

**Quantifying Ecosystem Functions of Shellfish Habitats within the South-East  
England**

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For the Department of Life Sciences at University of Essex**

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## List of Abbreviations

Abbreviation	Meaning
AOA	Ammonia-Oxidizing Archaea
<i>amoA</i>	Ammonia Monooxygenase gene
AOB	Ammonia-Oxidizing Bacteria
C	Carbon
CG	<i>Crassostrea gigas/C. gigas</i>
CON	Control
CTAB	Cetyltrimethyl ammonium bromide
DNA	Deoxyribonucleic Acid
DON	Dissolved Organic Nitrogen
ENORI	Essex Native Oyster Restoration Initiative
LS	Live Oyster and Shell Layer
LO	Live Oyster Only
MCZ	Marine Conservation Zone
N	Nitrogen
<i>nirS</i>	Nitrate Reductase S Gene
NMDS	Non-Metric Multidimensional Scaling
NO <sub>2</sub> <sup>-</sup>	Nitrite
NO <sub>3</sub> <sup>-</sup>	Nitrate
NH <sub>4</sub> <sup>+</sup>	Ammonium
O <sub>2</sub>	Oxygen
OE	<i>Ostrea edulis/O. edulis</i>

PO <sub>4</sub> <sup>3-</sup>	Phosphate
qPCR	Qualitative Polymerase Chain Reaction
RO	Reverse osmosis (water)
rRNA	Ribosomal Ribonucleic Acid/Ribosomal RNA
TC	Total Carbon
TDON	Total dissolved organic nitrogen
TN	Total nitrogen
TOC	Total organic carbon
TIC	Total inorganic carbon
TOxN	Total oxidised nitrogen

## **Abstract**

Decline in Flat Oyster (*Ostrea edulis*) populations since the 1960s by 90% globally has resulted in momentum to restore this species to coastal areas. One justification for Native Flat Oyster restoration is ecosystem function and services shellfish can provide, but restoration of this species is questioned as Pacific Oyster (*Crassostrea gigas*) populations continue to rise on UK coastlines (Beck et al., 2011). The *Crassostrea* species has been the primary focus of oyster-based studies, as a result ecosystem function data are heavily assumed for *O. edulis* with no evidence to support the possibility for species differentiation. Mesocosm and field experiments were conducted to quantify these ecosystem functions. Mesocosms involved a comparison between live oyster with a shell layer, live oyster alone, and bare sediment in relation to nutrient (nitrogen) cycling for *C. gigas* only. Mesocosm results for microbial communities inconclusive but did however show a significant change in N compounds within sediments, based on presence of oyster communities dependent on day. *In-situ* field experiments compared species *C.gigas* vs *O.edulis* and density of oysters in 10x10m<sup>2</sup> plots (living oyster to dead shell ratio = 1:4 - densities of living oyster 1, 5, 10, 15 per m<sup>2</sup>). Field experiments showed high variation in sediment-stored inorganic carbon content for both species at all densities with overall no significant difference. Biodiversity measured as animal abundance showed insignificant difference based on density and based on base oyster species, but showed variation in abundance as *C. gigas* displays overall higher abundance with *O. edulis* having higher richness. *C. gigas* show trend of higher functionality than *O. edulis* in higher densities potentially due to size and site placement, but data inconclusive.

## **1.0 Introduction**

In recent years, shellfish habitat restoration has been a 'hot topic' for discussion after close to 95% of all shellfish habitats within the UK and Ireland have depleted, with 85% of oyster reefs being lost globally (Beck et al., 2011, Gamble et al., 2020). Of the current remaining habitats, < 5% of those remain for the Native Oyster in comparison to the historical distribution of oyster reefs (ENORI, 2021). Oyster populations, especially Native Oyster (*Ostrea edulis*) populations, have consistently depleted as commercial fishing has increased, but with the introduction of the invasive species (*Crassostrea gigas*) in UK waters, the population has shown a more rapid decline (Beck et al., 2011). There is evidence to suggest that even though the invasive species is present due to functional similarities such as water filtration and carbon stores, the *C. gigas* can compensate where *O. edulis* is now missing (Zwerschke et al., 2019). Whilst restoration and rehabilitation of shellfish, more specifically oyster reefs are still in the early stages of development, the acknowledgement of the benefits of aquaculture has helped increase the attention of research towards oysters, especially in recent years.

The decline in native Oyster populations have been primarily affected by overfishing, as well as the introduction of the invasive species *Crassostrea gigas*, as well as other invasive species and predators (ENORI, 2021, Smyth et al., 2009). One of these predatory species includes the Atlantic Oyster Drill (*Urosalpinx cinerea*), which actively drills through the *O. edulis* shell, which is another cause for population decline – if the oyster dies before maturity because of the drill, then there is no chance for increase in oyster population (Hayer et al., 2019). In addition to the *U. cinerea* there are other invasive species such as the Slipper Limpet (*Crepidula fornicata*) which has had an impact on *O. edulis* populations, with some observing that in areas of a 96%

decrease in the Flat Oyster species there has been a 441% increase in *C. fornicate* populations within a 19 year time frame – suggesting that the presence of the limpet is preventing recovery of *O. edulis* populations through lack of habitat heterogeneity or reducing the suitability of substrate settlement (Helmer et al., 2019).

*Ostrea edulis* as a species operates without stress within the temperate matrix of 5°C to 25°C, but in recent years with an impact on climate there has been a noticeable flux in sediment surface temperatures and water surface temperatures due to heatwaves (Hutchinson & Hawkins, 1992). With climate impacts in the last few decades, especially within the South-East of the UK, this has had the potential to decrease reproductive rates, growth rates, impact biodiversity, and prevent successful reintroduction of the oyster species within the Essex area due to unpredictable temperatures (Officer et al., 1982). With *O. edulis* being a temperature-sensitive organisms, *C. gigas* has been seen to be more resilient, as the invasive species of *C. gigas* was originally thought to not be able to spawn or have a high mortality rate within the naturally cooler region of the South-East of England in comparison to their natural habitat within the Pacific (Davidson, 1976). This initial theory surrounding *C. gigas* was disproved due to their high populations in current coastal areas within the UK after their introduction in the 1970's and are seeing their populations increase more with the rising climates and the decline in *O. edulis* habitats. With *C. gigas* naturally residing in tidal zones whilst *O. edulis* are generally found in subtidal zones, and therefore would have no habitat competition under natural conditions, in Essex there is an overlap in habitat for commercial populations and with *C. gigas* being an invasive species (Ezgeta-Balić et al., 2020).

## **1.1 Oyster Influence on Macrofaunal Biodiversity in an Oyster Reef Environment**

Increased biodiversity has been heavily correlated with a complex and stable habitat (May 1973), which a bivalve reef system is categorised as, indicating the hypothesis of an increased complex shellfish reef would have an increased level of biodiversity. Some previous work has suggested that a reef system made of oyster shell has a notably higher biodiversity in comparison to areas with a less complex habitat or that of bare sediment – this was regardless of oyster maturity, reef dispersion, and initial substratum type (e.g., mud or silt) (Smyth and Roberts, 2010). Additionally, it has been previously suggested that oysters act as a keystone species – a species that has a collateral effect on what surrounding interacting species then become present within the given environment – with the species effected most usually being smaller creatures seeking refuge within the complex shell network from environmental stress such as predators or atmospheric exposure (Rodriguez-Perez et al., 2019, Smyth and Roberts, 2010). One notable way in which biodiversity naturally increases with the presence of oysters is through the invasive and predatory species of the Atlantic Oyster Drill (*Urosalpinx cinerea*), which actively seek other molluscs – specifically oysters – as a means for sustenance by drilling into the oyster and as a place to reproduce by laying eggs both internally and externally on the oyster shell. *Urosalpinx cinerea* have only been observed while in areas made of shellfish habitats and are unlikely to be observed naturally in areas without shellfish like oysters as a base in the environment, therefore biodiversity is naturally increased already with the presence of oysters. Whilst the *U. cinerea* species already poses a threat to oysters in general, the predatory species can consume up to 3 oysters daily which creates a larger threat for declining or smaller populations of *O. edulis* communities (Joseph, 2004). This has already been observed in *O. edulis* populations, with areas consisting of a naturally

lower population of *O. edulis* there is a naturally occurring higher population of *U. cinerea* (egg sacks, larvae, and mature) that is observed within the habitat (Preston et al., 2020).

Shellfish, specifically oysters, have been heavily implicated to be a keystone species due to providing a habitat and substratum, and acting as a place of refuge for varying fauna, naturally increasing the biodiversity within the habitat of an oyster reef system as opposed to a less complex area without the bivalve structure (Grabowski & Peterson, 2007, Smyth & Roberts, 2010, Coen et al., 2017, Rodriguez-Perez et al., 2019).

Experimental approaches have also been used to examine if there are differences in biodiversity provision services between native flat oysters and non-native rock oysters. In these studies, it has been noted that the overall biodiversity function of both oyster species is similar, as species richness and benthic assemblage structure is similar but varied based on vertical or horizontal formation of the shellfish as well as location in the tidal zone (Zwerschke et al. 2016). Overall this work suggests that whilst the biodiversity may differ between the two species based on epibiota, overall, the species biodiversity would remain equal due to what habitat each oyster species creates.

What is missing from current studies is an understanding of how biodiversity provisioning, or indeed any ecosystem function, is related to shellfish density. This is relevant as shellfish habitats are looking to be restored around coastal areas of the UK and would therefore follow a protocol similar to the methodologies presented within the Habitat restoration and monitoring guidelines provided by Mitchley et al., for Natural England. The suggested habitat guidelines provide a measure of frequency and abundance – the DAFOS Scale – which assesses the frequency based on visible

abundance with a dominant area being usually 50% or more cover, abundance being no more than 50% cover, frequent being regularly observed, occasional being present but hard to find and sparse being extremely hard to find (Mitchley et al., 1997). Following this DAFOS Scale, naturally the *O. edulis* species would fall within the sparse or occasional category as their current naturally observed abundance is closer to 0-4 oysters per m<sup>-2</sup> (Lown et al., 2021), which most restorative plans would follow due to this being their expected density in a natural environment. However, due to many factors that would naturally prevent habitat restoration in *O. edulis*, such as *C. fornicata* populations being one example, it is hard to determine the success of restoration projects, as well as the lack of knowledge on historical distributions of *O. edulis* reefs (Lown et al., 2021).

It is known that density of a given species can influence associated species richness in other coastal systems, with medium coverage plots of *C. gigas* having an overall increase in associated biodiversity, whilst a maximum or higher rock oyster density – where high or maximum density acts roughly as 15 or more oyster per one meter squared covering, and medium being between 5 to 10 oysters per one meter squared - coverage shows a net decrease in biodiversity (Green et al. 2013). It has also been noted that assemblages are dependent on nutrient content and fluxes, showing a complex relationship between biodiversity functions, individual density and nutrient cycling properties of oysters (Green et al. 2013). Similar density dependent relationships have also been observed in blue mussel seabed-aquaculture systems (Beadman et al., 2004). Little is known about density dependent ecosystem function of European flat oysters (zu Ermgassen et al., 2020). It is known that aggregations of this shellfish were associated with many other species in the past when historical co-catch records are analysed (Bennema et al., 2020), and compared to surrounding

shellfish free habitats aggregations of mixed native and rock oyster reefs support significantly greater biodiversity in the east of the North sea (Christianen et al., 2018). High density caged densities of native oyster have been shown to support a great deal of biodiversity, significantly more than cages without shellfish or rocks, and live shellfish more so than rocks alone (Preston in press). But these are raised cages and not natural shellfish aggregations. Lown et al (2021) measured a biased component of associated species richness with flat oysters off the Essex coast. Dredges capture oysters in areas that varied in density and found that biodiversity increased with native oyster density, but the patterns were heavily compounded by live slipper limpet abundance which are known to reduce associated species abundance via competition for space and antagonistic interactions (Lown et al., 2021).

Oysters also naturally create space within a tidal area, as previously mentioned one way is as a hard substrate for epibiota. Another way is also by creating complex environments with reefs, which generate 'safe spots' for creatures such as small fish, small crabs, and snails from predation from larger creatures (McAfee & Bishop, 2019). Which whilst may only temporarily increase biodiversity temporarily (depending on time of day, tide height/time, weather, etc.), can help increase it in general. When creating space, it is suggested that when rocks are present only a low density of oyster is needed to replicate the protection of a high-density oyster reef (Green & Crowe, 2013). Overall, with contradictory evidence showing the compensation of *C. gigas* for *O. edulis* loss, it is hard to truly determine if there is a drastic effect on coastal biodiversity based on the species presence alone. Furthermore, we know little of density dependent ecosystem functionalities of flat oysters in natural settings. Here, the research aims of this project fill this knowledge gap.



Figure 1A (left) intact *Crassostrea gigas* shell left to be dried. Credit: L McGinley

Figure 1B (right) live *Ostrea edulis* with *Urosalpinx cinerea* egg sacks attached. Credit: L McGinley

## **1.2. Ecological Properties and Non-Biodiversity Services of Oysters**

Oysters have many ecological properties and benefits, including habitat services, gas regulation, disturbance regulation, water purification, sediment retention, nutrient cycling and a food source (Pollack et al., 2013). Previous models have hypothesised that with a reintroduction and increase in abundance of oysters, there will be an increase to benthic primary production, zooplankton densities, and fish stocks, as well as mitigating eutrophication (Ulanowicz & Tuttle, 1992). This has been seen primarily within Atlantic waters on the East Coast of America, as there has been an attempt to increase oyster yield and natural growth around the Chesapeake Bay area (Gamble et al., 2020, zu Ermgassen et al., 2020). Studies vary in location when observing *Crassostrea virginica*, however, as some are based around the Gulf of Mexico and others closer to the Atlantic Ocean – which have different sediment types and hydrology, suggesting the environment does not affect the oyster’s overall ability

to perform expected ecosystem functions (Zimmerman et al. 1979, Bordeaux et al. 2006, Stunz et al. 2010, Humphries et al. 2016). However, with little studies performed within Europe in comparison to the various parts of North America, and the prime species of focus for oyster-based studies usually being *Crassostrea virginica*, this means that most proposals for the benefits of flat oyster restoration are extrapolated from other species (zu Ermgassen et al., 2020). The *C. virginica* species has a different morphology, and potential functionality to that of the flat oyster this is a dangerous extrapolation. Known ecological functions of *C. virginica* include high production (which contributes to the shellfish industry), water filtration, providing habitat for epibenthic invertebrates (increasing biodiversity), carbon sequestration, shore stabilisation, and diversifying landscapes (increasing biodiversity) (Grabowski and Peterson, 2007). Whilst these ecosystem functions are the same for *O. edulis*, it is unknown if the Native species performs at a lower rate in comparison to *C. gigas*. The lack of information surrounding *O. edulis* is demonstrated in a basic “web of science” literature search, which shows that *C. virginica* has 4,871 results, whilst *C. gigas* has 7,423 and *O. edulis* has 1,367. Further, when looking for sources to support ecosystem functions there is a severe lack of literature to support ideas surrounding *O. edulis* - one example being that there are only two published studies attempting to link the Flat Oysters to the microbial communities in their environment (Arfken et al. 2017., Cafferey et al., 2016), although others are now in progress. Additionally, most studies conducted on quantifying oyster functionality, usually lack clarity in both the hypotheses they are looking for and the success of the goals at hand (Baggett et al., 2015). The only speculated benefits *O. edulis* has been said to offer is strong shellfish harvest, sediment stabilisation, and increasing biodiversity (zu Ermgassen et al., 2020). The functionality of *O. edulis* across six ecosystem functions (filtration,

production of associated species, coastal protection, sediment processes, cultural value, and ecosystem services) was found to be undocumented, whilst the mussel species *Perna canaliculus* was found to have all six (zu Ermgassen et al., 2020). This is potentially due to knowledge gaps in the lack of studies performed with *O. edulis*, a bias due to data primarily being performed in the east coast of America, where shellfish restoration is much further ahead than in Europe, as well as that it is hard to quantify the ecosystem services themselves. This is unlike the *C. virginica* which has been shown to perform burial for nitrogen, phosphorus, and carbon, as well as assist in denitrification through microbial associations (Fodrie et al., 2017, Kellogg et al., 2014). However, whether this is also applicable to other oyster species like *O. edulis* is currently unknown.

Whilst there has been uncertainty around whether oysters contribute towards being a source or sink of carbon, it has been shown that in areas with net less carbon and higher carbon rich sediments, there was a higher population of shellfish communities, mainly consisting of oysters (Fodrie et al., 2017). Furthermore, in most studies the oysters are often placed in the intertidal, leaving the areas of subtidal where the Pacific Oyster and Native Oyster usually reside, which still leaves a large misconception and interpretation of what these oyster species are capable of.

Studies showing that oysters act as incredible filter feeders, filtering water at rates of up to 200 L in 24 h (Gamble et al, 2020). Further, it has been shown that where the oysters primarily feed on phytoplankton, zooplankton and bacterioplankton, there has been a control of eutrophication and seasonal blooms through oyster interaction (Officer et al., 1982, Ulanowicz & Tuttle, 1992). In some studies nitrogen fluxes have been equally divided as going to the bottom of the water column through benthic

feeding and through hydrodynamics forcing it out of the localised area, but this is excluding any potential for nutrient recycling (Officer et al., 1982).

Quantifying functionality is always a problem, especially with oysters. Oysters, and other bivalves alike, have been associated with the ecosystem functions of nutrient cycling, sedimentation and biodeposition of nutrients (zu Ermgassen et al., 2020). Within the literature when searched together 'oyster' and 'nutrient cycling' within the last 5 years, only obtain a total 73 search results, with only 3 of these being related to *Ostrea edulis* and 23 being for *Crassostrea gigas*. Similarly results when the following were paired with 'biodeposition' instead of 'nutrient cycling' the results found 108 for oysters overall, two for *Ostrea edulis* (with one of the searches primarily focusing on *C. gigas*), and 42 overall for *Crassostrea gigas*. This once again emphasises the lack of research for not just oysters and ecosystem functions overall, but specifically the Native Flat Oyster.

Some research has suggested that with oysters being available in the ecosystem, there is an increase in nutrients within sediments, with one field study suggesting a 136% increase in ammonium, 78% increase in total nitrogen (TN), 46% increase in total phosphorus, and a 75% increase in overall organic matter concentrations, with a positive correlation between oyster density, oyster shell length and reef height for an increase in overall sediment nutrient content (Locher et al., 2020). Additionally, the study also found that age influenced rate of nutrient cycling, with juvenile or younger oysters having a higher rate of chlorophyll-a removal and higher sedimentary biodeposits of total dissolved organic carbon, nitrate, and ammonium in comparison to older or mature oysters (Locher et al., 2020). This has also been tested on a estuarine-sized plot where phytoplankton levels are considered, with oysters displaying a large control in phytoplankton blooms, and a large removal

of  $\text{NO}_3^-$  from the water column in March, but a large addition of  $\text{NO}_3^-$  to the water column in October due to oysters out-competing the removal processes (Pan et al., 2021). Some studies have cited that living oysters are capable of increasing sediment denitrification by up to 61% and an overall increase in  $\text{O}_2$  demand and  $\text{NH}_4^+$  production, often through pseudo-faeces or deposits – where pseudo-faeces is the deposit of filtered plankton that cannot be digested – and shell by itself being capable of roughly a 24% increase (Smyth et al., 2017). This suggests that oysters, functionally through their metabolic processes, act as a process and ecosystem function to remove nitrogen compounds, as well as secondarily through sedimentary deposits through reef formations.

Oysters, whilst having a solid control on phytoplankton communities through grazing, can encourage phosphorus and nitrogen recycling through pseudo-faeces and the microbial communities present on and within the oyster (Newell et al., 2005). With bivalve biodeposition, however, there can be a net loss of both phosphorus and nitrogen (Newell et al., 2005), which suggests that with the presence of oysters there is the potential for an overall equilibrium of nitrogen within the environment as there would be some nitrogen compounds immediately recycled and some stored or lost. With oyster reefs dominated by *Crassostrea virginica* it has been seen to remove up to  $502.5 \text{ kg N km}^{-2}$  through denitrification, whilst acting as a sink of nutrients (Pollack et al., 2013). This however can still be density dependent or species dependent as this hasn't been qualified for the Native Species or Invasive Species within the UK (ref here). Overall, the key ecological functions of *O. edulis* have yet to be fully quantified for the species itself outside of its function for fisheries purposes, whilst *C. gigas* has evidence to support its ecological functions of water filtration, support of an

environmental community, fisheries services, and enabling various nutrient cycle pathways.

### **1.3. Microbial cycling of nitrogen (N) in estuarine environments**

Within estuaries and mudflats, microorganisms are fundamental to the cycling of nitrogen (N) (Li et al 2015), especially where there may be high anthropogenic N inputs (Nedwell et al. 2016). The N cycle (Fig. 2) is mediated by metabolically diverse groups of microorganisms, and in coastal environments the occurrence of the different processes is spatially determined by the redox state of the sediments (Underwood et al 2022). Two key processes (i.e. nitrification and denitrification), are especially important in this context (Fig 3). Nitrification involves the autotrophic oxidation of ammonia to nitrite, as well as the conversion of nitrite to nitrate (Prosser and Nicol 2008). Nitrite is then subsequently oxidised to nitrate ( $\text{NO}_3^-$ ), resulting in a net loss of N from the ecosystem, via leaching, and/ or denitrification (Li et al 2015; Prosser and Nicol 2008; Erguder et al 2009).

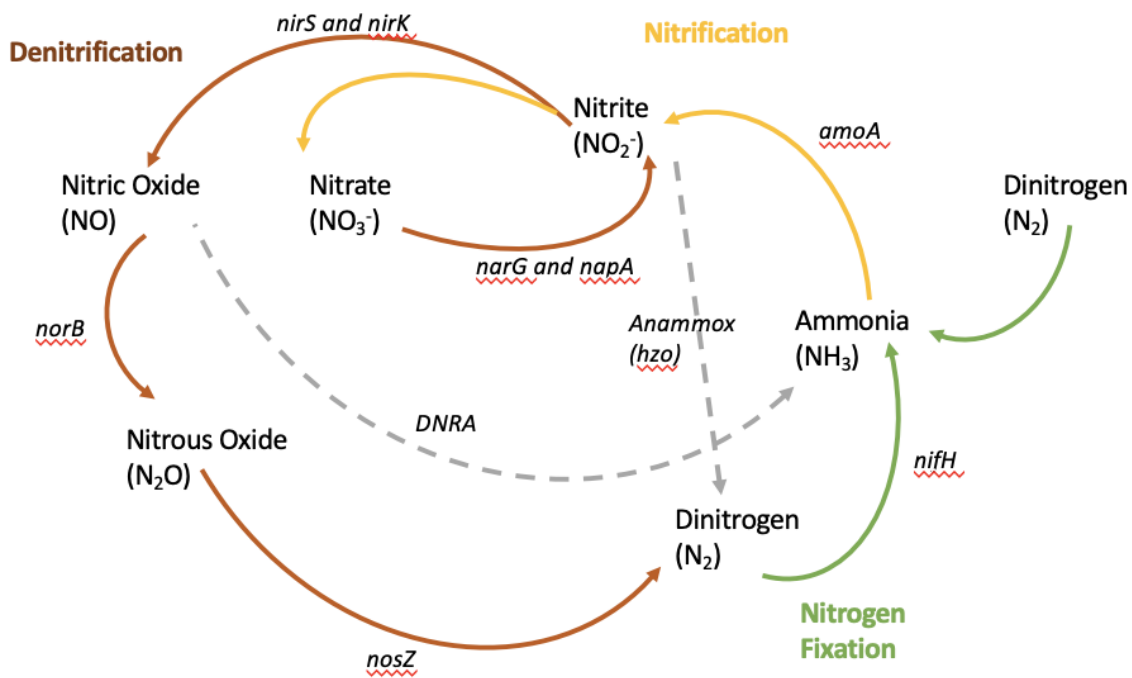


Fig 2. Adapted figure of the nitrogen cycle and key functional genes involved (Griffith 2016).

The autotrophic oxidation of ammonium is mediated by aerobic ammonia-oxidizing bacteria (AOB) and archaea (AOA), which is catalysed by ammonia monooxygenase, (encoded by the *amoA* gene) (McTavish et al. 1993). Whilst aerobic ammonia-oxidizers occur in most environments, there is evidence of niche differentiation, linked to various environmental factors (e.g. temperature, nitrite, ammonium, phosphate, oxygen, pH, salinity, light, macrofaunal activity) (Stehr et al. 1995; Caffrey et al. 2007; Scarlett et al. 2020; Martens-Habbena et al. 2009; Clark et al. 2020; Cao et al. 2012). In some coastal environments and estuaries AOA have been found to be abundant (Lipsewers et al. 2014; Smith et al. 2014) a, Jiang et al. 2009), whereas in others, AOB dominated (Caffrey et al. 2007). The oxidation of ammonia via nitrite to nitrate was previously considered a two-step process, performed by ammonia-oxidisers and nitrite oxidisers. However, the complete oxidation of ammonia to nitrate (comammox) showed that this was not the case (Daims et al. 2015)

(Fig. 3). Indeed, comammox bacteria have been found in various habitats and high proportions in estuarine environments (Xia et al. 2018).

Nitrate produced from nitrification can be reduced in a stepwise manner by denitrification to nitric oxide (NO), nitrous oxide (N<sub>2</sub>O), and dinitrogen gas (N<sub>2</sub>) and involves *napA*, *narG* (nitrate reductase), *nirS*, *nirK* (nitrite reductases) and *nosZ* (nitrous oxide reductase) (Nogales et al. 2002) (Fig. 3). Denitrification is an important process, especially in eutrophic estuaries, where denitrification can mediate N load reduction and contribute to eutrophication control (Nogales et al. 2002). Indeed, in estuarine sediments, denitrification can remove >50% of inorganic N inputs from terrestrial ecosystems (Nedwell et al. 2016) and denitrification rates are generally higher in coastal and estuarine sediments compared to shallower waters (Herbert, 1999). Nitrogen removal by denitrification may lead to a decrease in N availability, which in coastal environments can severely impact primary producers and levels of eutrophication (Seitzinger, 1998; Herbert 1999). Numerous environmental factors, (e.g. N availability and concentration, temperature, oxygen, organic matter, and bioturbation), influence the distribution and abundance of denitrifiers (Braker et al. 2000; Dang et al. 2009; Pappaspyrou et al. 2014; Prokopenko et al. 2013).

Given, the ubiquity of nitrifier and denitrifiers, it is possible that these microorganisms may thrive on the shells of oysters or even harbour a greater biodiversity, on the shells of the living oyster *Ostrea edulis*. It has been previously suggested that there is no difference in rates of nitrification between living shells and dead shells (Caffrey et al., 2016). However, the study by Caffrey et al (2016) was performed on a species that is not present within the UK. Yet, in some non-native oysters, it has been shown that there is a denitrification potential within the genetics of the oyster, as microbial communities varied within different areas, such as digestive

glands, when compared to just the exterior of the shell (Arfken et al., 2017). This may be a similar situation for the *Ostrea edulis*, however, due to the knowledge gap amongst oyster species it is hard to determine if this is applicable to other oyster species. Further, the knowledge gap is also prevalent not just for *Ostrea edulis*, but for the invasive species of *Crassostrea gigas*, suggesting if the native flat oyster shows no effect on nutrient cycling rates, then it is possible that the invasive species is responsible for such. In species that aren't the Flat Oyster there has been a connection between a change in microbial communities and the presence of said species.

Changes in microbial diversity, and in turn changes to ecosystem functions, were noted to include significantly greater microbial activity, increased chlorophyll content, increased CO<sub>2</sub> and CH<sub>4</sub> emissions when there was 40%-80% coverage of *C. gigas* in comparison to when not present at all (Green et al., 2012). Whilst at 10%, it was displayed that *C. gigas* generated a total increase in Total Oxidised Nitrogen (TOxN), as well as altering the assemblage structure of ammonia-oxidisers and methanogens. This suggests that with a change in oyster density, there is likely to be a change in ability and ecosystem function. It has also been seen that pore-water CH<sub>4</sub> was seen to increase with presence of *C. gigas* regardless of population percentage coverage. Thus, suggesting the presence of the 'invasive' oyster species can have direct and indirect effects on the ecosystem functions of a mudflat based on microbial communities present when the *C. gigas* is present, which are shown to be density dependent also. Density dependence has also been displayed when comparing nutrient cycling and changes in benthic chemical assemblages, in *Magallana gigas* (also known as *Crassostrea gigas*) when compared to *O. edulis* - with no changes being noted between the individual species, but both species showing improved or

changes in performance when the overall density was changed (Zwerschke et al., 2019).

Microbial communities can vary just based on location within the same area, for example, Crayford et al. (2017), reported that different corrosion layers of the same pipe were swabbed for corrosion levels based on changes in microbial communities, with wall samples being dominated by Xanthomonadales and Hydrogenophilales and ceiling samples being dominated by Rhodospirillales and Acidithiobacillales, whilst tidal zones were composed of a singular archaeal order and eight bacterial orders with their only common trait being anaerobic metabolisms (Crayford et al., 2017). This helps suggest the idea that with a change in species and an increase in density changing the overall environment and habitat that it would in turn change the microbial community present, thus impacting the overall ability of denitrification or nitrification.

#### **1.4 The Colne Estuary**

One way in which the UK has tried to encourage a natural and less “hands on” approach to restoring populations is with the introduction of Marine Conservation Zones (MCZ). MCZ's discourage fishing in the areas, as well as general interacting within the waters, which is meant to allow fish stocks to replenish more easily and at a higher rate than if not protected. The MCZ for the Essex area, is located on the River Colne, and is the largest inshore Marine Conservation Zone within the UK, totalling 284km<sup>2</sup> in area (Figure 4).

In recent years with partnerships between the Essex Wildlife Trust and Blackwater Oystermen Association, as well as the introduction of controlled fisheries, there has been a better understanding of how to encourage oyster reef rehabilitation in the estuary (Allison et al., 2019). The connection of Essex Wildlife Trust and Blackwater Oystermen Association was also aided by the UK Marine and Coastal

Access Act of 2009. Historically, it has also been known that bad spat falls (also known as reproduction periods where oyster spawn attaches to shell) have caused concern, and has been highly correlated to the fall in population size amongst *O. edulis* from as early as the 1850's which has carried over into modern times (Baggs et al., 1994). Populations, whilst originally at 700 million in the mid 1800's, rapidly declined due to overfishing, a large in-flux of toxins, invasive species (both competition and predatory) and extreme weather, causing a rapid decline to only 3 million in Colchester by 1898, to 3 million total in the UK alone by 1964. This is without taking into account 'freak weather' occurrences, such as a large freeze in 1963 which killed 85% of oyster stocks just in Pyefleet Creek alone. Not only this, but from an economic standpoint oyster markets suffered as continental markets have had multiple fluctuations due to transportation availability such as ferries, as well as fluxes in exchange rates which affect what oyster stocks are bought in the UK and redistributed.

The sediment within the Blackwater and Colne is a mixture of mudflat and relatively low quantities of fine sediments but consisting of sediments of mostly muddy texture. Within the Colne, roughly 19% of total dissolved organic nitrogen (TDON) is from dissolved organic nitrogen as a result of eutrophication from surface run-off and anthropogenic inputs of sewage treatment wastewaters, with between 2.2-5.2% of dissolved organic nitrogen (DON) being bioavailable, with DON often being typically around 20% of total dissolved nitrogen towards the top of the estuary and around 70% at the mouth of the estuary – with nitrate being the dominant form of dissolved inorganic nitrogen at the top of the estuary (Nedwell et al., 2009).

The Colne estuary is described as a hypernutrified temperate estuary, that exhibits gradients of salinity and ammonia (Li et al 2015). It was previously shown that benthic AOB (notably *Nitrosomonas* spp.) were 100-fold greater in abundance than AOA, both

spatially and temporally, suggesting AOB were the main contributors to nitrification in the estuary (Li et al. 2015). Seasonal differences in nitrification rates have also been reported, with maximal rates often in the summer months (Li et al. 2015).



Figure 3. Map of Mersea Island, Essex, UK, showing sites 1 at Pyefleet Creek (red) and 2 and 3 at Saltcott Creek. Made in ArcGIS Online.

## **1.5 Rationale**

Universal metrics and rationale as defined by Baggett et al. (2015) raises the question of density as a variable within a restored oyster reef, where it was previously thought not to be a factor at all. This implies that with a higher density of living oysters present, there would be a higher rate of various ecosystem functions including nutrient up-cycling and recycling through the established reef and biodiversity provisioning. To test this theory, a biphasic approach was applied. First in Chapter 2, the effect on presence of shellfish habitat and/or live shellfish on nutrient cycling in an enclosed estuarine mesocosm system was investigated. Secondly in Chapter 3, field experiments were performed, using both the non-native rock oyster and the native flat

oyster and examined the effect of density from low density (i.e. 1 live oyster per area 1m<sup>2</sup>) to high density (i.e. 15 live oysters per area 1m<sup>2</sup>) in a total experimental area of 5m<sup>2</sup>×5m<sup>2</sup> plot per species. In these plots, habitat rugosity was examined as a measure of complexity, animal abundance and diversity to explore species and density dependent biodiversity provisioning to understand what processes might be leading to any differences observed.

Following the guidelines of the 40 Questions of Importance to Policy and Practice of Native Oyster Research (zu Ermgassen et al., 2020), the study has attempted to take into account as many of the 10 categories of question (1 - baseline, 2 - restoration methods, 4 - biosecurity, 8 - quantifying benefits, 9 - policy and management.) to ensure validity and purpose of results. This is firstly attempted to be achieved by choosing private sites along the Blackwater Estuary, with one of the sites being of a higher placement compared to the other two, allowing for some comparison of variation of oyster bed positioning. In conjunction with the in-field experiment, a mesocosm experiment was run alongside to collect controlled data and to measure microbial communities that collect on just the oyster species shells alone. By prioritising these ecosystem functions, they will either fill in blanks within the grey areas of literature or reaffirm the already assumed and believed functions of both the native and non-indigenous oyster species.

### **1.6 Aims and Objectives**

The aim of this study is to explore ecosystem functions of oysters which have been observed or perceived in the Eastern Pacific Oyster (*Crassostrea virginica*) and see if the same functions are observed for the Native Flat Oyster (*Ostrea edulis*) and non-native rock oyster (*Crassostrea gigas*). The grey area of literature surrounding

oysters, but more specifically *Ostrea edulis* is vast, especially for such a fast-growing 'hot topic' of aquaculture and conservation. Where there is literature, there is no clear or concise answer, or there is too much contradictory evidence. By going ahead with the research proposed of determining the ecosystem functions, such as nitrogen cycling (Chapter 1) and carbon cycling and biodiversity provisioning (Chapter 2), this research will provide clarity on the benefit (if any) of Native Oyster restoration (Figure 1b). This will also provide better clarity on whether they outperform their non-indigenous counterpart *Crassostrea gigas* (Figure 1a), and what benefits could be accrued from non-native rock oyster in an aquaculture setting, or where they become naturalised. This will be achieved with the following specific objectives:

- 1) To conduct a literature search to determine whether the presence of live oysters has any effect on the rate of nitrification and denitrification and biodiversity services (Introduction chapter).
- 2) To determine if presence of oysters will obtain an observable change in nitrogen-based compounds within sediments in a mesocosm environment (Section 2)
- 3) To quantify denitrifying and nitrifying bacterial communities on oyster shell exteriors in comparison to bare sediment in a mesocosm environment (Section 2)
- 4) To determine if there is any carbon store generated within sediment when live oysters are present in an *in-situ* environment and if this is affected by density. (Section 3)
- 5) To ascertain if there is an observable change in species present based on density-dependence when live oysters are in the monitored area. (Section 3)

All the changes being examined are a comparison between the indigenous (*O. edulis*) and non-indigenous species (*C. gigas*), with exception to mesocosm studies which are *C. gigas* only.

### **1.7. Hypotheses:**

Here, it was hypothesised that:

(i) Where oysters are present, the concentration of nitrogen will increase, which in turn will lead to an increase in denitrifiers abundance,

(ii) In the presence of an oyster bed within the estuary (as opposed to bare sediment) there will be an increase in macrofauna biodiversity, as there is a new complex habitat and food source with oysters being available. Specifically, *Crassostrea gigas* will harbour increased macrofauna biodiversity than *Ostrea edulis* as the shell structure of the *C. gigas* will create a more complex habitat in comparison to the flatter and less rigid *O. edulis* shell.

(iv) Changes in macrofauna biodiversity will be density dependent - with higher densities having more macrofaunal biodiversity regardless of oyster species

(v) Total organic carbon content will be density dependent, with *C. gigas* storing more total organic carbon compared to *O. edulis* and bare sediment

## 2.0 Mesocosm Materials, Methods, and Results

### 2.1.1 Mesocosm Experimental Set Up

Ten mesocosms were set up using a PVC pipe approximately 12-inch in diameter and 38-inch height (Figure 5A) which had a base of same diameter attached secured by wedging on to the bottom of the PVC and placed within a larger PVC pipe roughly 15-inches in diameter (Figure 5B). The inner mesocosm base also had rope attached to make it easier to lift the inner mesocosm out and ensure the base did not separate from the tube (Figure 5B).

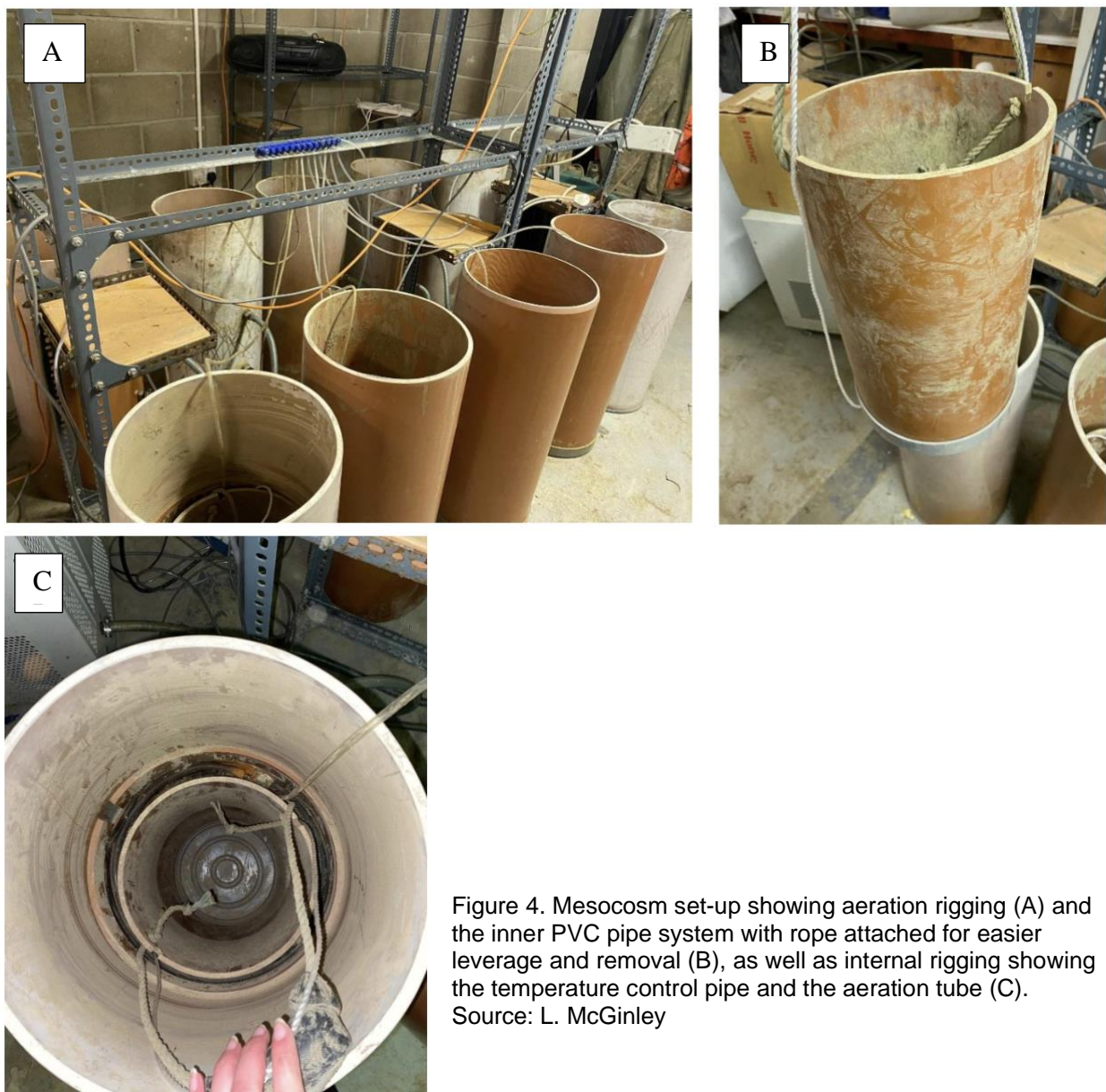


Figure 4. Mesocosm set-up showing aeration rigging (A) and the inner PVC pipe system with rope attached for easier leverage and removal (B), as well as internal rigging showing the temperature control pipe and the aeration tube (C).  
Source: L. McGinley

The inner PVC pipe was surrounded by a larger PVC pipe which had a heating coil secured around the centre to keep the temperature of the water controlled and constant. The mesocosms were filled individually with 35 L of unfiltered seawater obtained from the Colne Estuary and left for a minimum of 24 h to equilibrate. All ten mesocosm pipes were connected by a cooling system (ThermoChill II Recirculating Chiller) which pumped antifreeze through a silicone tubing system attached to the outer PVC pipe and allowed the cooling system to circulate the outer wall of the inner PVC tube (Figure 5C), which kept the temperature at a constant of 16°C to mimic the spring sea surface temperature. The mesocosms were also connected by a water aeration pump so the mesocosms remained oxygenated, and to also mimic the conditions of tidal mixing, encouraging the mesocosm environment to maintain roughly 5-7 mg/L of compressed air within the mesocosm to replicate the optimal dissolved oxygen levels of an estuarine environment. All internal casings of PVC, where the simulated environment was placed, were filled with five centimetres of sediment obtained from the River Colne.

For the initial experiment to ensure set up worked and to practice data collection methods five of the ten mesocosms were filled with a layer of sun-bleached shell, whilst for the second experiment involving live oyster four of the ten mesocosms would then be filled with a shell. Reverse osmosis (RO) water was used to replenish the mesocosms individually to ensure the salinity remained consistently within the limits of 33-35ppm, (i.e., the salinity of the natural estuary). Sediment cores were added to the mesocosms within 3 h of collection and allowed to equilibrate for 24 h. Large infauna were also carefully removed from the cores before use to prevent interference to the experiment.

### **2.1.2 Mesocosm Treatment - Live Oyster Trials**

For the ten mesocosms involving the live oysters, there was one control of sediment only and two of a simulated environment of layers as follows: sediment alone (CON, and sediment + dead shells + living oysters (L+S), and four of just the sediment + living oysters (LO). There was a total of two living native oysters, *Ostrea edulis*, per L+S treatment and per LO treatment, which mimics a higher density oyster habitat of one live oyster per 10m<sup>2</sup> to 15m<sup>2</sup>. 16 oysters were used in total for the experiment, with the shell layer following a similar thickness to that used in the prior treatments set-up of shell only when learning technique application of roughly 5-8cm thickness of shell layer. Dead *C. gigas* shells could vary in size but were usually selected to be within the range of 10-20 cm in length, where shell length was a standardised measure from shell lip to umbo, through the centre point of the shell. Mesocosms remained undisturbed except for infauna removal and for data collection on sampling days (once every seven days). The direct comparison of sediment only and a highly dense treatment will show the effect of a restored oyster reef and in turn the effect of a healthy *Ostrea edulis* reef – follows the BACI (Before-After-Controlled-Impact) sampling procedure suggested by NORA. Dead shells were stacked to mimic flow and sediment deposition disruption and to act as the base for the living oysters to attach to, whilst living oysters contribute to bio-deposition of N towards sediment.

This experiment only used *C. gigas* due to time and availability of live *O. edulis* for the experiment set up. The experiment lasted for a total of 28 days, with data collection every seven days from initial day set up (day 1) to the final day of collection (day 28). The data collected for this experimental set up consisted of a mixed sediment cores for nutrient sampling and shell swabs of the live oyster for qPCR analysis. Shell

swabs were standardised at 10 seconds total, (5 seconds per side of oyster). Swabs were stored in a sterile falcon tube at -20°C.

Sterile cotton swabs were used to swab the outer shells and the surface biofilm layer of the sediment and stored in separate falcon tubes. 10 ml of water was taken from the water column for total nitrogen and total carbon measurements. Sediment (approximately 3g) was sampled from directly under the oyster's position to account for any burial of N or C. The only data collected from this treatment was from day 0, day 1, and day 10 (the end of the experiment).

For microbial community analysis, a sterile cotton swab was used to swab a randomly selected area of oyster shell internally for 15 s and externally for 15 s. However, it was found that this made the swab gain too much water and lose its structural integrity, so the swabbing time was reduced to a total of 10 s total, (i.e. 5 s internal; 5 s external sides). Swabs were stored in a sterile falcon tube at -20°C. A control atmospheric swab was also taken on each day of data collection to account for any air contamination and subtracted from the final data set. Sterile conditions were met by maintaining no falcon tubes were open prior to using when collecting data, and that swabs were kept stored in a sterile bag. In addition, all utensils and equipment used were sterilised with 70% (v/v) ethanol.

### **2.1.3 Nutrient Analysis – Nitrogen**

To measure ammonium, nitrate, nitrite, and phosphate concentrations, 10 mL of sterile MilliQ water was added to 1 g of sediment and sonicated for 20 mins in a Decon F5 Minor Sonicator. The sample was filtered using a 0.2 µm filter (sartorius stedim Minisart) and a minimum of 8 ml of sample, was injected into a Total Nitrogen SEAL Auto-Analyser AA3 HR with a FP-2020plus Jasco Intelligent Fluorescence

detector. Total Organic Nitrogen content was calculated by summing the ammonium, nitrate, nitrite values obtained.

#### **2.1.4 DNA Extraction of Sediment and Swab Samples**

DNA was extracted from swabs and sediment samples. For sediments, 0.5 g of sediment was placed into a sterile 2ml screw-cap tube containing 0.5 g of sterile 0.1mm silica beads and 0.65 ml of Cetyltrimethyl ammonium bromide (CTAB) buffer. Phenol chloroform isoamyl alcohol (ration 25:24:1) pH 7.0 (0.5 ml) was added to the tubes and the samples were placed into a beadbeater (Bertin Technologies: Precellys Evolution) and bead-beated for 5 mins. Samples were centrifuged at 13,000 rpm for 5 mins. The upper aqueous layer was removed and placed into a sterile eppendorf, containing 0.5 ml phenol chloroform isoamyl alcohol (pH 7.0), and centrifuged at 13,000 rpm for 5 mins. The upper-most layer was removed and placed into a sterile eppendorf, with 1 vol of 30% (w/v) polyethylene glycol (PEG) solution and incubated overnight at room temperature. The sample was centrifuged at 20,784×g for 5 mins to pellet the DNA. The DNA pellet was then washed with a 70% (v/v) ethanol, air dried and resuspended in 50 µl RNase free water (Ambion: The RNA Company). For swab samples, DNA was extracted as described above except 5 mL of sterile water was added to the swabs placed in sterile tubes and vortexed for 5 mins to remove the biomass. Samples were centrifuged at 20,784×g for 5 mins to pellet the cells and resuspended in CTAB buffer and phenol chloroform isoamyl alcohol (pH 7.0) and bead-beated as described above for the sediments.

### **2.1.5 qPCR Amplification of Taxonomic and Key Functional Genes**

Gene abundances were quantified by qPCR with a SensiFAST SYBR No-ROX Kit (Bioline) on a CFX96 Real-Time PCR Detection System (BioRad). The 16S rRNA genes were targeted using the primer pairs Bakt\_341F and Bakt\_805R for Bacteria (Herlemann et al., 2011), and 344F (Raskin et al., 1994) and 915R (Stahl et al., 1991) for Archaea. For key N-cycle functional genes, the AOA *amoA* genes were targeted using the primers CrenamoA-23F and CrenamoA-616R for AOA (Tourna et al., 2008). For AOB *amoA* gene abundance, the primers *amoA* 1F and *amoA* 2R (Rotthauwe et al. 1997) were used. For denitrifier abundance, the *nirS* gene was targeted using the primers *nirS* F and *nirS* R (Throback et al 2014). Gene abundances were quantified with an absolute quantification method against an internal standard calibration curve using DNA standards of each target gene from  $10^2$  to  $10^7$  copies in 20  $\mu$ l reactions containing 200 nM of primers and 1  $\mu$ l of DNA template. Cycle conditions for all genes were 95 °C for 3 min followed by 40 cycles at 95 °C for 10s then 60 °C for 30 seconds. Amplification specificity was confirmed by melting curve analysis.

However, due to the DNA extraction method using phenol-chloroform to obtain a DNA pellet, the end pellet sample may not always be a usable sample as the presence of phenol decreases the detection of DNA by UV absorbance (Javadi et al., 2014). This was observed in some of our data as the readings for copies were below the minimum target of  $10^2$  or read as a 0 meaning no detection whatsoever (figure 6.). Therefore, for the purpose of this study the microbial analysis is deemed unusable and will be evaluated in a future study.

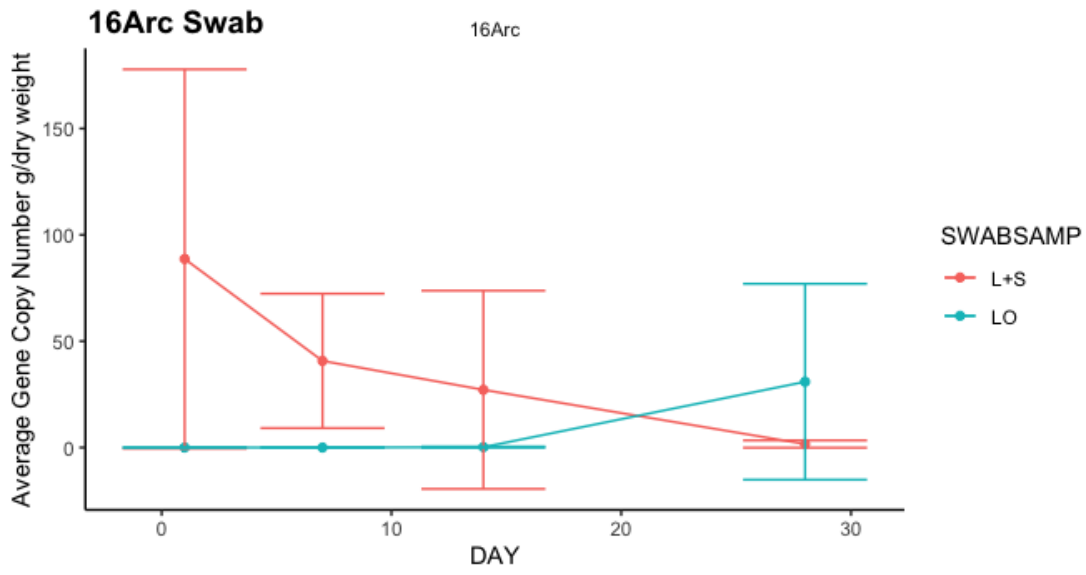


Figure 5. Average copies for 16Arc presence in swab samples from oyster shell exterior of living oyster with shell layer (L+S) and from living oyster only (LO) conditions across a 28-day period.

### 2.2.1 Nutrient Analysis

For the mesocosm experiments, sediment cores were taken from the mesocosm sediment and shell swabs were labelled as follows: sediment alone (CON), live oyster (LO) and sediment and a mix of sediment, dead shell and live oyster (L+S). LO is an average of four samples from four separate mesocosms (1 per mesocosm) L+S is an average of four mesocosm also, CON average of two mesocosms.

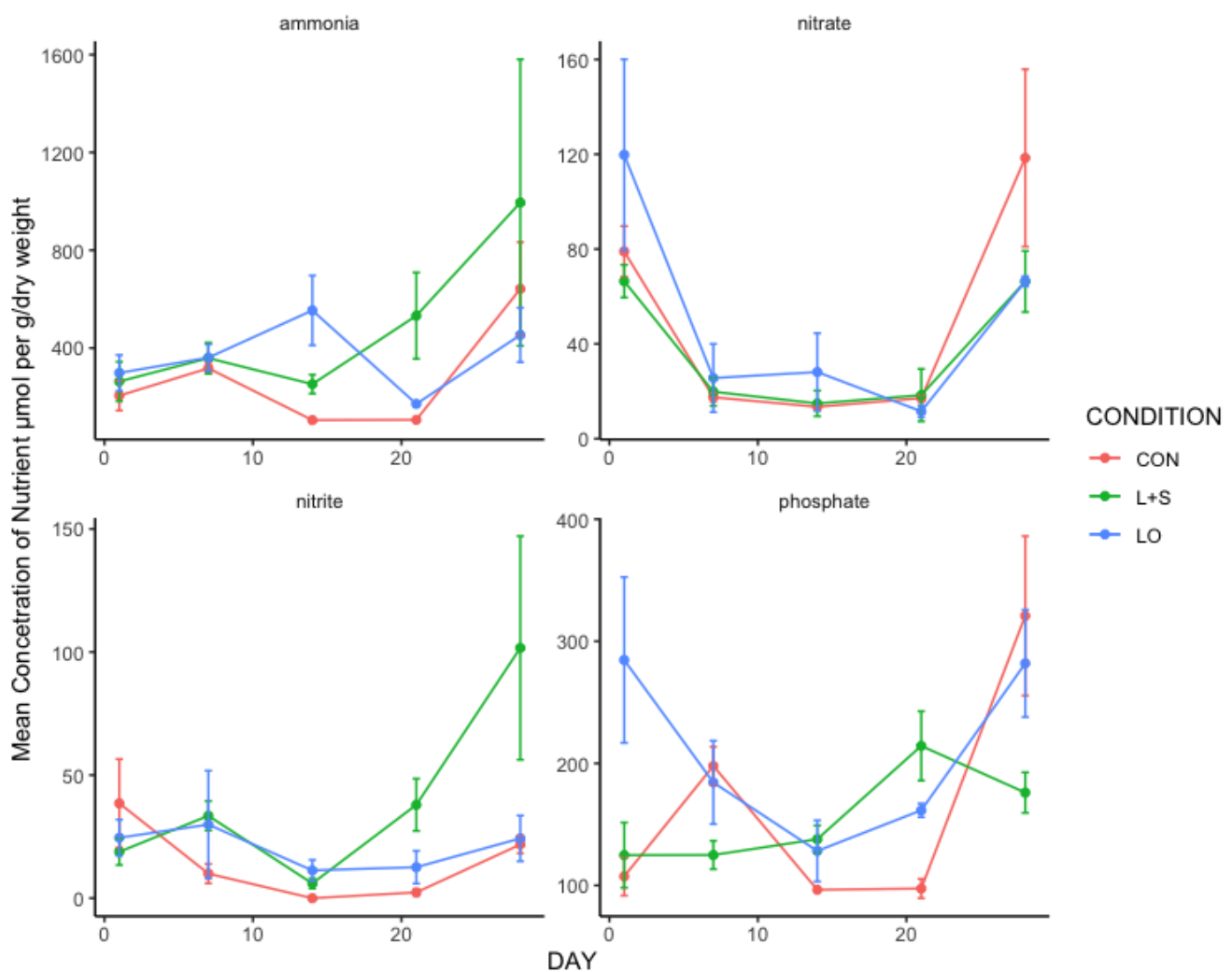


Figure 6. Average change in Ammonia ( $\text{NH}_4^+$ , top left), Nitrate ( $\text{NO}_2^-$ , bottom left), Nitrite ( $\text{NO}_3^-$ , top right), and Phosphate ( $\text{PO}_4^{3-}$ , bottom right) in  $\mu\text{mol per gram}$  of dry weight of sediment across a 28-day period for mesocosm sediment cores for three conditions of sediment alone (CON), live oyster and sediment only (LO), and a mix of sediment, dead shell and live oyster (L+S) where data is collected

every seven days. Two-Way ANOVA for Nutrients and Day [df:3 , F-Value: 3.406, *p*-value: 0.01 at *p*<0.05]. Two-Way ANOVA for Nutrients interacting with Day and Condition [df:6, F-Value: 1.14, *p*-value: 0.36 at *p*<0.05]

Table 1. Paired T-Test at *p*<0.05 for Figure 6 to display difference between Phosphate (PO<sub>4</sub><sup>3-</sup>) based on day between conditions Live Oyster only (LO), Shell Layer and Live Oyster (LS) and a sediment only control (CON), where first row is for day 1, second row is day 7, third row is day 14, fourth row is day 21, and fifth row is day 28, where *df* = 4 for all conditions.

<b>PHOSPHATE (PO<sub>4</sub><sup>3-</sup>)</b>	Live Oyster Only	Live Oyster and Shell	Sediment Only
	-	0.17*	0.08*
Live Oyster Only	-	0.07**	0.73
	-	0.65	0.29
	-	0.18*	0.01**
	-	0.05*	0.67
Live Oyster and Shell	0.17*	-	0.66
	0.07**	-	0.02**
	0.65	-	0.03**
	0.18*	-	0.03**
	0.05*	-	0.26
Sediment Only	0.08*	0.66	-
	0.73	0.02**	-
	0.29	0.03**	-
	0.01**	0.03**	-
	0.67	0.26	-

Table 2. Paired T-Test at  $p < 0.05$  for Figure 6 to display difference between Ammonia ( $\text{NH}_4^+$ ) based on day between conditions Live Oyster only (LO), Shell Layer and Live Oyster (LS) and a sediment only control (CON), where first row is for day 1, second row is day 7, third row is day 14, fourth row is day 21, and fifth row is day 28, where  $df = 4$  for all conditions.

<b>AMMONIA (<math>\text{NH}_4^+</math>)</b>	Live Oyster Only	Live Oyster and Shell	Sediment Only
	-	0.82	0.18
Live Oyster Only	-	0.98	0.45
	-	0.15	0.05**
	-	0.13	0.01**
	-	0.41	0.54
Live Oyster and Shell	0.82	-	0.68
	0.98	-	0.62
	0.15	-	0.03**
	0.13	-	0.09*
	0.41	-	0.53
Sediment Only			
	0.18	0.68	-
	0.45	0.62	-
	0.05**	0.03**	-
	0.01**	0.09*	-
	0.54	0.53	-

Table 3. Paired T-Test at  $p < 0.05$  for Figure 6 to display difference between Nitrite ( $\text{NO}_3^-$ ) based on day between conditions Live Oyster only (LO), Shell Layer and Live Oyster (LS) and a sediment only control (CON), where first row is for day 1, second row is day 7, third row is day 14, fourth row is day 21, and fifth row is day 28, where  $df = 4$  for all conditions.

<b>NITRITE (<math>\text{NO}_3^-</math>)</b>	Live Oyster Only	Live Oyster and Shell	Sediment Only
	-	0.59	0.56
Live Oyster Only	-	0.90	0.46
	-	0.1	0.07*
	-	0.21	0.21*
	-	0.14*	0.77
Live Oyster and Shell	0.59	-	0.33
	0.90	-	0.02**
	0.1	-	0.06*
	0.21	-	0.05**
	0.14*	-	0.16
Sediment Only	0.56	0.33	-
	0.46	0.02**	-
	0.07*	0.06*	-
	0.21*	0.05**	-
	0.77	0.16	-

Table 4. Paired T-Test at  $p < 0.05$  for Figure 6 to display difference between Nitrate ( $\text{NO}_2^-$ ) based on day between conditions Live Oyster only (LO), Shell Layer and Live Oyster (LS) and a sediment only control (CON), where first row is for day 1, second row is day 7, third row is day 14, fourth row is day 21, and fifth row is day 28, where  $df = 4$  for all conditions.

<b>NITRATE (<math>\text{NO}_2^-</math>)</b>	Live Oyster Only	Live Oyster and Shell	Sediment Only
Live Oyster Only	-	0.23	0.45
	-	0.71	0.64
	-	0.52	0.43
	-	0.64	0.19
	-	0.99	0.27
Live Oyster and Shell	0.23	-	0.40
	0.71	-	0.68
	0.52	-	0.43
	0.64	-	0.91
	0.99	-	0.18
Sediment Only	0.45	0.40	-
	0.64	0.68	-
	0.43	0.43	-
	0.19	0.91	-
	0.27	0.18	-

Concentrations of nitrate ( $\text{NO}_3^-$ ), nitrite ( $\text{NO}_2^-$ ), ammonium ( $\text{NH}_4^+$ ) and phosphate ( $\text{PO}_4^{3-}$ ) were measured over the 28-day period (Figure 6) with data intervals of every seven days, collecting sediment samples on Day 1, Day 7, Day 14, Day 21, and Day 28. Observed trend shown for CON treatment showed an overall increase for  $\text{NO}_3^-$  at Day 1 being  $79.00(\pm 10.68)$   $\mu\text{mol/g}$  dry weight to  $118.43(\pm 37.42)$ , and an increase for  $\text{NH}_4^+$  of  $205.13(\pm 60.84)$   $\mu\text{mol/g}$  dry weight on Day 1 to  $642.05(\pm 191.07)$   $\mu\text{mol/g}$  dry weight to Day 28,  $\text{NO}_2^-$  at Day 1 is at  $38.56(\pm 17.93)$   $\mu\text{mol/g}$  dry weight and decreases to Day 28 at  $21.81(\pm 3.56)$   $\mu\text{mol/g}$  dry weight, and  $\text{PO}_4^{3-}$  increasing from 205.13

( $\pm 60.84$ )  $\mu\text{mol/g}$  dry weight to  $320.82$  ( $\pm 65.34$ )  $\mu\text{mol/g}$  dry weight. Overall trend for nutrient content within mesocosms for LO treatment displayed decrease in  $\text{NO}_3^-$  from  $119.74$  ( $\pm 40.32$ )  $\mu\text{mol/g}$  dry weight to  $66.42$  ( $\pm 2.14$ )  $\mu\text{mol/g}$  dry weight,  $\text{NO}_2^-$  decreasing from  $24.56$  ( $\pm 7.34$ )  $\mu\text{mol/g}$  dry weight to  $24.28$  ( $\pm 9.28$ )  $\mu\text{mol/g}$  dry weight,  $\text{NH}_4^+$  increases from Day 1 readings of an average of  $298.24$  ( $\pm 73.16$ )  $\mu\text{mol/g}$  dry weight to  $453.25$  ( $\pm 111.06$ )  $\mu\text{mol/g}$  dry weight by Day 28, and  $\text{PO}_4^{3-}$  decreased from  $284.68$  ( $\pm 67.92$ )  $\mu\text{mol/g}$  dry weight on Day 1 to  $281.88$  ( $\pm 43.96$ )  $\mu\text{mol/g}$  dry weight at Day 28. Observed trend shown in L+S condition display barely any change in  $\text{NO}_3^-$  as Day 1  $66.46$  ( $\pm 6.89$ )  $\mu\text{mol/g}$  dry weight and  $66.33$  ( $\pm 12.88$ )  $\mu\text{mol/g}$  dry weight by Day 28,  $\text{NO}_2^-$  increased from  $18.83$  ( $\pm 5.35$ )  $\mu\text{mol/g}$  dry weight to  $101.03$  ( $\pm 45.42$ )  $\mu\text{mol/g}$  dry weight,  $\text{NH}_4^+$  increased from  $263.06$  ( $\pm 80.58$ )  $\mu\text{mol/g}$  dry weight on Day 1 to  $995.10$  ( $\pm 586.14$ )  $\mu\text{mol/g}$  dry weight,  $\text{PO}_4^{3-}$  increased from  $124.82$  ( $\pm 26.69$ ) to  $176.03$  ( $\pm 16.57$ )  $\mu\text{mol/g}$  dry weight by Day 28 (Figure 6). Average nutrient content data per gram of dry weight overall insignificant when showing interaction between 'Day', 'Condition', and 'Nutrients',

Total Nitrogen (TN) for purpose of study is calculated by summing the  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{NH}_4^+$  values. There was an observed significant change in TN to  $\text{PO}_4^{3-}$  ratios over the 28 days both between the conditions (Figure 7), with L+S conditions having a greater positive ratio between TN to  $\text{PO}_4^{3-}$ , showing there is overall more TN than  $\text{PO}_4^{3-}$  in L+S conditions by day 28, whilst there is a decrease in TN to  $\text{PO}_4^{3-}$  in LO and NA. L+S started at  $1.15\mu\text{mol/g}$  ( $\pm 0.10$ ) of TN to  $1\mu\text{mol/g}$  of  $\text{PO}_4^{3-}$  at day 1, and increased to  $3.62\mu\text{mol/g}$  ( $\pm 0.01$ ) by day 28. LO TN started at  $1.23\mu\text{mol/g}$  ( $\pm 0.02$ ) and decreased to  $1.02\mu\text{mol/g}$  ( $\pm 0.09$ ) to  $1\mu\text{mol/g}$  of  $\text{PO}_4^{3-}$  by day 28. NA TN started at  $1.14\mu\text{mol/g}$  ( $\pm 0.03$ ) and decreased to  $0.05\mu\text{mol/g}$  ( $\pm 0.02$ ) by day 28 to  $1\mu\text{mol/g}$  of  $\text{PO}_4^{3-}$  (Figure 8).

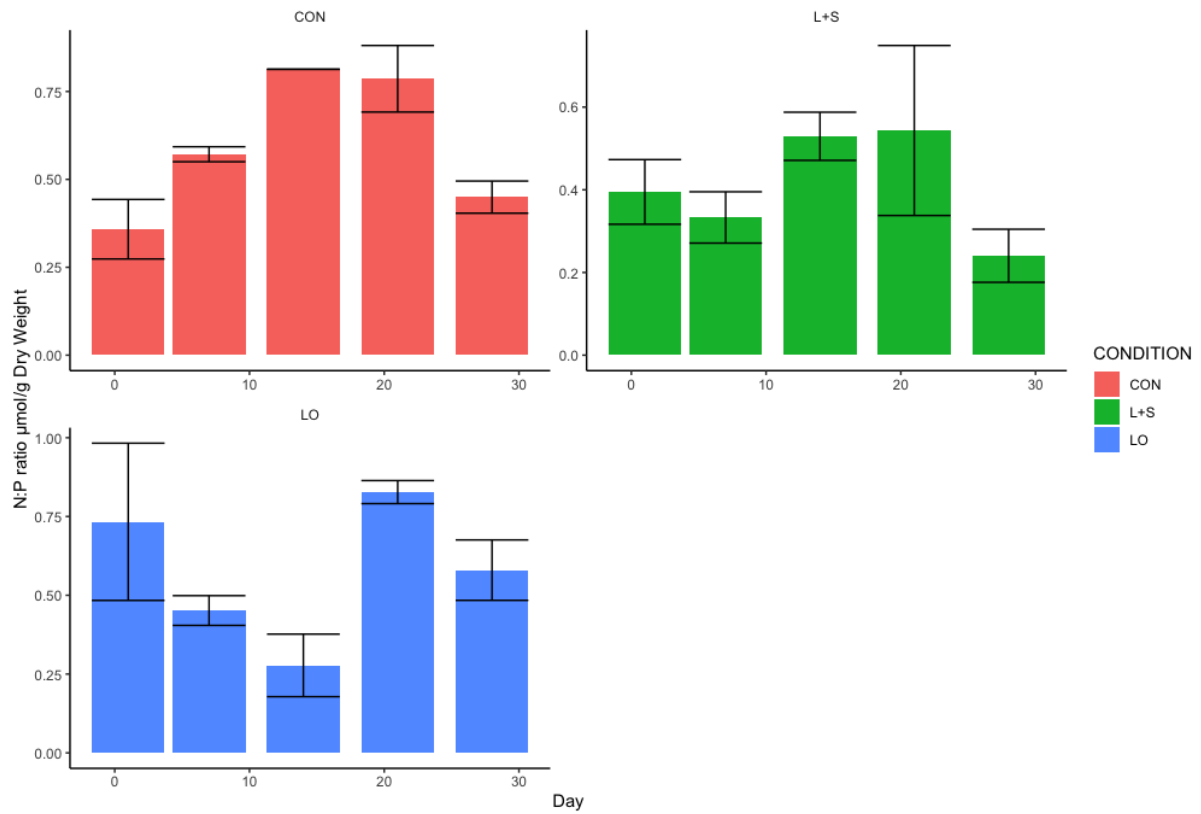


Figure 7. Total Nitrogen (sum of  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ) to  $1 \mu\text{mol/g}$  of phosphate ( $\text{PO}_4^{3-}$ ) ratio in a gram of sediment over a 28-day period for Live Oyster only (LO, blue), Live Oyster and a layer of shell (L+S, green) and sediment only (CON, red). Two-Way ANOVA for Day interacting with Condition [df:2, F-Value: 0.128,  $p$ -value: 0.82 at  $p < 0.05$ ],

Table 5. Paired T-Test at  $p < 0.05$  for Figure 7 to display difference between the Nitrogen : Phosphate ratio per one  $\mu\text{mol}$  per gram of dry weight based on day between conditions Live Oyster only (LO), Shell Layer and Live Oyster (LS) and a sediment only control (CON), where first row is for day 1, second row is day 7, third row is day 14, fourth row is day 21, and fifth row is day 28, where  $df = 4$  for all conditions.

<b>NITRATE (NO<sub>2</sub><sup>-</sup>)</b>	Live Oyster Only	Live Oyster and Shell	Sediment Only
	-	0.34	0.17
Live Oyster Only	-	0.12*	0.14
	-	0.13*	0.01**
	-	0.25	0.72
	-	0.03**	0.41
Live Oyster and Shell	0.34	-	0.02**
	0.12*	-	0.68
	0.13*	-	0.01**
	0.25	-	0.36
	0.03**	-	0.06*
Sediment Only	0.17	0.02**	-
	0.14	0.68	-
	0.01**	0.01**	-
	0.72	0.36	-
	0.41	0.06*	-

### **3.0 Artificial Reefs Materials and Methods**

#### **3.1 Artificial Reef Density Gradient Design**

The experiment was conducted in both West and East Mersea at Pyefleet Creek, close to the Colchester Oyster Fishery company, and Saltcott Creek (Figure 4). Both locations have soft clay-like estuarine mud sediments. At Pyefleet Creek the artificial reefs were built on a sloped area of the lowest intertidal as it meets the subtidal compared to the beds at West Mersea, which were on a flat plateau of the lowest intertidal and inundated by seawater and sediments much more regularly.

Each field sites consisted of two 10 x 10 metre grids, one for the *Crassostrea gigas*, the other for the *Ostrea edulis*. The 10 x 10 plots were split into four density gradients for live oysters: low = 1 oyster per m<sup>2</sup>, low-medium = 5 oysters per m<sup>2</sup>, high-medium = 10 oysters per m<sup>2</sup>, high = 15 oysters per m<sup>2</sup> (Figure 9). To mimic a real reef scenario, for every individual live oyster there were four empty valves (shells) beneath,. The densities were allocated by having the minimum and maximum parallel to and closest to the river, Figure 9), with this being 15 live oysters per m<sup>2</sup> and 1 live oyster per m<sup>2</sup>. - this pattern was used for both oyster species at all sites.

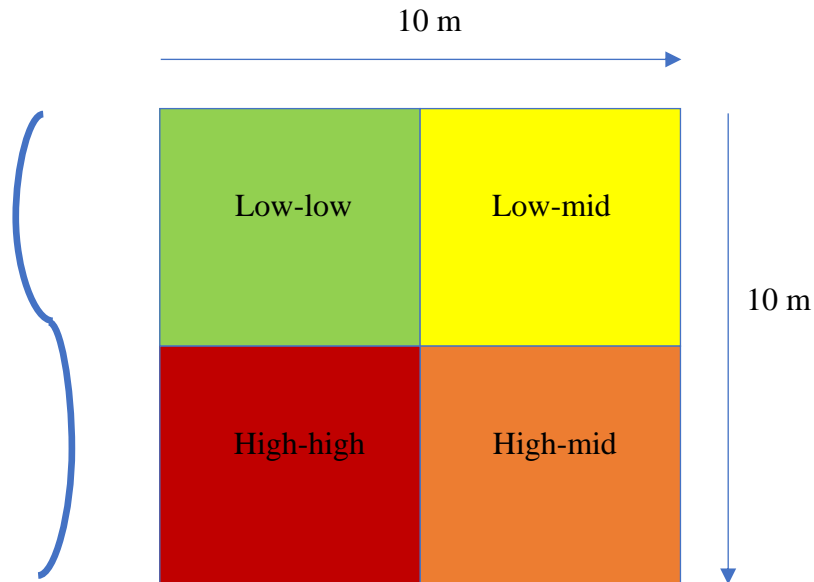


Figure 8. Density dependent oyster reefs set up displaying the rotation of low to high densities, with low-low being 1 live oyster per metre squared, low-mid 5 per metre squared, mid-high 10 per metre squared, high-high 15 per metre with the lowest and highest densities positioned parallel to the river (blue line)

The 10 x 10m grids were situated on a low tidal zone, to mimic a natural oyster reef setting, especially for that of the Native Oyster which is usually found further down the intertidal zone in comparison to the Pacific Oyster. Site one at Pyefleet Creek has an extremely soft sediment, consisting mainly of mud and clay, with little interference due to being on private land, and the main disturbance being from nearby dredging boats. Site two was at Saltcott Creek in West Mersea, which is still soft clay and muddy sediments, was more disturbed due to a higher usage of the estuary from recreational boats, as well as dredging boats and fishing boats.

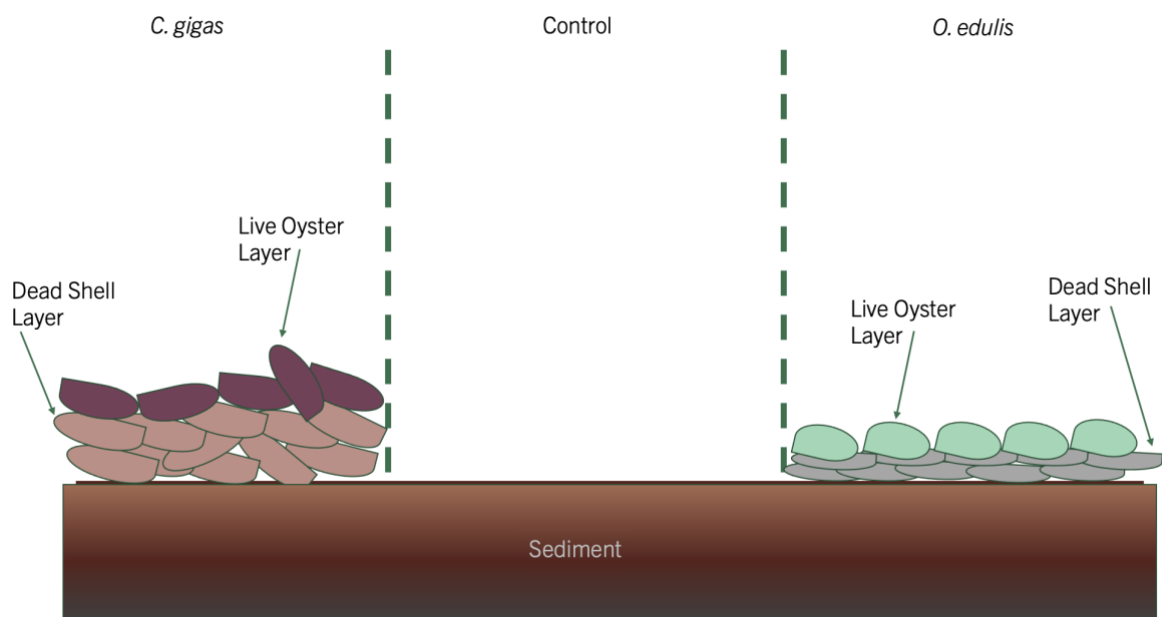


Figure 9. Diagram showing the experimental oyster reef set up, in field, no density applied, displaying the layer of dead shells beneath the layer of living oyster in comparison to each species and bare sediment.



Figure 10. Image of Site 2 at Saltcott Creek of *Crassostrea gigas* 10x10 plot with layers of dead shell and live oyster, site is marked out using wooden canes – credit: L McGinley

The experiment was initially planned to follow the sample trays method, to show potential other taxonomic groups present in oyster colonies collected within the trays (Baggett et al., 2014). However, the oysters would possibly be restricted within the trays, as it would not allow for fauna to naturally migrate between plots easily and could influence the results, as well as forcing the oysters into a closed environment where the potential for more sedimentation could occur would potentially skew the results for microbial data collection and nutrient analysis. Further, if placed within the trays, then the data for carbon burial would not be obtainable as there would be no direct sediment contact due to the tray restricting the interaction between the sediment of the estuary and the oyster habitat within the tray (Baggett et al., 2014). There was also concern that due to the deep intertidal nature of the sites that trays would be dislodged by currents unless pinned in place, thus making them difficult to sample overall. Instead, the individual oyster beds were placed directly onto a 5x5 meter plot of existing estuarine sediment to be left to adapt to the surrounding environment and a destructive method of data collection was used instead. The plots of land, due to already having a natural *Crassostrea gigas* population in the areas, required raking the ground and physical removal of pre-existing live oyster or shell within the mostly bare plots of the estuary used for the experiment. Live shellfish and shell existing under sediment surfaces were left in place to reduce major disruption of the habitat itself. *Crassostrea gigas* that were originally in the habitat were of a much larger size due to being there for years prior, making them distinguishable from *the C. gigas* used for the study and could be identified and not be included in future analysis.

### **3.2 Nutrient Analysis - Carbon**

Sediment mini cores were collected by pushing a Falcon tube into the sediment directly, collecting approximately 15 g of sediment. The cores were stored at 4°C and processed within 24-72 h from collection. Total organic carbon was performed by placing the sediment into crucibles, weighed and placed into an oven at 100°C for 24 h to dry to a consistent weight. Once baked, the crucibles were left to cool to room temperature for 12 h and re-weighed, to calculate the pure sediment weight without moisture. After cooling, the sediment was homogenised using a morsel and pestle and 50 mg placed into a smaller glass crucible, with a layer of thin fibreglass material on top to prevent sediment escaping whilst analysing to obtain Total Carbon level. The sample was then analysed using a SKALAR Formacs<sup>HT</sup> TOC Analyzer. Total Organic Carbon obtained by dropping 0.5ml of phosphoric acid onto sample until completely settled, then baked again for 24 h and left to cool, then placed into TOC Analyser following same procedure for Total Carbon level.

### **3.3 Macrofauna Biodiversity**

Following a semi-destructive method of measurement, the macrofauna biodiversity was measured by random quadrats of roughly 50 cm by 50 cm, with a minimum of 3 quadrats per density per site for each species. The quadrat area will have a surface-level observation followed by being dug down by a maximum of 5cm in depth, with contents being placed through a sieve of 1mm mesh size and directly into a white tray to observe all possible macrofauna without full destruction of plots and to minimise disturbance (Larsen., 1985). Macrofauna was identified on site only by visual assessment using trained individuals, and using identification booklets where needed. All large fauna which can be identified will be identified to species level, when not

possible individuals will be identified to level of family or classified as “Unidentified Species”. Comparison between densities and relevant oyster reef species will use a Shannon Index ( $H'$  value). Shannon  $H'$  value was used in conjunction to a Species Richness Index ( $S$  value) as abundance should be considered as well as richness as there were large quantities of individual species in a given density and location whilst other densities were minimal but showed a variance in species present. Simpson Index ( $S_i$ ) was also used so evenness could be measured by taking species richness into account. Shannon Index used to measure alpha ( $\alpha$ ) diversity by combining the richness and diversity, whilst Simpson Index used to measure gamma ( $\gamma$ ) as it describes the total number of species within the area. NMDS used to visualise the beta ( $\beta$ ) diversity between communities based on density and base species additionally. All values are obtained through three quadrats at each individual sites, obtaining nine sets of data per density per species with exception of June S3CGD10, as this site remained flooded on all days of data collection, and August data for D5 and D10 for all sites, as flooding occurred on site so a priority of the highest (D15) and lowest (D1) densities were made.

### **3.4 Habitat Complexity**

A 1 m length chain was laid upon the given oyster species and density-dependent artificial reef loosely so that it can settle on the top layer of the reef. A measuring tape was used to measure the final point of the chain's length after it has fallen into place. This follows a suggested and adapted method by Luckhurst and Luckhurst (1978) which uses a bronze chain on top of a material sheet which is held in place to gauge habitat rugosity of coral reefs. As this method does not take into account side profiles and depth profiles, but only the final chain length it is a better

representation of space utilisation and total surface area of the shell surface instead of a measure of heterogeneity or reef patchiness, instead acting as a measure of actual distance against linear distance. This will therefore be compiled into average surface area per density per oyster species, with a higher surface area signifying a flatter surface and therefore less complex habitat, using an ANOVA as a statistical test as it is a comparison of means.

### **3.5 Statistics**

ANOVA was used for all statistical analysis as it is a comparison of means between experimental plots of species and densities.. Two-Way ANOVA used when comparing densities and another variable, One-Way ANOVA used when only looking at one variable between base species of *Ostrea edulis* or *Crassostrea gigas*.

Mean abundance data performed in conjunction to Non-Metric Multidimensional Scaling plot due to sparseness of data collected (e.g., an individual species being present but only having one count for all plots) and to identify key species within the communities.

Non-Metric Multidimensional Scaling plot (NMDS) was used to visualise the spatial quadrat data of species presence and abundance for density and oyster species and obtain comparable community structure for all densities. NMDS is created using the 'vegan' package in R, using a stress plot of a Shephard's Test to determine validity of species dissimilarity. Stress for plots run to 20 or 0.2 as reviewers from earlier publications deem anything past this stress value as unreadable, uninterpretable, or less accurate (Kruskal 1964, Clarke 1993) (Figure 11). Distance method used to determine community matrix and distances is the Bray-Curtis method. Shannon Index is normally distributed with the Shapiro-Wilks Test, obtaining  $W = 0.95196$  and  $p\text{-value} = 0.2391$ , so parametric ANOVA performed. Two-Way

perMANOVA performed on NMDS, with a perMADONIS performed after as a post-hoc test. R packages used for figures include 'vegan', 'ggplo2', 'ADONIS', 'tidyverse' on R version 3.03.06.

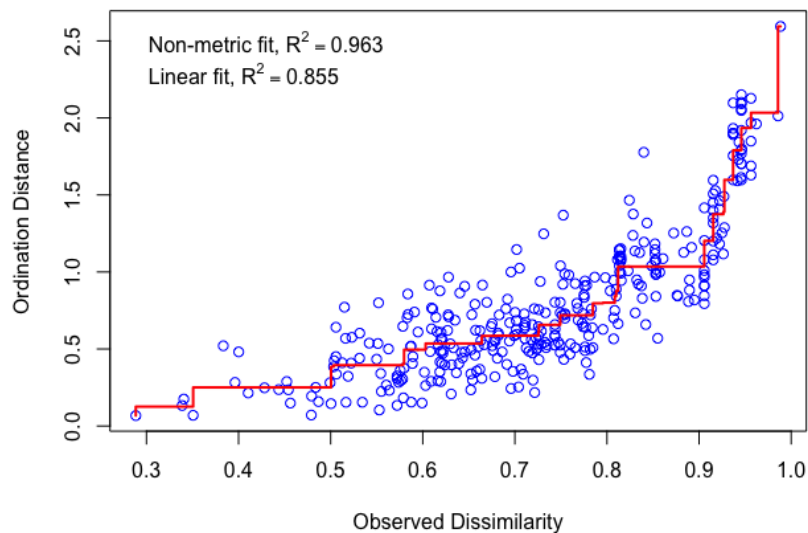


Figure 11. An example of a Shepherd's Test of Fit for the June Biodiversity data. Non-metric fit shows significance, with a strong positive correlation between ordination distance and observed dissimilarity, Linear fit also shows significance as it is  $0.8 <$ . Red line signifies a perfectly linear relationship between observed distance and observed dissimilarity for species present.

## **4.0 Artificial Reef Results**

### **4.1 Habitat Rugosity**

There was a significant interaction between shellfish species present and densities in a higher more complex habitat, with low complexity being associated with low densities and significantly higher rugosities being with higher densities of rock oyster (*C. gigas*) habitats as opposed to the native flat oyster (*O. edulis*). Average final chain length was determined only for highest and lowest oyster densities as data for middle ranges of densities 5 and 10 were unobtainable at every site visit due to tide. Some information

from intermediate densities will be discussed. *Ostrea edulis* (OE) are flatter overall compared to *Crassostrea gigas* (CG), and I found them to provide less complex habitat with a lower density, but higher density is more complex with a final average rugosity index of 92.50cm ( $\pm 0.76$ ) compared to the lower density of 94.67cm ( $\pm 0.44$ ). Lowest density of 1 live oyster per m<sup>2</sup> shown to have average longer chain length for their respective species treatments with CG still having the structurally complex habitat with 6cm ( $\pm 0.54$ cm) on average as it is a higher average than the OE D1 treatment of 5.33cm ( $\pm 0.53$ cm). Highest density of 15 live oyster per m<sup>2</sup> also shows that CG was on average more complex as it used more chain, using on average 17.17cm ( $\pm 0.69$ cm) of chain, whilst the max density for OE only used 10.25cm ( $\pm 0.37$ cm). Control reefs had the overall more complex site as it used on average 31.33cm ( $\pm 0.92$ cm) of the total 100cm chain length to cover the pre-established CG reef. One-Way ANOVA shows that the rugosity data is significant at  $p < 0.05$  as the overall  $p$ -value is  $2.54e^{-11}$  (Figure 12).

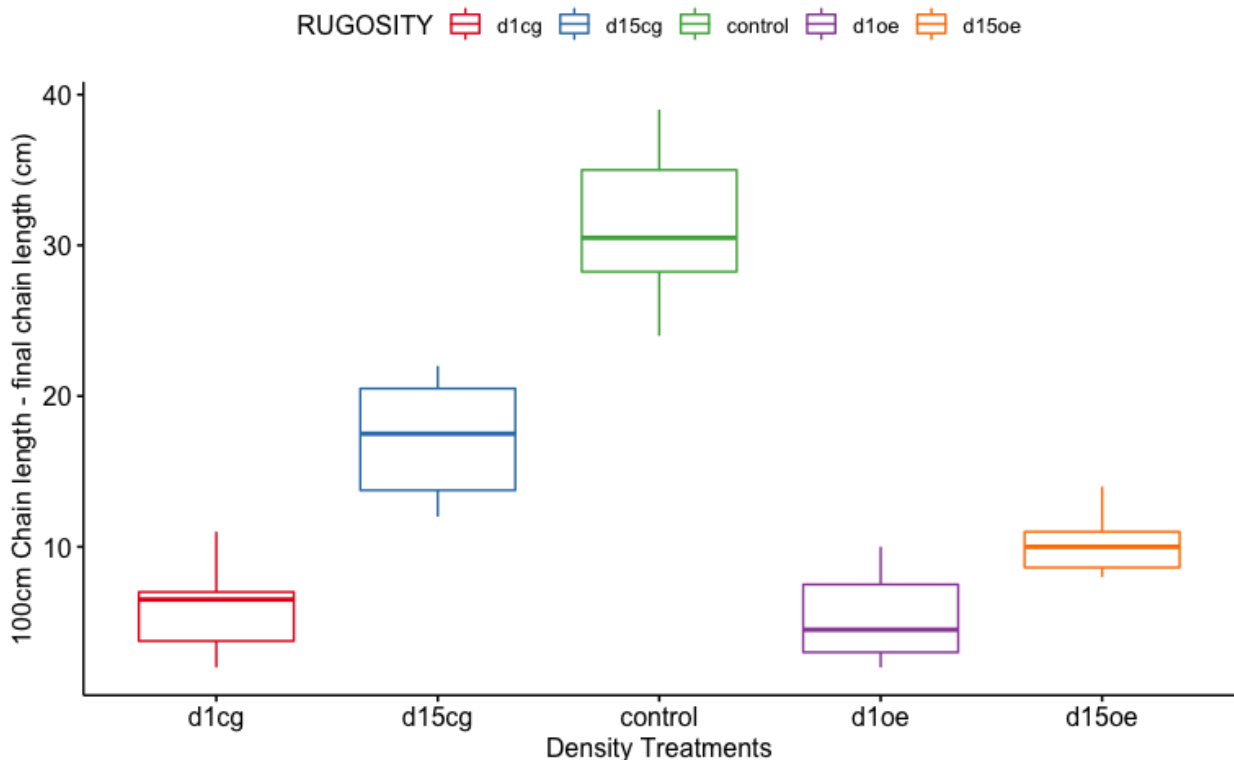


Figure 12. Average final chain length of a 1 metre length chain after being placed on a low-density reef (1 live oyster per meter<sup>2</sup>) and a high-density reef (15 live oysters per meter<sup>2</sup>) for *Crassostrea gigas* (CG) and *Ostrea edulis* (OE) compared to a pre-established localised rock oyster reef. *N*=6 per density treatment. ANOVA [F-value: 47.46, df=4, *p*<0.05, *p*-value: 0.001]

## **5.2 Biodiversity**

Individual species abundance and total species present displayed that whilst *C. gigas* overall had the higher abundance of individuals, *O. edulis* had overall a larger variety of species present (Table 1). Species present in *C. gigas* plots that were not observed in *O. edulis* plots for June dataset include: *Carcinus maenus*, *Mercenaria mercenaria*, *Achelia echinata*, *Charybdis japonica*, and *Urticina felina*. Species present in *O. edulis* plots for June dataset only was Orange Ascidiens, and for the control was a Star Ascidian. Species present in *O. edulis* plots that were not observed in *C. gigas* for August dataset include: *Styela clava*, Unidentified species 1, *Urosalpinx cinerea* egg sacks, *Halichondria sp.*, and *Hydrozoa sp.*

Species present in *C. gigas* plots that were not observed in *O. edulis* plots for August dataset include: *Urosalpinx cinerea*, *Acanthocardia tuberculata*, *Porcellana platycheles*, and *Malmgrenia lunulate*.

Identifiable key species among all reefs for June data included: Periwinkles (*Littorina littorea*), *Gammarus sp.*, American Drill (*Crepidula fornicata*), and Clear Sea Squirrels (*Asciidiella scabra*) (Figures 16). Identifiable key species among all reefs for August data included: Amphipods, American Drill (*Crepidula fornicata*), (*Achaellia echinate*) and (*Corella eumyota*) (Figures 17).

Non-Metric Multidimensional Scaling plot (NMDS) visualises data in terms of ordination, distance and dissimilarity. Any biodiversity data obtaining less than 3 individuals for any given base species plot was removed to form the NMDS as it didn't represent the community structure properly. This reduced the total 22 observed

species to 11 species present in both base species plots. June biodiversity where only density is a factor produces a stress value of 0.2311435 which is close to 0.2 meaning the data displays a weak positive correlation between distance and dissimilarity ( $R^2 = 0.698$  where  $p < 0.05$ , Metric Linear Fit), with overlaps in community structure being in. June biodiversity where base species is taken into consideration obtains a stress value of 0.1078825 ( $R^2 = 0.953$  where  $p < 0.05$ , Metric Linear Fit) for *C. gigas*, with overlaps in community being seen in D5, D10 and D15 for the following species. June biodiversity where base species is taken into consideration obtains a stress value of 0.1952663, which is  $< 0.2$  ( $R^2 = 0.862$  where  $p < 0.05$ , Metric Linear Fit) for *O. edulis*, with overlaps in community being seen in D1 and D15 for the following species.

Table 6 Species present and total individuals are based on a total of three quadrats per species and density. 'All' density is a combined total for D1, D5, D10 and D15 densities so only the base species of *C. gigas* and *O. edulis* are compared as one factor and does not account for density dependence.

Density/Species	Total Species Present	Total Individuals
All <i>C. gigas</i>	19	1197
All <i>O. edulis</i>	27	921
D1 <i>C. gigas</i>	12	146
D5 <i>C. gigas</i>	9	159
D10 <i>C. gigas</i>	9	181
D15 <i>C. gigas</i>	21	711
D1 <i>O. edulis</i>	11	118

D5 <i>O. edulis</i>	16	281
D10 <i>O. edulis</i>	19	310
D15 <i>O. edulis</i>	15	212
Control	9	56

Table 7. A full table of all identified base plot species for *Crassostrea gigas* plots (CG), *Ostrea edulis* plots (OE), and Control plots (CON) at varying densities of 1 oyster per m<sup>2</sup> (\*), 5 oysters per m<sup>2</sup> (†), 10 oysters per m<sup>2</sup> (•), and 15 oysters per m<sup>2</sup> (‡) – total count obtained as sum of three random quadrats in June, per site, per density, per base species.

Species Identified	Total Count Found at Given Density - CG	Total Count Found at Given Density – OE	Total Count Found at Given Density - CON
<i>Ostrea edulis</i>	0 - * 0 - † 0 - •. 0 - ‡	2 - * 14 - † 18 - •. 23 - ‡	0
<i>Crassostrea gigas</i>	4 - * 15 - † 34 - •. 46 - ‡	0 - * 0 - † 0 - •. 0 - ‡	3
Amphipoda	0 - * 0 - † 0 - •. 0 - ‡	0 - * 0 - † 0 - •. 0 - ‡	0
<i>Littorina littorea</i>	14 - * 52 - † 94 - •. 48 - ‡	20 - * 28 - † 26 - •. 40 - ‡	18
<i>Cerastoderma edule</i>	3 - * 0 - † 0 - •. 1 - ‡	0 - * 1 - † 1 - •. 0 - ‡	0
<i>Carcinus maenus</i>	0 - * 2 - † 6 - •. 2 - ‡	0 - * 0 - † 0 - •. 0 - ‡	0
<i>Gammarus sp.</i>	24 - * 38 - † 35 - •. 25 - ‡	11 - * 26 - † 31 - •. 32 - ‡	6
<i>Mytilus edulis</i>	0 - * 0 - † 3 - •. 5 - ‡	0 - * 2 - † 0 - •. 0 - ‡	0
<i>Sphaerium corneum</i>	0 - * 0 - † 0 - •. 0 - ‡	0 - * 0 - † 1 - •. 0 - ‡	1

<i>Lajonkairia</i>	0 - * 0 - † 0 - •. 1 - ‡	0 - * 0 - † 0 - •. 0 - ‡	0
<i>lajonkairii</i>			
<i>Urosalpinx</i>	0 - * 0 - † 2 - •. 0 - ‡	0 - * 0 - † 1 - •. 0 - ‡	0
<i>cinerea</i>			
<i>Mercenaria</i>	0 - * 0 - † 0 - •. 0 - ‡	1 - * 0 - † 0 - •. 0 - ‡	0
<i>mercenaria</i>			
Chiton	1 - * 3 - † 5 - •. 4 - ‡	0 - * 6 - † 1 - •. 2 - ‡	1
Annelida	2 - * 2 - † 1 - •. 10 - ‡	1 - * 3 - † 5 - •. 7 - ‡	1
Isopoda	1 - * 0 - † 0 - •. 1 - ‡	1 - * 0 - † 0 - •. 0 - ‡	0
<i>Urticina felina</i>	0 - * 0 - † 1 - •. 0 - ‡	0 - * 0 - † 0 - •. 0 - ‡	0
<i>Charybdis</i>	0 - * 0 - † 0 - •. 2 - ‡	0 - * 0 - † 0 - •. 0 - ‡	0
<i>japonica</i>			
<i>Ascidella scabra</i>	1 - * 25 - † 0 - •. 33 - ‡	6 - * 60 - † 19 - •. 11 - ‡	1
<i>Halichondria sp.</i>	0 - * 0 - † 0 - •. 0 - ‡	0 - * 1 - † 0 - •. 0 - ‡	0
<i>Botrylloides</i>	0 - * 0 - † 0 - •. 0 - ‡	0 - * 0 - † 1 - •. 1 - ‡	0
<i>violaceus</i>			
<i>Crepidula</i>	6 - * 3 - † 0 - •. 16 - ‡	3 - * 20 - † 0 - •. 0 - ‡	11
<i>fornicate</i>			
<i>Nymphon sp.</i>	4 - * 0 - † 0 - •. 0 - ‡	0 - * 0 - † 2 - •. 6 - ‡	2
<i>Botryllus</i>	0 - * 0 - † 0 - •. 0 - ‡	0 - * 0 - † 0 - •. 0 - ‡	1
<i>schlosseri</i>			
<i>Necora puber</i>	2 - * 1 - † 0 - •. 0 - ‡	1 - * 4 - † 3 - •. 0 - ‡	0

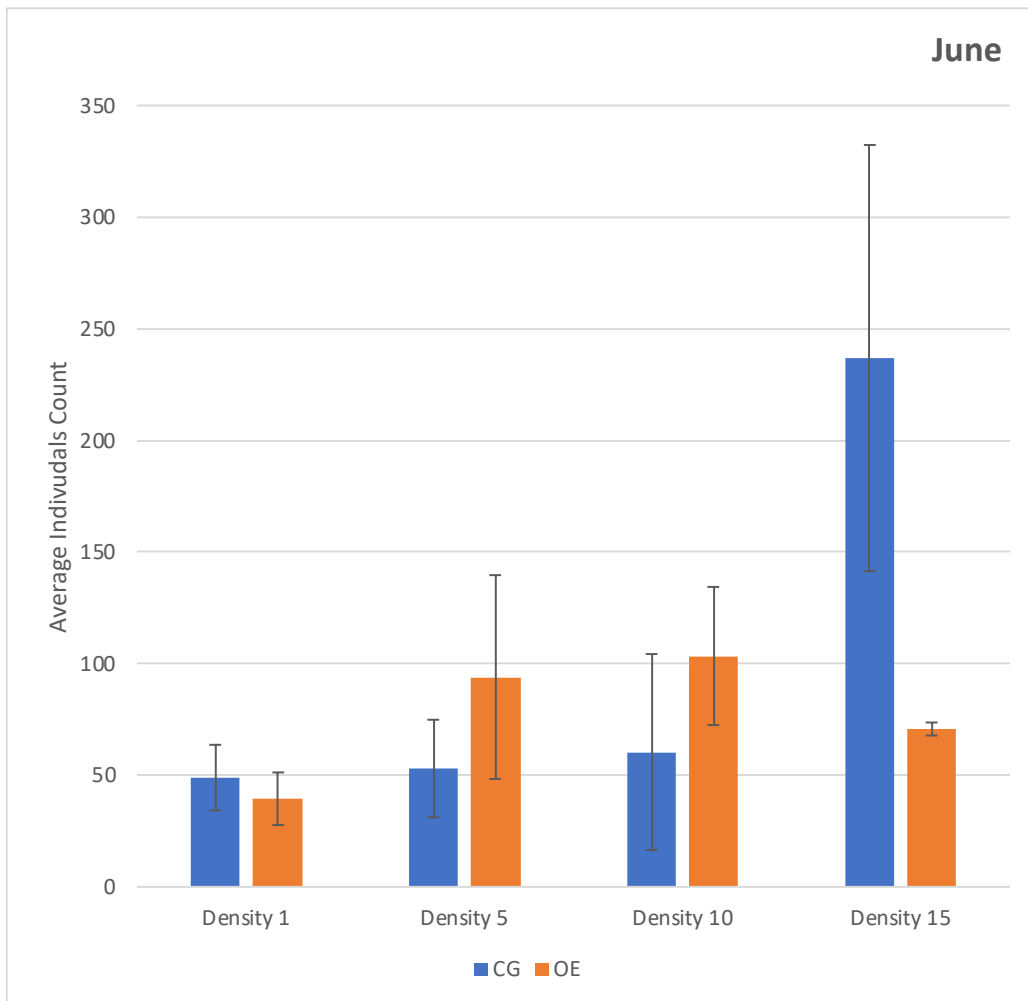


Figure 13. Mean species count for June Biodiversity data, for 1 live oyster per m<sup>2</sup>, live oysters 5 per m<sup>2</sup>, 10 live oysters per m<sup>2</sup>, and 15 live oysters per m<sup>2</sup> for both *Ostrea edulis* (orange) and *Crassostrea gigas* (blue) plots across all three sites T-Test used to compare variation based on species per density. One-Way T-Test for *C. gigas* vs *O. edulis* at density 1 [T-value: 1.35, df=2, 3 observations, p<0.05, p-value: 0.15]. One-Way T-Test for *C. gigas* vs *O. edulis* at density 5 [T-value: -0.70, df=2, 3 observations, p<0.05, p-value: 0.28]. One-Way T-Test for *C. gigas* vs *O. edulis* at density 10 [T-value: -0.27, df=2, 3 observations, p<0.05, p-value: 0.41]. One-Way T-Test for *C. gigas* vs *O. edulis* at density 15 [T-value: 1.71, df=2, 3 observations, p<0.05, p-value: 0.11].

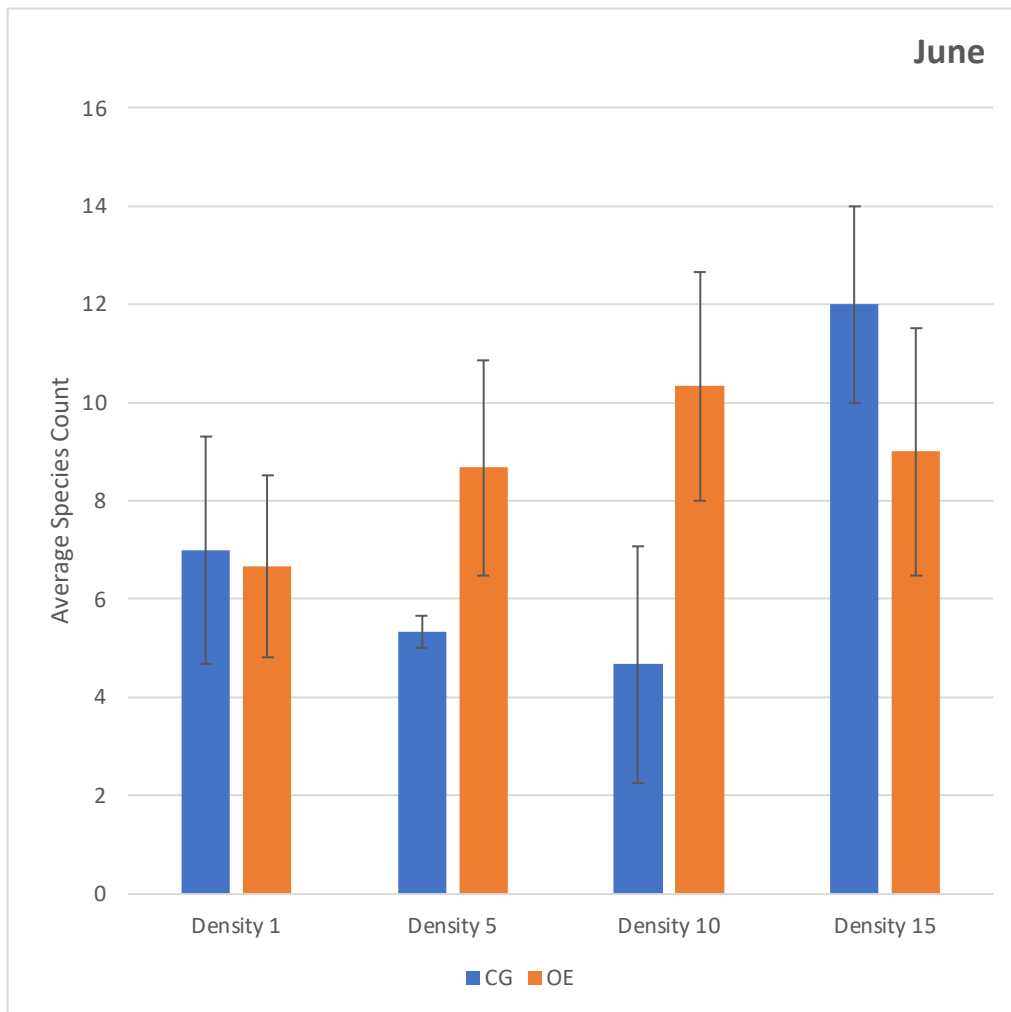


Figure 14. Mean species count for June Biodiversity data, for 1 live oyster per m<sup>2</sup>, live oysters 5 per m<sup>2</sup>, 10 live oysters per m<sup>2</sup>, and 15 live oysters per m<sup>2</sup> for both *Ostrea edulis* (orange) and *Crassostrea gigas* (blue) plots across all three sites T-Test used to compare variation based on species per density. One-Way T-Test for *C. gigas* vs *O. edulis* at density 1 [T-value: 0.38, df=2, 3 observations, p<0.05, p-value: 0.37]. One-Way T-Test for *C. gigas* vs *O. edulis* at density 5 [T-value: -1.79, df=2, 3 observations, p<0.05, p-value: 0.10]. One-Way T-Test for *C. gigas* vs *O. edulis* at density 10 [T-value: -1.22, df=2, 3 observations, p<0.05, p-value: 0.17]. One-Way T-Test for *C. gigas* vs *O. edulis* at density 15 [T-value: 1.19, df=2, 3 observations, p<0.05, p-value: 0.17].

Table 8. A full table of all identified base plot species for *Crassostrea gigas* plots (CG), *Ostrea edulis* plots (OE), and Control plots (CON) at varying densities of 1 oyster per m<sup>2</sup> (\*), 7.5 oysters per m<sup>2</sup> (“), and 15 oysters per m<sup>2</sup> (‡) – total count obtained as sum of three random quadrats in August, per site, per density, per base species.

<b>Species Identified</b>	<b>Total Count Found at Given Density - CG</b>	<b>Total Count Found at Given Density – OE</b>	<b>Total Count Found at Given Density - CON</b>
<i>Ostrea edulis</i>	0 - * 0- “ 0 - ‡	2 - * 7- “ 19 - ‡	0
<i>Crassostrea gigas</i>	9 - * 9- “ 14 - ‡	0 - * 0- “ 0 - ‡	3
Amphipoda	19 - * 29- “ 29 - ‡	22 - * 21- “ 29 - ‡	0
<i>Littorina littorea</i>	0 - * 1- “ 0 - ‡	2 - * 0- “ 0 - ‡	18
<i>Carcinus maenus</i>	0 - * 2- “ 1 - ‡	0 - * 1- “ 3 - ‡	0
Malmgrenia lunulata	1 - * 1- “ 0 - ‡	0 - * 1- “ 2 - ‡	6
<i>Achelia echinata</i>	0 - * 1- “ 0 - ‡	1 - * 0- “ 4 - ‡	0
<i>Corella eumyota</i>	10 - * 3- “ 27 - ‡	1 - * 0- “ 19 - ‡	1
<i>Styela cleva</i>	0 - * 0- “ 0 - ‡	0 - * 0- “ 3 - ‡	0
Unidentified species 2	0 - * 0- “ 0 - ‡	0 - * 0- “ 1 - ‡	0
Unidentified species 3	0 - * 1- “ 1 - ‡	0 - * 0- “ 1 - ‡	0
<i>Botrylloides violaceus</i>	0 - * 0- “ 1 - ‡	1 - * 0- “ 3 - ‡	1
<i>Ursolapinx cinerea</i>	0 - * 0- “ 6 - ‡	0 - * 0- “ 0 - ‡	1
<i>Ursolapinx cinerea</i> egg sacks	0 - * 0- “ 0 - ‡	0 - * 0- “ 50 - ‡	0

<i>Acanthocardia</i>	1 - * 1 - " 0 - ‡	0 - * 0 - " 0 - ‡	0
<i>tuberculata</i>			
<i>Porcellana</i>	1 - * 0 - " 0 - ‡	0 - * 0 - " 0 - ‡	0
<i>platycheles</i>			
Chiton	0 - * 2 - " 0 - ‡	0 - * 0 - " 1 - ‡	0
<i>Halichondria sp.</i>	0 - * 0 - " 0 - ‡	1 - * 0 - " 2 - ‡	0
<i>Botrylloides</i>	0 - * 0 - " 1 - ‡	0 - * 0 - " 1 - ‡	0
<i>violaceus</i>			
<i>Hydraso asp.</i>	0 - * 0 - " 0 - ‡	0 - * 0 - " 5 - ‡	0
<i>Nymphon sp.</i>	0 - * 0 - " 0 - ‡	0 - * 0 - " 0 - ‡	0
Unidentified	0 - * 0 - " 0 - ‡	1 - * 0 - " 2 - ‡	0
species 4			
Unidentified	1 - * 3 - " 0 - ‡	1 - * 0 - " 0 - ‡	0
species 5			

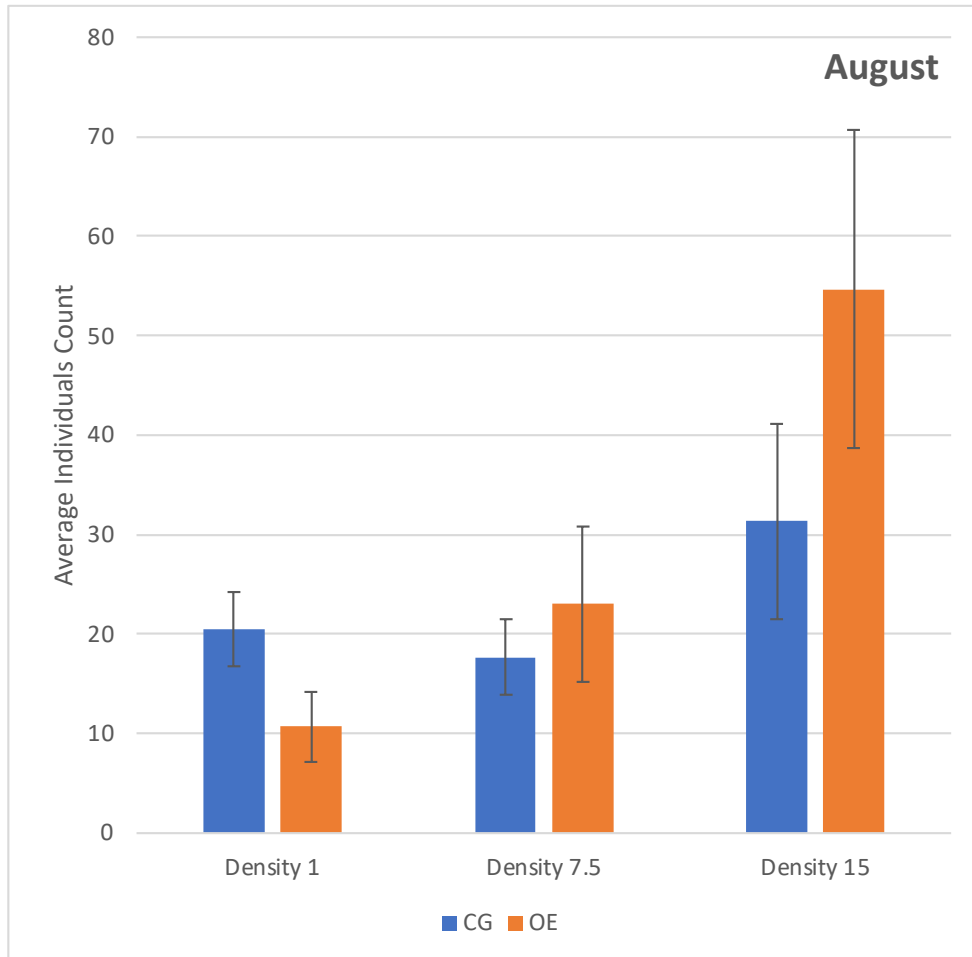


Figure 15. Mean individual count for August Biodiversity data, for 1 live oyster per m<sup>2</sup>, a combination of 5 and 10 live oyster per m<sup>2</sup>, and 15 live oysters per m<sup>2</sup> for both *Ostrea edulis* (orange) and *Crassostrea gigas* (blue) plots across all three sites T-Test used to compare variation based on species per density. One-Way T-Test for *C. gigas* vs *O. edulis* at density 1 [T-value: 0.93, df=2, 3 observations,  $p < 0.05$ ,  $p$ -value: 0.23]. One-Way T-Test for *C. gigas* vs *O. edulis* at density 7.5 [T-value: -0.54, df=2, 3 observations,  $p < 0.05$ ,  $p$ -value: 0.32]. One-Way T-Test for *C. gigas* vs *O. edulis* at density 15 [T-value: -3.67, df=2, 3 observations,  $p < 0.05$ ,  $p$ -value: 0.03].

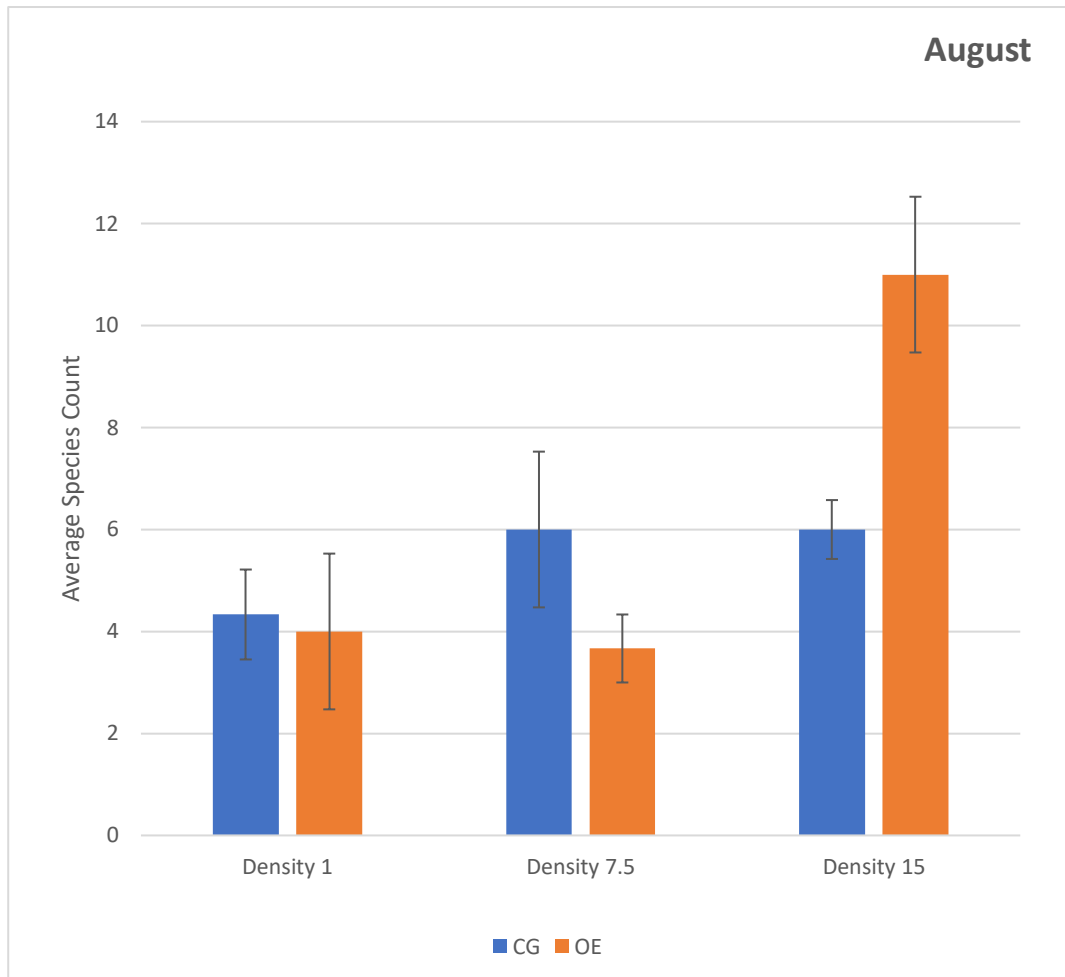


Figure 16. Mean species count for August Biodiversity data, for 1 live oyster per m<sup>2</sup>, a combination of 5 and 10 live oyster per m<sup>2</sup>, and 15 live oysters per m<sup>2</sup> for both *Ostrea edulis* (orange) and *Crassostrea gigas* (blue) plots across all three sites T-Test used to compare variation based on species per density. One-Way T-Test for *C. gigas* vs *O. edulis* at density 1 [T-value: 0.23, df=2, 3 observations,  $p < 0.05$ ,  $p$ -value: 0.42]. One-Way T-Test for *C. gigas* vs *O. edulis* at density 7.5 [T-value: 1.26, df=2, 3 observations,  $p < 0.05$ ,  $p$ -value: 0.16]. One-Way T-Test for *C. gigas* vs *O. edulis* at density 15 [T-value: -4.33, df=2, 3 observations,  $p < 0.05$ ,  $p$ -value: 0.02].

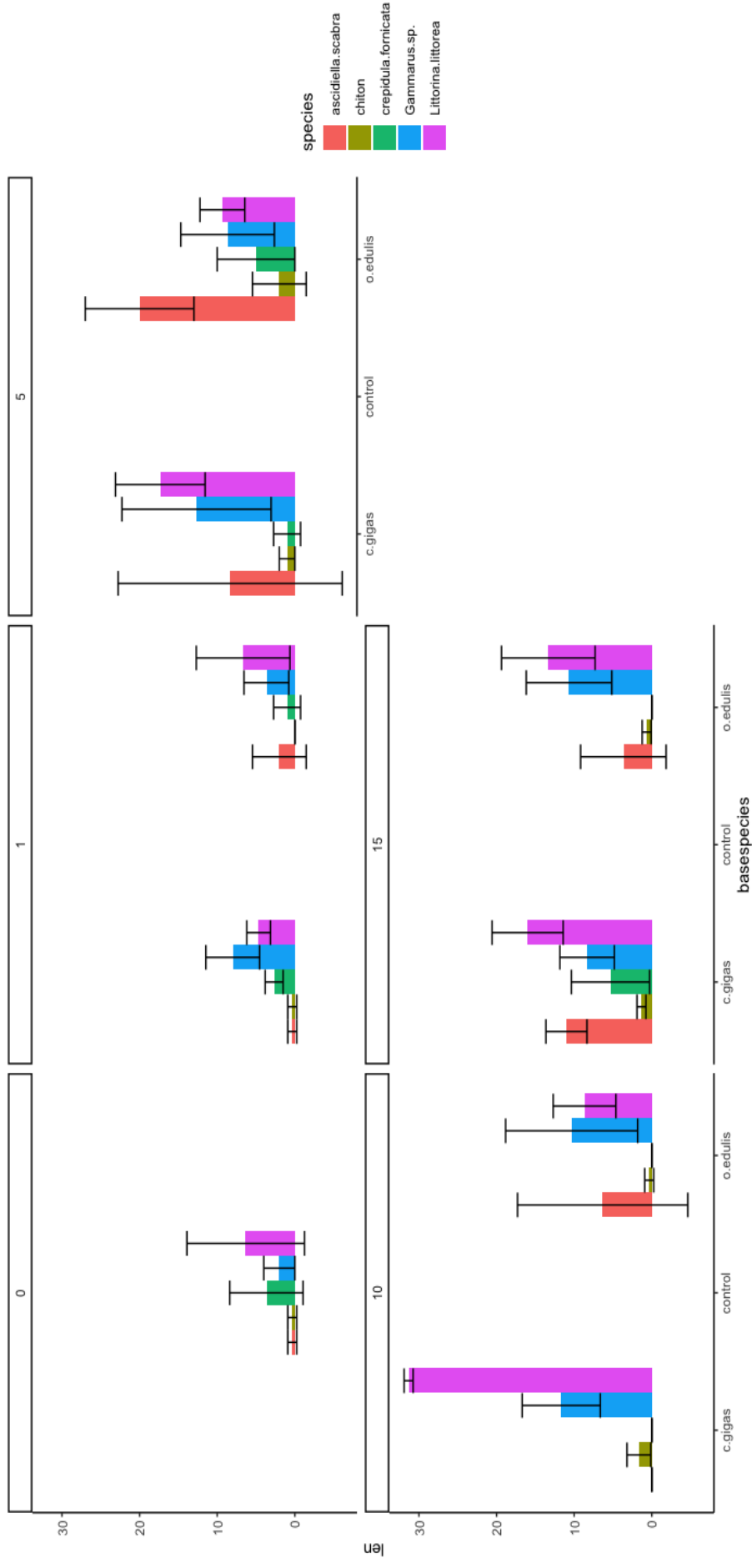


Figure 17. Average abundance (len) for top 5 most abundant species per density per reef condition for

June biodiversity data, faceted into density. Species present: Red = *Ascidella scabra*, Gold = Chiton, Green = *Crepidula fornicata*, Blue = *Gammarus sp.*, Pink = *Littorina littorea*, with left column displaying community for *C. gigas* plots and right column displaying communities for *O. edulis*. Kruskal-Wallis[FOR Mean Abundance ~ BaseSpecies, df: 2, Chi-Squared value = 1.4011, p-value = 0.4963 at  $p < 0.05$ , FOR Mean Abundance ~ Density, df = 5, Chi-Squared value = 6.3731, p-value = 0.17 at  $p < 0.05$ ] Two-Way ANOVA[Density: df = 4, F-value: 4.054, p-value = 0.05 at  $p < 0.05$  – significant based on density, Base Oyster Species: df = 2, F-Value = 0.840, p-value = 0.4396 at  $p < 0.05$  - insignificant ].

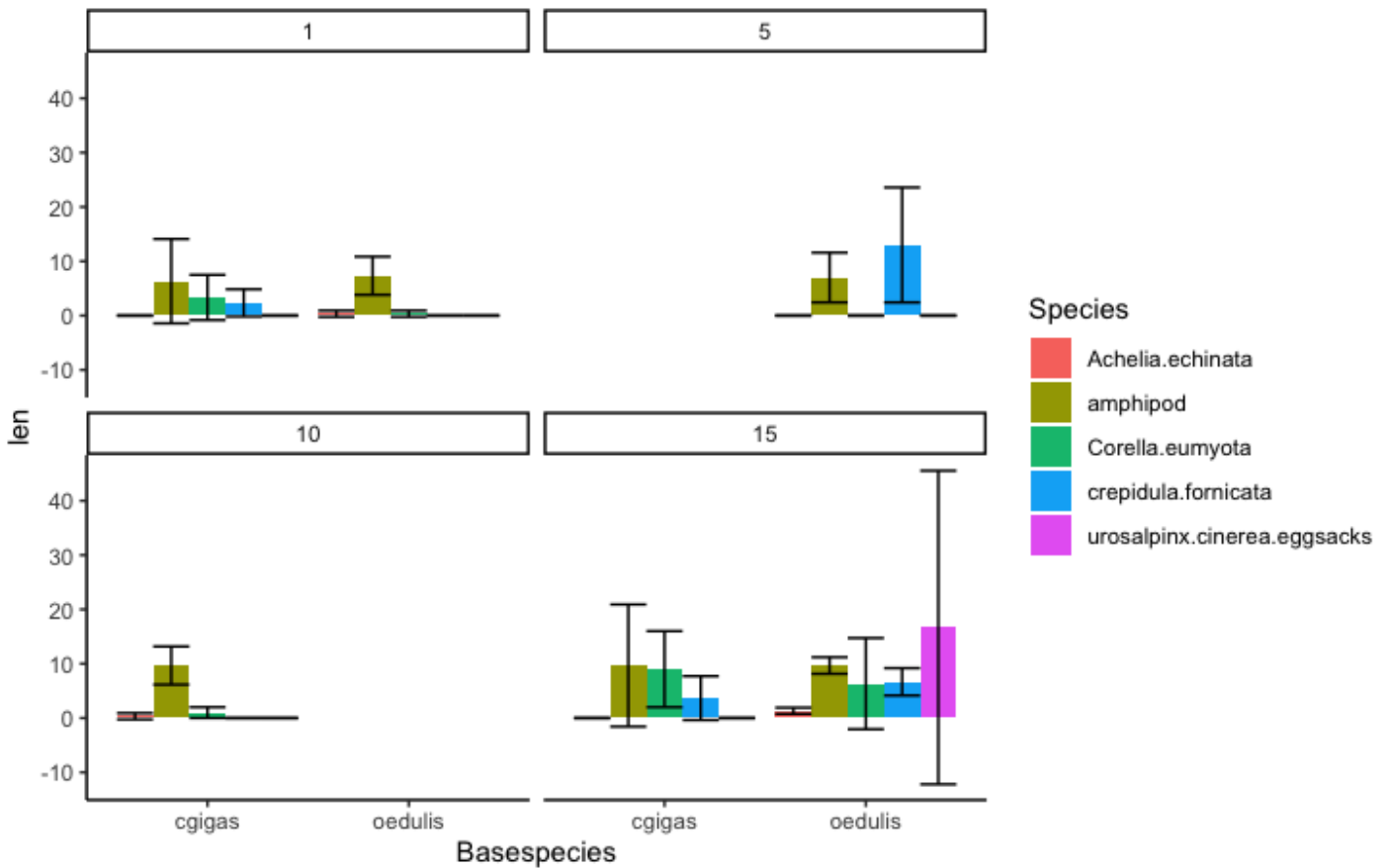


Figure 17. Average abundance (len) for top 5 most abundant species for all available data in August data collection at varying densities. Species present: Red = *Achelia echinata*, Gold = Amphipods, Green = *Corella eumyota*, Blue = *Crepidula forincata*, Pink = *Urosalpinx cinerea* eggsacks, with left column displaying community for *C. gigas* plots and right column displaying communities for *O. edulis*. Kruskal-Wallis [For Mean Abundance ~ Base species –  $df:1$ , F-value: 0.40788,  $p$ -value: 0.523 at  $p<0.05$ , FOR Mean Abundance ~ Density –  $df: 3$ , F-value: 3.9095,  $p$ -value: 0.2714]. Two-Way ANOVA [FOR Base species – F-Value: 1.5981,  $p$ -value: 0.21738 at  $p<0.05$ . FOR Density – F-value: 4.0698,  $p$ -value: 0.04129 at  $p<0.05$ . FOR Base species \* Density – F-value: 1.4375,  $p$ -value: 0.24135 at  $p<0.05$ ].

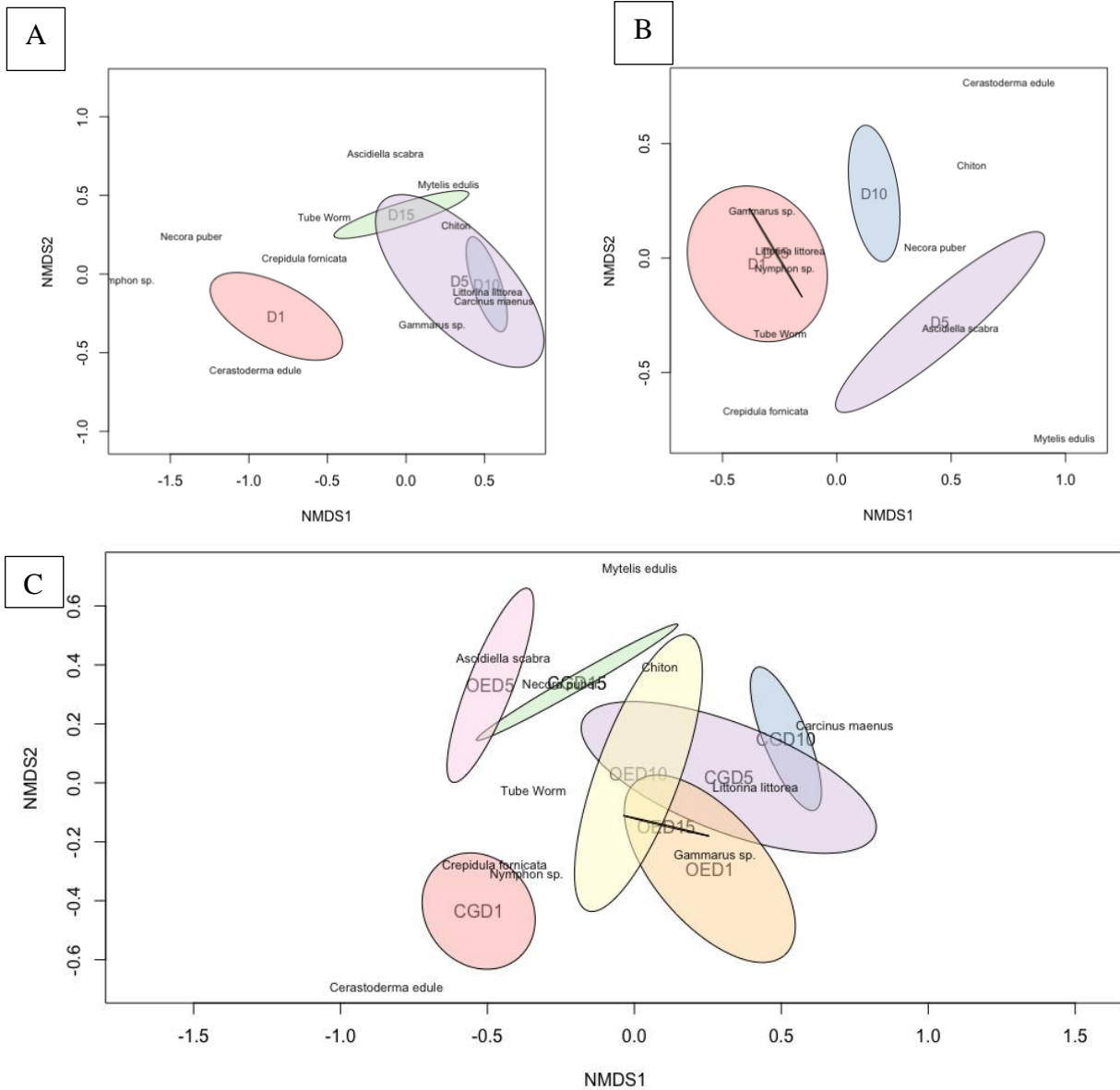


Figure 18a and 18b. Non-Metric Multidimensional Scaling Plot of June biodiversity for *Crassostrea gigas* (18a) and *Ostrea edulis* (18b) all densities and sites. 16a. stress runs to 20 obtaining a stress value of 0.1078825. perMANOVA[df:3, F-value:3.6094,  $p$ -value:0.021, data significant at  $p<0.05$ ], post-hoc - permADONIS[data insignificant for all densities at  $p<0.05$ ]. 16b stress runs to 20 obtaining a stress value of 0.1952663. perMANOVA[df:3, F-value: 1.2848,  $p$ -value: 0.241 at  $p<0.05$  - data insignificant. Post-hoc - permADONIS[data insignificant for all densities at  $p<0.05$ ].

Figure 18c. Non-Metric Multidimensional Scaling Plot of June biodiversity for *Crassostrea gigas*, *Ostrea edulis* and a control for all densities and sites. Stress runs to 20 obtaining a stress value of 0.1935022. perMANOVA[df:4, F-value: 2,0885,  $p$ -value:0.004, data significant at  $p<0.05$ ], post-hoc -

permADONIS[data insignificant for all densities vs controls, data significant for all densities vs each other at  $p < 0.05$ ]

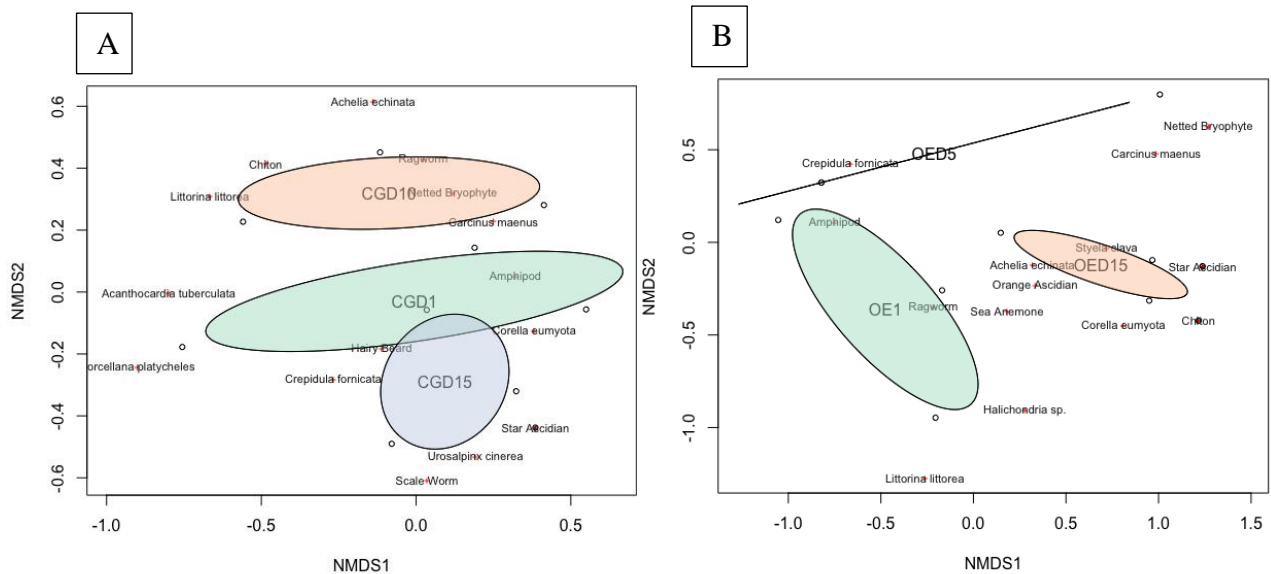


Figure 19. Non-Metric Multidimensional Scaling plot for August Biodiversity data for *Crassostrea gigas* (19a) and *Ostrea edulis* quadrats (19b).  $N$  quadrats = 3. Stress runs at 20, producing stress value: 0.1874851 for Fig17a. and 0.1745807 for 17b. Two-Way perMANOVA[df:1, F-value: 1.5963,  $p$ -value: 0.142, data insignificant at  $p < 0.05$ ].

### **5.3 Total Inorganic Carbon**

Overall trends saw that at a longer, more established site (Pyefleet Creek) density dependence is visible with *Crassostrea gigas* peaking at storing carbon within sediments at 10 live oyster per square meter performing lowest in densities of 1 per meter squared, and *Ostrea edulis* being more solitary and peaking at 1 live oyster per square meter and performing lowest at 10 live oyster per meter squared. Trends not as visible for sites 2 and 3 (Saltcott Creek 1 and 2) but are beginning to establish.

Total Inorganic Carbon (TIC) was measured from a mixed sediment core and averaged at density and site level. Average Total Carbon (TC) was labelled as species and density and colour-coded by site, for example CG or OE signifying *C. gigas* or *O.*

*edulis* and D1 meaning the density level of one live oyster per meter<sup>2</sup>. Sites were referred to by number correlating to the chronological order in which they were set up - Site 1 being Pyefleet Creek, Site 2 being Saltcott 1 and Site 3 being Saltcott 2. Site 1 displays clear density dependent trends, whilst sites 2 and 3 show inconsistent patterns of density influenced carbon storage. Site 1 displays high level of carbon store in sediment of Density 1 for *Crassostrea gigas* (CGD1) with 20.15 ( $\pm 5.60$ ) mg, which is seen to gradually increase to 26.16 ( $\pm 1.11$ ) in CGD5, and peaks at Site 1 for CG at CGD10 with 30.49 ( $\pm 0.19$ ) mg and drastically decreases to 18.86 ( $\pm 1.26$ ) mg in CGD15. Total Inorganic Carbon percentage at Site 1 for *Ostrea edulis* peaks at Density 1 (OED1) at 31.57 ( $\pm 0.63$ ) mg, decreases to 30.07 ( $\pm 0.71$ ) mg for OED5 and decreases further in OED10 to 20.63 ( $\pm 2.88$ ) mg only to increase in OED15 to 26.24 ( $\pm 0.67$ ) mg. All conditions for Site 1 remain above the control of 16.30 ( $\pm 1.77$ ) mg. TIC for Site 2 for CGD1 is at 19.70 ( $\pm 2.00$ ) mg, decreased to 10.78 ( $\pm 0$ ) mg in CGD5, peaks at CGD10 at 18.64 ( $\pm 2.74$ ) mg and decreases again to 19.37 ( $\pm 2.65$ ) mg in CGD15. TIC for *Ostrea edulis* conditions in Site 2 display 13.45 ( $\pm 0.61$ ) mg in OED1, peaking at 14.06 ( $\pm 0.61$ ) mg in OED5, dropping to 13.36 ( $\pm 0.45$ ) mg in OED10 and increasing slightly in OED15 to 12.79 ( $\pm 0.43$ ) mg. For Site 2 all OE conditions and CGD5 were significantly lower than the control of 16.30 ( $\pm 1.77$ ) mg with the remaining CG conditions being above the control.

At Site 3 TIC peaks for CG conditions at CGD1 is at 16.64 ( $\pm 0.76$ ) mg which decreases to 14.46 ( $\pm 0.52$ ) mg in CGD5 and decreases further to 8.83 ( $\pm 0.73$ ) mg in CGD10 and increases slightly to 16.95 ( $\pm 0.86$ ) mg in CGD15. Site 3 TOC for OE conditions has 15.76 ( $\pm 1.11$ ) mg in OED1, 12.47 ( $\pm 0.95$ ) mg in OED5, increases in OED10 to 14.30 ( $\pm 0.54$ ) mg and peaks at 16.02 ( $\pm 0.76$ ) mg in OED15. For Site 3, all conditions remain

higher than the control of 13.09 ( $\pm 1.02$ ) mg with exception of CGD10, OED5 AND OED10 (Figure 20).

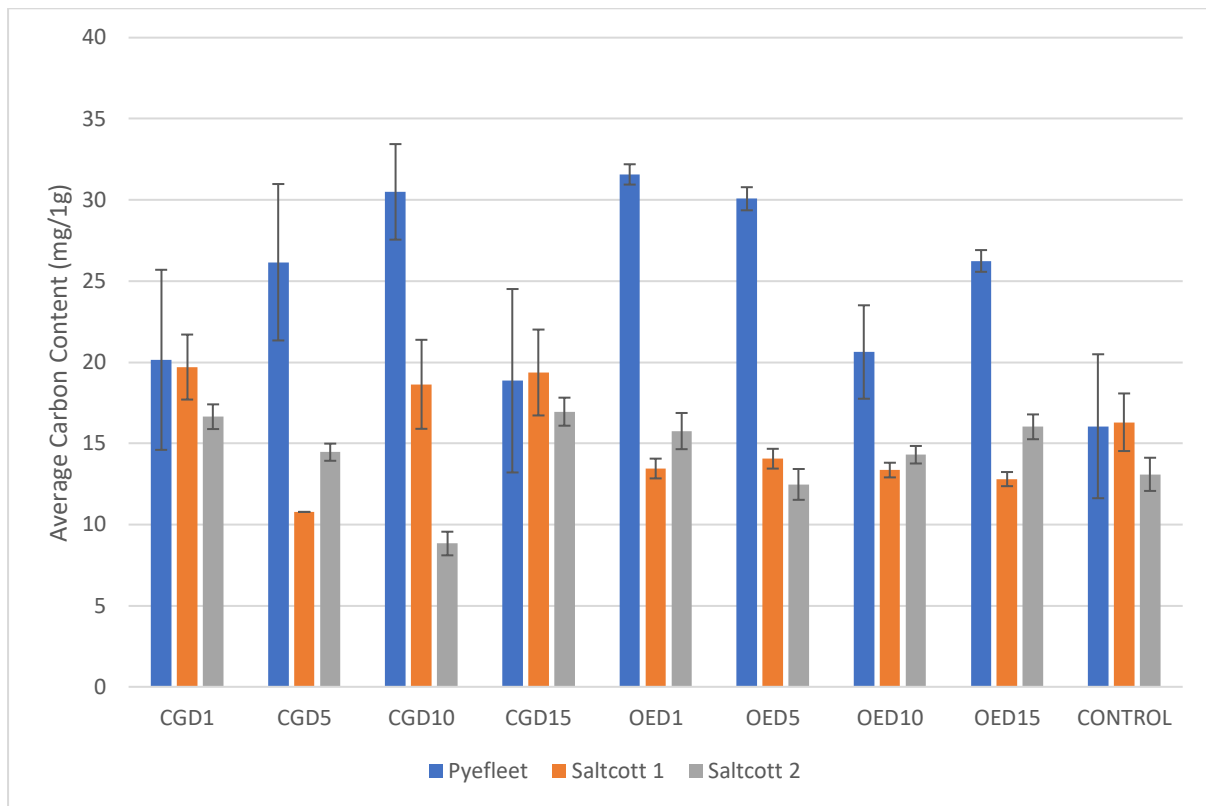


Figure 20. Average Total Inorganic Carbon in milligrams of Carbon per gram of sediment, content of mixed sediment cores containing either *Crassostrea gigas* (CG) and *Ostrea edulis* (OE) at all four density dependent categories (D1, D5, D10, D15) compared against each other and a control area for Pyefleet Creek (Blue), Saltcott Creek plot 1 (Red), and Saltcott Creek plot 2 (Yellow). ANOVA: [For all Species, Densities and Sites - F-value: 1.62711, df = 26, at  $p < 0.05$ ,  $p$ -value: 0.15059]

## **5.0 Discussion**

### **5.1 Sediment Nutrient Content in Presence of Bivalve Habitats**

Initial readings of nutrient content from the mesocosms on day 1 display naturally higher readings of  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$ . Phosphate and Ammonium levels increased greatly with presence of live oyster - suggesting that the environment had higher anaerobic conditions as there was a higher reduction of nitrogen and suggests the presence of anaerobic archaea and bacteria habiting the oyster shell, as a change in nitrogen content within the sediment overall will affect the denitrification rates within both sediment and water column (Nedwell and Raffaelli, 1999, Beutel et al., 2008). Nitrogen content - including  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{NH}_4^+$  - is affected by anoxic and anaerobic conditions and the microbes found within the environment (Nedwell and Raffaelli, 1999, Beutel et al., 2008). By adding oysters to the mesocosm environment, it allowed a larger surface area for microbes, a potentially different microbial community on the shell exterior consisting of more denitrifiers, and the use of pseudofeces to deposit nitrogen compounds – more specifically  $\text{NH}_4^+$  (Hoellein et al., 2015, Klawonn et al., 2015). Anoxic conditions for the study however can also be attributed to poor aeration problems – as aeration tubes did not fully penetrate when weighed down making air flow rates inconsistent within the individual chambers, therefore making it hard to determine if the change in nitrogen and phosphate is based on the lack of oxygenation within the water column or from the oysters itself. Overall, whilst there is significant observed change based on day and condition for certain conditions, the study itself held too many external factors such as nutrient deficiency and aeration issues to fully conclude the effect of the oyster itself on the nitrogen and phosphate contents within the sediment.

## **5.2 Inorganic Carbon Content in Sediments of Density-Dependent Bivalve**

### **Habitats**

Site 1 - Peewit Island - was the most established site of the three as that had been placed in the March, whilst Sites 2 and 3 at Saltcott Creek were only down for a month from the May to the June when data collection took place. This could potentially explain the unclear trends in carbon storage for the latter two sites, whilst the first site has a distinguished trend of carbon found in sediment samples based on density dependence and oyster species present. To ensure a clearer and more consistent trend between pseudo-replicate sites as well as individual plots, for a future study the beds would need to be left for a minimum of six weeks at an accessible site - accessibility would include ability to get to site on tides between 0.3-0.7m heights and minimal flooding to be able to access the plots and sediment for data collection, as well as getting to the site itself. However, it is worth noting that whilst for accessibility reasons the low tides and exposure would be effective for data collection, the utilisation of natural flooding even at low tides would be more similar to that of a natural reef setting for *Ostrea edulis* as they usually reside in the low intertidal, which may also be a reasoning for the low performance of *Ostrea edulis* as it is not their optimal conditions to perform (Ezgeta-Balić et al., 2020, Stagličić et al., 2020).

The expected results for carbon content were to be higher in plots 10 for both species, as a higher density community of oysters has shown better performance before, with the *Crassostrea gigas* plots having a higher carbon content due to their naturally larger shell size and therefore need for more carbon for shell growth and through pseudofaeces deposits (Dame et al., 1989, Grabowski and Peterson, 2007, Bayne 2009, Fodrie et al., 2017, Peterson et al., 2021). When observing the more established site of Site 1 - where there is a clear pattern - the hypotheses of more carbon stored

at a density of 10 live oysters per m<sup>2</sup> are both correct (Figure 20). However, the hypothesis does not apply to sites 2 and 3 as there is little to no change for *O. edulis* and *C. gigas* at both sites regardless of density and are almost outperformed by the control.

### **5.3 Biodiversity of Density-Dependent Bivalve Habitats**

Functional similarity in provision for a habitat for varying species was observed due to the volume of individuals found within plots with an oyster habitat in comparison to a control area of muddy sediment, however there was no functional dissimilarity in performance between all densities of *O. edulis* and lower densities of *C. gigas*.

Due to sites 2 and 3 being more susceptible to remaining flooded at low tide in comparison to site 1, it allows for *O. edulis* to have a high biodiversity as they usually remain covered in water, unlike the *C. gigas*. However, this did pose an issue with data collection as the site was harder to access. In plots where flooding is less susceptible, utilisation of *C. gigas*'s complex shell structure is clearly used by species that need shelter or access to water pools as water gets trapped easier within the *C. gigas* shells seen in a lot of amphipods and ascidians being found within these sites.

NMDS for June data displayed that certain species such as Ascidians, Clams and Anemone's whilst present were often observed further from other invertebrates - this is potentially due to these species relying on hard substrata to attach to, meaning they utilised the lower densities emptier shell patches where live oyster did not reside (Zwerschke et al., 2018, Preston et al., 2020, Rodriguez-Perez et al., 2020). Further, with a soft, muddy sediment and a high level of flooding, this leaves for more infauna and animals that bury beneath the beds to be present in the artificial

reefs, such as smaller amphipods, but due to their burying nature it also means that they are often hard to account for when performing a species richness or abundance count in comparison to other species which are more accountable such as Green Shore Crab (*Carcinus maenas*) which stays on the surface (Grabowski and Peterson, 2007).

Not only this, but where *C. gigas* have a faster growth rate they were observed more to begin fusing together with the layers of dead shell used to establish the experimental reefs, providing less original space for other species to hide or utilise – especially in comparison to *O. edulis* (Pogoda et al., 2011).

Some species which are dependent on the oyster species present such as the American Tingle (*Urosalpinx cinerea*) which is an invasive species and predator for the bivalves was found that they were more present in *C. gigas* plots overall with *U. cinerea* being observed in lower densities in *O. edulis*, potentially due to higher oxygen consumption rates and optimal reef conditions for the given oyster species, as *U. cinerea* often are attracted to species with higher respiratory metabolism (Blake, 1960). However, it is worth noting that whilst no live *U. cinerea* were found in *O. edulis* plots for August, 50 egg sacks for American Tingle were found on shell exterior of a live *O. edulis*, which were not found for *C. gigas* at any site. There is a clear change in both spatial variation and species present at the sites from the June to the August, suggesting the potential for seasonality in use of the oyster reefs as a habitat due to more environmental stressors, and a higher need for refuge within the shell system (Grabowski and Peterson, 2007, Pogoda et al., 2011). Additionally, through the August data collection there was a nationwide heatwave, which could be the reasoning for the change in species present within the sites due to the need for refuge, natural mortalities from stress, or other external factors (Lonhart et al., 2019).

The heatwave suggests that varying species utilise the different oyster reefs for a means of protection – with more free-moving creatures using the *C. gigas* plots as their complex shells create air pockets or water capture pockets, whilst the *O. edulis* plots generate more of a sediment build up, which links up to the habitat complexity of species-based reefs and its effect on biodiversity (Grabowski and Peterson, 2007, Pogoda et al., 2011, Zwerschke et al., 2016).

#### **5.4 Habitat Complexity of Density Dependent Bivalve Habitats**

A limitation of the study when attempting to quantify habitat complexity is that not only are the habitats not a true representation of a fully developed oyster reef habitat – where complex habitats for oysters would usually take over a year to develop, allowing for the oysters to fuse and grow not just to other hard-surfaced structures but to each other, creating a more rigid reef surface (Petersen et al., 2021) In addition, for a future study it would be beneficial to have two separate controls consisting of a naturally occurring *O. edulis* and *C. gigas* reef to fully compare how the artificial reefs differ from a long-standing and established site for the respective species. Biodiversity is influenced heavily by the habitat provided and its complexity from the environment of study, and therefore rugosity plays a large factor in helping quantify the ecosystem functionalities of oyster species. Rugosity for plots was only determined for minimum and maximum densities as they were the only accessible densities for all three sites and gave the largest range of comparison. The *C. gigas* plots were naturally higher in complexity than *O. edulis* just based on shell structure alone as *O. edulis* has the flatter and less rigid shell and are smaller in size overall (Figure 1). Observed lower mean (Figure 13) displays that there is less habitat complexity as the chain has had to fill more gaps than the substrata generated,

showing that *C. gigas* has the confirmed significant higher habitat complexity for both D1 and D15 against *O. edulis*. Control plots for rugosity were from localised *C. gigas* reefs, which were pre-established and untouched, and therefore are more inclined to provide a naturally more structured environment from the complex shell structure as well as size from maturity of oysters. Increased biodiversity based on increased habitat complexity has been observed in *Crassostrea virginica* oyster reefs where with increased internal oyster density there was a greater observed biodiversity of macrofauna (Karp et al., 2018), suggesting that overall *O. edulis* would not provide the ecosystem function of a complex habitat system or a means of shelter via hard substrata in comparison to *C. gigas*.

Habitat complexity is also affected by the layer of dead shell under the living oyster, as the hollowed shell creates an open space for either sessile creatures to attach or a pocket for species to hide from predators, as the shell space generates more surface area and space – overall allowing for a variety of substrata and space for more species (Tomatsuri and Kon, 2017). As previously mentioned, shell structure would affect the utilisation of the dead shell layer as the *Ostrea edulis* shell is flatter with a less prominent depression in the inside of the shell, unlike *Crassostrea gigas* which has a larger depression in both parts of the valves, generating a more complex structure (Figure 13). The idea of oyster species causing a difference in habitat complexity is also further proven due to *C. gigas* having a higher growth rate than *O. edulis* - meaning that the former is able to offer a larger habitat of a rigid shell in a shorter space of time, whilst *O. edulis* maintains a slow growth rate and therefore a minimally varying habitat of less structured shell (Mann, 1979).

Additionally, with *C. gigas* having a high growth rate, their complex shells fuse together to generate more of a maze-like reef structure than that of a flowing shell

layer and living oyster layer - which is more like the non-reef-building oyster of *O. edulis* (Grabowski and Peterson, 2007). It has been observed before that biodiversity in fish species based on substrata present is often more due to predation than food availability (Risk, 1972), suggesting that with a more complex habitat offering more relief from predation, there will be a greater biodiversity – yet this has been disproved as with the flatter and less complex substrata of *O. edulis* often has the greater species richness and diversity (Figure 13 & 14). Overall, it was overserved that *C. gigas* provides a naturally more complex habitat structure in comparison to *O. edulis*, which offers the opportunity for higher biodiversity and greater community structure.

## **6.0 Summary**

Overall functionality between *C. gigas* and *O. edulis* are equal, with optimal densities often being the peak for each ecological function – density of 10 live oysters per m<sup>2</sup> for *C. gigas* and 5 live oysters per m<sup>2</sup> for *O. edulis*. Significant difference between any condition with oysters compared to an area without oyster for nitrogen cycling, specifically during days 7 and days 21. No significant difference between live oyster environments without a shell layer and with a shell layer for nitrogen compounds but significant when looking at phosphate. Density dependence affects biodiversity within oyster reefs, with *Ostrea edulis* having the highest species abundance at a density of 5 live oysters per m<sup>2</sup>, and *Crassostrea gigas* having the highest species abundance at 10 live oysters per m<sup>2</sup>. Biodiversity shown to have higher richness in *Ostrea edulis* reefs but higher abundance in *Crassostrea gigas* reefs – but overall, no significant difference. Oyster reef complexity of a natural and pre-established *C. gigas* reef is significantly more complex compared to a low density (1 live oyster per m<sup>2</sup>) and high density (15 live oyster per m<sup>2</sup>) for both *O. edulis* and *C. gigas*, with *C. gigas* at high density being more complex overall of the experimental design reefs. Further studies for density-dependent experimental reefs would need longer time to allow reefs to equilibrate for nutrient cycling data, time for in-situ microbial communities to develop and swabs to be collected, and to observe seasonality. Additionally, use of a different method for DNA extraction to guarantee a usable pellet for qPCR analysis of microbial communities.

## References

- Allison, S. 2019. The endangered European native oyster *Ostrea edulis* (L) and creation of Marine Conservation Zones: a win – win scenario for fisheries and conservation?. University of Essex Research Repository.
- Allison, S., Hardy, M., Hayward, K., Cameron, T., Underwood, G. 2020. Strongholds of *Ostrea edulis* populations in estuaries in Essex, SE England and their association with traditional oyster aquaculture: Evidence to support a MPA designation. *Journal of the Marine Biological Association of the United Kingdom*, 100(1), pages 27-36.
- Baggs, A., Board, B., Crummy, P., Dove, C., Durgan, S., Goose, N., Pugh, R., Studd, P., Thornton C. 1994. 'Mills', in *A History of the County of Essex: Volume 9, the Borough of Colchester*, ed. Janet Cooper and C R Elrington London, 1994, pages 259-264. *British History Online*
- Baggett, L., Powers, S., Brumbaugh, R., Coen, L., DeAngelis, B., Greene, J., Hancock, B., Morlock, S., Allen, B., Breitburg, D., Bushek, D., Grabowksi, J., Grizzle, R., Grosholz, E., La Peyre, M., Luckenbach, M., McGraw, K., Piehler, M., Westby, S., zu Ermgassen, P. .2015. Guidelines for Evaluating Performance of Oyster Habitat Restoration. *Restoration Ecology* 23, 6, pages 737-745.
- Bayne, B. 2009. Carbon and nitrogen relationships in the feeding and growth of the Pacific oyster, *Crassostrea gigas* (Thunberg). *Journal of Experimental Marine Biology and Ecology*, 374, 1, pages 19-30
- Beck, M., Brumbaugh, R., Airoidi, L., Carranza, A., Coen, L., Crawford C., Defeo, O., Edgar, G., Hancock, B., Kay, M., Lenihan, H., Luckenbach, M., Toropova, C., Zhang, G., Guom X. 2011. Oyster Reefs at Risk and Recommendations for Conservation, Restoration, and Management. *Bioscience* 61, 2, Pages 107-116

- Bennema, F., Engelhard, G., Lindeboom, H. 2020. *Ostrea edulis* beds in the Central North Sea: Delineation, ecology and restoration. *ICES Journal of Marine Science*, Fsaal134.
- Beutel, M., Leonard, T., Dent, S., Moore, B. 2008. Effects of aerobic and anaerobic conditions on P, N, Fe, Mn, and Hg accumulation in waters overlaying profundal sediments of an oligo-mesotrophic lake. *Water Res* Apr, 42, 8-9, pages 1953-1962.
- Blake, J. 1960. Oxygen Consumption of Bivalve Prey and Their Attractiveness to the Gastropod, *Ursoalpinx cinerea*. *Limnology and Oceanography*, pages 273-280.
- Braker, G., Zhou, J., Wu, L., Devol, A., Tiedje, J. 2000. Nitrite reductase genes (*nirK* and *nirS*) as functional markers to investigate diversity of denitrifying bacteria in Pacific Northwest marine sediment communities. *Appl Environ Microbiol* 66, pages 2096–2104
- Bromley, C., McGonigle, C., Ashton, EC., Roberts, D. 2016. Bad moves: pros and cons of moving oysters – a case study of global translocations of *Ostrea edulis* Linnaeus, 1758 (Mollusca: Bivalvia). *Ocean & Coastal Management* 122, pages 103-115. Elsevier.
- Caffrey, J., Bano, N., Kalanetra, K., Hollibaugh, J. 2007. Ammonia oxidation and ammonia-oxidizing bacteria and archaea from estuaries with differing histories of hypoxia. *ISME Journal*, 1, pages 660–662
- Cao, H., Hong, Y., Li M., Gu, JD. 2012. Community shift of ammonia-oxidizing bacteria along an anthropogenic pollution gradient from the Pearl River Delta to the South China Sea. *Applied Microbiology Biotechnology* 94:247–259
- Christianen, M. J. A., Lengkeek, W., Bergsma, J. H., Coolen, J. W. P., Didderen, K., Dorenbosch, M., van der Have, T. M. (2018). *Return of the native facilitated by the invasive? Population composition, substrate preferences and epibenthic species richness of a recently discovered shellfish reef with native European flat oysters*

(*Ostrea edulis*) in the North Sea, **14**(6), 590– 597. <https://doi.org/10.1080/17451000.2018.1498520>

- Clark, DR., McKew, BA., Dong, LF., Leung, G., Dumbrell, AJ., Stott, A., Nedwell, DB., Trimmer, M., Whitby, C. (2020). Mineralization and nitrification: Archaea dominate ammonia-oxidising communities in grassland soils.
- Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Aust. J. Ecol.* 18: 117–143. doi:[10.1111/j.1442-9993.1993.tb00438.x](https://doi.org/10.1111/j.1442-9993.1993.tb00438.x)
- Crayford, B., Jiang, G., Keller, J., Tyson, G., Bond, P. 2017. Comparison of microbial communities across sections of corroding sewer pipe and the effects of wastewater flooding. *Biofouling: The Journal of Bioadhesion and Biofilm Research*, Volume 33, Issue 9, Pages 780-792.
- Colden, A., Latour, R., Lipcius, R. 2017. Reef height drives threshold dynamics of restored oyster reefs. *Marine Ecology Progress Series*, Volume 582, pages 1-13. <https://doi.org/10.3354/meps12362>
- Culloty, S., Cronin, M., Mulcahy, M. 2004. Potential resistance of a number of populations of the oyster *Ostrea edulis* to the parasite *Bonamia ostreae*. *Aquaculture*, Volume 237, Issues 1-4, pages 41-58. Elsevier.
- Dang, H., Wang, C., Li, J., Li, T., Tian, F., Jin, W., Ding, Y, Zhang, Z. 2009. Diversity and distribution of sediment nirS-encoding bacterial assemblages in response to environmental gradients in the eutrophied Jiaozhou Bay, China. *Microb Ecol* 58:161–169
- Davidson, P. 1976. *Oyster Fisheries of England and Wales*. Ministry of Agriculture Fisheries and Food, Directorate of Fisheries Research. Laboratory Leaflet No.31, Fisheries Laboratory Lowestoft, Suffolk.
- Erguder, T., Boon N., Wittebolle, L., Marzorati, M., Verstraete, W. 2009. Environmental factors shaping the ecological niches of ammonia-oxidizing archaea. *FEMS Microbiology Reviews* 2009; **33**: 855–869.

- Fodrie, J., Rodriguez, A., Gittman, R., Grabowski, J., Lindquist, N., Peterson, C., Piehler, M., Ridge, J. 2017. Oyster reefs as carbon sources and sinks. *Proceedings of the Royal Society B, Biological Science*, 284, 1859.
- Frost, N., Burrows, M., Johnson, M., Hanley, M., Hawkins, S., 2005. Measuring surface complexity in ecological studies, *Limnol. Oceanogr. Methods*, 3, doi:10.4319/lom.2005.3.203.
- Gamble, C., Ashton, E., Bromley, C., Fariñas-Franco, J., Hancock, B., Hayden-Hughes, M., Helmer, L., Lee, H., Preston, J., Sanderson, W., Thurstan, R., zu Ermgassen, P. 2020. European Native Oyster Restoration Handbook: An introduction – in J Preston, C Gamble, A Debney, L Helmer, B Hancock & P zu Ermgassen (eds). *European Native Oyster Habitat Restoration Handbook: UK & Ireland*. The Zoological Society of London, Pages 2-11.
- Grabowski, J., Peterson, C. 2007. Restoring Oyster Reefs to Recover Ecosystem Services. *Ecosystem Engineers*, 15, pages 281-298
- Green, D., Boots, B., Crowe, T. 2012. Effects of Non-Indigenous Oysters on Microbial Diversity and Ecosystem Functioning. *PLoS ONE* 7, 10: e48410.
- Green, D., Rocha, C., Crowe, T. 2013. Effects of Non-indigenous Oysters on Ecosystem Processes Vary with Abundance and Context. *Ecosystems*, 16, pages 831-833
- Green, D., Crowe, T. 2013. Physical and Biological effects of introduced oysters on biodiversity in an intertidal boulder field. *Marine Ecology Progress Series*, 482, pages 119-132.
- Griffith, J. 2016. Insights into the soil microbial communities in New Zealand's indigenous tussock grasslands. PhD Thesis. University of Otago.
- Guy, C., Blight A., Smyth, D., Roberts, D. 2018. The world is their oyster: Differences in epibiota on sympatric populations of native *Ostrea edulis* and non-native *Crassostrea gigas* (*Magallana gigas*) oysters. *Journal of Sea Research*, 140, pages 52-58. Elsevier.

- Hayer, S., Bick, A., Brandt, A., Ewers-Saucedo, C., Fiege, D., Fütting, S., Krause-Kyora, B., Michalik, P., Reinicke, G-B., Brandis, D. 2019. Coming and going – Historical distributions of the European oyster *Ostrea edulis* Linnaeus, 1758 and the introduced slipper limpet *Crepidula fornicata* Linnaeus, 1758 in the North Sea. PLoS ONE 14 (10)
- Herbert, R. 1999. Nitrogen cycling in coastal marine ecosystems. FEMS Microbiol Rev 5: pages563-590
- Herlemann, D.P.R., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J.J., Andersson, A.F., 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME Journal 5, pages 1571-1579.
- Hoellein, T., Zarnoch, C., Grizzle, R. 2015. Eastern oyster (*Crassostrea virginica*) filtration, biodeposition, and sediment nitrogen cycling at two oyster reefs with contrasting water quality in Great Bay Estuary (New Hampshire, USA). Biogeochemistry, 122, pages 113-129.
- Javadi A, Shamaei M, Mohammadi Ziazi L, Pourabdollah M, Dorudinia A, Seyedmehdi SM, Karimi S. 2014. Qualification study of two genomic DNA extraction methods in different clinical samples. Tanaffos. 2014;13(4): pages 41-7.
- Jiang, H., Dong, H., Yu, B., Lv, G., Deng, S., Berzins, N., Dai, M. 2009. Diversity and Abundance of Ammonia-Oxidizing Archaea and Bacteria in Qinghai Lake, Northwestern China, Geomicrobiology Journal, 26:3, pages 199-211.
- Joseph, Nancy. 2014. An Overabundance of Oyster Drills. College of Arts & Sciences, University of Washington. <https://artsci.washington.edu/news/2004-07/overabundance-oyster-drills>
- Joyce, P., Dickey, J., Cuthbert, R., Dick, J., Kregting, L. 2019. Using functional responses and prey switching to quantify invasion success of the Pacific Oyster, *Crassostrea gigas*. Marine Environmental Research 145, pages 66-72. Green Open Access.

- Joyce, P., Dick, J., Kregting, L. 2020. Lack of biotic resistance to an invasive bivalve irrespective of season or hydrodynamic disturbance. *Journal of Experimental Marine Biology and Ecology* 528.
- Klawonn I., Bonaglia S., Brüchert V., Ploug H. 2015. Aerobic and anaerobic nitrogen transformation processes in N<sub>2</sub>-fixing cyanobacterial aggregates. *ISME J.* Jun;9(6): pages 1456-66.
- Kruskal, J. 1964. Nonmetric multidimensional scaling: A numerical method. *Psychometrika* 29: 115–129.
- Larsen, P. 1985. The Benthic Macrofauna Associated with the Oyster Reefs of the James River Estuary, Virginia, U.S.A. *Int. Revue ges. Hydrobiol. Hydrogr.*, 70: pages 797-814.
- Li, J., Nedwell, D., Beddow, J., Dumbrell, A., McKew. B., Thorpe, E., Whitby, C. 2015. *amoA* gene abundances and nitrification potential rates suggest that benthic ammonia-oxidizing bacteria (AOB) not archaea (AOA) dominate N cycling in the Colne estuary, UK. *Appl. Environ. Microbiol.* 81: pages 159-165.
- Lipsewers, Y., Vasquez-Cardenas, D., Seitaj, D., Schauer, R., Hidalgo-Martinez, S., Sinninghe Damsté, J., Meysman, F., Villanueva, L., Boschker, H. 2017. Impact of Seasonal Hypoxia on Activity and Community Structure of Chemolithoautotrophic Bacteria in a Coastal Sediment. *Appl Environ Microbiol.* 2017 May 1;83(10)
- Locher, B., Hurst, N., Walters, L., Chambers, L. 2020. Juvenile Oyster (*Crassostrea virginica*) Biodeposits Contribute to a Rapid Rise in Sediment Nutrients on Restored Intertidal Oyster Reefs (Mosquito Lagoon, FL, USA). *Estuaries and Coasts*, Volume 44, 5, pages 1363-1379. Springer.
- Lonhart, S., Jeppesen, R., Beas-Luna, R., Crooks, J., Lorda, J. 2019. Shifts in the distribution and abundance of coastal marine species along the eastern Pacific Ocean during marine heatwaves from 2013 to 2018. *Mar Biodivers Rec* 12, 13

- Lown, A., Hepburn, L., Heywood, J., Cameron, T. 2021. European native oysters and associated species richness in the presence of non-native species in a southern North Sea estuary complex. *Conservation Science and Practice*.
- Luckhurst, B.E., Luckhurst, K. (1978). *Analysis of the influence of substrate variables on coral reef fish communities*. *Marine Biology*, 49(4), 317–323. doi:10.1007/bf00455026
- Mann, R. 1979. Some biochemical and physiological aspects of growth and gametogenesis in *Crassostrea gigas* and *Ostrea edulis* grown at sustained elevated temperatures. *Journal of the Marine Biological Association of the United Kingdom*, 59(1), 95-110. doi:10.1017/S0025315400046208
- Martens-Habbena W, Berube PM, Urakawa H, Torre, J. Stahl, D. 2009. Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. *Nature* 461:976–979
- May, R. 1973. *Stability and complexity in model ecosystems*. Princeton Univ. Press, 1973.
- McAfee, D., Bishop, M. 2019. The mechanisms by which oysters facilitate invertebrates vary across environmental gradients. *Oecology*, 149, 4, pages 1095-1106.
- McTavish H., Fuchs J., Hooper, A. 1993. Sequence of the gene coding for ammonia monooxygenase in *Nitrosomonas europaea*. *J Bacteriol* 175: pages 2436–2444.
- Mitchley, J., Burch, F., Buckley, P., Watt, T. 2000. *Habitat Restoration Monitoring Handbook*. English Nature. English Nature Research Reports – No. 378.
- Nedwell, D., Underwood, G. McGenity, T., Whitby, C., Dumbrell, A. 2016. [Advances in Ecological Research] *Large-Scale Ecology: Model Systems to Global Perspectives* Volume 55, The Colne Estuary, pages 227–281.
- Newell, R., Fisher, T., Holyoke, R., Cornwell, J. 2005. INFLUENCE OF EASTERN OYSTERS ON NITROGEN AND PHOSPHORUS REGENERATION IN CHESAPEAKE BAY, USA. [NATO Science Series IV: Earth and Environmental Series]

The Comparative Roles of Suspension-Feeders in Ecosystems Volume 47, Influence of Eastern Oysters on Nitrogen and Phosphorus Regeneration in Chesapeake Bay, USA. pages 93–120.

- Nogales B., Timmis K., Nedwell D., Osborn, A. 2002. Detection and Diversity of Expressed Denitrification Genes in Estuarine Sediments after Reverse Transcription-PCR Amplification from mRNA. *Appl Environ Microbiol* 68, pages 5017-5025
- Officer, C.B., Smayda, T.J., Mann, R. 1982. Benthic Filter Feeding: A Natural Eutrophication Control. *Marine Ecology – Progress Series*, Volume 9, pages 203-210. Inter-Research, F.R. Germany.
- Ouyang, Y., Zhang, J.E., Ou, L.-T. 2006. Temporal and Spatial Distributions of Sediment Total Organic Carbon in an Estuary River. *Journal of Environment Quality*, 35(1), 93.
- Papaspyrou S., Smith C., Dong L., Whitby, C., Dumbrell, A., Nedwell, D. 2014. Nitrate reduction functional genes and nitrate reduction potentials persist in deeper estuarine sediments Why? *PLoS One* 9:e94111
- Peterson, J., Titschack, J., Groeneveld, J., Wehrmann, A., Hebbeln, D., Freiwald, A. 2021. Reef-building Pacific oysters record seasonal variations in water mass-properties of tidal basins from the Central Wadden Sea (North Sea). *Palaeogeography, Palaeoclimatology, Palaeoecology*, 577, 110534
- Pogoda, B., Buck, B. H., & Hagen, W. 2011. Growth performance and condition of oysters (*Crassostrea gigas* and *Ostrea edulis*) farmed in an offshore environment (North Sea, Germany). *Aquaculture*, 319(3-4), pages 484–492.
- Pollack, J., Yoskowitz, D., Kim, H-C., Montagna, P. 2013. Role and Value of Nitrogen Regulation Provided by Oysters (*Crassostrea virginica*) in the Mission-Aransas Estuary, Texas, USA. *PLoS One*.
- Preston, J., Fabra, M., Helmer, L., Johnson, E., Harris-Scott, E., Hendy, I. 2020. Interactions of larval dynamics and substrate preference have ecological significance

for benthic biodiversity and *Ostrea edulis* Linnaeus, 1758 in the presence of *Crepidula fornicata*

- Prokopenko M., Sigman D., Berelson W., Hammond, D., Barnett, B., Chong, L., Townsend-Small, A. 2011. Denitrification in anoxic sediments supported by biological nitrate transport, 75(22), pages 0–7199.
- Prosser, J., Nicol, G. 2008. Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. *Environmental Microbiology*; **10**: pages 2931-2941.
- Raskin, L., Stromley, J., Rittmann, B., Stahl, D. 1994. Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens. *Applied and Environmental Microbiology* 60, pages 1232-1240.
- Risk, M.J. 1972. Fish diversity on a coral reef in the Virgin Islands. *Atoll Res. Bull.* 153, pages 1-6
- Rodriguez-Perez, A., James, M., Donnan, D., Henry, T., Møller, L., Sanderson, W.G. 2019. Conservation and restoration of a keystone species: Understanding the settlement preferences of the European oyster (*Ostrea edulis*). *Marine Pollution Bulletin*, 138(), pages 312–321.
- Rodriguez-Perez, A., Sanderson, W., Møller, L., Henry, T., James, M. 2020. Return to sender: The influence of larval behaviour on the distribution and settlement of the European Oyster *Ostrea edulis*. *Aquatic Conservation, Marine and Freshwater Ecosystems*, 30, 11, special issue: UNLOCKING THE BLUEPRINT FOR NATIVE OYSTER RESTORATION IN EUROPE, page 2116-2132.
- Rotthauwe J., Witzel K., Liesack W. 1997. The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. *Appl Environ Microbiol*; **63**: pages 4704-12.
- Sas, H., Deden, B., Kamermans, P., zu Ermgassen, P., Pogoda, B., Preston, J., Helmer, L., Holbrook, Z., Arzul, I., van der Have, T., Villalba, A., Colsoul, B., Lown, A., Merk, V., Zwerschke, N. and Reuchlin, E. 2020. *Bonamia* infection in native oysters

(*Ostrea edulis*) in relation to European restoration Projects. Aquatic Conservation-Marine and Freshwater Ecosystems, 30 (11), pages 2150-2162.

- Scarlett, K., Denman, S., Clark, D. R., Forster, J., Vanguelova, E., Brown, N., Whitby, C. (2020). Relationships between nitrogen cycling microbial community abundance and composition reveal the indirect effect of soil pH on oak decline. The ISME Journal.
- Seitzinger S.P. (1988). Denitrification in fresh-water and coastal marine ecosystems-ecological and geochemical significance. Limnol Oceanogr 33: pages 702–724
- Smith, J., Casciotti, K., Chavez, F., Francis, C. 2014. Differential contributions of archaeal ammonia oxidizer ecotypes to nitrification in coastal surface waters. The ISME Journal, 8(8), pages 1704–1714.
- Smyth, A., Geraldi, N., Thompson, S., Piehler, M. 2017. Biological activity exceeds biogenic structure in influencing sediment nitrogen cycling in experimental oyster reefs. Marine Ecology Progress Series, 560, pages 173-183. Inter-Research.
- Smyth, D., Roberts, D. 2010. The European oyster (*Ostrea edulis*) and its epibiotic succession. Hydrobiologia, 655(1), pages 25–36.
- Stahl, D.A., Amann, R., 1991. Development and application of nucleic acid probes, p. 205-248. In E. Stackebrandt and M. Goodfellow (ed.), Nucleic acid techniques in bacterial systematics. John Wiley & Sons Ltd.
- Stagličić, N., Šegvić-Bubić, T., Ezgeta-Balić, D., Varezić, D., Grubišić, L., Žuvić, L., Lin, Y., Briski, E. 2020. Distribution patterns of two co-existing oyster species in the northern Adriatic Sea: The native European flat oyster *Ostrea edulis* and the non-native Pacific oyster *Magallana gigas*. Ecological Indicators, 113.
- Stehr G., Biittcher B., Dittberner P., Rath, G., Koops, H-P. 1995. The ammonia-oxidizing nitrifying population of the River Elbe estuary. FEMS Microbiol Ecol 17: pages 177–186
- Throbäck I.N., Enwall, K., Jarvis, Å., Hallin, S. 2004. Reassessing PCR primers targeting nirS, nirK and nosZ genes for community surveys of denitrifying bacteria with DGGE. , 49(3), pages 401–417.

- Tomatsuri, M., Kon, K. 2017. Effects of dead oyster shells as a habitat for the benthic faunal community along rocky shore regions. *Hydrobiologia* 790, pages 225–232.
- Ulanowicz, R., Tuttle, J. 1992. The Trophic Consequences of Oyster Stock Rehabilitation in Chesapeake Bay. *Estuaries*, 15(3), pages 298–306.
- Underwood, G., Dumbrell, A., McGenity, T., McKew, B., Whitby, C. 2022. The Microbiome of Coastal Sediments, pages 479-534.
- Xia, F., Wang, J-G., Zhu, T., Zou, B., Rhee, S-K., Quan, Z-X. 2018. Ubiquity and diversity of complete ammonia oxidizers (comammox). *Applied and Environmental Microbiology*,
- Zwerschke, N., Hollyman, P., Wild, R., Stringer, R., Turner, J., King, J.W. 2018. Limited impact of an invasive oyster on intertidal assemblage structure and biodiversity: the importance of environmental context and functional equivalency with native species. *Mar Biol* 165, 89.
- Zwerschke, N., Emmerson, M., Roberts, D., O'Connor, N. 2016. Benthic assemblages associated with native and non-native oysters are similar. *Marine Pollution Bulletin*, 111(1-2), pages 305-319.
- Zwerschke, N., Eagling, L., Roberts, D., O'Connor, N. 2019. *Can an invasive species compensate for the loss of a declining native species? Functional similarity of native and introduced oysters. Marine Environmental Research.*
- zu Ermgassen, P., Thurstand, R., Corrales, J., Alleway, H., Carranza, A., Dankers, N, DeAngelis, B., Hancock, B., Kent, F., McLeod, I., Pogoda, B., Liu, Q., Sanderson, W. 2020. The benefits of bivalve reef restoration: A global synthesis of underrepresented species. *Aquatic Conservation: Marine Freshwater Ecosystems*. Issue 30, pages 2050-2065.
- zu Ermgassen, P., Bonačić, K., Boudry, P., Bromley, C., Cameron, T., Colsoul, B., Coolen, J., Frankić, A., Hancock, B., Have, T., Holbrook, Z., Kamermans, P., Laugen,

A.T., Nevejan, N., Pogoda, B., Pouvreau, S., Preston, J., Ranger, C., Sanderson, W., Sas, H. 2020. Forty questions of importance to the policy and practice of native oyster reef restoration in Europe. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 30(11), pages 2038–2049.