.17
C. maenas count	2018	summer	Outer Colne	0	0	4569339	0
C. maenas count	2018	summer	ALL	0.003919	0.005131	39516808	154856.7

Supplementary Information Table 2.2. Tukey's *post-hoc* comparisons of *Ostrea edulis* biomass between oyster bed areas from dredge surveys between 2016 and 2018. A positive coefficient estimates indicates the first site listed has higher biomass rates and a negative coefficient indicating the second site listed has higher biomass with statistically significant results highlighted in grey.

Site comparison	Coefficient	Std. Error	z value	Pr(> z)
Crouch - Blackwater	2.2151	0.5998	3.693	0.00384
Eagle - Blackwater	-4.1474	0.7417	-5.592	<0.001
Outer Colne - Blackwater	-1.3273	0.7316	-1.814	0.51503
Raysand - Blackwater	0.4836	0.4592	1.053	0.93466
Wallet Spitway - Blackwater	-0.9742	0.5999	-1.624	0.6449
Whitaker - Blackwater	-1.5701	0.7683	-2.044	0.36492
Eagle - Crouch	-6.3625	0.7437	-8.555	<0.001
Outer Colne - Crouch	-3.5424	0.7337	-4.828	<0.001
Raysand - Crouch	-1.7315	0.4624	-3.744	0.00303
Wallet Spitway - Crouch	-3.1893	0.6024	-5.295	<0.001
Whitaker - Crouch	-3.7852	0.7702	-4.914	<0.001
Outer Colne - Eagle	2.8201	0.8536	3.304	0.01497
Raysand - Eagle	4.631	0.6357	7.284	<0.001
Wallet Spitway - Eagle	3.1732	0.7438	4.266	<0.001
Whitaker - Eagle	2.5773	0.8852	2.912	0.05082
Raysand - Outer Colne	1.8109	0.624	2.902	0.05252
Wallet Spitway - Outer Colne	0.3531	0.7337	0.481	0.99894
Whitaker - Outer Colne	-0.2428	0.8768	-0.277	0.99996
Wallet Spitway - Raysand	-1.4578	0.4625	-3.152	0.02471
Whitaker - Raysand	-2.0537	0.6666	-3.081	0.03096
Whitaker - Wallet Spitway	-0.5959	0.7703	-0.774	0.98572

Supplementary Information Table 2.3. Tukey's *post-hoc* comparisons of *Ostrea edulis* abundance (counts) between oyster bed areas from dredge surveys between 2016 and 2018. Positive coefficient estimates indicate the first site listed has higher abundances with statistically significant results highlighted in grey.

Site comparison	Coefficient	Std. Error	z value	Pr(> z)
Crouch - Blackwater	1.6563	0.4127	4.014	0.00114
Eagle - Blackwater	-4.2217	0.5988	-7.051	<0.001
Outer Colne - Blackwater	-1.6166	0.5098	-3.171	0.02283
Raysand - Blackwater	-0.3149	0.3165	-0.995	0.94903
Wallet Spitway - Blackwater	-2.0416	0.4192	-4.871	<0.001
Whitaker - Blackwater	-2.8597	0.5542	-5.16	<0.001
Eagle - Crouch	-5.878	0.5995	-9.805	<0.001
Outer Colne - Crouch	-3.2728	0.5106	-6.41	<0.001
Raysand - Crouch	-1.9712	0.3178	-6.202	<0.001
Wallet Spitway - Crouch	-3.6979	0.4202	-8.801	<0.001
Whitaker - Crouch	-4.516	0.555	-8.137	<0.001
Outer Colne - Eagle	2.6052	0.6701	3.888	0.00175
Raysand - Eagle	3.9068	0.5378	7.264	<0.001
Wallet Spitway - Eagle	2.1801	0.604	3.61	0.00507
Wallet Spitway - Eagle	1.362	0.7044	1.933	0.43112
Raysand - Outer Colne	1.3016	0.4366	2.981	0.04069
Wallet Spitway - Outer Colne	-0.425	0.5159	-0.824	0.97987
Whitaker - Outer Colne	-1.2432	0.6306	-1.972	0.40647
Wallet Spitway - Raysand	-1.7267	0.3262	-5.292	<0.001
Whitaker - Raysand	-2.5448	0.4877	-5.218	<0.001
Whitaker - Wallet	-0.8182	0.5598	-1.461	0.74659

Supplementary Information Table 2.4. Tukey's *post-hoc* comparisons of *Crepidula fornicata*

biomass between oyster bed areas from dredge surveys between 2016 and 2018. Negative coefficients indicate the second site listed has higher biomass with statistically significant results highlighted in grey.

Site comparison	Coefficient	Std. Error	z value	Pr(> z)
Crouch - Blackwater	-2.3178	0.5107	-4.539	<0.001
Eagle - Blackwater	-10.6899	0.7725	-13.838	<0.001
Outer Colne - Blackwater	-5.6986	0.6239	-9.134	<0.001
Raysand - Blackwater	-0.669	0.3909	-1.711	0.542
Wallet Spitway - Blackwater	-9.0976	0.5299	-17.169	<0.001
Whitaker - Blackwater	-28.2346	1848.712	-0.015	1
Eagle - Crouch	-8.3721	0.774	-10.817	<0.001
Outer Colne - Crouch	-3.3808	0.6256	-5.404	<0.001
Raysand - Crouch	1.6488	0.3937	4.188	<0.001
Wallet Spitway - Crouch	-6.7798	0.532	-12.745	<0.001
Whitaker - Crouch	-25.9168	1848.712	-0.014	1
Outer Colne - Eagle	4.9913	0.8529	5.852	<0.001
Raysand - Eagle	10.0209	0.7007	14.301	<0.001
Wallet Spitway - Eagle	1.5923	0.7868	2.024	0.336
Whitaker - Eagle	-17.5447	1848.712	-0.009	1
Raysand - Outer Colne	5.0296	0.5324	9.448	<0.001
Wallet Spitway - Outer Colne	-3.399	0.6414	-5.299	<0.001
Whitaker - Outer Colne	-22.536	1848.712	-0.012	1
Wallet Spitway - Raysand	-8.4286	0.4183	-20.148	<0.001
Whitaker - Raysand	-27.5656	1848.712	-0.015	1
Whitaker - Wallet Spitway	-19.1371	1848.712	-0.01	1

Supplementary Information Table 2.5. Tukey's *post-hoc* comparisons of *Asterias rubens* abundances between biannual dredge surveys between 2016 and 2018. Positive coefficients indicate the first survey listed has higher *A. rubens* abundances and a negative coefficient indicating the second survey listed has higher biomass with statistically significant results highlighted in grey.

Site comparison	Coefficient	Std. Error	z value	Pr(> z)
Post-winter 2017 - Post-winter 2016	-0.27816	0.25568	-1.088	0.88612
Post-winter 2018 - Post-winter 2016	-0.11989	0.25409	-0.472	0.99709
Post-summer 2016 - Post-winter 2016	-0.9548	0.26754	-3.569	0.00479
Post-summer 2017 - Post-winter 2016	-1.11202	0.27429	-4.054	<0.001
Post-summer 2018 - Post-winter 2016	-1.14588	0.27533	-4.162	<0.001
Post-winter 2018 - Post-winter 2017	0.15826	0.25448	0.622	0.98943
Post-summer 2016 - Post-winter 2017	-0.67664	0.26789	-2.526	0.11628
Post-summer 2017 - Post-winter 2017	-0.83387	0.27431	-3.04	0.02849
Post-summer 2018 - Post-winter 2017	-0.86772	0.27533	-3.152	0.02024
Post-summer 2016 - Post-winter 2018	-0.83491	0.26643	-3.134	0.0212
Post-summer 2017 - Post-winter 2018	-0.99213	0.27284	-3.636	0.00374
Post-summer 2018 - Post-winter 2018	-1.02598	0.27387	-3.746	0.00249
Post-summer 2017 - Post-summer 2016	-0.15722	0.28515	-0.551	0.99394
Post-summer 2018 - Post-summer 2016	-0.19108	0.28612	-0.668	0.98538
Post-summer 2018 - Post-summer 2017	-0.03386	0.29204	-0.116	1

Supplementary Information Table 2.6. Tukey's *post-hoc* comparisons of *Asterias rubens* abundances between oyster bed areas from dredge surveys between 2016 and 2018. Positive coefficients indicate the first site listed has higher *A. rubens* abundances and a negative coefficient indicating the second site listed has higher biomass with statistically significant results highlighted in grey.

Site comparison	Coefficient	Std. Error	z value	Pr(> z)
Crouch - Blackwater	-0.3395	0.3454	-0.983	0.95301
Eagle - Blackwater	2.3266	0.3787	6.143	<0.001
Outer Colne - Blackwater	-0.9619	0.4637	-2.075	0.34729
Raysand - Blackwater	-0.5259	0.2611	-2.014	0.38364
Wallet Spitway - Blackwater	1.4769	0.3181	4.643	<0.001
Whitaker - Blackwater	0.6626	0.4136	1.602	0.66079
Eagle - Crouch	2.6661	0.3883	6.867	<0.001
Outer Colne - Crouch	-0.6224	0.4712	-1.321	0.82879
Raysand - Crouch	-0.1864	0.2743	-0.68	0.99288
Wallet Spitway - Crouch	1.8164	0.3293	5.517	<0.001
Whitaker - Crouch	1.0021	0.4229	2.37	0.19644
Outer Colne - Eagle	-3.2884	0.4964	-6.625	<0.001
Raysand - Eagle	-2.8525	0.3154	-9.043	<0.001
Wallet Spitway - Eagle	-0.8497	0.3636	-2.337	0.21071
Whitaker - Eagle	-1.664	0.449	-3.706	0.00364
Raysand - Outer Colne	0.4359	0.4134	1.055	0.93463
Wallet Spitway - Outer Colne	2.4388	0.4518	5.398	<0.001
Whitaker - Outer Colne	1.6245	0.5236	3.102	0.02862
Wallet Spitway - Raysand	2.0028	0.2392	8.373	<0.001
Whitaker - Raysand	1.1885	0.3567	3.332	0.01374
Whitaker - Wallet Spitway	-0.8143	0.3995	-2.038	0.36918

Supplementary Information Table 2.7. Tukey's *post-hoc* comparisons of *Crassostrea gigas* abundances between oyster bed areas from dredge surveys between 2016 and 2018. Positive coefficients indicate the first site listed have higher *Crassostrea gigas* abundances and a negative coefficient indicating the second site listed has higher biomass with statistically significant results highlighted in grey.

Site comparison	Coefficient	Std. Error	z value	Pr(> z)
Crouch - Blackwater	5.0663	0.5168	9.803	<0.001
Eagle - Blackwater	0.8926	0.6586	1.355	0.80326
Outer Colne - Blackwater	3.5995	0.5917	6.083	<0.001
Raysand - Blackwater	1.9032	0.4472	4.256	<0.001
Wallet Spitway - Blackwater	0.6931	0.5613	1.235	0.86357
Whitaker - Blackwater	-0.79	0.9326	-0.847	0.97607
Eagle - Crouch	-4.1737	0.5794	-7.203	<0.001
Outer Colne - Crouch	-1.4668	0.5022	-2.921	0.04708
Raysand - Crouch	-3.1632	0.3194	-9.902	<0.001
Wallet Spitway - Crouch	-4.3732	0.4659	-9.386	<0.001
Whitaker - Crouch	-5.8563	0.8785	-6.666	<0.001
Outer Colne - Eagle	2.7069	0.6472	4.183	<0.001
Raysand - Eagle	1.0106	0.5183	1.95	0.4143
Wallet Spitway - Eagle	-0.1994	0.6194	-0.322	0.99989
Whitaker - Eagle	-1.6826	0.9687	-1.737	0.55766
Raysand - Outer Colne	-1.6964	0.4302	-3.943	0.00123
Wallet Spitway - Outer Colne	-2.9064	0.5479	-5.305	<0.001
Whitaker - Outer Colne	-4.3895	0.9246	-4.748	<0.001
Wallet Spitway - Raysand	-1.21	0.3873	-3.124	0.02572
Whitaker - Raysand	-2.6931	0.8395	-3.208	0.01973
Whitaker - Wallet	-1.4831	0.9054	-1.638	0.62586

Appendix II:

Supplementary Information for Chapter 3: Density and seasonally dependent associations of biodiversity with the European flat oyster (*Ostrea edulis*): evidence for marine planning.

Supplementary Information 3.1

Standardising and centering predictors

Figure S3.1: Comparative plots of coefficients extracted from negative binomial ManyGLM using centred vs uncentred predictors and using *O. edulis* weight (kg m⁻²) vs counts.

Table S1: Likelihood ratios and P values of predictors comparing centred vs un centred and use of *O. edulis* count vs weight in the negative binomial manyglm models.

Supplementary Information 3.2

Using mixed models as opposed to manyglm

Table S3.2: Coefficients and significant values for various mixed models incorporating spatial and temporal repeated measures of the native oyster survey between 2016 and 2017

Supplementary Information 3.3

Figure S2: Average weight of native oysters per m² in a 100m dredge adjusted to 20% dredge efficiency vs number of different species within the dredge

Supplementary Information 3.4

Figure S3: Distribution of number of species in a dredge grouped by weight of native oyster.

Supplementary Information 3.5

Results from full species matrix presence/absence from multivariate analysis

Figure S4: Unstandardized coefficients for environmental variables for each species from negative binomial manyglm using total shell weight (kg m^{-2} adjusted to 10% dredge efficiency), live *C. fornicata* weight (kg m^{-2} adjusted to 20% dredge efficiency), *O. edulis* density (m^{-2} adjusted to 20% dredge efficiency) predictors.

Supplementary Information 3.6

R code for ManyGLM R analysis

Supplementary Information 3.7

References for Supplementary Information

Supplementary Information 3.1

With number of oysters per m^2 defining what an oyster bed is, initial analysis incorporated solely the number of native oysters m^2 as opposed to weight of oyster m^2 . All analysis in the main paper, therefore, was completed using counts of live oysters. This highlighted problems regarding comparisons on increased biodiversity associated with 1kg *C. fornicata*, 1kg shell and 1 individual oyster. With individual oysters ranging in size and weight it was deemed 1 oyster is likely to have a smaller effect on biodiversity than 1kg live oyster therefore analysis was completed again using weight of live *O. edulis* (kg m^{-2}). Significant values remained similar (Table S1).

Coefficients to highlight the respective impact each type of hard substrate has on species diversity were plotted using both standardised and centred predictors. Standardizing involved using the weight of native oysters as opposed to counts with all predictors measured in kg. Coefficient results are found in figure S2 reference. Centering predictors were found to have very little effect on predictor coefficients therefore uncentred values were used in all analysis. Using weight of oysters rather than number was found to seemingly increase the impact of oysters on species diversity. This was due to individual oysters weighing much less than 1kg therefore the impact was reduced when only a single oyster was taken into account. This highlights the effect that larger oysters may have a larger impact on species diversity however full analysis regarding the effect of size structure of the population is required for this. With OSPAR definition of an oyster bed said to be 5 oysters m^{-2} this can be interpreted widely. It may therefore be more appropriate to incorporate weight of oyster mass within the environment as an additional prerequisite to define an oyster bed. I.e. 5 juvenile oysters of combined mass 0.2kg are likely to have a smaller effect on species diversity than 5 adult oysters of mass of 1kg however this analysis cannot specifically state that larger oysters do have more impact yet.

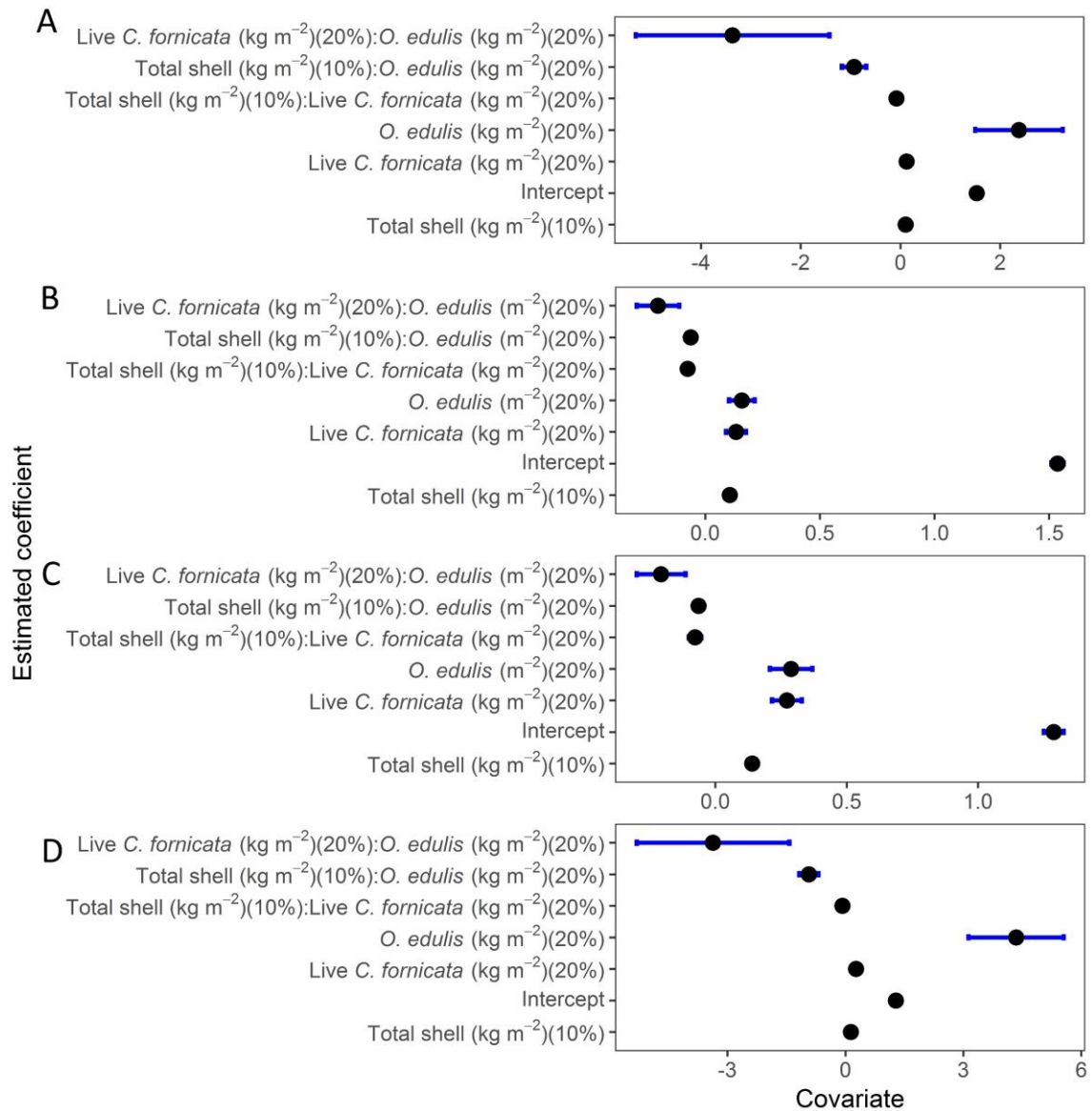


Figure S1: Comparative plots of coefficients extracted from negative binomial ManyGLM using centred vs uncentred predictors and using *O. edulis* weight (kg m⁻²) vs counts. A: Centred predictors and *O. edulis* weight, B: Centred predictors and *O. edulis* counts, C: Uncentred predictors and *O. edulis* count, and D: Uncentred predictors and *O. edulis* weight.

Table S1: Likelihood ratios and P values of predictors comparing centred vs un centred and use of *O. edulis* count vs weight in the negative binomial manyglm models.

Predictor	Model							
	Centred variables, <i>O. edulis</i> count		Uncentred variables, <i>O. edulis</i> count		Centred variables, <i>O. edulis</i> weight		Uncentred variables, <i>O. edulis</i> weight	
	LR	P value	LR	P value	LR	P value	LR	P value
Intercept	1074.54	<0.001	537.67	<0.001	1081.23	<0.001	537.70	<0.001
Total shell (10%)	54.19	<0.001	75.87	<0.001	54.72	<0.001	75.87	<0.001
Live <i>Crepidula fornicata</i> (20%)	8.78	0.003	20.44	<0.001	8.13	0.004	20.44	<0.001
<i>Ostrea edulis</i> (20%)	7.50	<0.001	12.03	<0.001	6.96	<0.001	12.03	<0.001
Total shell (10%): Live <i>Crepidula fornicata</i> (20%)	10.91	0.003	10.91	0.003	11.05	0.002	10.91	0.002
Total shell (10%): <i>Ostrea edulis</i> (20%)	15.242	0.005	15.24	<0.002	15.80	<0.001	15.24	0.003
Live <i>Crepidula fornicata</i> (20%): <i>Ostrea edulis</i> (20%)	5.15	<0.001	5.14	<0.001	3.05	0.003	5.15	0.002

Supplementary Information 3.2

Analysis presented in the main paper used a manyglm model with a negative binomial distribution however this type of analysis is primarily designed for multivariate analysis using full species matrices. In addition, due to logistical constraints, unequal numbers of dredges were performed between surveys. This resulted in surveys being unbalanced and block resampling by area or by survey were not possible and case resampling was required. Mixed models were therefore completed to assess differences between analysis types and the subsequent effect using area and individual survey as random effects however, due to ease in resampling and the use of predict functions the use of manyglm was used within the main paper. Results from the mixed models are presented in Table S2.

Table S3.2: Coefficients and significant values for various mixed models incorporating spatial and temporal repeated measures of the native oyster survey between 2016 and 2017

Model	AIC	Effect type	Effect name	Coefficient estimate/ variance	Coefficient standard error/ standard deviation of variance	Z value	P value
Number.species~ Total.shell + C.fornicata + O.edulis20 + Total.shell:C.fornicata + C.fornicata:O.edulis20 + Total.shell:O.edulis20 + (1 Season)	1798.4	Random	Season	0.008056 (variance)	0.08975 (std. dev)		
		Fixed	Intercept	1.2546	0.07421	16.905	<0.001
		Fixed	Total shell weight	0.14485	0.01362	10.634	<0.001
		Fixed	<i>C. fornicata</i> weight	0.28446	0.05604	5.076	<0.001
		Fixed	<i>O. edulis</i> count	0.29837	0.07816	3.818	<0.001
		Fixed	Total shell weight: <i>C. fornicata</i> weight	-0.07444	0.02349	-3.169	0.001
		Fixed	weight: <i>O. edulis</i> count	-0.20732	0.08983	-2.308	0.021
		Fixed	Total shell weight: <i>O. edulis</i> count	-0.06367	0.01645	-3.871	<0.001
Number.species~ Total.shell + C.fornicata + O.edulis20 + Total.shell:C.fornicata + C.fornicata:O.edulis20 + Total.shell:O.edulis20 + (1 Survey)	1783.2	Random	Survey	0.0214 (variance)	0.1463 (std. dev)		
		Fixed	Intercept	1.25161	0.08216	15.234	<0.001
		Fixed	Total shell weight	0.1422	0.01302	10.921	<0.001
		Fixed	<i>C. fornicata</i> weight	0.27414	0.05382	5.094	<0.001
		Fixed	<i>O. edulis</i> count	0.32179	0.07539	4.268	<0.001
		Fixed	Total shell weight: <i>C. fornicata</i> weight	-0.0679	0.02277	-2.983	0.003
		Fixed	weight: <i>O. edulis</i> count	-0.22793	0.08686	-2.624	0.009
		Fixed	Total shell weight: <i>O. edulis</i> count	-0.06815	0.01595	-4.273	<0.001
Number.species~ Total.shell + C.fornicata + O.edulis20 + Total.shell:C.fornicata + C.fornicata:O.edulis20 + Total.shell:O.edulis20 + (1 Area)	1753	Random	Area	0.05885 (variance)	0.2426 (std. dev)		
		Fixed	Intercept	1.4466	0.10258	14.103	<0.001
		Fixed	Total shell weight	0.1029	0.0548	6.648	<0.001
		Fixed	<i>C. fornicata</i> weight	0.37044	0.05095	7.271	<0.001
		Fixed	<i>O. edulis</i> count	0.34686	0.07901	4.39	<0.001
		Fixed	Total shell weight: <i>C. fornicata</i> weight	-0.07558	0.02194	-3.594	0.003
		Fixed	weight: <i>O. edulis</i> count	-0.27169	0.08642	-3.144	0.002
		Fixed	Total shell weight: <i>O. edulis</i> count	-0.05795	0.01533	-3.781	0.002
Number.species~ Total.shell + C.fornicata + O.edulis20 + Total.shell:C.fornicata + C.fornicata:O.edulis20 + Total.shell:O.edulis20 + (1 Area) + (1 survey)	1727.3	Random	Area	0.06207 (variance)	0.2491 (std. dev)		
		Random	Survey	0.2368 (variance)	0.1539 (std. dev)		
		Fixed	Intercept	1.41837	0.13016	10.897	<0.001
		Fixed	Total shell weight	0.10813	0.01551	6.972	<0.001
		Fixed	<i>C. fornicata</i> weight	0.36155	0.05035	7.181	<0.001

Fixed	<i>O. edulis</i> count	0.37267	0.07875	4.732	<0.001
Fixed	Total shell weight: <i>C. fornicata</i> weight	-0.07004	0.02185	-3.205	0.002
Fixed	<i>C. fornicata</i> weight: <i>O. edulis</i> count	-0.29004	0.02592	-3.376	<0.001
Fixed	Total shell weight: <i>O. edulis</i> count	-0.06243	0.01511	-4.133	<0.001

Supplementary Information 3.3

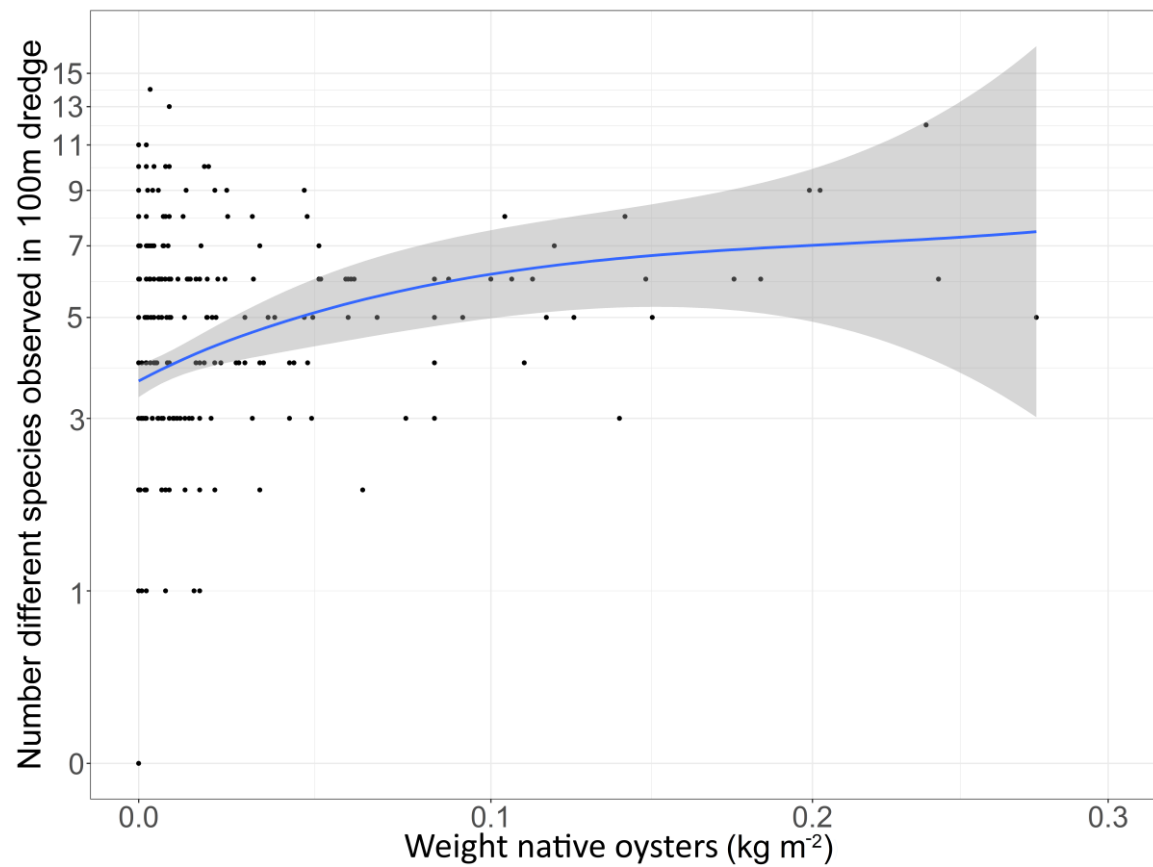


Figure S2: Average weight of native oysters kg m⁻² in a 120m dredge adjusted to 20% dredge efficiency vs number of different species within the dredge (not including native oysters or fish species). A loess smoother with span=1 and 95% CI have been added for ease in observation.

Supplementary Information 3.4

Negative binomial glms with Tukey's HSD *post hoc* analysis were used to identify differences in the average number of observed species by grouped weight of shell and by grouped weight of native oyster (kg m^{-2}), to identify where greatest differences in the number of species observed occur.

To better understand the density at which no other further change is observed with increasing oyster abundance we created three biomass categories of 0 kg oysters per dredge (group A), 0.0003-2.0 kg oysters ($1.25\text{E}^{-5} - 0.083 \text{ kg m}^{-2}$) (group B) and 2.01+ kg oysters per ($0.084+ \text{ kg oysters m}^{-2}$) (group C).

There was a statistical difference in the number of species observed between groups (deviance=24.407, $\text{df}=3$, $P<0.001$). *Post hoc* analysis showed a significant difference between A vs B and A vs C (Tukey $z=2.859$, $P=0.0105$ and $z=2.675$, $P=0.0181$ respectively) however no difference between groups B vs C were found (Tukey, $z=1.395$, $P=0.3283$ Figure S5).

To better understand the density at which no other further change is observed with increasing shell density we created 3 biomass categories of zero shell (group 1), 0.001-30kg shell (group 2) and 30+ kg shell (group 3) there was a statistical difference in the number of species observed between groups (LR=69.5697, $P<0.001$) with *post hoc* analysis showing significant differences between all groups (group 1 v group 2 Tukey, $z=4.876$, $P<0.001$; group 2 v group 3: Tukey, $z=7.735$, $P<0.001$ and group 1 v group 3 Tukey, $z=6.343$, $P<0.001$).

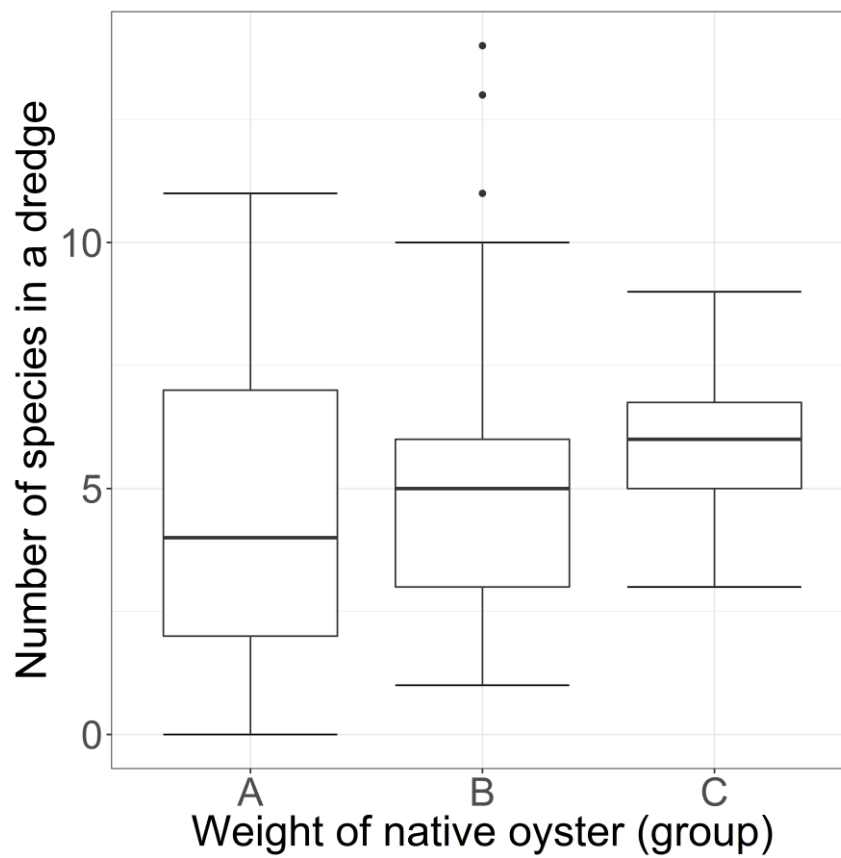


Figure S3: Distribution of number of species in a dredge grouped by weight of native oyster. either 0 kg oysters (group A), 0.0003-2.0 kg oysters per dredge ($1.25\text{E}^{-5} - 0.083 \text{ kg m}^{-2}$) (group B) and 2.01+ kg oysters per dredge ($0.084+ \text{ kg oysters m}^{-2}$) (group C).

Supplementary Information 3.5

Using a full matrix of all species presence/absence, highlighted significant effects of total shell, live *C. fornicata* and *O. edulis* count, on the presence/absence of all individual species (Total shell (kg m^{-2}) LR=196.0 $P=0.045$, Live *C. fornicata* (kg m^{-2}) LR=106.5, $P=0.039$ and *O. edulis* density (m^{-2}) LR=105.7, $P=0.021$) showing each type of hard substrate is significant in determining community structure. There were no significant interactions and so these were removed from the model.

Unstandardized coefficients for the model for individual species presence/absence response are plotted in Figure S12 highlighting increases in *Actinatia spp.*, the less widely distributed *C. gigas*, *Aequipecten opercularis*, *Cancer pagarus*, *Necora puber* and *Veneroidea* species with increasing native oyster abundance (Figure S6).

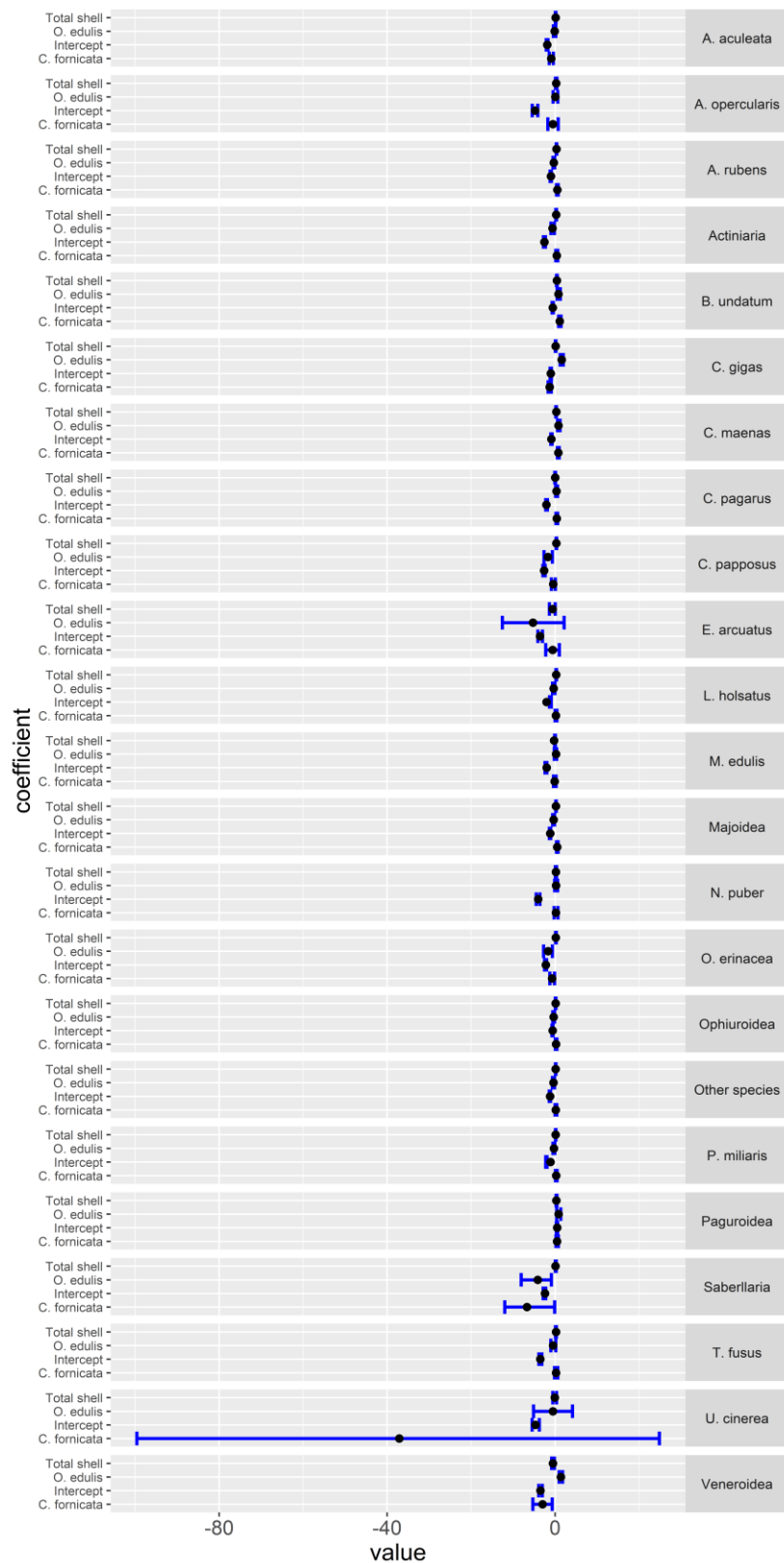


Figure S4: Unstandardized coefficients for environmental variables for each species from negative binomial manyglm using total shell weight (kg m^{-2} adjusted to 10% dredge efficiency), live *C. fornicata* weight (kg m^{-2} adjusted to 20% dredge efficiency), *O. edulis* density (m^{-2} adjusted to 20% dredge efficiency) predictors.

Supplementary Information 3.6

```
library(mvabund)
```

```
library(ggplot2)
```

```
library(plyr)
```

```
Number.species=Number_species_total_nofish
```

```
TS_m2_10<-Total.shell.10
```

```
SL_m2_20<-C.fornicata20
```

```
Oedulis_m2_20_kg<-O.edulis20_weight
```

```
Oedulis_count<-O.edulis_20_count
```

```
dat<-data.frame(Number.species,SL_m2_20, Oedulis_m2_20_kg, TS_m2_10,Oedulis_count)
```

```
####weight o.edulis uncentred
```

```
Full1_m20_weight=manyglm(Number.species~TS_m2_10*SL_m2_20*Oedulis_m2_20_kg,family="negative.binomial", show.coef = TRUE)
```

```
drop1(Full1_m20_weight)
```

```
updatefull1_m2<-update(Full1_m20_weight,~.-TS_m2_10:SL_m2_20:Oedulis_m2_20_kg)
```

```
updatefull1summary1<-
```

```
summary(updatefull1_m2,test="LR",resamp="case",data=dat,show.est=TRUE)
```

```
#####Centred
```

```
SL_m2_20.c = scale(dat$SL_m2_20, center=TRUE, scale=FALSE)
```

```

Oedulis_m2_20_kg.c = scale(dat$Oedulis_m2_20_kg, center=TRUE, scale=FALSE)

Oedulis_COUNT.c = scale(dat$Oedulis_count, center=TRUE, scale=FALSE)

TS_m2_10.c = scale(dat$TS_m2_10, center=TRUE, scale=FALSE)

centred_dat<-data.frame(SL_m2_20.c,Oedulis_m2_20_kg.c,TS_m2_10.c)

centred_weight_m1=manyglm(Number.species~TS_m2_10.c*SL_m2_20.c*Oedulis_m2_20_kg.c,fa
mily="negative.binomial", show.coef = TRUE)

drop1(centred_weight_m1)

centred_weight_m2<-update(centred_weight_m1,~.-TS_m2_10.c:SL_m2_20.c:Oedulis_m2_20_kg.c)

coef(centred_weight_m2)

centred_weight_m2_summary<-
summary(centred_weight_m2,test="LR",resamp="case",data=centred_dat,show.est=TRUE)

####Count oedulis uncentred

Full1_m20_Count=manyglm(Number.species~TS_m2_10*SL_m2_20*Oedulis_count,family="negati
ve.binomial", show.coef = TRUE)

drop1(Full1_m20_Count)

updatefull1_m2_Count<-update(Full1_m20_Count,~.-TS_m2_10:SL_m2_20:Oedulis_count)

updatefull1summary1_Count<-
summary(updatefull1_m2_Count,test="LR",resamp="case",data=dat,show.est=TRUE)

####Centred COUNT

```

```

centred_count_m1=manyglm(Number.species~TS_m2_10.c*SL_m2_20.c*Oedulis_COUNT.c,family
="negative.binomial", show.coef = TRUE)

drop1(centred_count_m1)

entred_count_m2<-update(centred_count_m1,~.-TS_m2_10.c:SL_m2_20.c:Oedulis_COUNT.c)

coef(centred_count_m2)

centred_count_m2_summary<-
summary(centred_count_m2,test="LR",resamp="case",data=centred_dat,show.est=TRUE)

#####full binomial presence absence mvabund

mvfull<-mvabund(NoFISH3)

mvfull1=manyglm(mvfull~TS_m2_10*SL_m2_20*Oedulis_count,family="binomial")

drop1(mvfull1)


mvfull2<-update(mvfull1,~.-TS_m2_10:SL_m2_20:Oedulis_count)

drop1(mvfull2)


mvfull2=manyglm(mvfull~TS_m2_10+SL_m2_20+Oedulis_count,family="binomial")

#summary(mvfull2, block=Survey)


summary.manyglm(mvfull2, test="LR", block=Survey,show.est=TRUE,p.uni="unadjusted")

```


Supplementary Information 3.7

Defra. (2013). *Blackwater, Crouch, Roach and Colne Marine Conservation Zone*.

Hancock, D.A. (1969) Oyster pests and their control. *Ministry of Agriculture, Fisheries and Food*. **19**, 1-30.

Tully, O. & Clarke, S., (2012) The status and management of Oyster (*Ostrea edulis*) in Ireland. *Irish Fisheries Investigations*. **24** 1-40.

UKGoverment. (2013). Ministerial order: Wildlife environmental protection marine management, The Blackwater, Crouch, Roach and Colne estuaries Marine Conservation Zone Designation Order 2013.

Appendix III

Supplementary Information for Chapter 4: A novel method for tracking growth rate and survival of individual native oysters at low density.

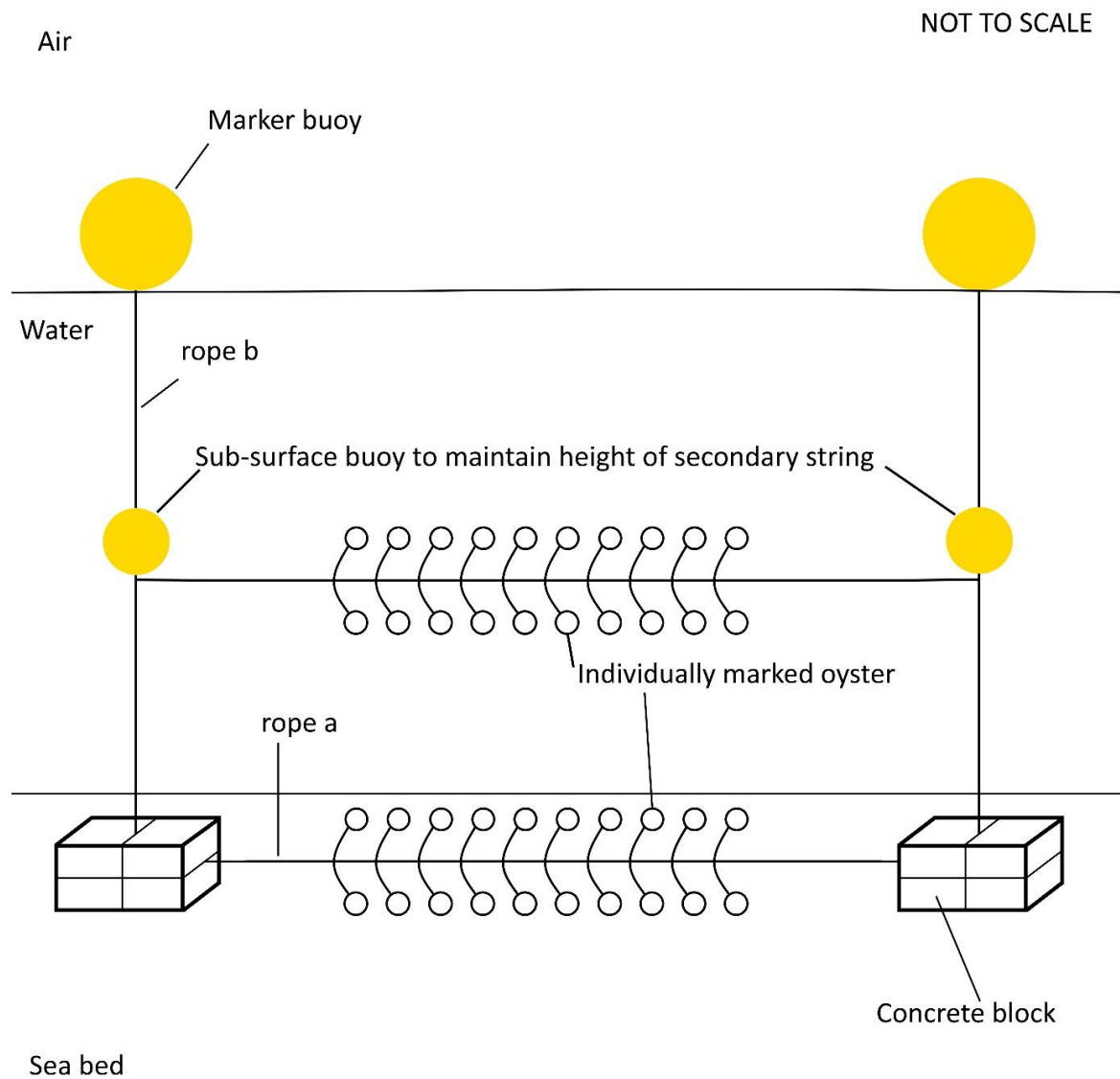


Figure S4.1. Alterations to experimental design completed in Chapter 4 with suspended oyster line above the sea floor.

Appendix IV:

Chapter 5: Demographic modelling of *Ostrea edulis* in Essex, UK.

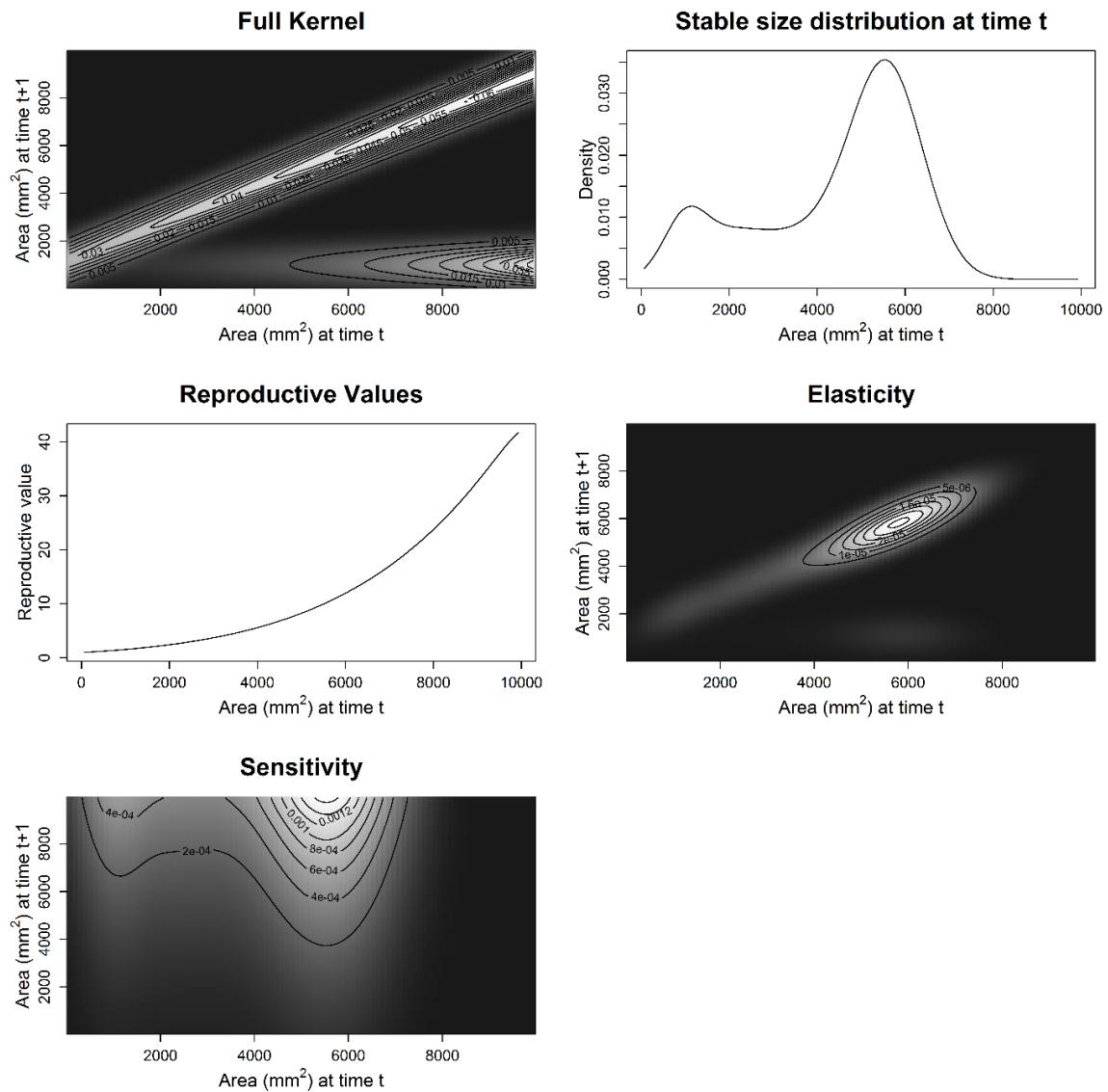


Figure S5.3. Population summary plots model output from a size structured IPM by area (mm^2) for *Ostrea edulis* for the Crouch where $\lambda = 0.589$.

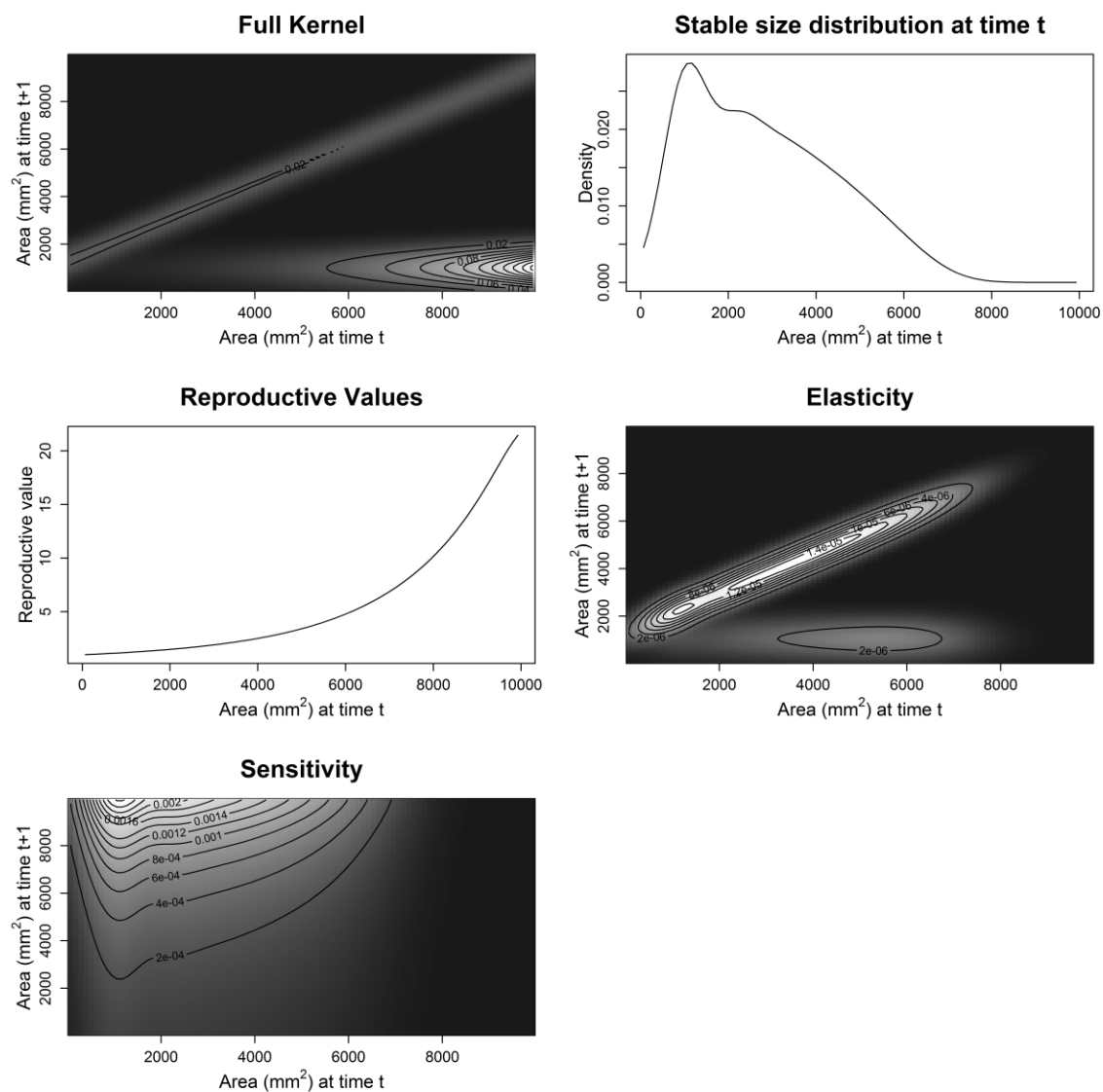


Figure S5.2. Population summary plots model output from a size structured IPM by area (mm²) for *Ostrea edulis* for the Blackwater where $\lambda = 0.263$.

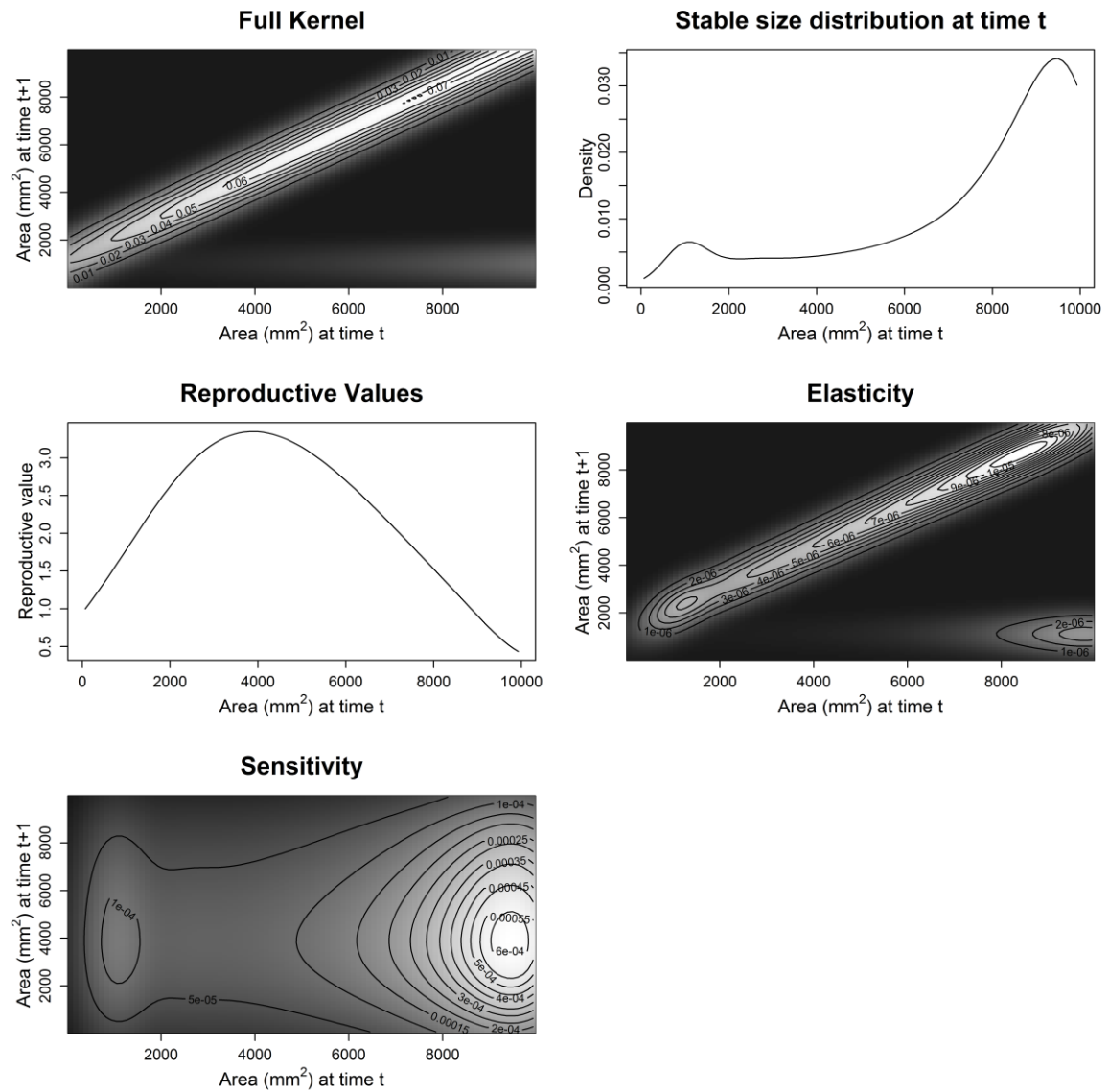


Figure S5.3. Population summary plots model output from a size structured IPM by area (mm²) for *Ostrea edulis* for the Ray Sand where $\lambda = 0.842$.

Table S5.1. Breakdown of calculation of P_{estab2} based on total populations measured in dredge surveys (n0) and estimated length-based fecundity (f_{eggst}) as calculated in Chapter 4. MCZ = all MCZ, Ray = Ray Sand, Black = Blackwater and Crou = Crouch.

Site	year	Sum n0	Sum 40+	Mean length adult	Total eggs for oysters in dredge survey	total year	ratio of juv to total (JA)	Ratio of adults to total (RA)	Total estimated pop (Tot)	Total adults (RA*n0)	Total 1 year olds (JA*n0)	total eggs (f_{eggst} * total number of adults)	P_{estab2} w (total eggs from previous year/total 1 year olds)	P_{estab2}
MCZ	2018	384	366	73.19	40028988	6	0.015625	0.953125	3479060	3315979	54360.31	3.63E+11	1.49E-07	4.40E-07
MCZ	2017	496	477	67.9	40548877	4	0.008065	0.961694	4455334	4284666	35930.11	3.64E+11	1.28E-07	3.78E-07
MCZ	2016	444	389	64.84	28323450	18	0.040541	0.876126	4397924	3853136	178294.2	2.81E+11	6.63E-07	1.95E-06
MCZ	2015	651	493	63.73	33873204	63	0.096774	0.757296	5171152	3916095	500434	2.69E+11	3.02E-06	8.91E-06
MCZ	2014	359	332	66.82	26743047	16	0.044568	0.924791	2221607	2054523	99013.14	1.65E+11		
Ray	2018	118	114	78.24	15599969	1	0.008475	0.966102	1154772	1115627	9786.203	1.53E+11	8.47E-08	2.50E-07
Ray	2017	142	138	71.12	13706394	2	0.014085	0.971831	1196470	1162766	16851.69	1.15E+11	1.36E-07	4.00E-07
Ray	2016	150	138	68.38	12011988	5	0.033333	0.92	1552184	1428009	51739.47	1.24E+11	3.51E-07	1.03E-06
Ray	2015	100	83	77.43	10967738	10	0.1	0.83	1344232	1115713	134423.2	1.47E+11	3.06E-06	9.03E-06
Ray	2014	37	36	72.19	3759489	0	0	0.972973	432032.4	420355.8	0	4.39E+10		
Black	2018	28	24	66.16	1869841	1	0.035714	0.857143	410967.7	352258	14677.42	2.74E+10	4.59E-07	1.35E-06
Black	2017	59	50	58.56	2585658	0	0	0.847458	728908.6	617719.2	0	3.19E+10	0	0.00E+00
Black	2016	100	66	51.64	2237228	10	0.1	0.66	1193314	787587.1	119331.4	2.67E+10	2.64E-06	7.80E-06
Black	2015	276	175	55.78	7689605	31	0.112319	0.634058	1619336	1026753	181881.9	4.51E+10	7.7E-06	2.27E-05
Black	2014	30	29	63.07	1924077	0	0	0.966667	368084.2	355814.8	0	2.36E+10		
Crou	2018	236	226	71.23	22563523	4	0.016949	0.957627	1851451	1773000	31380.53	1.77E+11	1.76E-07	5.18E-07
Crou	2017	290	284	67.96	24214035	1	0.003448	0.97931	2140880	2096586	7382.345	1.79E+11	7.61E-08	2.24E-07
Crou	2016	181	173	65.95	13352285	3	0.016575	0.955801	1315907	1257745	21810.61	9.7E+10	1.53E-07	4.51E-07
Crou	2015	173	169	67.56	14126180	3	0.017341	0.976879	1747376	1706974	30301.32	1.43E+11	3.38E-07	9.97E-07
Crou	2014	133	132	63.7	9055167	1	0.007519	0.992481	1315873	1305979	9893.782	8.96E+10		

Integrated Projection Model code

Part 1 Finding coefficients:

```
###getting n0

setwd("C:/Users/aelown/Documents/PhD 2015/Experiments/Dredge surveys/Master data sheets")

data<-read.csv("Individual size measurements.csv")

#to calculate number of samples of each value in data$factor

splitdat<-split(data, data$Year_season)

S2016<-splitdat$`2016S`
W2016<-splitdat$`2016W`
S2017<-splitdat$`2017S`
W2017<-splitdat$`2017W`
S2018<-splitdat$`2018S`
W2018<-splitdat$`2018W`

#break up population into a hist of 98 bins
n0<-hist((pi*(S2017$Length/2)*(S2017$Width_2016/2)), breaks=98)$counts
S2018$area<-(pi*(S2018$Length/2)*(S2018$Width_2016/2))
#n0<-S2018$Length

setwd("C:/Users/aelown/Documents/PhD 2015/Experiments/Slab experiment/Data")

#####

#IPM for Ostrea edulis

#####

library(ggplot2)

setwd("C:/Users/aelown/Documents/PhD 2015/Experiments/Slab experiment/Data")

datfull<-read.csv("ALL_mortality_year_growth.csv")

#to calculate number of samples of each value in data$factor

splitdat<-split(datfull, datfull$Site)

#datfull<-splitdat$Blackwater

###datfull<-splitdat$Brightlingsea

###datfull<-splitdat$Colne

###datfull<-splitdat$`Colne Raft`

#datfull<-splitdat$Crouch

datfull<-splitdat$Raysand
```

```

###datfull<-splitdat$`Several Order`
###
#MCZ<-rbind(datfull1,datfull2, datfull3)
#datfull<-MCZ

d<-data.frame("ID"=c(datfull$Oyster),
              "z"=c(pi*(datfull$Length1_year/2)*(datfull$Width1_Year/2)),
              "z1"=c(pi*(datfull$Length2/2)*(datfull$Width2/2)),
              "Surv"=c(datfull$Opposite1_year_mortality),
              "Repr"=c(datfull$fec0_NA10),
              "site"=c((datfull$Site)),
              "SumLength"=c(datfull$Summer_Length),
              "Eggs"=c(round(datfull$fec1_13.4)),
              "RL"=c(pi*(datfull$Recruit_length/2)*(datfull$Recruit_width/2)))

# -----
# Build regressions for vital rate functions
# -----

m_par=c(
  surv.int=NA,
  surv.slope=NA,
  growth.int=NA,
  growth.slope=NA,
  growth.sd=NA,
  seed.int=NA,
  seed.slope=NA,
  recruit.z.mean=NA,
  recruit.z.sd=NA,
  establishment.prob=NA
)

```



```

# 1. survival regression
surv.reg=glm(Surv~z,data=d,family=binomial())
summary(surv.reg)
m_par["surv.int"]=coefficients(surv.reg)[1]
m_par["surv.slope"]=coefficients(surv.reg)[2]

# 2. growth regression
growth.reg=lm(z1~z,data=d)
summary(growth.reg)
m_par["growth.int"]=coefficients(growth.reg)[1]
m_par["growth.slope"]=coefficients(growth.reg)[2]
m_par["growth.sd"]=sd(resid(growth.reg))

# 3. eggs regression
seed.reg=glm(Eggs~z,data=d,family=poisson())
summary(seed.reg)
m_par["seed.int"]=coefficients(seed.reg)[1]
m_par["seed.slope"]=coefficients(seed.reg)[2]

# 4. z distribution of recruits
#m_par["recruit.z.mean"]=mean(na.omit(d$z1[is.na(d$z)]))
#m_par["recruit.z.sd"]=sd(na.omit(d$z1[is.na(d$z)]))
m_par["recruit.z.mean"]=1000
m_par["recruit.z.sd"]=500

# 5. establishment probability
# these data represent a single year's worth of data, hence establishment probability can be estimated
# by dividing the number of observed recruits by the number of eggs. hence the growth/survival
# measurements were taken in year t which the recruit zs were measured in year t+1.
m_par["establishment.prob"]=(length(d$RL[!is.na(d$RL)]))/sum(d$Eggs,na.rm=TRUE)
#All sites average
m_par["establishment.prob"]= 4.40E-07

```

```
#####m_par_MANUAL###  
#m_par=c(  
# surv.int=-7.18E-01,  
# surv.slope=2.66E-04,  
# growth.int=1.17E+03,  
# growth.slope=8.34E-01,  
# growth.sd=5.15E+02,  
# seed.int=9.80E+00,  
# seed.slope=4.34E-04,  
# recruit.z.mean=1.00E+03,  
# recruit.z.sd=5.00E+02,  
# establishment.prob=4.400E-07)  
#  
#
```

Part 2: Vital rates plots

```
setwd("C:/Users/aelown/Documents/PhD 2015/Experiments/Population models/IPMs/Model
docs/Final model codes")

source("Finding coefficients Run 1.R")

#GROWTH

#par(mfrow=c(2,2),mar=c(4,4,2,1))

library(ggplot2)

Growthplot <- ggplot(d, aes(z, z1)) +
  geom_point() +
  stat_smooth(method = lm, col="Red", se=F, fullrange=T)+theme_bw()+geom_abline(intercept=0,
col="darkgrey")+
  labs(y=expression(paste("Area at time t+1 (m",m^{2}, ")")), x=expression(paste("Area at time t+1
(m",m^{2}, ")")), title="Growth")+
  theme(text = element_text(size=20),
        axis.text.x = element_text(angle=0,size=20, hjust=1),
        axis.text.y = element_text(angle=0,size=20, hjust=1))

Growthplot

#png("Growthplot_ggplot_AREA_ALL.png", width = 10, height = 10, units = 'in', res = 800)

#print(Growthplot)

### Make plot

#dev.off()

#SURVIVAL

Survplot <- ggplot(d, aes(z, Surv)) +
  geom_jitter(height = 0.1) +
  stat_smooth(method = lm, col="Red", se=F, fullrange=T)+theme_bw()+
  labs(y="Survival", x=expression(paste("Area at time t (m",m^{2}, ")")), title="Survival")+
  theme(text = element_text(size=20),
        axis.text.x = element_text(angle=0,size=20, hjust=1),
        axis.text.y = element_text(angle=0,size=20, hjust=1))

Survplot

#png("Survivalplot_ggplot_AEAALL.png", width = 10, height = 10, units = 'in', res = 800)
```

```

#print(Survplot)

## Make plot
#dev.off()

#FECUNDITY

Fecplot <- ggplot(d, aes(z, Eggs)) +
  geom_point() +
  geom_smooth(slope=growth.reg$coefficients[2], intercept=growth.reg$coefficients[1], se=F,
col="Red", fullrange=T)+
  theme_bw()+geom_vline(xintercept = (pi*40/2*38/2), col="darkgrey")+
  labs(y = "Predicted number of Eggs", x=expression(paste("Area at time t (m",m^{2}, ")")),
title="Fecundity")+
  theme(text = element_text(size=20),
        axis.text.x = element_text(angle=0,size=20, hjust=1),
        axis.text.y = element_text(angle=0,size=20, hjust=1))
Fecplot
#png("Fecundityplot_ggplot_AREALL.png", width = 10, height = 10, units = 'in', res = 800)
#print(Fecplot)

## Make plot
#dev.off()

#RECRUITSIZE

recs<-data.frame(na.omit(d$RL))
recs<-recs$na.omit.d.RL.
FECS<-data.frame(recs)

gg <- ggplot(FECS, aes(x=recs)) + geom_histogram(binwidth=300, colour="black", fill="lightgrey",
        aes(y=..density..))+

stat_function(fun=dnorm,color="red",lwd=1,args=list(mean=m_par["recruit.z.mean"],sd=m_par["recr
uit.z.sd"]))+theme_bw()+
  scale_x_continuous(limits=c(0,2500))+
  labs(y = "Density", x=expression(paste("Area (m",m^{2}, ")")), title="Recruit size")+
  theme(text = element_text(size=20),

```

```
axis.text.x = element_text(angle=0,size=20, hjust=1),  
axis.text.y = element_text(angle=0,size=20, hjust=1))  
  
gg  
#  
#png("Recruitsize_ggplot_AREALL.png", width = 10, height = 10, units = 'in', res = 800)  
#print(gg)  
## Make plot  
#dev.off()
```

Part 3: Functions kernels sensitivity and elasticity

```
setwd("C:/Users/aelown/Documents/PhD 2015/Experiments/Population models/IPMs/Model  
docs/Final model codes")
```

```
source("Vital rates plot Run 2.R")
```

```
# 1. probability of surviving
```

```
s.z=function(z,m_par) {  
  u=exp(m_par["surv.int"]+m_par["surv.slope"]*z)  
  return(u/(1+u))  
}
```

```
# 2. growth function
```

```
G.yz=function(z1,z,m_par) {  
  dnorm(z1,mean=m_par["growth.int"]+m_par["growth.slope"]*z,sd=m_par["growth.sd"])  
}
```

```
f.yz=function(z1,z,m_par) {
```

```
  if (any(z> 1193.805)) {
```

```
    m_par["establishment.prob"]*
```

```
    dnorm(z1,mean=m_par["recruit.z.mean"],sd=m_par["recruit.z.sd"])*
```

```
    exp(m_par["seed.int"]+m_par["seed.slope"]*z)
```

```
  } else {
```

```
    0*z
```

```
  }}
```

```

## Functions to build IPM kernels P, F, and K

## Define the survival/growth kernel
P_z1z = function(z1,z,m_par) {s.z(z,m_par)*G.yz(z1,z,m_par)}

## Define the fecundity kernel
F_z1z = function(z1,z,m_par) {f.yz(z1,z,m_par)}

L=.5*min(c(d$z,d$z1),na.rm=T)
U=1.1*max(c(d$z,d$z1),na.rm=T)
L=14.13717
U=9981.939

# number of cells in the discretized kernel
m=length(n0)

## #boundary points (the edges of the cells defining the kernel)
b=L+c(0:m)*(U-L)/m

## mesh points (midpoints of the cells)
y=0.5*(b[1:m]+b[2:(m+1)])

## width of the cells
h=y[2]-y[1]

G=h*outer(y,y,G.yz,m_par=m_par) # growth kernel
S=s.z(y,m_par=m_par) # survival
P=G # placeholder; we're about to redefine P on the next line
for(i in 1:m) P[,i]=G[,i]*S[i] # growth/survival kernel
F=h*outer(y,y,f.yz,m_par=m_par) # reproduction kernel
K=P+F #full kernel

lam=Re(eigen(K)$values[1])
w.eigen=Re(eigen(K)$vectors[,1])
stable.dist=w.eigen/sum(w.eigen)
v.eigen=Re(eigen(t(K))$vectors[,1])
repro.val=v.eigen/v.eigen[1]

```

```

# 2. compute elasticity and sensitivity matrices

v.dot.w=sum(stable.dist*repro.val)*h

sens=outer(repro.val,stable.dist)/v.dot.w

elas=matrix(as.vector(sens)*as.vector(K)/lam,nrow=m)


#model function
mk_K <- function(m, m_par,meshpts, L, U) {
  # mesh points
  h <- (U-L)/m; meshpts <- L + ((1:m) - 1/2) * h
  P <- h * (outer(meshpts, meshpts, P_z1z, m_par = m_par))
  F <- h * (outer(meshpts, meshpts, F_z1z, m_par = m_par))
  K <- P + F
  return(list(K = K, meshpts = meshpts, P = P, F = F))
}

IPM_sys <- mk_K(m=m,meshpts=meshpts, m_par=m_par, L=L, U=U)
names(IPM_sys)
library(fields)
#Check kernel plot
image.plot(IPM_sys$meshpts,IPM_sys$meshpts, t(IPM_sys$K))

```


Part 4: Population summary plots of model

```
#####

###Population summary plots of model output

#####

setwd("C:/Users/aelown/Documents/PhD 2015/Experiments/Population models/IPMs/Model
docs/Final model codes")

source("Functions kernels sensitivity and elasticity Run 3.R")

n=1000

#All one plot

png("Population summary all_RAW_RAY.png", width = 10, height = 10, units = 'in', res = 800)
par(mfrow=c(3,2))

image(y,y,t(K), xlab=expression(paste("Area (m",m^{2}, ") at time t")),cex.lab=1.5,cex.axis=1.25,
      ylab="",col=grey.colors(n, start = 0.1, end = 1, gamma = 2.2, alpha = NULL), main="Full
Kernel", cex.main=2)+
  contour(y,y,t(K), add = TRUE, drawlabels = TRUE)+
  title(ylab=expression(paste("Area (m",m^{2}, ") at time t+1")), line=2.15, cex.lab=1.5)

##Fecundity kernel

#image(y,y,t(F), xlab=expression(paste("Area (m",m^{2}, ") at time t")),cex.lab=1.5,cex.axis=1.25,
#  ylab="",col=topo.colors(100), main="Fecundity Kernel", cex.main=2)+
#  contour(y,y,t(F), add = TRUE, drawlabels = TRUE)+
#  title(ylab=expression(paste("Area (m",m^{2}, ") at time t+1")), line=2.15, cex.lab=1.5)

##Growth kernel

#image(y,y,t(P), xlab=expression(paste("Area (m",m^{2}, ") at time t")),cex.lab=1.5,cex.axis=1.25,
#  ylab="",col=topo.colors(100), main="Growth and survival Kernel", cex.main=2)+
#  contour(y,y,t(P), add = TRUE, drawlabels = TRUE)+
#  title(ylab=expression(paste("Area (m",m^{2}, ") at time t+1")), line=2.15, cex.lab=1.5)

#stable dist

plot(y,stable.dist, xlab=expression(paste("Area (m",m^{2}, ") at time t")),cex.lab=1.5,cex.axis=1.25,
     ylab="", main="Stable size distribution at time t",type="l", cex.main=2)+
  contour(y,y,t(F), add = TRUE, drawlabels = TRUE)+
  title( ylab="Density", line=2.15, cex.lab=1.5)
```

```

#Reproductive value
plot(y, repro.val, xlab=expression(paste("Area (m", m^{2}, ") at time t")), cex.lab=1.5, cex.axis=1.25,
     ylab="", main="Reproductive Values", type="l", cex.main=2)+
  title(ylab="Reproductive value", line=2.25, cex.lab=1.5)

#Elasticity
image(y, y, t(elas), xlab=expression(paste("Area (m", m^{2}, ") at time t")), cex.lab=1.5, cex.axis=1.25,
     ylab="", col=grey.colors(n, start = 0.1, end = 1, gamma = 2.2, alpha = NULL), main="Elasticity",
     cex.main=2)+
  title(ylab=expression(paste("Area (m", m^{2}, ") at time t+1")), line=2.15, cex.lab=1.5)+
  contour(y, y, t(elas), add = TRUE, drawlabels = TRUE)

#Sensitivity
image(y, y, t(sens), xlab=expression(paste("Area (m", m^{2}, ") at time t")), cex.lab=1.5, cex.axis=1.25,
     ylab="", col=grey.colors(n, start = 0.1, end = 1, gamma = 2.2, alpha = NULL), main="Sensitivity",
     cex.main=2)+
  title(ylab=expression(paste("Area (m", m^{2}, ") at time t+1")), line=2.15, cex.lab=1.5)+
  contour(y, y, t(sens), add = TRUE, drawlabels = TRUE)

# Make plot
dev.off()

```

Part 5: Density plots of model validation

```
library(dplyr)
library(broom)
library(tidyr)
library(ggplot2)
library(stats)

setwd("C:/Users/aelown/Documents/PhD 2015/Experiments/Population models/IPMs/Model
      docs/Final model codes")

source("Population summary plots of model output Run 4.R")

)
```

```
#Iterate 2017 summer data forward 1 year
Nt_store<-numeric(2)
nt<-matrix(NA,nrow=length(n0),ncol=2)
nt[,1] <- n0;
for(k in 2:2) {
  m.par.use <- m_par
  Kt <- mk_K(m=m,meshpts=meshpts, m_par=m.par.use, L=L, U=U)$K
  nt[,k] <- Kt%*%nt[,k-1]
  Nt_store[k]<-h*sum(nt[,k])
}
plot(nt[,1])
graphics.off()
plot(nt[,2])
sum(nt[,2])

#this is the number in each bin predicted in the dredge survey
full.v<-as.vector(nt[,2])

#vector of sizes from h
lengthsseq<-seq(L+h,U,by=h)
```

```

#change dredge prediction to density
densest<-full.v/(sum(nt[,2]))

#breaks of centre points to make hists of 2018 data
L=.5*min(c(d$z,d$z1),na.rm=T)
U=1.1*max(c(d$z,d$z1),na.rm=T)

L=14.13717
U=9981.939

# number of cells in the discretized kernel
m=length(n0)

## #boundary points (the edges of the cells defining the kernel)
b=L+c(0:m)*(U-L)/m

## mesh points (midpoints of the cells)
y=0.5*(b[1:m]+b[2:(m+1)])

setwd("C:/Users/aelown/Documents/PhD 2015/Experiments/Dredge surveys/R analysis/Individual
oysters")
data1<-read.csv("Size_measurements_natives_2014_2015_2016_2017_2018_simple.csv")
CI2018S<-split(data1, data1$Year_season)
data1<-CI2018S$`2018S`
data1$area<-((pi*(data1$Length/2)*(data1$Width/2)))

#adding 2.5% to length and width of 2018 oysters
data1$Length10<-data1$Length*1.025
data1$Width10<-data1$Width*1.025
data1$area25<-((pi*(data1$Length10/2)*(data1$Width10/2)))

#

```

```

dens18<-hist((data1$area), breaks=b)
dens18_25<-hist((data1$area25), breaks=b)

library(stats)
measure<-rep("Dredge survey", length=91)
measured_S18<-data.frame(measure, "density"=c(dens18$density), "area"=c(y))
plot(measured_S18$density~measured_S18$area)
measure<-rep("IPM estimate", length=91)
estimated_218<-data.frame(measure, "density"=c(densest), "area"=c(y))
measure<-rep("Dredge survey + 2.5%", length=91)
measureplus5_218<-data.frame(measure, "density"=c(dens18_25$density), "area"=c(y))
comparison_S2018<-rbind(measured_S18, estimated_218,measureplus5_218)
Wide_comparison18<-data.frame(measured_S18,
                               "Estimated density"=c(densest),
                               "Measuredplus2.5"=c(dens18_25$density))

##### Bootstrap for 99% CI
data_frame(bs = 1:1000) %>% group_by(bs) %>%
  mutate(data = list(data1 %>% group_by(Year_season) %>%
                      sample_frac(size = 1, replace = T)))
#compute within sample density
densities.within <-
  data_frame(bs = 1:1000) %>% group_by(bs) %>%
  mutate(data = list(data1 %>% group_by(Year_season) %>%
                      sample_frac(size = 1, replace = T))) %>%
  unnest() %>%
  group_by(bs, Year_season) %>%
  do(tidy(density(.$area,
                from = min(68.90531),
                to = max(9927.17086),
                n = 91)))

```

```

densities.qtiles <-
  densities.within %>%
  rename(area = x, dens = y) %>%
  ungroup() %>%
  group_by(Year_season, area) %>%
  summarise(q05 = quantile(dens, 0.025),
            q50 = quantile(dens, 0.5),
            q95 = quantile(dens, 0.975))

View(densities.qtiles)

##### Bootstrap oysters 2.5% larger

data_frame(bs = 1:1000) %>% group_by(bs) %>%
  mutate(data = list(data1 %>% group_by(Year_season) %>%
    sample_frac(size = 1, replace = T)))
#compute within sample density
densities.within <-
  data_frame(bs = 1:1000) %>% group_by(bs) %>%
  mutate(data = list(data1 %>% group_by(Year_season) %>%
    sample_frac(size = 1, replace = T))) %>%
  unnest() %>%
  group_by(bs, Year_season) %>%
  do(tidy(density(.$area25,
    from = min(68.90531),
    to = max(9927.17086),
    n = 91)))

```

```

densities.qtiles5 <-
  densities.within %>%
  rename(area25 = x, dens = y) %>%
  ungroup() %>%
  group_by(Year_season, area25) %>%
  summarise(q05 = quantile(dens, 0.025),

```

```

q50 = quantile(dens, 0.5),
q95 = quantile(dens, 0.975))

View(densities.qtiles5)

bootS20185<-split(densities.qtiles5, densities.qtiles$Year_season)
bootS20185<-data.frame(bootS20185$`2018S`)
bootS2018<-split(densities.qtiles, densities.qtiles$Year_season)
bootS2018<-data.frame(bootS2018$`2018S`)
measure<-rep("Dredge survey + 2.5%", length=91)
Midbound_2185pc<-data.frame(measure, "density"=c(bootS20185$q50*100),
  "area"=c(bootS20185$area))
measure<-rep("q95 2.5%", length=91)
upperbound_2185pc<-data.frame(measure, "density"=c(bootS20185$q95*100),
  "area"=c(bootS20185$area))
measure<-rep("q05 2.5%", length=91)
Lowerbound_2185pc<-data.frame(measure, "density"=c(bootS20185$q05*100),
  "area"=c(bootS20185$area))
measure<-rep("q05", length=91)
Lowerbound_218<-data.frame(measure, "density"=c(bootS2018$q05*100),
  "area"=c(bootS2018$area))
measure<-rep("q95", length=91)
Upperbound_S2018<-data.frame(measure, "density"=c(bootS2018$q95*100),
  "area"=c(bootS2018$area))
measure<-rep("Dredge survey", length=91)
Midbound_S201<-data.frame(measure, "density"=c(bootS2018$q50*100),
  "area"=c(bootS2018$area))
ALL<-rbind(Upperbound_S2018, Lowerbound_218, estimated_218,Midbound_S201,
  Midbound_2185pc,
  upperbound_2185pc, Lowerbound_2185pc)
ALL$Legend<-as.factor(ALL$measure)
#plot which has 95% CI as lines rather than ribbons
Est_real_density_plot<-ggplot(ALL) +geom_line(aes(area, density, colour= Legend,
  size=Legend))+theme_bw()+
  scale_size_manual(values=c(1,1,2,2,2, 1, 1))+
  scale_color_manual(values= c("grey", "grey", "red", "black", "blue", "grey", "grey"))+

```

```

labs(y = "Density", x=expression(paste("Area (m",m^{2}, ")")))+
theme(text = element_text(size=20),
      axis.text.x = element_text(angle=0,size=20, hjust=1),
      axis.text.y = element_text(angle=0,size=20, hjust=1))

#####putting CI into wide format
bootS20185$Year_season<-rep("Dredge survey + 2.5%", length=91)
bootS2018$Year_season<-rep("Dredge survey", length=91)
bootS20185_2<-data.frame("q50"=c(bootS20185$q50*100), "q05"=c(bootS20185$q05*100),
                        "q95"=c(bootS20185$q95*100), "Year_season"=bootS20185$Year_season,
                        "area"=bootS20185$area)
bootS2018_2<-data.frame("q50"=c(bootS2018$q50*100), "q05"=c(bootS2018$q05*100),
                        "q95"=c(bootS2018$q95*100), "Year_season"=bootS2018$Year_season,
                        "area"=bootS2018$area)
estimated_218_wide<-data.frame("q50"=estimated_218$density,
                              "q05"=estimated_218$density,
                              "q95"=estimated_218$density,
                              "area"=estimated_218$area,
                              "Year_season"=estimated_218$measure)
wide_dat<-rbind(bootS20185_2, estimated_218_wide, bootS2018_2)
wide_dat$Legend<-as.factor(wide_dat$Year_season)
Est_real_density_plot<-ggplot(wide_dat, aes(area, q50, group=Legend)) +
  geom_ribbon(aes(ymin = q05, ymax = q95, fill=Legend), alpha = 0.5) +
  theme_bw()+
  geom_line(size = 1.5, aes(color = Legend)) +
  scale_color_manual(values= c("blue", "red", "black"))+
  scale_fill_manual(values = alpha(c("grey", "white", "grey50"), 0.1))+
  labs(y = "Density", x=expression(paste("Area (m",m^{2}, ")")))+
  theme(text = element_text(size=20),
        axis.text.x = element_text(angle=0,size=20, hjust=1),
        axis.text.y = element_text(angle=0,size=20, hjust=1)+
        scale_colour_discrete(name = "class"))

```



```
#png("MCZ_Est_real_density_CI_plot_WEIGHTEDAVERAGE_2.5pc_ribbonCI_WAEPWIDE.png", width = 15, height = 10, units = 'in', res = 800)

#print(Est_real_density_plot)

## Make plot

#dev.off()
```

Part 6: Projecting forward 50 years with no stochasticity

```
setwd("C:/Users/aelownd/Documents/PhD 2015/Experiments/Population models/IPMs/Model
      docs/Final model codes")

source("Predict density plot Run 5.R")

#Not stochastic

Nt_store<-numeric(50)

#newlam<-numeric(1000)

nt<-matrix(NA,nrow=length(n0),ncol=50)

nt[,1] <- n0;

for(k in 2:50) {

  m.par.use <- m_par

  Kt <- mk_K(m=m,meshpts=meshpts, m_par=m.par.use, L=L, U=U)$K

  nt[,k] <- Kt%*%nt[,k-1]

  Nt_store[k]<-h*sum(nt[,k])

}

plot(nt[,1])

sum(nt[,1])

plot(log(Nt_store))

matplot(nt)

tot_pop<-colSums (nt, na.rm = FALSE, dims = 1)

plot(log(tot_pop))

plot((tot_pop))

#multiply dredge survey data up to make estimated full population

tot_pop_real<-tot_pop*8982.528

#year numbers (could also use actual year numbers)

years<-seq(1,50,1)

pred_pop_1<-data.frame(years, tot_pop, tot_pop_real)

Est_real_population_plot<-ggplot(pred_pop_1, aes(years, tot_pop_real)) + geom_point()+
  geom_line()+theme_bw()+
  labs(y = "Population", x="Years")+
  theme(text = element_text(size=20),
        axis.text.x = element_text(angle=0,size=20, hjust=1),
```

```

axis.text.y = element_text(angle=0,size=20, hjust=1))
Est_real_population_plot
#png("MCZ_Est_dredge_population_25years_sq.png", width = 10, height = 10, units = 'in', res = 800)
#print(Est_real_population_plot)
## Make plot
#dev.off()
#then want to extract lamda for each pairs of columns
#new_Ntstore<-Nt_store[-278:-1000]
Lambda.spt1<-(Nt_store[-1]/Nt_store[-50])
Lambda.s<-mean(log(Lambda.spt1[-1]) )
exp(Lambda.s) # this is the actual lamda value overall

```

Part 7: Summarised coefficients for stochastic runs

```
#####HIDE INDIVIDUAL SITE COEFFICIENTS AS REQUIRED DO NOT RUN ALL #####
#####
setwd("C:/Users/aelown/Documents/PhD 2015/Experiments/Dredge surveys/Master data sheets")
data<-read.csv("MASTER individual size measurements all oysters 2015_2018_only_ages_est.csv")

data$Age_are<-as.factor(data$Age_are)
#to calculate number of samples of each value in data$factor
splitdat<-split(data, data$Year_season)
S2016<-splitdat$`2016S`
W2016<-splitdat$`2016W`
S2017<-splitdat$`2017S`
W2017<-splitdat$`2017W`
S2018<-splitdat$`2018S`
W2018<-splitdat$`2018W`
#break up population into a hist of 98 bins
n0<-hist((pi*(S2017$Length/2)*(S2017$Width_2016/2)), breaks=98)$counts
S2018$area<-(pi*(S2018$Length/2)*(S2018$Width_2016/2))
#n0<-S2018$Length
setwd("C:/Users/aelown/Documents/PhD 2015/Experiments/Slab experiment/Data")

#####Crouch
#splitdat<-split(S2017, S2017$Area.1)
#Crouch_n0<-splitdat$RC_mouth
#n0<-hist((pi*(Crouch_n0$Length/2)*(Crouch_n0$Width_2016/2)), breaks=98)$counts
#
#m_par=c(
#  surv.int   =   -7.25E-01,
#  surv.slope =    1.75E-04,
#  growth.int=    1.14E+03,
#  growth.slope=  7.91E-01,
```

```

# growth.sd=      4.96E+02,
# seed.int=       9.77E00,
# seed.slope=     4.35E-04,
# recruit.z.mean=  1E+3,
# recruit.z.sd=    5E+2,
# establishment.prob= 4.78E-07)
#
#list of establishment probabilities
#eplist<-c(5.18E-07,
# 2.24E-07,
# 4.51E-07,
# 0.00E+00)

###Blackwater
#splitdat<-split(S2017, S2017$Area.1)
#Blackwater_n0<-splitdat$Blackwater_mouth
#n0<-hist((pi*(Blackwater_n0$Length/2)*(Blackwater_n0$Width_2016/2)), breaks=98)$counts
#
#m_par=c(
# surv.int =    -1.35E+00,
# surv.slope =  -1.63E-05,
# growth.int=   1.28E+03,
# growth.slope=  8.17E-01,
# growth.sd=    4.16E+02,
# seed.int=     9.46E+00,
# seed.slope=    5.51E-04,
# recruit.z.mean= 1E+03,
# recruit.z.sd=  5E+02,
# establishment.prob= 8.45E-07)
#list of establishment probabilities

```

```

#eplist<-c(1.35E-06,
#      0.00E+00,
#      7.80E-06,
#      2.27E-05)
#

###Raysand
#splitdat<-split(S2017, S2017$Area.1)
#Raysand_n0<-splitdat$Raysand
#n0<-hist((pi*(Raysand_n0$Length/2)*(Raysand_n0$Width_2016/2)), breaks=98)$counts
#
#m_par=c(
#  surv.int  =    -3.19E-1,
#  surv.slope =     5.99E-4,
#  growth.int=    2.11E+3,
#  growth.slope=   5.80E-1,
#  growth.sd=    6.05E+02,
#  seed.int=     9.82E00,
#  seed.slope=    4.74E-04,
#  recruit.z.mean=  1E+3,
#  recruit.z.sd=   5E+2,
#  establishment.prob= 1.31E-6)
#
#list of establishment probabilities
#eplist<-c(2.50E-07,
#      4.00E-07,
#      1.03E-06,
#      9.03E-06)
#

```

```

#####m_par_MANUAL###
m_par=c(
  surv.int=-7.18E-01,
  surv.slope=2.66E-04,
  growth.int=1.17E+03,
  growth.slope=8.34E-01,
  growth.sd=5.15E+02,
  seed.int=9.80E+00,
  seed.slope=4.34E-04,
  recruit.z.mean=1.00E+03,
  recruit.z.sd=5.00E+02,
  establishment.prob=4.40E-07)

n0<-hist((pi*(S2017$Length/2)*(S2017$Width_2016/2)), breaks=98)$counts
#list of establishment probabilities
eplist<-c(4.40E-07,
          3.78E-07,
          1.95E-06,
          8.91E-06)

#For all survival slope stochastic runs
sslist<-c(-1.63E-05,
          1.75E-04,
          5.99E-04,
          2.66E-04)

# 1. probability of surviving
s.z=function(z,m_par) {
  u=exp(m_par["surv.int"]+m_par["surv.slope"]*z)
  return(u/(1+u))
}

```

```

# 2. growth function
G.yz=function(z1,z,m_par) {
  dnorm(z1,mean=m_par["growth.int"]+m_par["growth.slope"]*z,sd=m_par["growth.sd"])
}

f.yz=function(z1,z,m_par) {
  if (any(z> 1193.805)) {
    m_par["establishment.prob"]*
    dnorm(z1,mean=m_par["recruit.z.mean"],sd=m_par["recruit.z.sd"])*
    exp(m_par["seed.int"]+m_par["seed.slope"]*z)
  } else {
    0*z
  }
}

#IPM kernel functions
## Define the survival/growth kernel
P_z1z = function(z1,z,m_par) {s.z(z,m_par)*G.yz(z1,z,m_par)}

## Define the fecundity kernel
F_z1z = function(z1,z,m_par) {f.yz(z1,z,m_par)}

L=14.13717
U=9981.939

# number of cells in the discretized kernel
m=length(n0)
## #boundary points (the edges of the cells defining the kernel)
b=L+c(0:m)*(U-L)/m
## mesh points (midpoints of the cells)
y=0.5*(b[1:m]+b[2:(m+1)])
## width of the cells
h=y[2]-y[1]
#h

```



```

#View(m_par)
G=h*outer(y,y,G.yz,m_par=m_par) # growth kernel
S=s.z(y,m_par=m_par) # survival
P=G # placeholder; we're about to redefine P on the next line
for(i in 1:m) P[,i]=G[,i]*S[i] # growth/survival kernel
F=h*outer(y,y,f.yz,m_par=m_par) # reproduction kernel
K=P+F #full kernel
lam=Re(eigen(K)$values[1])
w.eigen=Re(eigen(K)$vectors[,1])
stable.dist=w.eigen/sum(w.eigen)
v.eigen=Re(eigen(t(K))$vectors[,1])
repro.val=v.eigen/v.eigen[1]

# compute elasticity and sensitivity matrices
v.dot.w=sum(stable.dist*repro.val)*h
sens=outer(repro.val,stable.dist)/v.dot.w
elas=matrix(as.vector(sens)*as.vector(K)/lam,nrow=m)
#y=meshpts

mk_K <- function(m, m_par,meshpts, L, U) {
  # mesh points
  h <- (U-L)/m; meshpts <- L + ((1:m) - 1/2) * h
  P <- h * (outer(meshpts, meshpts, P_z1z, m_par = m_par))
  F <- h * (outer(meshpts, meshpts, F_z1z, m_par = m_par))
  K <- P + F
  return(list(K = K, meshpts = meshpts, P = P, F = F))
}

IPM_sys <- mk_K(m=m,meshpts=meshpts, m_par=m_par, L=L, U=U)
names(IPM_sys)
library(fields)
image.plot(IPM_sys$meshpts,IPM_sys$meshpts, t(IPM_sys$K))

```

Part 8: single stochastic run

```
setwd("C:/Users/aelown/Documents/PhD 2015/Experiments/Population models/IPMs/Model
      docs/Final model codes")

source("Short_IPM_for_stochastic_runs.R")

three_d_arraymcz <- array(
  NA,
  dim = c(length(n0), 50, 40),)

Nt_store<-numeric(50)
#newlam<-numeric(1000)

nt<-matrix(NA,nrow=length(n0),ncol=50)
nt[,1] <- n0;
for(k in 2:50) {
  m.par.use <- m_par
  m.par.use["establishment.prob"]<-sample(eplist,1)
  #m.par.use["surv.slope"]<-sample(sslist,1) # or for stochastic SS show this
  #m.par_store[k-1]<-m.par.true
  Kt <- mk_K(m=m,meshpts=meshpts, m_par=m.par.use, L=L, U=U)$K
  #newlam[k-1]<-Re(eigen(Kt)$values[1])
  nt[,k] <- Kt%*%nt[,k-1]
  Nt_store[k]<-h*sum(nt[,k])

}

three_d_arraymcz[,1] <- nt;
```

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