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MSc Applied Aquatic Biology

MSc Thesis

Survivorship, growth and reproductive potential of protected broodstock flat oyster, *Ostrea edulis*, populations attached to existing floating structures within the Solent, UK

By

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Supervisor

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August 2016

In association with

LAND ROVER BAR

MDL Marinas

11TH HOUR RACING

SOLENT FORUM

BLUE MARINE FOUNDATION

Southern IFCA Inshore Fisheries and Conservation Authority

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"*I love oysters. It's like kissing the sea on the lips*"

Léon-Paul Fargue (1876-1947)

Artwork by Catherine Helmer

University of Portsmouth

Abstract

School of Biological Sciences

Institute of Marine Science

Thesis for the degree of Master of Science in Applied Aquatic Biology

Survivorship, growth and reproductive potential of protected broodstock flat oyster, *Ostrea edulis*, populations attached to existing floating structures within the Solent, UK

by Luke David Helmer

The recent decline in *Ostrea edulis* stocks within its native habitat, due to a variety of detrimental influences, has prompted numerous restoration efforts to reverse the population collapse. This project is one such attempt, with the aim of protecting broodstock populations in an effort to increase larval output and address the issues associated with successful recruitment. Cages were suspended from both the University of Portsmouth Research Raft, in Langstone Harbour (Hampshire, UK), and the Land Rover Ben Ainslie Racing pontoon, situated in the Camber Dock within Portsmouth Harbour (Hampshire, UK). At each location a total of 675 oysters, in both high (50 per 0.5 m^2) and low density (25 per 0.5 m^2) populations, were placed into a three-tier cage environment in December 2015, with monitoring occurring on a monthly basis until July 2016. Percentage mortalities experienced between locations were significantly different (PERMANOVA main test, $F_{1, 142} = 54.8$, $p = < 0.001$), with the Portsmouth population experiencing a total of 49 (7.3 %) mortalities, compared to 312 (46.2%) experienced within the population in Langston Harbour. Environmental parameters average temperature and dissolved oxygen concentration, as well as fluctuations in these parameters, were show to influence the mortality within Langstone Harbour. In comparison, average dissolved oxygen concentration, turbidity and total dissolved solids were shown to influence the mortality in Portsmouth Harbour. The success of the project within Portsmouth Harbour was confirmed with the discovery of veliger 'D' larvae within the broodstock females sampled in July 2016.

Key Words

Oyster; *Ostrea edulis*; Restoration Project; Population Structures; Protected Broodstock; Aquaculture; Conservation; Bonamiosis; *Bonamia ostreae*; Larval recruitment; Solent.

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List of Abbreviations

BGA: Blue green algae

BLAST: Basic Local Alignment Search Tool

bp: Base pairs

CD: Compact disc

CHI: Chichester Harbour

CI: Condition index

COI gene: Cytochrome c oxidase subunit I gene

DNA: Deoxyribonucleic acid

EDTA: Ethylenediaminetetraacetic acid

IMS: Institute of Marine Sciences

Kb: Kilobase

LRBAR: Land Rover Ben Ainslie Racing

MLS: Minimum landing size

nLF: non-linear function

NOSAP: Native Oyster Species Action Plan

ODO: Optical dissolved oxygen

PC: Principle component ordination

PCR: Polymerase chain reaction

PORT: Portsmouth Harbour

PRIMER: Plymouth Routines in Multivariate Ecological Research package

psu: practical salinity units

rDNA: Ribosomal DNA

RFU: Relative fluorescence unit

RNA: Ribonucleic acid

SD: Standard deviation

SE: Standard error

Southern IFCA: Inshore Fisheries & Conservation Authority

SSU: Small sub unit

TAE: Tris-acetate-EDTA

TBT: Tributyltin

TDS: Total dissolved solids

UK: United Kingdom

UKBAP: UK Biodiversity Action Plan

UoPRR: The University of Portsmouth Research Raft

UV: Ultraviolet

1. Introduction

1.1. The Native Oyster

Ostrea edulis (L.) is a sessile bivalve mollusc that is native to brackish and marine subtidal waters around Europe, with salinity tolerances of 18 – 40 ‰ reported by Jackson (2007). The species is known by various common names such as native, common, edible, flat, European, mud and Colchester native oyster. Individuals have an oval shaped rough shell consisting of two valves (ventral (left) valve convex, dorsal (right) valve flat) formed of calcite and that express phenotypic polymorphism (Figure 1).

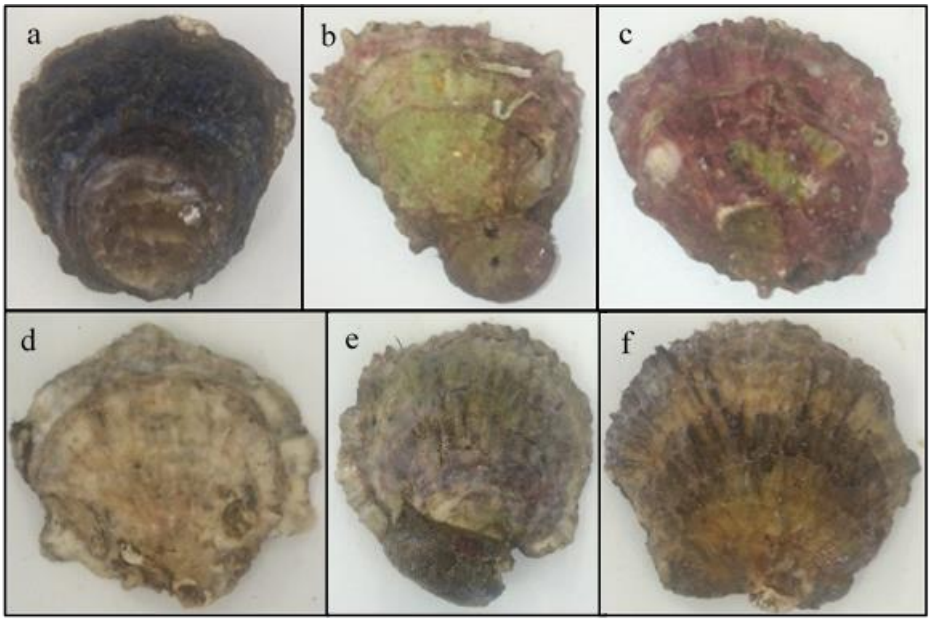


Figure 1. Array of *Ostrea edulis* shell colourations. (a) Blue iridescence on dorsal valve. (b) Pale green colouration on ventral valve. (c) Red/Pink colouration on ventral valve. (d) White colouration on ventral valve (e) Pale green, white, purple and blue colouration on ventral valve. (f) Brown and yellow colouration on ventral valve.

As protandrous hermaphrodites, individuals mature during the male phase (Orton 1927a, Laing et al. 2005), after eight to ten months' from release, and henceforth alternate between male and female stages (Orton 1927a, Laing et al. 2005). Throughout the UK, *O. edulis* typically spawn once a year, between May and August (Hayward et al. 1996),

depending on temperature (minimum 16°C) (Laing et al. 2005) and food availability (Ruiz et al. 1992). Peak spawning usually occurs during the presence of a full moon (Jackson 2007), however, if conditions are favourable multiple spawning can occur in a season (Laing et al. 2005).

Sperm released by males are drawn into the female mantle cavity, via the inhalant siphon, where eggs are released and fertilisation occurs. Following this, brooding ensues for seven to ten days, the fertilised eggs first develop into trochophore (Andrews 1979), then into veliger ('D' shaped) larvae (Orton 1927a, Walne 1974, Andrews 1979). The larvae are released and enter the early umbo and umbo stages, with the final larval phase being pediveliger (Acarli & Lok 2009). In total between seven and sixteen days are spent within the water column before settling on suitable substrata (Korringa 1941, Cole & Knight-Jones 1949, Galtsoff 1964, Bayne 1969, Walne 1974, Andrews 1979) (Figure 2). *O. edulis* express high fecundity with larvae released in numbers relative to the age and size of the mature individual (Walne 1974, Table 1), the progeny of larger individuals can exceed two million (Jackson 2007). The expected brood quantities for an oyster of a size between 65 - 80 mm would be between 0.75 - 1.2 million (Cole 1941, Walne 1964, 1974, Eagling 2012).

Table 1. Fecundity (number of larvae) of native oysters in relation to approximate age and size. (Source: Adapted from Walne 1974, cited in Laing et al. 2005).

Age (years)	Mean shell diameter (mm)	Number of larvae
1	40	100,000
2	60	540,000
3	70	840,000
4	80	1,100,000
7	90	1,500,000

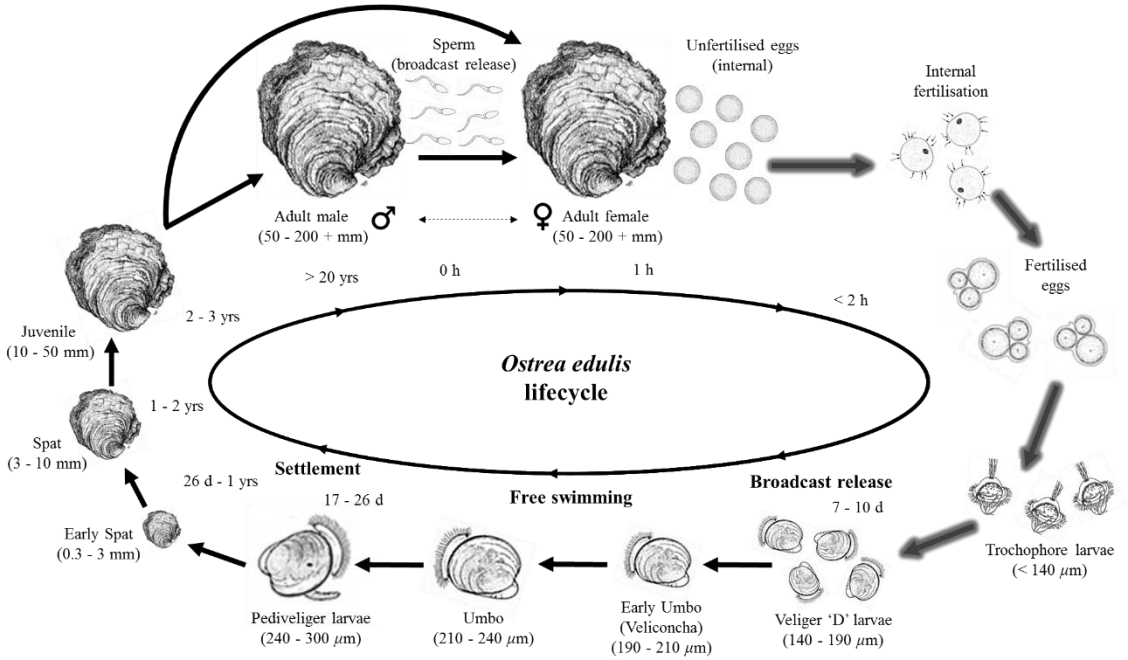


Figure 2. Overview of the lifecycle of *Ostrea edulis*. Arrows with glow effect indicate stages that occur internally within the female oyster pallial (mantle) cavity. Approximate sizes and timings are based upon information from Hu et al. (1993), Acarli & Lok (2009), FAO (2016) and, Loosanoff et al. (1966), Pascual (1972) and Tanaka (1981), cited within Hu et al. (1993). Images obtained and edited from Environment Canada (2011, p43), YouTube (2011), Pangea Shellfish & Seafood Company, Inc (2016), FAO (2016). Images of life stages are not to scale.

1.2. Trends in Native Oyster populations and the extent of their decline

Native oyster beds once dominated many estuarine and coastal areas within Europe, both ecologically and economically. These oysters can be described as ecosystem engineers, as the biogenic reef structure they form and the habitat they provide creates an integrated ecosystem, with increased biodiversity compared to similar habitats where they are absent (Wells 1961, Lenihan & Peterson 1998). Oysters have been a source of food for many civilizations, and exploitation dates back to the Roman Empire (Gunther 1897). In the 1870's, 700 million European Native oysters were consumed in London alone, employing approximately 120,000 workers throughout Britain (Gardner & Elliot 2001).

Recently, Beck et al. (2011) sampled over 140 bays and estimated that global oyster reefs and beds have been reduced by some 85 %. Many of these bays (37 % of areas sampled) were shown to have lost more than 99 % of their reefs and have become functionally extinct, predominantly the case in North America, Australia and Europe. Lallias et al. (2008) has shown that remaining oyster populations occur in select, geographically small and localised areas, with low economical production. This is particularly relevant regarding areas around the Solent where recent estimations suggest low densities of 1 oyster per 20.9 m^2 (Kamphausen 2012).

Until recently the Solent had one of the largest remaining *O. edulis* fisheries in Europe, up to 450 fishing boats employing more than 700 people in 1978. Since the 1980's catch rates have fluctuated, with an acute decline from 2007 - 2011, experienced after a peak in 2006, in areas that typically record the highest catch levels (Figure 3) (Lockwood 1985, Vanstaen & Palmer 2010, Kamphausen, 2012). After a rapid decline in total annual harvest, from 200 tonnes to 20 tonnes over a five-year period, the Southern Inshore Fisheries and Conservation Authority (Southern IFCA) introduced closed fishery seasons for 2013/14, 14/15 and 15/16 (1st November – 28th February) for the wider Solent, a minimum landing size (MLS - individuals must not pass through a 70 mm ring) and specifications for gear use (1.5 m maximum front edge or blade, 3.0 meters for use of two or more dredges). The fishery was opened for a brief two-week period at the beginning of November 2015 in Langstone and Portsmouth harbours, then closed 15th November – 28th February, to allow for relaying and fishing of oysters within the planned capital dredge area. Current global aquaculture production was at only 2,809 tonnes in 2014 (FAO 2016).

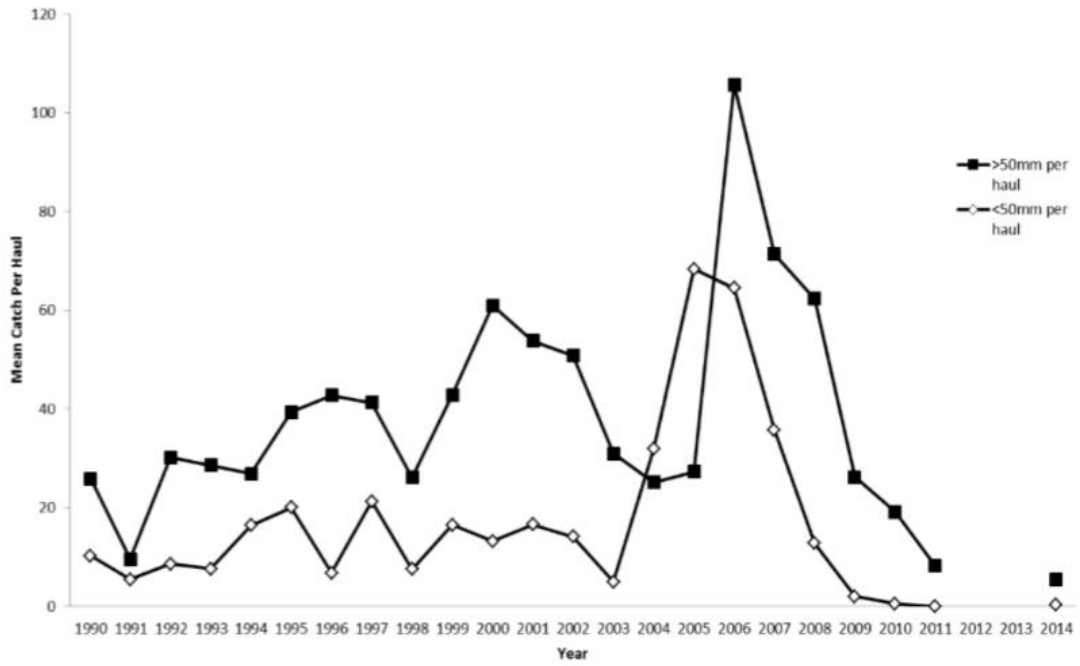


Figure 3. The 1990 – 2014 trend in both small (< 50 mm) and large (> 50 mm) *Ostrea edulis* catch rates for Ryde Middle (located in the eastern Solent). Data presented as average positive haul (containing oysters) catch. Source: Southern IFCA 2014, cited in Gravestock et al. 2014.

1.3. Potential explanations for the population decline and collapse of the fishery

1.3.1. Overfishing

The native oyster can be relatively long lived (on average 6 years, up to 15 - 20 years (Joint Nature Conservation Committee 2015)) however, due to their intermittent and irregular reproduction patterns (Orton 1927b, Spärck 1951), populations are susceptible to overfishing which decreases their average life expectancy (Laing et al. 2005). The continuous removal of mature individuals, accompanied with the lack of additional recruits means the population composition moves towards that of one with extremely low density beds that lack the resources to regenerate a sustainable fishery (Kamphausen 2012). Overfishing is a worldwide problem for many species and has been attributed, amongst other factors, to the population decline of the Eastern oyster, *Crassostrea virginica*, in Chesapeake Bay, USA (Rothschild et al. 1994, Wilberg et al. 2011).

1.3.2. Recent quantities of larvae in the Solent

Recently, in 2009 and 2010, Kamphausen (2012) observed total bivalve larval densities within the Solent, which varied from 20 - 40 per 100 l^{-1}, with the highest percentage of *O. edulis* larvae occurring during August 2010, 59% (9 larvae per 100 l^{-1}) of the sample. Historic data, 1984 - 1987 (Key 1987), also showed peak spawning occurring at this time of the year, however, the densities observed by Kamphausen (2012) were lower than that of Key (1987). The difference in larval density may be attributed to a difference in sampling, a single plankton tow was used by Kamphausen (2012) compared with multiple 100 l^{-1} samples average by Key (1987), therefore comparison between the two data sets could result in false assumptions.

1.3.3. Larval retention and settlement

The larvae of *O. edulis* are subject to, and influenced by, hydrological and biological parameters such as current, temperature and salinity. A review of field studies on the effects of temperature on larval development period by Korringa (1941) concluded that temperature was the determining factor in the length of free-swimming period. Within this, Walne (1964) found that an increase in temperature, from 17°C - 25°C, decreased the length of time, from fourteen days to five days, for an increase in growth from 175 - 250 µm of the pelagic larval phase. Similarly, Davis & Calabrese (1969) observed a correlation between temperature and average growth rate. When temperatures were increased from 10°C to 25°C the average growth rate increased from 19.0% to 93.6%, alternatively, a decrease in average growth rate of 82.5% and 25.1% was recorded when temperatures were decreased from 30°C to 32.5°, respectively.

It has been estimated that one year after the releases of one million individual larvae, by a four-year-old oyster in a female state, only two individuals will survive (Guerra 2002 cited in Laing et al. 2005). Low survival rates, of below 10 %, are experienced even when larvae are subject to ideal hatchery environments (Walne 1974, Jackson 2007). Despite this estimation, the high quantities of offspring produced could allow for a considerable increase in population size with a single successful recruitment of a large number of larvae (Underwood & Fairweather 1989).

The larvae of *O. edulis* display gregarious behaviour (Cole & Knight-Jones 1939, 1949, Walne 1964, Bayne 1969, Jackson 2007) with a preference for surfaces with a greater topographical variance (Walne 1974), such as living or recently deceased oysters (Woolmer et al. 2011). This behaviour often results in larval congregation in areas containing high densities of mature oysters, in turn increasing the success of fertilisation and breeding by decreasing the distance travelled by male sperm as well as limiting the Allee effects associated with isolated populations (Allee et al. 1949, Gascoigne & Lipcius 2004). The Allee effect occurs in low population densities due to a reduction in gene mixing which leads to a decrease in fitness levels of the organisms (Allee et al. 1949).

The current low abundances of large mature *O. edulis* within the Solent provide almost exactly the opposite of what is required for successful settlement of large quantities of larvae to repopulate the area.

1.3.4. Predators, Pests and Parasites

1.3.4.1. Oyster drills *Ocenebra erinacea* and *Urosalpinx cinerea*

The European oyster drill or tingle, *Ocenebra erinacea*, and the invasive American oyster drill, *Urosalpinx cinerea*, are both predatory gastropod molluscs. Known to prey upon

juvenile oysters and larvae, they have caused severe mortalities within *O. edulis* populations in the past (Hancock 1954, Laing et al. 2005). *U. cinerea* is limited to waters in Essex and Kent (Laing et al. 2005). *O. erinacea* is, however, present in the Solent with recent observations showing 12 *O. erinacea* in 24 separate 0.25 m^2 areas with only a single oyster valve exhibiting a drill hole (Kamphausen 2012). Populations have fluctuated, spatially and temporally, throughout the 20th Century as a result of imposex induced by tributyltin (TBT) exposure, which is characterised by malformation of the oviduct in females (Gibbs 1996) (Feral & Legall 1982, Hawkins & Hutchinson 1990, Gibbs 2009, cited in Kamphausen 2012)).

Conversations with local fishermen have led to the suggestion that, in recent years, the presence and predation of *O. erinacea* has increased and is having a large scale detrimental impact on *O. edulis* populations (Legg pers. comm., Breeze pers. comm.). However, no scientific studies currently back this up. This recent increase in tingle populations could be as a result of the restrictions on the use of TBT, discussed in section 1.3.5., which has historically suppressed the populations.

1.3.4.2. Boring Sponges *Cliona celata* and *Cliona viridis*

Cliona celata (Clionaidae) is a boring sponge known to infest various species within the family of true oysters (Ostreidae), including the Eastern oyster, *Crassostrea virginica* (Carver et al. 2010), the Sydney rock oyster, *Saccostrea commercialis* (Wesche et al. 1997), the Pacific oyster, *Crassostrea gigas* (Snowden 2007), and the Flat oyster, *O. edulis* (Rosell et al. 1999). As well as this, members of the feathered oyster family (Pteriidae) such as the Akoya pearl oyster, *Pinctada fucata* (Alagarswami & Chellam 1976), can also experience infestations. This species of sponge has been documented as a significant predator of *O. edulis* (Hancock 1960) and is present throughout the UK

(Snowden 2007), with populations reported in the Portsmouth area (Juniper & Steele 1969). The species was previously described by Arndt (1935, p44) (cited in Juniper & Steele 1969) *"The sponge had the appearance of several small, butter-yellow papillae about 2.5 mm tall projecting from the surface of the substrate whilst the main body of the sponge lay within."*.

Cliona viridis (Clionaidae), another species of boring sponge, is also known to infect species within the family Ostreidae such as *O. edulis* (Rosell & Uriz 1992, Rosell et al. 1999). This cryptic species (Rosell & Uriz 2002, p73) can only be distinguished from *C. celata* by microscopic observations of the spicules to determine differences between the megascleres and microscleres of each species (Rosell & Uriz 2002, Rützler 2002, p177, Evcen & Çinar 2015, p295). However, *C. viridis* is not included within the 'Sponges of Britain & Ireland' Guide (Picton et al. 2007) nor the 'Sponges of the British Isles ("SPONGE V")' (Ackers et al. 1992), therefore, further evidence is needed to determine the presence of this species within the UK.

1.3.4.3. Slipper limpet *Crepidula fornicata*

Early studies indicated that *Crepidula fornicata* competes with *O. edulis* for habitat and food, and was therefore extremely detrimental to oyster populations (Walne 1956). There are no natural predators of *C. fornicata* in the UK, allowing for extremely high density populations to occur, with the Solent and estuaries in Essex being known 'hot-spots' (Fitzgerald 2007). Excretion of pseudofaeces and the creation of 'mussel mud' by *C. fornicata* can smother oysters and alter the habitat they occupy, a combination of these effects can prevent the settlement of spat (Blanchard 1997, Hawkins et al. 2005, Laing et al. 2005). Contradictory to this, Key & Davis (1981) and Cooper (pers. comm.) indicate that the shells of *C. fornicata* are a suitable substratum upon which the spat of *O. edulis*

can settle, with a 1978 survey revealing that for every three *C. fornicata* there was one oyster spat. The contradiction in findings suggests further work is needed to determine their effects on both *O. edulis* beds and spat settlement (Key & Davis 1981).

1.3.4.4. Bonamiosis *Bonamia ostreae*

For the purpose of this thesis, bonamiosis is considered to be infection with *Bonamia ostreae* and excludes reference to other *Bonamia* spp., unless stated.

Since the 1970s, European countries (Hudson & Hill 1991, Edwards 1997, Carnegie et al. 2000) and the east and west coasts of North America (Elston et al. 1986, Friedman & Perkins 1994) have been subject to bonamiosis, an intrahaemocytic parasitosis with the etiological agent being the protozoan *B. ostreae* (*Haplosporidiidae*). The induction of mass mortalities up to, and occasionally over, 90% of commercial stocks has been noted throughout recent history (Figueras 1991, Cigarria et al. 1995, Culloty et al. 2004, Ling et al. 2005, Lallias et al. 2008, Kamphausen et al. 2011).

Slowed growth, gaping, yellow discoloration, excessive mortality and, in some cases, gill, mantle or digestive gland lesions and ulcers can often be indications that suggest the occurrence of advanced bonamiosis (Bucke & Feist 1985, OIE 2012). However, in the majority of cases, no clinical sign of infection can be observed until the proliferation of the parasite reaches a level whereby massive haemocyte infiltration, diapedesis and death occur (Laing et al. 2005). Once inside an *O. edulis* host, *B. ostreae* divide until the haemocytes they infect rupture, releasing the parasite into the haemolymph. This disease has been correlated with the destruction and infiltration of connective tissue in the mantle, gills and digestive gland (Cochennec-Laureau et al. 2003).

Histological and cytological analysis are the traditional techniques used to confirm the presence of the 2 - 5 µm long protozoans, however polymerase chain reaction (PCR)

protocols can detect small quantities of *B. ostreae* ribosomal DNA (rDNA) (Carnegie et al. 2000, Cochennec et al. 2000). Generally, the first incidences of mortality caused by *B. ostreae* are detected when oysters reach two years of age (Culloty & Mulcahy 1996), however Lynch et al. (2005) have shown that younger individuals at 0 + and 1 + are also susceptible.

Low mean infection levels of 8 % were described at Ryde Middle (Kamphausen 2012), alongside this, recent routine CEFAS surveys, from 1993 - 2007, have also described low mean infection levels of between 0 and 1.4 % within the Solent (Figure 4).

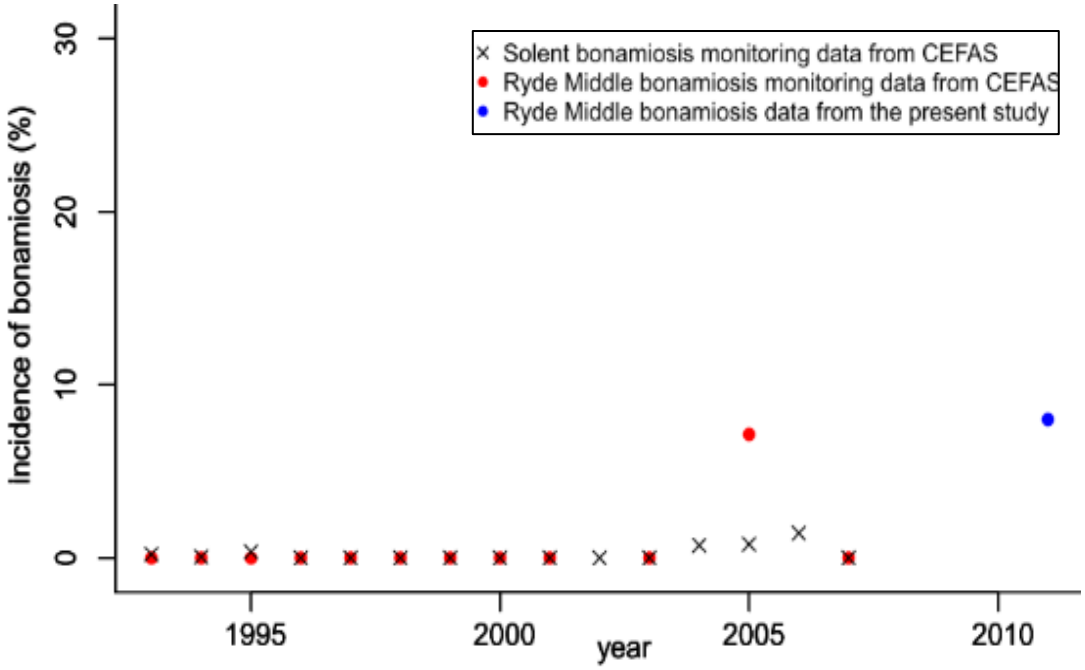


Figure 4. Incidences of *Ostrea edulis* infection with *Bonamia ostreae* within the Solent populations as a whole as well as Ryde Middle, an area within the Solent, measured by CEFAS' Fish Health Directorate (now Fish Health Inspectorate) from 1993 - 2007 and by Kamphausen in 2011, respectively. CEFAS data points were obtained from molecular analysis and haemolymph smears. The Solent data are means of samples sizes varying from 6 - 19 samples of 30 individuals from a range of locations around the Solent and the Ryde Middle data were obtained from a sample of 30 individuals. The Kamphausen data was from molecular analysis conducted on 25 individuals collected in the Ryde Middle area. Source: Kamphausen 2012, edited from original image.

Sawusdee (2015) has indicated that using histo-cytological and single-round PCR techniques may not be sensitive enough to detect very low intensities of infection, and

that nested PCR is required to confirm that individuals are completely uninfected. The study results and its authors suggest that although infection was found within 100 % of the 15 oysters from Poole Harbour, using nested PCR, the infection intensity was low, as the first round of less sensitive PCR provided no positive amplicons (Hauton pers. comm.).

Kamphausen (2012) only sampled oysters > 60 mm from Ryde Middle, therefore if prevalence is low in these larger individuals this would suggest that either, *B. ostreae* is not present in great quantities and that the oysters present within the areas are able to survive with a low prevalence or, that the oysters present have developed a mechanism of extruding large numbers of the parasites, indicating a degree of resistance. These assumptions have been made based on the data that shows higher prevalence of *B. ostreae* in older, higher density populations (Cigarria et al. 1995, Culloty & Mulcahy 1996, Hawkins et al. 2000).

These findings advocate that the recent failures in recruitment can not necessarily be attributed to bonamiosis and, that the low levels of infection within larger oysters may reflect low prevalence within the population, and the Solent as a whole. For this to be confirmed larger sample sizes from multiple locations throughout the Solent would be required.

1.3.5. Pollution

Alongside overfishing and diseases, anthropogenic introduction of pollution and chemical contaminants in the marine environment has also had a significant impact on oysters. TBT was used as a biocide in organotin-based antifouling paints from the 1950's until initial bans began in the 1980's (1987 - Ban on boats < 25 m, 2003 - Ban on European Union (EU) - flagged vessels, 2008 - Regulation prevents ships with TBT on hulls entering EU

ports). Irrespective of the bans, TBT remains one of the most potent chemicals present within harbour environments, with Champ et al. (1996) showing that TBT can remain within the marine environment for up to 30 years. There is an array of detrimental impacts associated with TBT, including masculinisation, digestive cell atrophy (Axiak et al. 1995), shell thickening (Axiak et al. 1995, 2000), lack of gonadal maturation (Thain et al. 1986), inhibition of larval production (Thain et al. 1986), reductions in growth rates, and, potentially, reductions in reproductive capability (Rees et al. 2001, Laing et al. 2006). Note: shell thickening may be difficult to distinguish from natural selection for a thicker shell to prevent natural predation by whelks or oyster drills.

The known effects of TBT will be particularly relevant to the proposed studies, as much of the sediment within Portsmouth Harbour will contain historic deposits that are likely to become re-suspended by the dredging work that is taking place (MOD & DIO 2015). The study by Rees et al. (2001) has, however, shown that populations of epifaunal species, including oysters, in the Crouch Estuary can recover and have been improving since the ban of TBT on small vessels in 1987.

1.4. Maintenance and capital dredge projects

As an extremely busy naval, commercial and recreational body of water, Portsmouth Harbour requires periodic maintenance dredging. In order to accommodate the arrival of HMS Queen Elizabeth, a Capital Dredge will be carried out from the end of 2015 into 2016 (MOD & DIO 2015). Aside from the physical damage caused to any oysters in the dredge area and the issues highlighted in section 1.3.5., the potential impacts of this dredging include smothering and burial of the oysters when the increase in suspended sediment settles, accumulation of metals as seen with the Sydney Rock Oyster, *Saccostrea glomerata* (Hedge et al. 2009), disturbances in spawning, fertilisation,

distribution of larvae and settlement behaviour. Determining the full extent and the range of impacts that will occur as a result of the capital dredging is outside the scope and remit of this project.

1.5. Current feasibility studies

Due to the long term bombardment of, the previously mentioned, detrimental influences, *O. edulis* has been named one of the nineteen species to be included within the UK Biodiversity Action Plan (UKBAP) (UKBAP 1999) and within this the Native Oyster Species Action Plan (NOSAP) was created (Hawkins et al. 2005). The plan aims to maintain and expand existing geographic distributions and abundances of the native oyster.

Oyster restoration feasibility studies have been conducted at various locations in the UK including South Wales (Woolmer et al. 2011), Northern Ireland (Strangford Lough) (Kennedy & Roberts 1999, Roberts et al. 2005), Shetland (Shelmerdine & Leslie 2009), Chichester (Vause 2010, Eagling 2012, cited in Gravestock et al. 2014), in the Solent (Key & Davidson 1981, Gravestock et al. 2014), the UK as a whole (Laing et al. 2005) and, most recently, a regeneration project begun in June 2016 in Milford Haven (Burton pers. comm.).

Due to the reproductive nature of this species fertilisation success settlement, growth and survival are positively influenced by high population densities (Lenihan & Peterson 1998, Lenihan et al. 1999, Serrão & Havenhand 2009). Vause (2010) and Eagling (2012) estimated that healthy beds containing 40 mature oysters m^{-2} are required to allow successful recruitment of veliger larvae. With regards to restoring populations, extremely high densities of 250 oyster m^{-2}, can actually result in adverse responses such as reduced

growth, weight and condition (Jackson 2007). At the other end of the scale poor recruitment can be an issue in low density populations (Lancaster et al. 2014), related to the Allee effect. It is therefore essential to consider the optimal quantities of broodstock oysters to be relayed within the areas in question.

1.6. Impact overview

Taking into consideration all of the known detrimental impacts, previously mentioned and summarised in Figure 5, as well as Appendix A (OSPAR Commission 2009) , it can be determined that the natural population within the Solent is struggling to survive and that without anthropogenic influence and intervention the species could become absent from the area in the not too distant future.

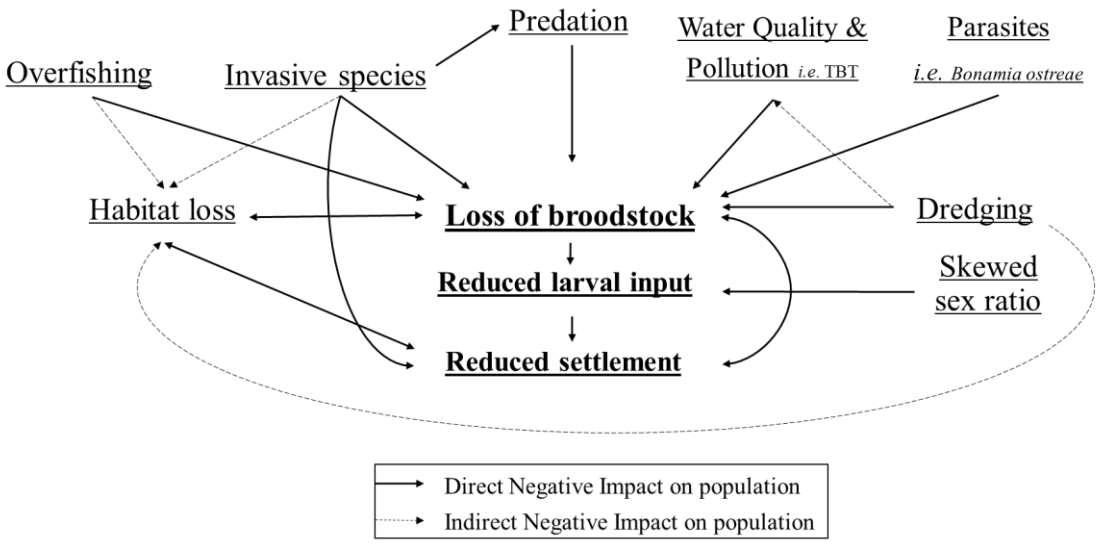


Figure 5. Illustration of the known detrimental impacts that are influencing the population of *Ostrea edulis* within the Solent and the interconnecting relationships between impacts.

1.7. Aims

The aims of the study are summarised in Table 2.

Table 2. Synopsis of the project aims.

The initial section of the study focuses on the conduction of a comprehensive oyster stock assessment from Chichester and Portsmouth Harbours, to determine their current state by: 1. Analysis of size class data, to estimate the age class structure (cohorts) of each harbour; 2. Determining the current *B. ostreae* prevalence in each of the harbours and; 3. Assessing the various attachment points present on oysters collected as an indication of the substrata utilised for settlement.
The collection of these oysters would allow for the selection of suitable broodstock populations for the main section of the study. The feasibility study, which is the main section, and aims to focus on protected broodstock cages containing mature oysters, as a potential source of larvae to contribute to the repopulation of the flat oyster within the Solent. If successful, the system will be replicated across the Solent as part of a broodstock scheme attached to various marinas. Therefore, the aims are: 1. Trial suspended, off bottom, cage designs to provide optimal environments attached to existing floating structures; 2. Determine the survivorship, growth and sexual maturation of high and low density populations of *O. edulis*; 3. To develop of a system of cages that contain optimal densities of oysters at tolerable depths and that are easily manageable.
The final study section will focus on both caged and natural populations with the aim of: 1. Determining timing and extent of the broodstock population sexual maturation and spawning in relation to various water parameters; 2. Determining veliger larval abundances within the surface water of study areas; 3. Determining settlement surface preferences.

2. Methods

2.1. Current fishery population structures of Chichester and Portsmouth Harbours

2.1.1. Sampling and study areas

Over 3,000 oysters from Chichester Harbour (CHI) (Figure 6) and Portsmouth Harbour (PORT) (Figure 7) fisheries, provided by the Southern IFCA, were delivered to the Institute of Marine Sciences (IMS) (Figure 12a) on 9th and 27th November 2015. A random selection of individuals (CHI n = 50, PORT n = 50. Total n = 100) were removed from the overall stock, cleaned and frozen at -20°C (CHI 21/01/16, PORT 26/11/15) for later condition indexing, as well as histological and molecular analysis. The remaining stocks were placed into two large holding aquaria with a flow through system to maintain a fresh supply of unfiltered sea water from Langstone Harbour.

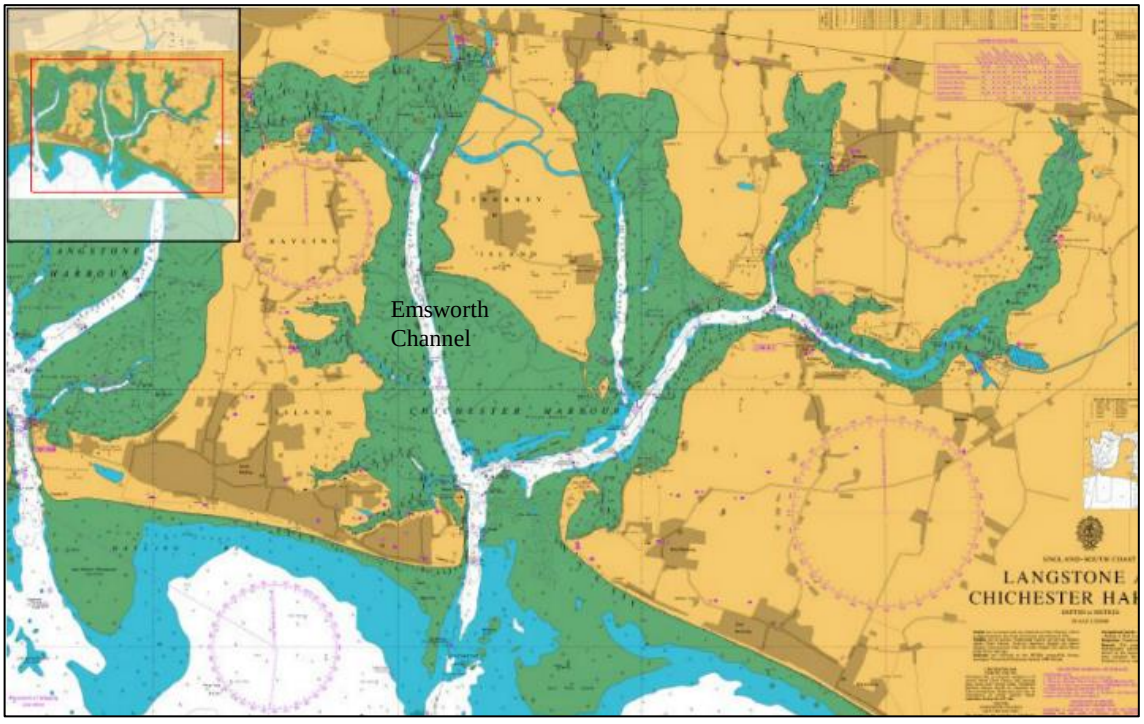


Figure 6. Chichester oyster fishery population collection site, the Emsworth Channel. Exact location was not provided. Source: Chichester Harbour Conservancy 2016.

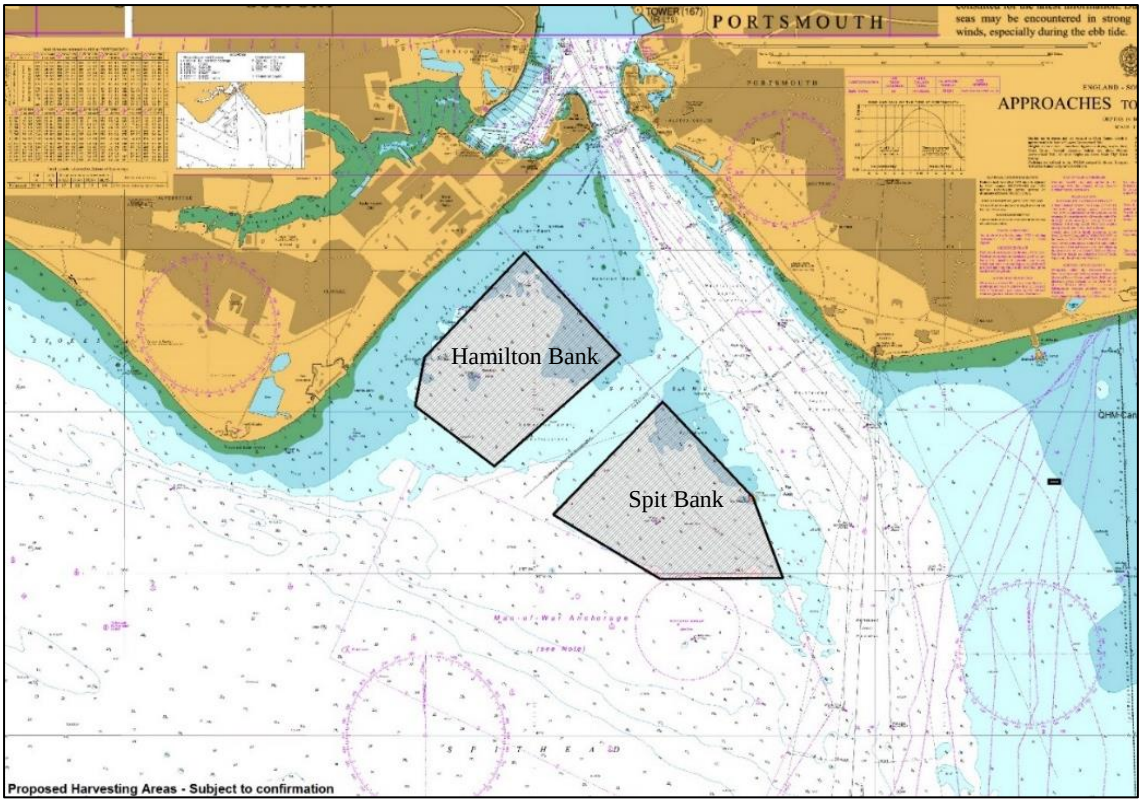


Figure 7. Portsmouth oyster fishery population collection sites, Hamilton Bank and Spit Bank. Source: Cooper pers. comm., Southern Inshore Fisheries and Conservation Authority (Southern IFCA).

2.1.2. Fishery Population comparisons

2.1.2.1. Morphometric measurements

Random samples from both locations (CHI n = 500, PORT = 500. Total n = 1000) had fouling organisms removed from the valves and were blotted dry, using paper towel. Morphometrics (Figure 8a) and attachment point, noted as the remains of organisms or material around the umbo area of the ventral valve (Figure 9), were then recorded.

Following this, further random samples of individuals from both locations (CHI n = 200, PORT n = 200. Total n = 400) underwent the same procedure, with the additional measurement of maximum shell depth (mm) (Figure 8b).

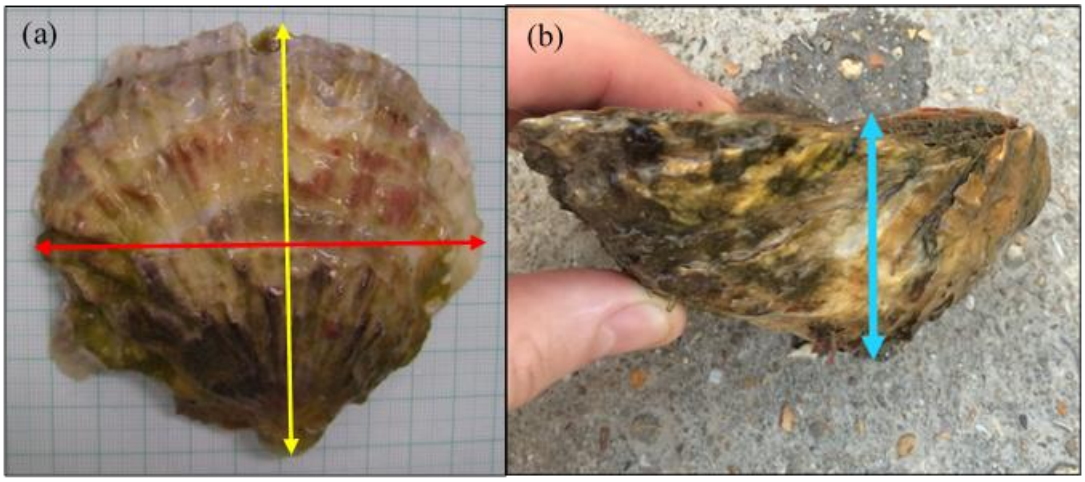


Figure 8. Morphometric measurements conducted on the fishery oyster populations of Chichester Harbour and Portsmouth Harbour. (a) Maximum length, taken as the greatest distance from the base of the umbo to the top of the shell margin (yellow arrow). Maximum width, taken as the greatest distance across the shell from anterior to posterior margins (red arrow). (b) Maximum depth (mm) taken as the greatest distance between the two valves (blue arrow).

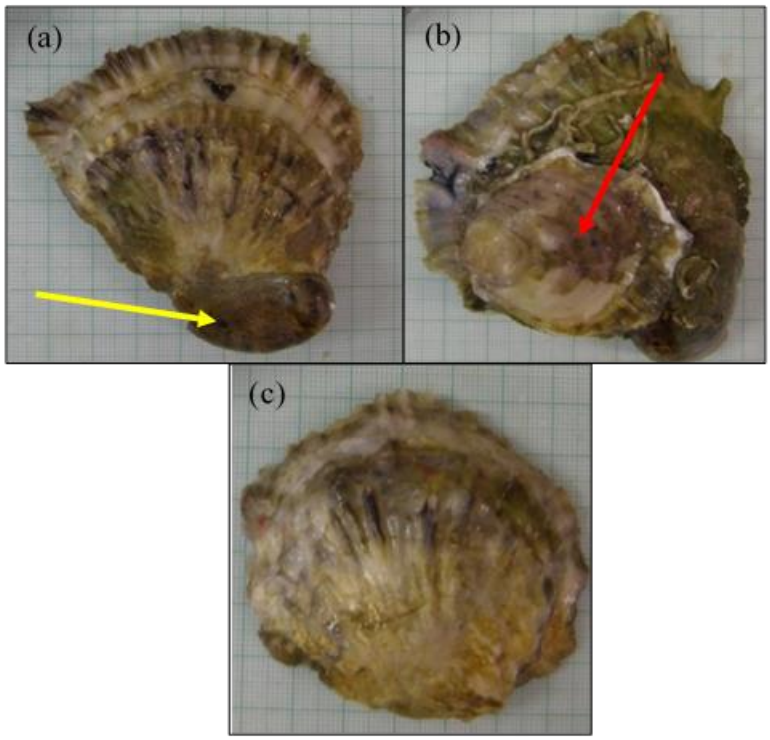


Figure 9. Attachment points observed on oysters used during morphometric measurements. (a) *Crepidula fornicata* shell (yellow arrow). (b) *Ostrea edulis* (red arrow) used as attachment point on the ventral valve. (c) No observed attachment point.

2.1.2.2. Condition Index (CI)

A proportion of the oysters stored at -20°C (CHI n = 24, PORT n = 24. Total n = 48) were removed, defrosted and morphometric data was recorded, as described in section 2.1.2.1. The oysters were prized open using an oyster knife and a small section of gill tissue was removed for molecular analysis, see section 2.1.2.3.

Condition index was performed according to the methodology conducted by Culloty et al. (2004, p45) with alterations to the temperature of, and duration in, the oven (105°C for 24 h, opposed to 60°C for 48 h). The calculation used by Walne & Mann (1975) and Lucas & Beninger (1985) (cited in Culloty et al. 2004) was used to determine condition index:

$$Condition\ index = \frac{DTW}{DSW} \times 100$$

where

DTW = Dry Tissue Weight (g)

DSW = Dry Shell Weight (g)

2.1.2.3. *Bonamia ostreae* prevalence: DNA extraction, polymerase chain reaction (PCR) and gel electrophoresis

In addition to the 48 oysters from section 2.1.2.2., morphometrics were recorded and gill tissue obtained, from a further 48 oysters stored at -20°C (CHI n = 24, PORT n = 24, second sample total n = 48. Overall total n = 96). The extraction of genomic DNA from an aliquot of the gill tissue was conducted by spin column purification using DNeasy® Blood & Tissue kits (QIAGEN™ product 69504) following the tissue protocol provided by the manufacturer (Qiagen 2006). Quantification of the DNA extractions was conducted using a NanoDrop® 1000 Spectrophotometer (NanoDrop®, Thermo Fisher Scientific Inc., Wilmington, USA).

Universal and species specific primers (LCO1490 / HCO2198 (Folmer et al. 1994) and Oe fw_1 / Oe rev_4 (Grecken & Schmidt 2014) (Table 3) were used to amplify the cytochrome c oxidase subunit I (COI) gene from the oyster DNA (Figure 10), as a positive control.

Family and species specific primers (BO / BOAS (Cochennec et al. 2000) and C_F / C_R (Carnegie et al. 2000, Figure 11) (Table 3) were used to amplify fragments of the nearly complete small sub unit (SSU) of 18S rDNA from all microcell members of the family *Haplosporidiidae* and *B. ostreae* specifically.

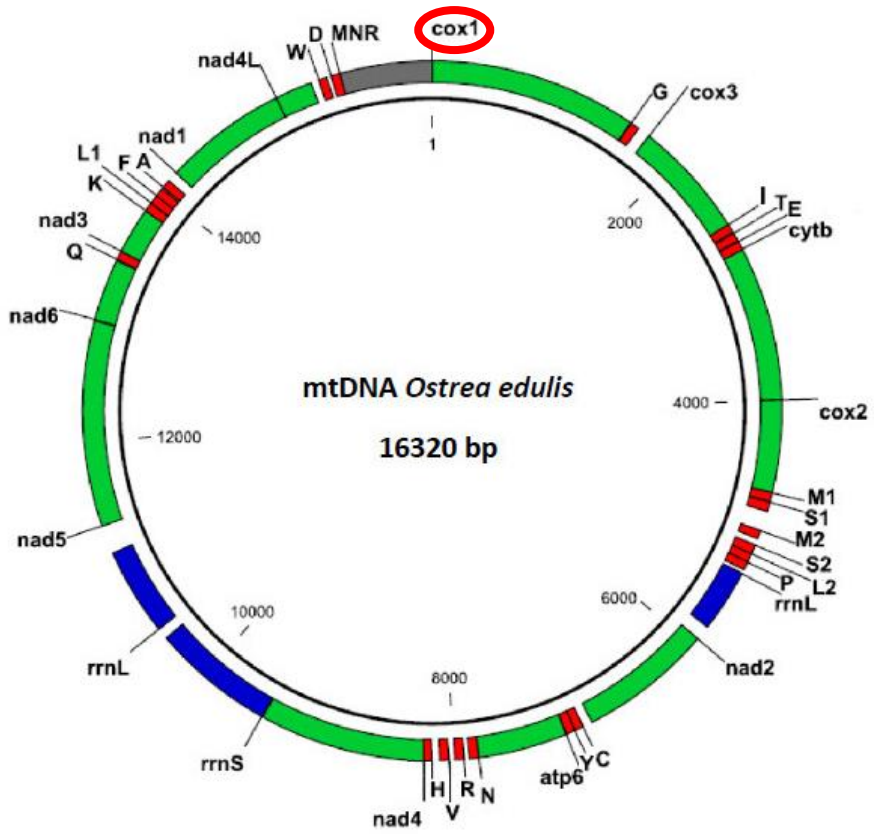


Figure 10. *Ostrea edulis* mitochondrial genome map. The COI gene location can be seen highlighted at the top of the image. Protein gene sequences are in green and rRNAs are in blue. Source: Danic-Tchaleu et al. 2011.

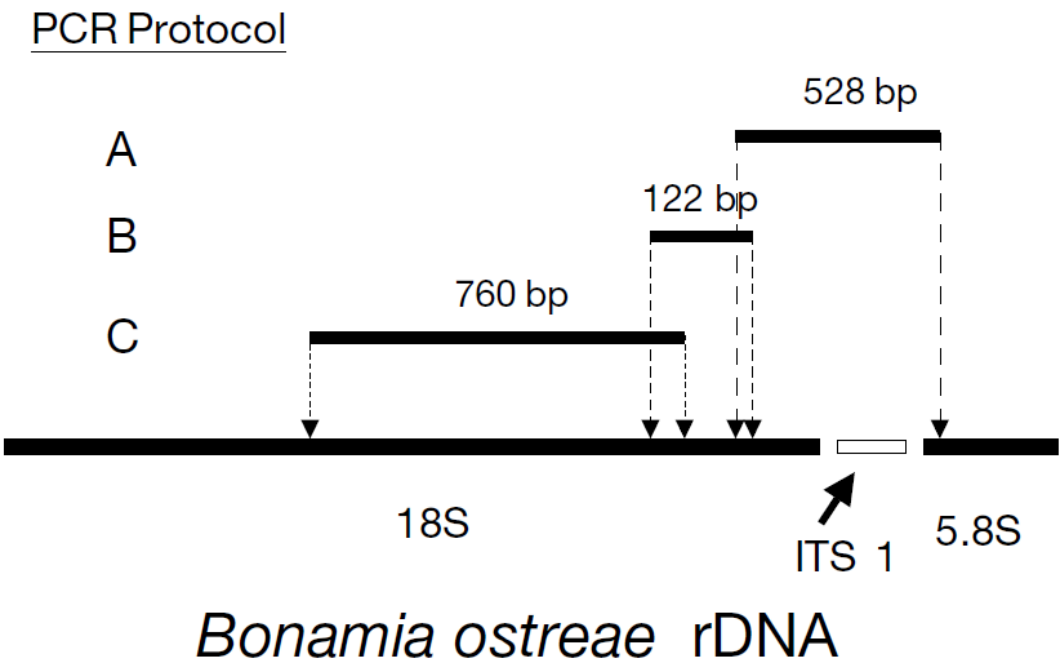


Figure 11. PCR protocols used by Carnegie et al. (2000) to detect *Bonamia ostreae* 18S rDNA, protocol C shows the 760 base pair section amplified by the primers C_F and C_R. Source: Carnegie et al. 2000.

Table 3. Universal and species specific PCR assays designed by Folmer et al. (1994) and Grecken & Schmidt (2014) to amplify the cytochrome c oxidase subunit I (COI) gene of *Ostrea edulis*, as a positive control. Family and species specific PCR assays designed by Cochennec et al. (2000) and Carnegie et al. (2000) to amplify fragments of the nearly complete small sub unit (SSU) of 18S rDNA from all microcell members of the family *Haplosporidiidae* and *Bonamia ostreae* specifically. Detailed are the forward and reverse primer DNA sequences, annealing temperature (T_m) and the expected amplicon base pair size for various metazoan invertebrates, *Ostrea edulis*, microcell Haplosporidians and *Bonamia ostreae*, respectively.

Name	**Primer sequences**	**T_m (°C)**	**Amplicon size (bp)**
LCO1490	5'-GGT-CAA-CAA-ATC-ATA-AAG-ATA-TTG-G-3'	46	~ 650
HCO2198	5'-TAA-ACT-TCA-GGG-TGA-CCA-AAA-AAT-CA-3'	48	
Oe fw_1	5'-ATG-GGA-CGA-TTT-GAT-AGA-GC-3'	45	~ 1100
Oe rev_4	5'-CCC-AAA-TAA-CGG-GAA-AAG-TGC-TAA-CCA-CCA-GAA-TGA-3'	59	
BO	5'-CAT-TTA-ATT-GGT-CGG-GCC-GC-3'	55	300
BOAS	5'-CTG-ATC-GTC-TTC-GAT-CCC-CC-3'	55	
C_F	5'-CGG-GGG-CAT-AAT-TCA-GGA-AC-3'	59	760
C_R	5'-CCA-TCT-GCT-GGA-GAC-ACA-G-3'	59	

Polymerase chain reaction (PCR) amplifications were all conducted in a final volume of 25 μl, consisting of 12.5 μl 2 x DreamTaq™ PCR Master Mix (Thermo Fisher Scientific Inc.), 0.2 μM forward and reverse primers, 1 - 5 μl extracted total DNA (20 - 200 ng), and 6.5 – 10.5 μl molecular H_2O. The PCR program ran in a G-STORM 482 - 48 Well Multi Block Thermal Cycler (Gene Technologies Ltd., Essex, England) as follows:

For the LCO1490 / HCO2198 and Oe fw_1 / Oe rev_4 primers:

- Initial denaturation for 5 min at 94°C;
- Denaturation at 94°C for 60 s;
- Primer annealing at 45°C for 60 s;
- Polymerase extension at 72°C for 60 s;

  } 35 Cycles
- Finishing with a final primer extension step at 72°C for 10 min.

For the BO / BOAS and C_F / C_R primers:

- Initial denaturation for 5 min at 94°C;
- Denaturation at 94°C for 60 s;
- Primer annealing at 55°C for 60 s;
- Polymerase extension at 72°C for 60 s;

  } 35 Cycles
- Finishing with a final primer extension step at 72°C for 10 min.

PCR products were loaded onto 1 % agarose gels (Fisher Scientific, UK) composed of 100 ml 1X Tris-acetate-EDTA (TAE) buffer and 4 μl ethidium bromide (Sigma-Aldrich®). Electrophoresis was conducted at 95 - 100 V for 45 min - 1 h, following this the samples were visualised by ultraviolet (UV) transillumination in a 'VWR® Gel Documentation Smart Version system', products were initially compared against a GeneRuler™ 100 base pair DNA ladder (Thermo Fisher Scientific Inc.). Upon finding the amplicon size of the Oe fw_1 / Oe rev_4 primers to be ~1100 bp, a GeneRuler™ 1kb DNA ladder (Thermo Fisher Scientific Inc.) was subsequently used.

2.2. Langstone and Portsmouth Harbour broodstock population study

2.2.1. Experiment locations and sample populations

Two existing floating structures were selected, the University of Portsmouth Research Raft (UoPRR) (50°48'23.73"N, 1°1'20.56"W), located in Langstone Harbour (Figure 12a & Figure 12c) and the pontoon at Land Rover Ben Ainslie Racing (LRBAR) (50°47'32.52"N, 1°6'25.93"W), located in Camber Dock, which is situated within Portsmouth Harbour (Figure 12a & Figure 12b). The three broodstock cage structures attached to the pontoon at LRBAR were kindly provided MDL Marinas.

Oysters bound for the UoPRR were selected for size (60 – 90 mm shell length and width, and 20 – 40 mm shell depth) and good general health, from both the CHI and PORT populations (Figure 6 & Figure 7). These oysters were placed in cages (section 2.2.2.) and deployed on 11th December, 2015. For the time prior to deployment, these populations were stored at the IMS (Figure 12a & Figure 12c) in large holding tanks with a flow through system to maintain a fresh supply of unfiltered sea water from Langstone Harbour. Those placed in cages at the LRBAR pontoon were sourced from Hamilton and Spit Bank (PORT) only (Figure 7). Initially stored in bags suspended from the pontoon, individuals were then sorted, as previously mentioned, and deployed in cages on 14th December, 2015.

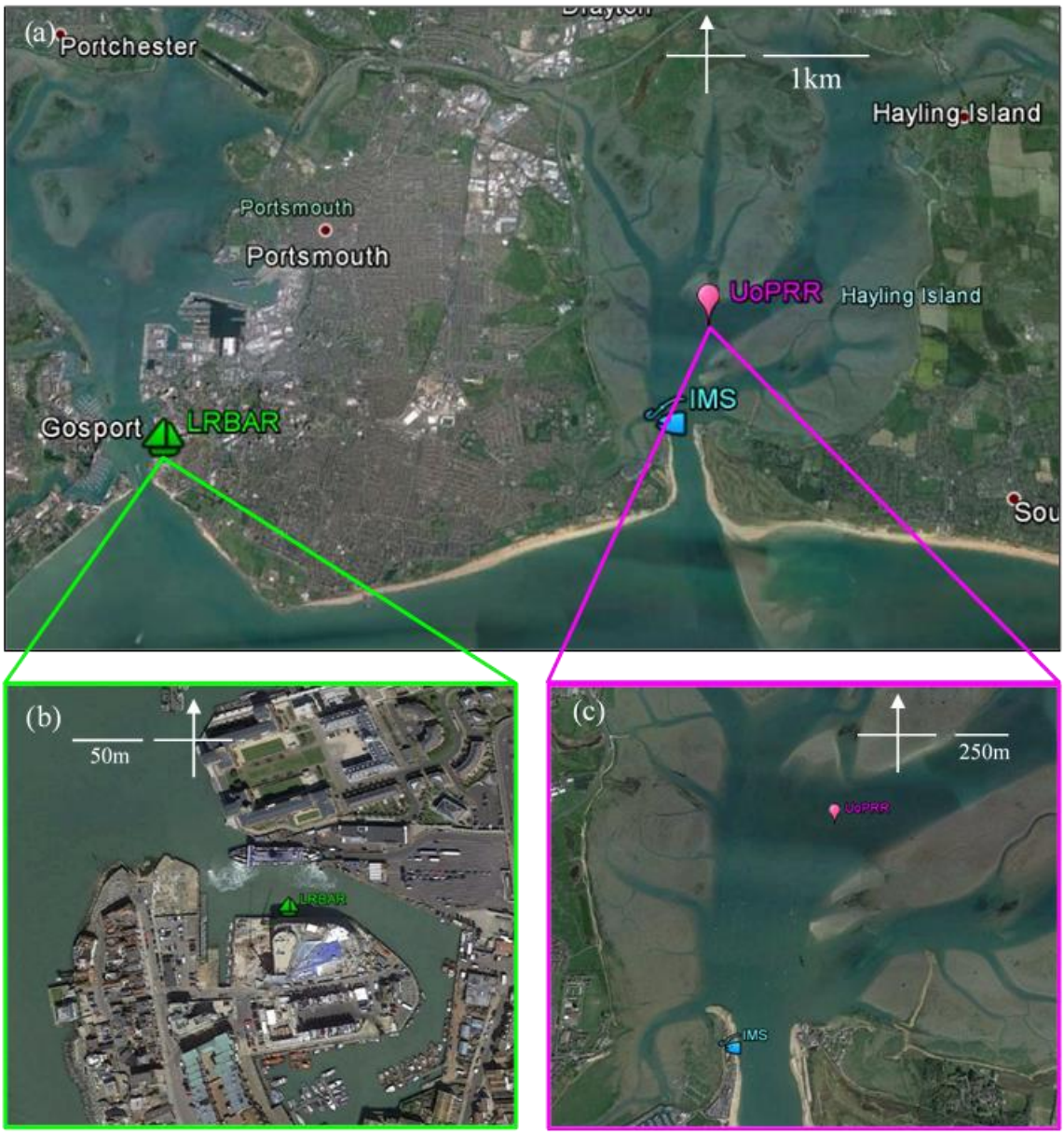


Figure 12. Google Earth image detailing the experimental locations for the protected broodstock cages. (a) Both the Land Rover Ben Ainslie Racing (LRBAR) pontoon (50°47'32.52"N, 1°6'25.93"W), in green, and the University of Portsmouth Research Raft (UoPRR) (50°48'23.73"N, 1° 1'20.56"W), in purple, are shown in relation to the location of the Institute of Marine Sciences (IMS) (50°47'40.07"N 1°1'47.60"W). (b) The LRBAR pontoon located in Camber Dock, which is situated within Portsmouth Harbour, is exposed to large changes in water depth with tide, low to moderate tidal flow rates, low to moderate wind energy, low to moderate wave energy, and periodic moderate wave energy from arriving and departing ferries on the opposite area of the dock (seen in image). (c) The UoPRR is anchored within the Langstone Channel, which is roughly central in Langstone Harbour. The site is exposed to large changes in water depth with tide, extreme tidal flow rates, high wind energy, moderate to high wave energy, and low wave energy from passing boats. In comparison to the LRBAR pontoon, this location can be described as exposed.

2.2.2. Cage design, population densities and recording

Broodstock cages (Figure 13a), provided by MDL Marinas, were constructed from 1" square Aquamesh®. Three cages were attached forming a single stack (Figure 13b). Two oyster population densities were tested, high density (cage oyster n = 50, stack oyster n = 150) and low density (cage oyster n = 25, stack oyster n = 75), and repeated three times at each location (each site: cage n = 18, stack n = 6). Cages were suspended at depths of 35 - 90 cm (top cage: 35 - 50 cm, middle cage: 55 - 70 cm and bottom cage 75 - 90 cm). Those initially on the UoPRR were suspended on a rope system (Figure 13c), whilst those at the LRBAR pontoon were attached to a specially designed pontoon structure (Figure 13d). After a period of extreme weather during April and March, 2016, three high density cages and one low density cage were lost from the UoPRR and replaced in June in a similar set up to those at the LRBAR pontoon. Within each cage a sub sample of oysters (n = 7) were marked using nail varnish (Figure 13a) or spray paint (Figure 14). On a monthly basis, from January to July, the morphometrics of these individuals were measured, as described in section 2.1.2.1. (including maximum depth, Figure 8). Total mortality, within each cage, was also recorded monthly with any deceased oysters replaced to maintain densities. Broodstock cages inevitably experienced fouling (Figure 15) which was removed periodically when possible.

Figure 13. (a) Marked oysters within a single high density broodstock cage. (b) Oysters within high density broodstock cages, three tier system forming a single stack. (c) Cages suspended on rope system at the University of Portsmouth Research Raft. (d) Oyster cages being placed into one of the research pontoons on the Land Rover Ben Ainslie Racing Pontoon.

Figure 14. Oysters marked using enamel spray paint with corresponding letter carved into dorsal valve (not visible in image). A total of seven colours were used to identify seven individuals in each cage on a monthly basis. Colours included white, pink, yellow, green, blue, red and metallic silver.

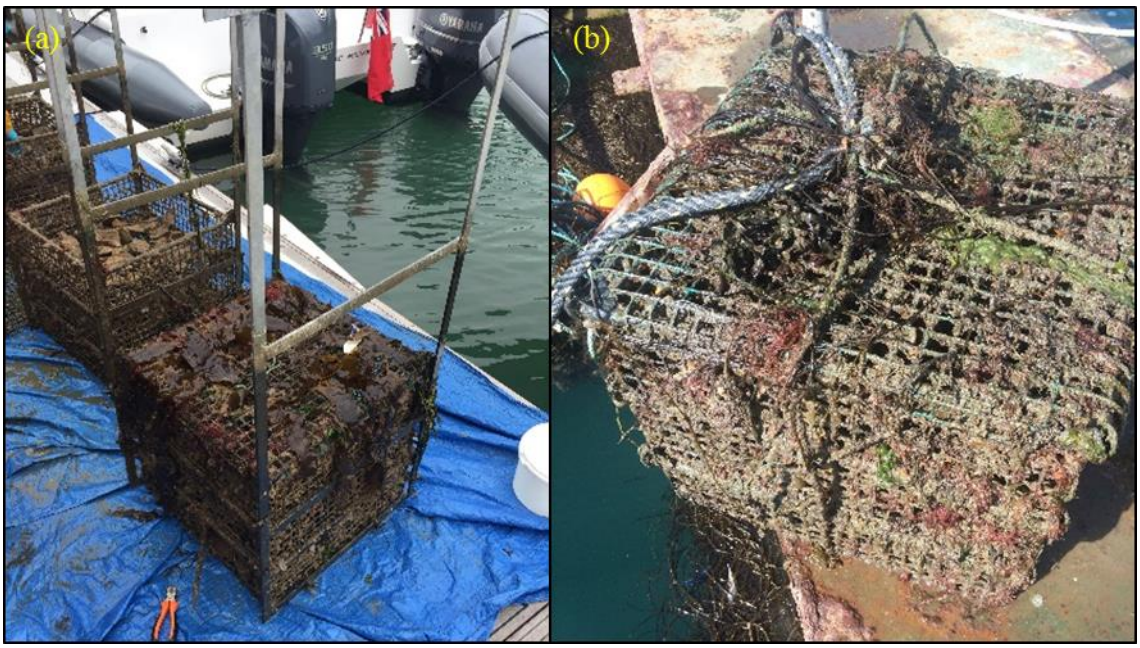


Figure 15. Fouling experienced on the broodstock cages at (a) the LRBAR pontoon during July 2016 (b) the UoPRR during August 2016.

2.2.3. Assessment of broodstock reproductive state

Subsequent to their final measurements on 27th and 28th July 2016, individuals that were marked and monitored for the broodstock cage experiment, section 2.2.2., were removed from the cages and transferred to the laboratory at the IMS. Those within the top cage of each stack were reserved for heavy metal analysis (LRBAR n = 42, UoPRR n =16). The contents of the pallial cavity from the marked oysters in the middle cages was pooled, as well as this, a 5 mm section of gonadal tissue was retained from those for brooding state and gonadal analysis (LRBAR n = 41, UoPRR n = 19). Individuals in the bottom cages were retained for condition index (LRBAR n = 42, UoPRR n = 16) and molecular analysis of *B. ostreae* prevalence (LRBAR n = 42, UoPRR n =16).

2.3. Larval assessment within the water column

2.3.1. Plankton tows and larval abundances

Plankton tows were conducted in surface waters on a weekly basis between April and August, from both Langstone Channel (Figure 12c) and Hamilton Bank (Figure 7), at high or low water ± 1 h. Three, 1 minute replicates were collected at each location using a plankton net with a diameter of 30 cm and a mesh size of 63 μm. Samples were placed into separate plastic containers and transferred to the laboratory at the IMS, where each sample was separated into two sub samples using a plankton splitter. One sub sample was fixed in 4 % borax buffered formalin, containing rose bengal, for analysis by microscopy. For analysis, known volumes of the sub samples were transferred to a Bogorov tray and all larvae present were noted to provide the community composition. *O. edulis* larval densities were also noted separately to this. The second sub sample was fixed in 70 % ethanol for quantitative PCR in future studies.

2.3.2. Settlement plates

Examination of the relevant literature provided numerous considerations to take into account such as geographic location, depth of collectors, temperature, water movement and other fouling species (Burke et al. 2008, cited in Van den Brink 2012). The literature also presented a diversity of experimental design ideas, taking into account factors such as settlement plate material, method of deployment and removal of settled spat (Korringa 1952, Yonge 1960, Kennedy 1999, Sawusdee 2015, Sawusdee et al. 2015) (Freeman & Denny 2003, Kamermans et al. 2004, Bataller et al. 2006, Nalesso 2008, cited in Van den Brink 2012).

"*Collectors should ideally be effective, inexpensive, reusable and easy to deploy in the sea. In addition, the young spat should be able to be easily detached from the surface for further breeding*" (Grecken & Schmidt 2014, p 22).

The settlement surfaces selected were modified from the University of Gothenburg "*Virtue: A science school project for children and young adults*", which consists of clear plastic CD disks arranged vertically with plastic spacers (University of Gothenburg 2013). A total of 15 disks were prepared for each location with five alternative substrata present on each structure, repeated three times and suspended between 0.8 - 1.8 m (Figure 16 & Figure 17a). In addition to this corrugated plastic tubing (Figure 17b), dipped in a cement mixture, and mesh bags containing oyster valves (Figure 17c) were also suspended at both the UoPRR and the LRBAR pontoon (Figure 12) at depths of 80 to 180 cm, in a similar manner to the cultch bags used by Kamermans et al. (2004) and Bataller et al. (2006).

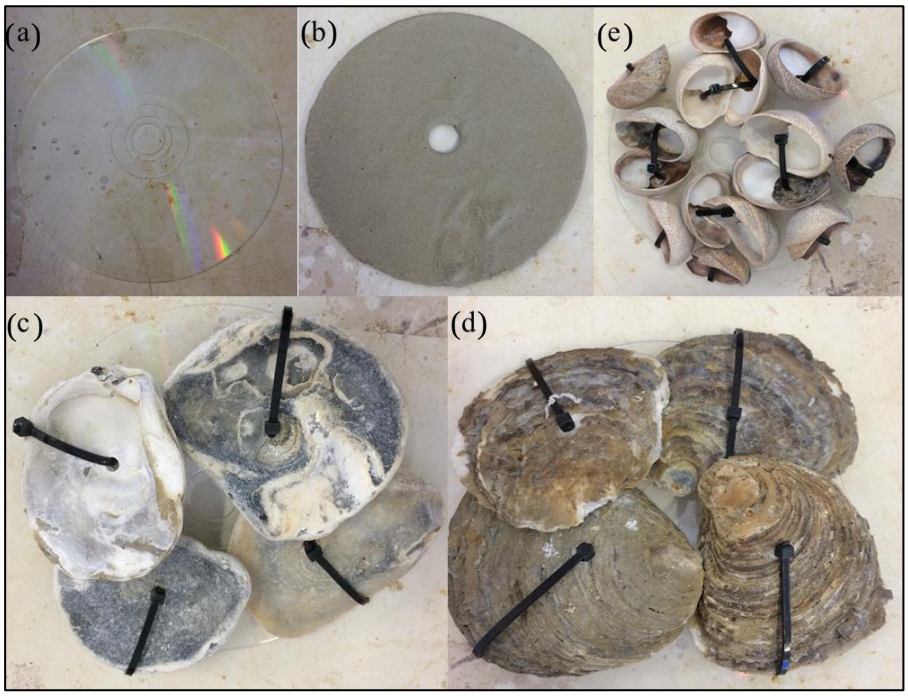


Figure 16. Settlement substrata used as settlement plates (a) Blank Virtue disk (b) Virtue disk dipped in cement (c) *Ostrea edulis* valves collected from the beach by the Institute of Marine Sciences (IMS) and attached to a blank Virtue disk (d) Recently deceased oyster valves collected from holding tanks at the IMS attached to a blank Virtue disk (e) *Crepidula fornicata* shells collected from the beach by the IMS attached to a blank Virtue disk. Figure 17a shows the final arrangement of all 5 substrata.

Figure 17. (a) The final arrangement of the 15 disks with the various substrata, described in Figure 15. (b) The arrangement of the corrugated plastic tubing before it was submerged in a cement mixture. (c) The arrangement of the mesh bags containing oyster valves on the University of Portsmouth Research Raft.

2.4. Abiotic water parameters

A multiparameter EXO2® Sonde (YSI Inc.) was deployed at the LRBAR pontoon from 12/02/16 – 29/05/16, then 01/07/16 - 27/07/16. A second EXO2® Sonde (YSI Inc.) was deployed on the UoPRR from 12/02/16 – 08/03/16, then 24/03/16 – 02/06/16, and between 23/06/16 - 28/07/16. The dates between deployments were due to removal for maintenance and unforeseen use elsewhere. Measurements of depth (cm), pressure (psi), temperature (°C), pH, conductivity (μS/cm), specific (sp) conductivity (μS/cm), non-linear function (NLF) conductivity (μS/cm), salinity (psu), total dissolved solids (TDS) (mg/L), chlorophyll *a* (RFU and μg/L), blue-green algae - phycocyanin (BGA-PC, RFU and μg/L) optical dissolved oxygen (ODO, % saturation and mg/L) and turbidity (NFU) were recorded every 15 minutes.

2.5. Statistical analysis

2.5.1. Current fishery population structures of Chichester and Portsmouth Harbours

2.5.1.1. Size class cohorts

Cohorts were estimated, from the maximum shell length of the 700 oysters measured from each population, by NORMSEP analysis using FAO-ICLARM Stock Assessment Tools II (FiSAT II). NORMSEP method "*applies the maximum likelihood concept to SEParation of the NORMally distributed components of size-frequency samples*" (Gayanilo 2005, p72).

2.5.1.2. Condition index

Data were tested for homogeneity of variance using Levene's test and were found to be 'normal' (F = 0.869, p > 0.05). As a result, the data were analysed using a one-way ANOVA. Statistical analysis was performed in IBM® SPSS® Statistics 22 (IBM Analytics)

2.5.2. Broodstock cage survivorship and growth

2.5.2.1. Broodstock oyster survivorship

Percentage mortality data from caged broodstock oysters on the UoPRR and LRBAR pontoon were analysed using the multivariate statistical analysis package, Plymouth Routines in Multivariate Ecological Research (PRIMER, version 6 Clarke & Gorley 2006). Percentage data were arcsine transformed, and differences of oyster mortalities between each location were tested using a Bray-Curtis similarity matrix (Bray & Curtis

1957). A Principle Component Ordination (PCO) was used to test the differences between the two locations, using cage replicates, cage density and cage position as factors. A PERMANOVA main test was used to test the differences between the total percentage mortalities at both locations. A PERMANOVA pairwise test was used to test the differences between percentage mortalities of the two densities at, and between, both locations. The PERMANOVA tests were based on 4999 permutations.

2.5.2.2. Broodstock oyster growth

Due to the large number of oysters lost from the UoPRR broodstock cages, the growth data from the individuals was not sufficient to allow statistical analysis, therefore multivariate analysis for growth was only conducted on the LRBAR population. Monthly change in growth (weight (g)) data were analysed using PRIMER (version 6 Clarke & Gorley 2006). Growth data were square root transformed, and the differences in oyster growth between top, middle and bottom cages were tested using a Bray-Curtis similarity matrix (Bray & Curtis 1957). A PCO was used to test the differences between the growth in each position (top, middle and bottom) using cage replication, cage density and cage position as factors. A PERMANOVA main test was used to test the differences between the growth in each position, and was based on 4999 permutations.

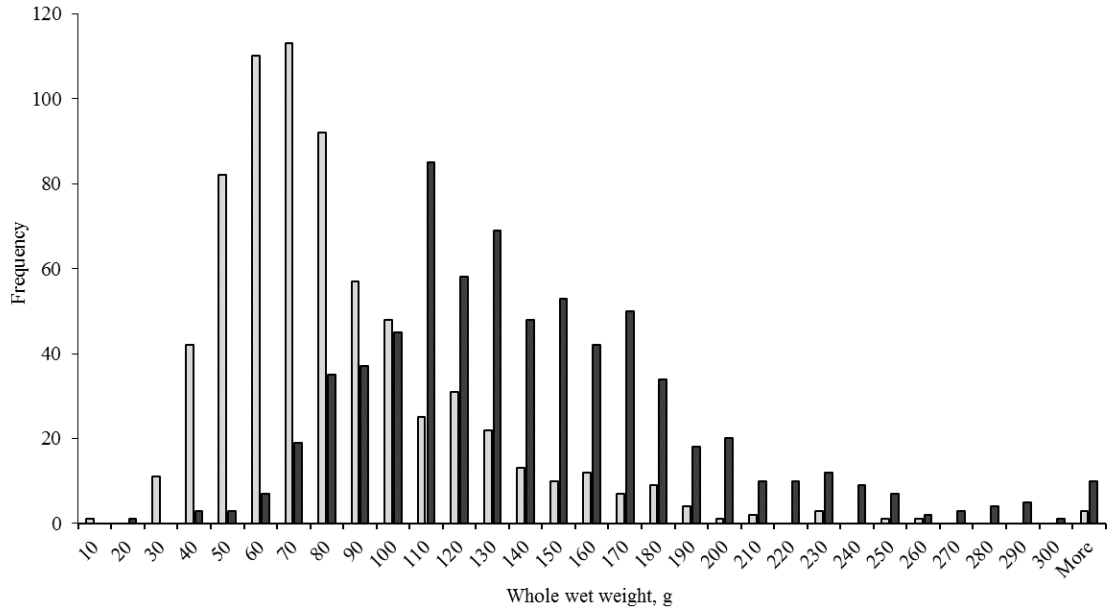
3. Results

3.1. Current fishery population structures of Chichester and Portsmouth Harbours

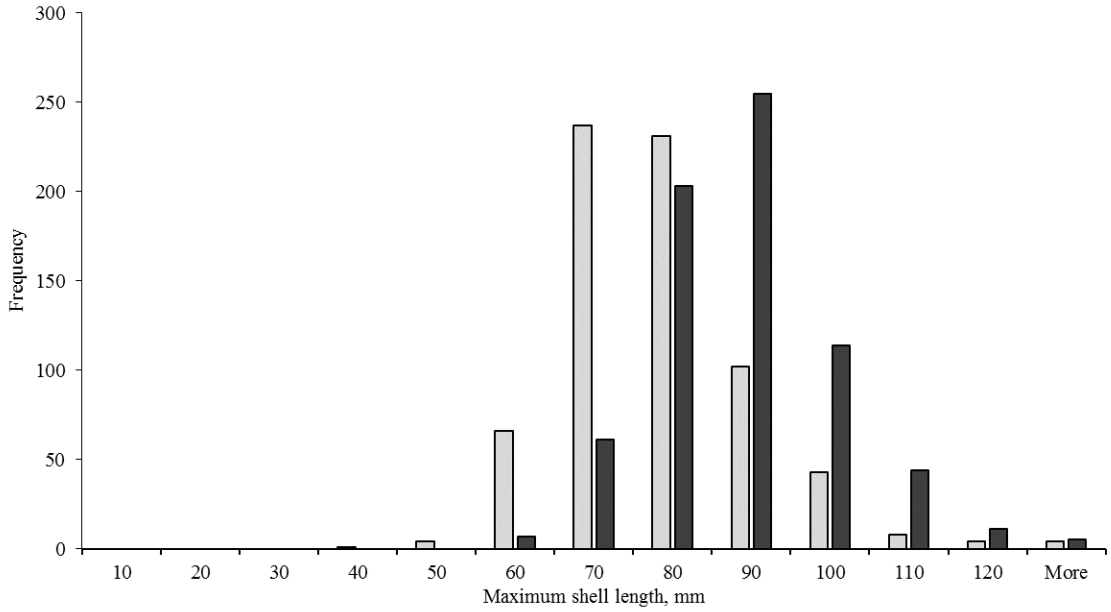
3.1.1. Morphometrics

There was a difference of 40 g between the most frequent wet weight groupings, the Portsmouth population's most frequent weights were between 110 - 120 g and the most frequent weights of the Chichester population were between 70 - 80 g (Figure 18a). There were also differences of 20, 0 and 17 mm between the most frequent groupings for maximum shell length, width and depth (Figure 18b, c & d). The Portsmouth population most frequent length, width and depth groupings were between 90 - 100, 80 - 90 and 35-36 mm, respectively. In comparison, the Chichester population dominant length, width and depth groupings were 70 - 80, 80 - 90 and 18 - 19 mm, respectively.

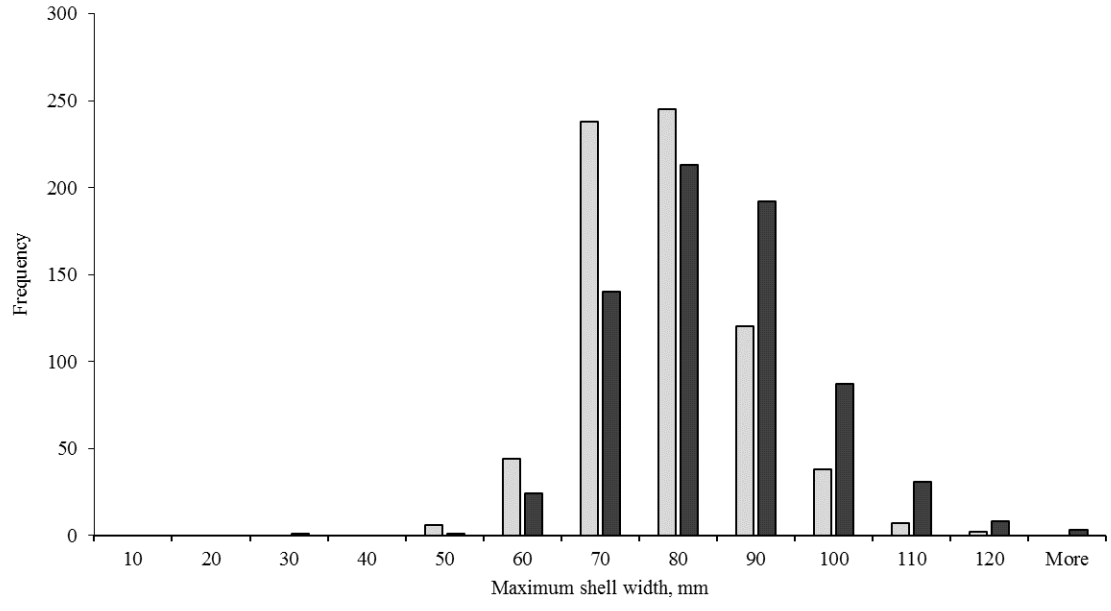
(a)



(b)


300
250
200
150
100
50
0
Frequency
10
20
30
40
50
60
70
80
90
100
110
120
More
Maximum shell length, mm

(c)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More
Maximum shell width, mm

(d)

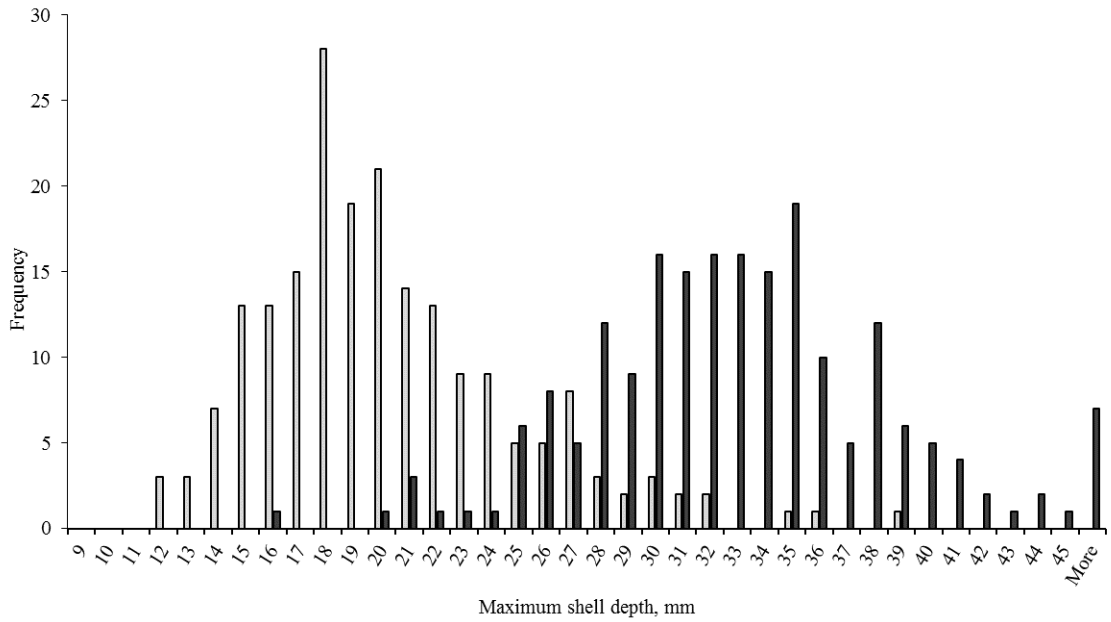
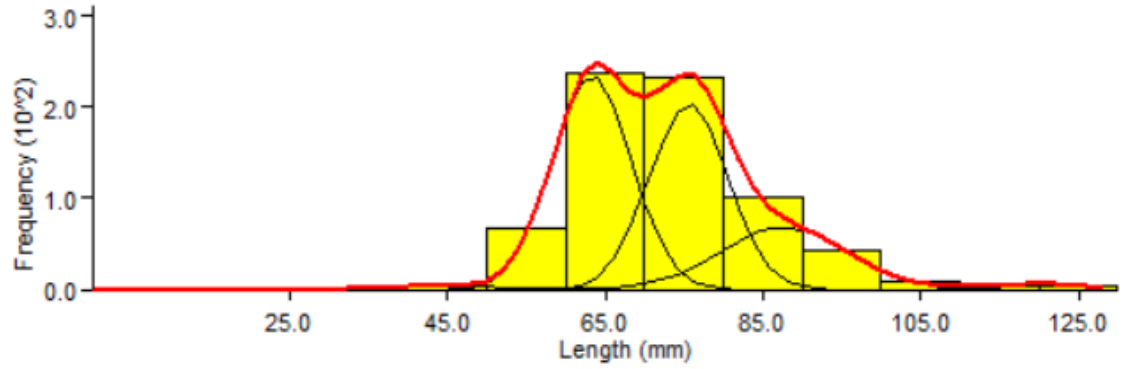


Figure 18. The size distribution of (a) whole wet weights for the Chichester (light grey, n = 700) and Portsmouth (dark grey, n = 700) *Ostrea edulis* fishery populations (total n= 1400), (b) maximum shell lengths for the Chichester (light grey, n = 700) and Portsmouth (dark grey, n = 700) *Ostrea edulis* fishery populations (total n= 1400), (c) maximum shell widths for the Chichester (light grey, n = 700) and Portsmouth (dark grey, n = 700) *Ostrea edulis* fishery populations (total n= 1400) and (d) maximum shell depths for the Chichester (light grey, n = 200) and Portsmouth (dark grey, n = 200) *Ostrea edulis* fishery populations (total n= 400).

The FiSAT II analysis, of the maximum shell lengths, produced 5 cohorts for the Chichester population: 44.78, 63.28, 75.6, 87.67 and 119.86 cm (Figure 20). Comparatively, the 5 cohorts for the Portsmouth population were: 74.26, 84.85, 95.03, 106.08 and 128.75 cm (Figure 19 & Figure 20). There was a 29.48 cm difference between the smallest size cohorts of the two populations, a 31.75 cm difference between the cohorts with the greatest frequency of each population and a difference of 8.89 cm between the cohorts with the greatest length (Figure 20).

(a)



(b)

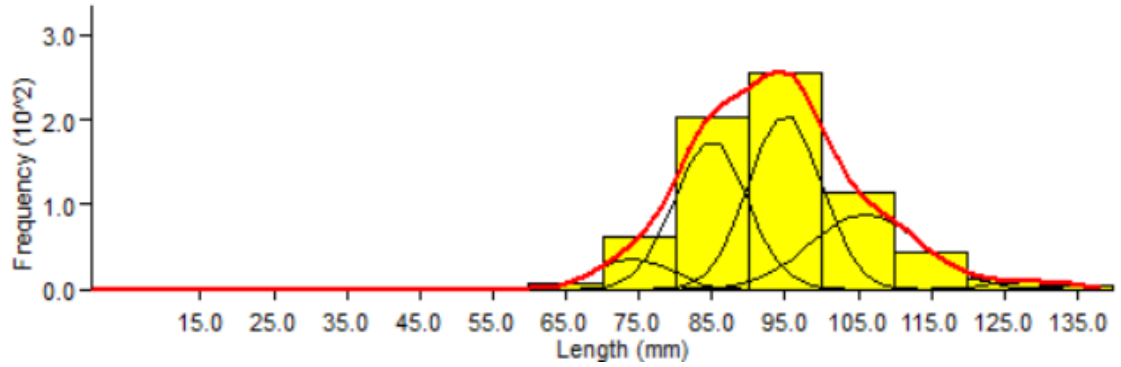


Figure 19. FAO-ICLARM Stock Assessment Tools II (FiSAT II) analysis of the lengths of the (a) Chichester *Ostrea edulis* fishery population sample (n = 700), providing 5 cohorts at 44.78, 63.28, 75.6, 87.67 and 119.86 cm, and the (b) Portsmouth *Ostrea edulis* fishery population sample (n = 700), providing 5 cohorts at 74.26, 84.85, 95.03, 106.08 and 128.75 cm.

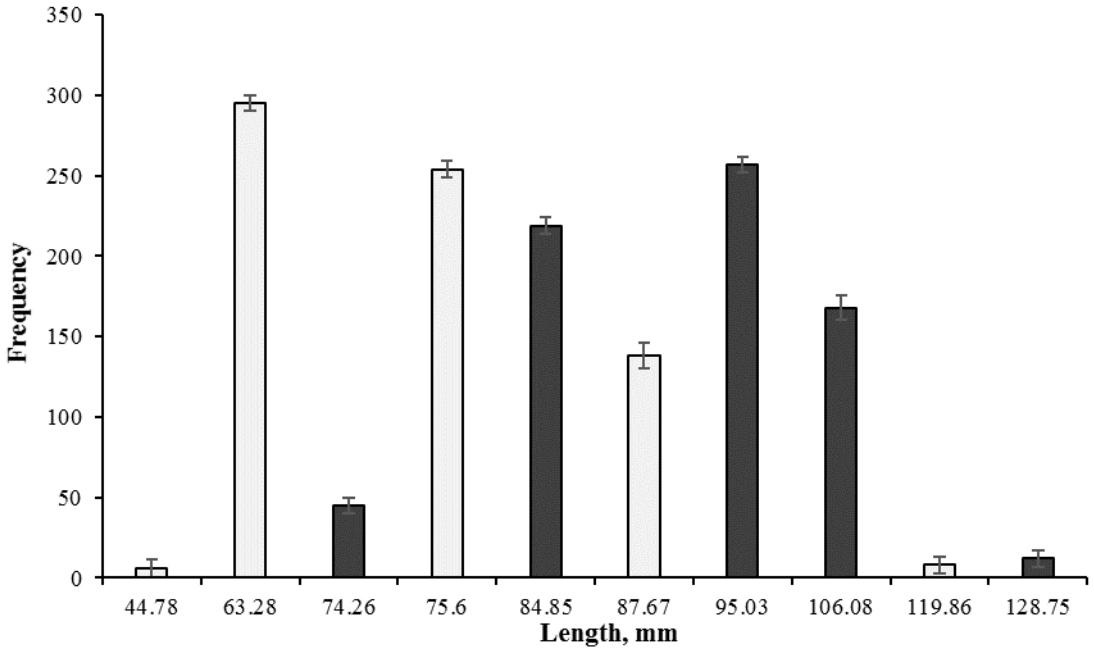


Figure 20. FAO-ICLARM Stock Assessment Tools II (FiSAT II) analysis of the maximum length distributions of the Chichester (light grey, n = 700) and Portsmouth (dark grey, n=700) fishery populations, providing 5 cohorts (±SD) for each population.

3.1.2. Attachment points

Of the 1,400 oysters measured for morphometrics, from both harbours, 27.1 % were found to have evidence of an attachment point on or around the umbo/hinge of the ventral valve. Of these individuals, 20.9 % were found with evidence that *C. fornicata* shell was the attachment point present and 6.2 % were found with evidence of another oyster valve as the attachment point (Table 4 and Figure 21). There was a 19.1 % difference between the Chichester and Portsmouth populations for the number of oysters with evidence of *C. fornicata* as the attachment, with 30.4 % and 11.3 %, respectively. For the number of oysters attached to another oyster valve there was a 4.4 % difference between the populations of Chichester (4.0 %) and Portsmouth (8.4 %).

Table 4. Percentage and number of the attachment points found on the oysters from the Chichester (CHI, n = 700) and Portsmouth (PORT, n = 700) fishery populations (total n = 1,400), from which morphometrics were recorded in section 2.1.2.1.

Attachment type		**Total**	**CHI**	**PORT**
Crepidula fornicata shell	%	20.9	30.4	11.3
	n	292	213	79
Oyster valve	%	6.2	4.0	8.4
	n	87	28	59
No attachment	%	72.9	65.6	80.3
	n	1021	459	562

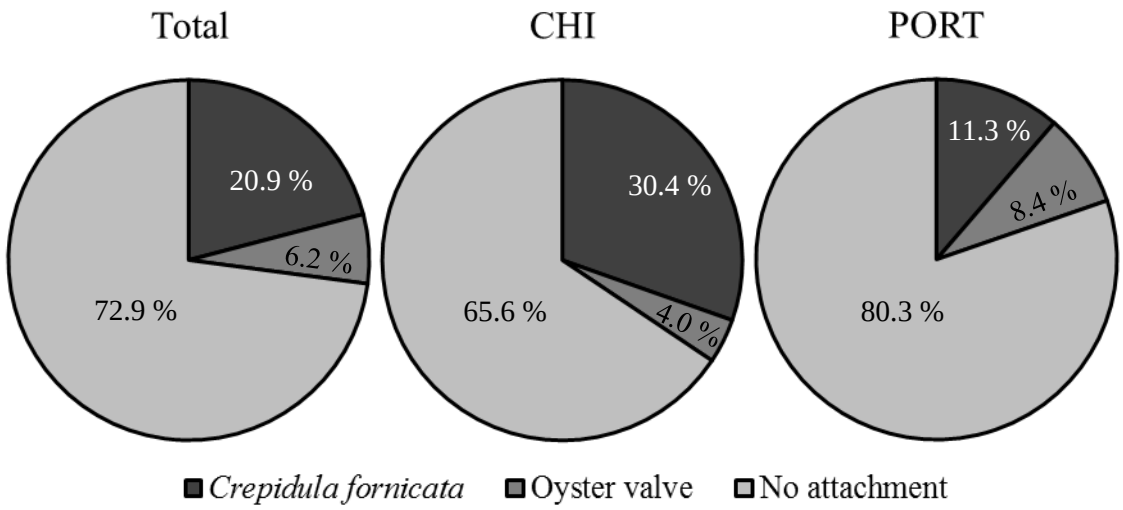


Figure 21. Percentage of each attachment point found on the oysters from the Chichester (CHI, n = 700) and Portsmouth (PORT, n = 700) fishery populations (total n = 1,400), from which morphometrics were recorded in section 2.1.2.1.

3.1.3. Condition Index (CI)

There was no statistically significant difference between the condition index (mean ± SE) of the Chichester population (3.30 ± 0.49) and the Portsmouth population (3.97 ± 0.45) (one-way ANOVA $F = 1.003$, $p = > 0.05$) (Figure 22).

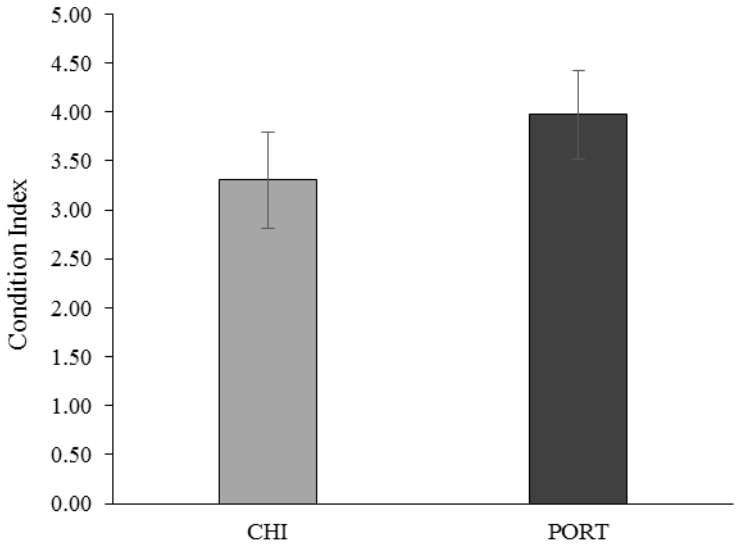


Figure 22. Condition index (mean ± SE) obtained from both Chichester (CHI, n = 24) and Portsmouth (PORT, n = 24) *Ostrea edulis* sample populations. No significant difference was observed between the two populations (one-way ANOVA, $F = 1.003$, $p > 0.05$).

3.1.4. *Bonamia ostreae* prevalence

DNA extraction was successful for all oysters sampled, quantities of template DNA acquired from the samples varied greatly, the exact quantity of DNA (ng/μl) extracted, as well as the 260/280 and 260/230 ratios, can be seen in Appendix B.

The LCO1490 / HCO2198 primer pair provided insufficient results for the *Ostrea edulis* COI gene amplification. As a result, only the primer pair Oe fw_1 / Oe rev_4, which produced clean PCR products with positive amplifications at 760 bp, was used as a positive control to amplify the *O. edulis* COI gene. Both the BO / BOAS and C_F / C_R primer pairs provided positive amplification so were used throughout.

The PCR provided 91 positive amplifications of the Oe fw_1 / Oe rev_4 positive control, those that did not provide positive amplifications were discarded from the results. Those that provided positive amplifications showed that 46.81 % of the CHI oysters, and 80.00 % of the PORT oysters, were not infected with microcell Haplosporidians or *B. ostreae*. Of the 53.19 % CHI oysters that were infected, 31.91 % were positive for a microcell Haplosporidian other than *B. ostreae* and 21.28 % were positive for *B. ostreae*. In comparison to this, the 20.00 % of the PORT oysters that were infected showed only positive amplifications for microcell Haplosporidians, with 0.00 % positive for *B. ostreae* (Table 5 & Figure 23). Figure 24 & Figure 25 illustrate the gel samples that were obtained, due to the large number of samples the remaining gel images can be seen in Appendix C.

Incidences of bonamiosis within the Chichester population occurred across a range of different sized oysters, however the majority of cases (n = 6 / 9) occurred in oysters < 82 mm in length, with a dry tissue weight of < 2 g (Figure 26a). Incidences of infection with microcell Haplosporidians, other than *B. ostreae*, within the Chichester population occurred in oysters < 87 mm in length, with a dry tissue weight of < 4 g. In comparison, the incidences of infection with microcell Haplosporidians, other than *B. ostreae*, within

the Portsmouth population occurred in oysters between 70 - 100 mm in length, with a dry tissue weights of 2 - 11 g (Figure 26b). No correlation between condition index, maximum shell length and infection with *B. ostreae* was observed for either location (Figure 27).

Table 5. Prevalence of microcell Haplosporidians, represented by the BO / BOAS primer pair (Cochennec et al. 2000), and *Bonamia ostreae*, represented by the C_F / C_R primer pair (Carnegie et al. 2000), within oysters collected from Chichester (CHI) and Portsmouth (PORT) Harbours.

Location	Negative		BO/BOAS		C_F / C_R		Total (n)
	%	n	%	n	%	n	
CHI	46.81	22	31.91	15	21.28	10	47
PORT	80.00	36	20.00	9	0.00	0	45

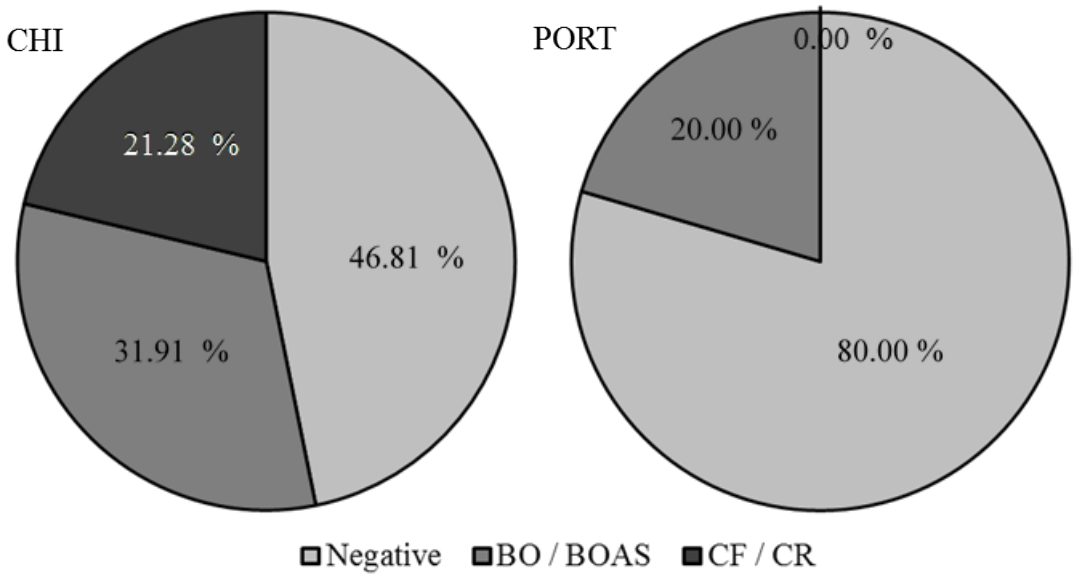


Figure 23. Prevalence of microcell Haplosporidians, shown with positive amplification by BO /BOAS primer pair, and *Bonamia ostreae*, shown with positive amplification by C_F / C_R primer pair, within the Chichester (CHI, n = 47) and Portsmouth (PORT, n = 45) oyster sample populations. Negative percentages represent those whereby only positive amplification by the positive control primer pair, Oe fw_1 / Oe rev_4, were obtained.

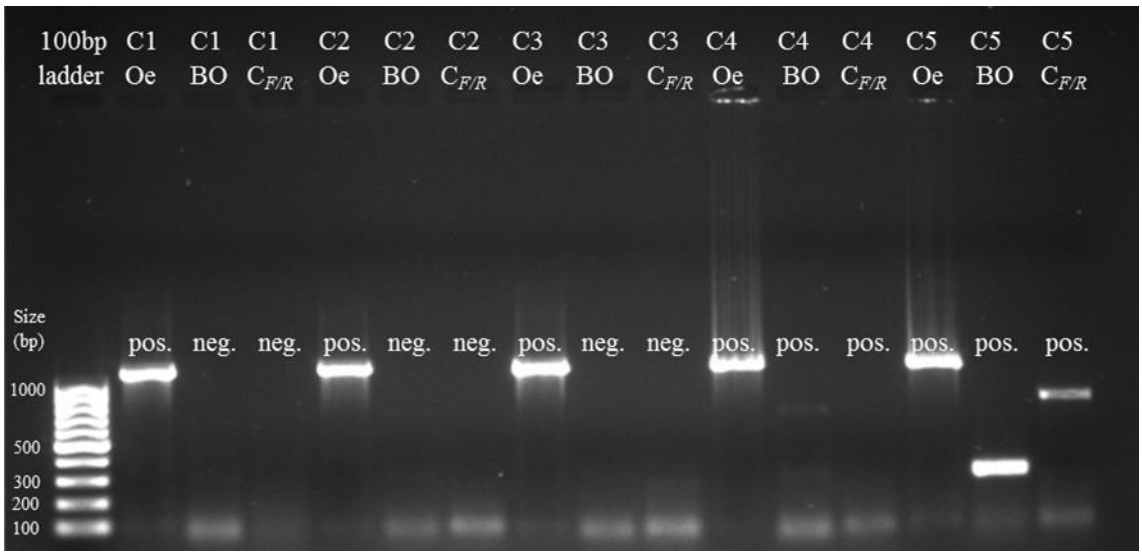


Figure 24. Electrophoresis gel under UV transillumination. Shown are the results for the PCR screening of gill tissue from Chichester oysters C1 - C5 for the *Ostrea edulis* COI gene, the nearly complete SSU microcell Haplosporidian DNA and the nearly complete SSU of *Bonamia ostreae* 18S rDNA. A 100 base pair (bp) ladder (100 - 1000 bp) was used as a reference for primer pairs Oe fw_1 / Oe rev_4 (Oe) (Grecken & Schmidt 2014), BO / BOAS (BO) (Cochennec et al. 2000) and 'C_F / C_R' ($C_{F/R}$) (Carnegie et al. 2000), amplifications. All Oe columns show positive results for *Ostrea edulis* COI gene amplification. Columns 15 (C5 BO) & 16 (C5 $C_{F/R}$) show positive identification for microcell Haplosporidian DNA at 300 bp and *Bonamia ostreae* 18S rDNA at 760 bp, respectively. Column 13 (C4BO) shows positive amplification of both microcell Haplosporidian DNA at 300 bp and *Bonamia ostreae* 18S rDNA at 760 bp as the sample from column 14 (C4 $C_{F/R}$) has migrated across.

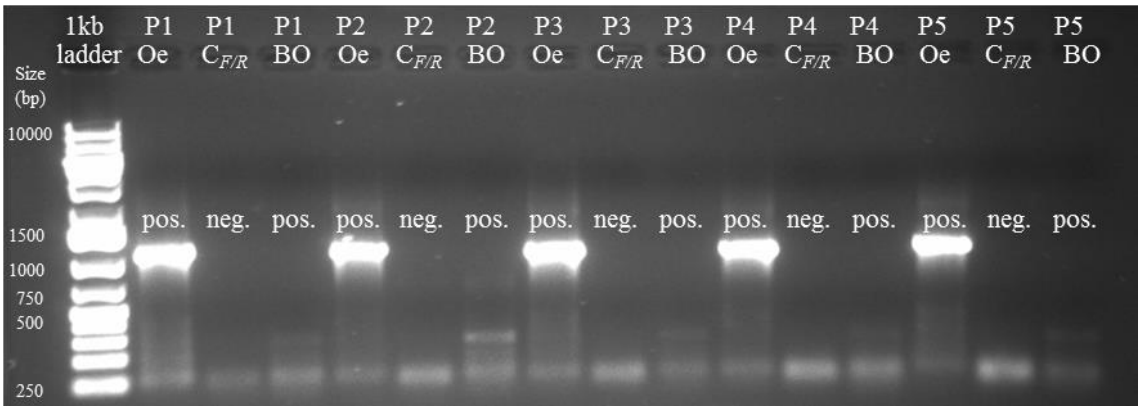
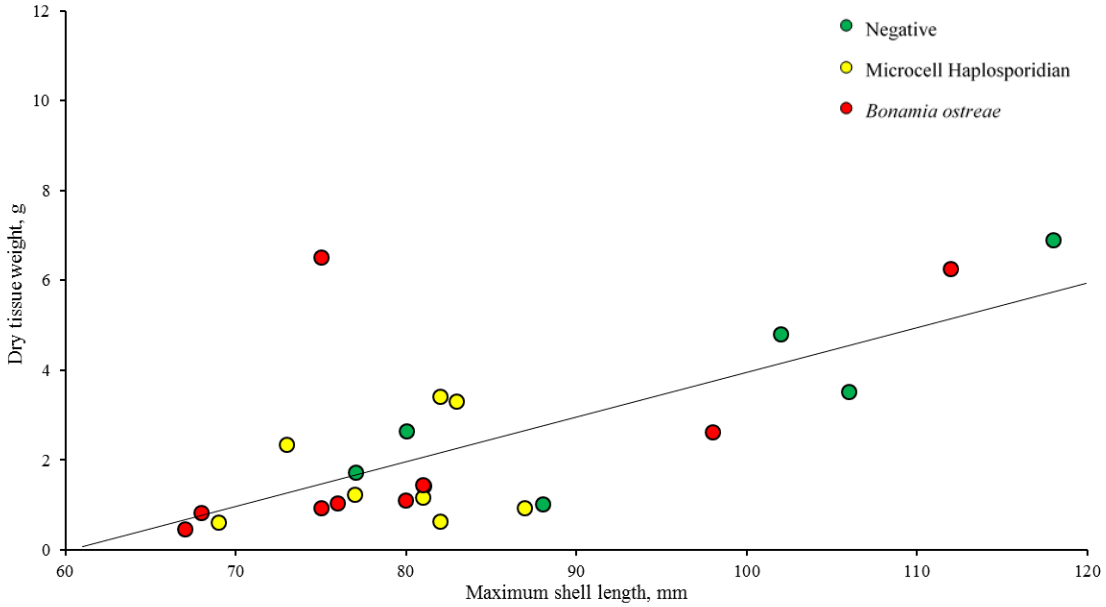


Figure 25. Electrophoresis gel under UV transillumination. Shown are the results for the PCR screening of gill tissue from Portsmouth oysters P1 - P5 for the *Ostrea edulis* COI gene, the nearly complete SSU microcell Haplosporidian DNA and the nearly complete SSU of *Bonamia ostreae* 18S rDNA. A 1 kilobase (kb) ladder (250 - 10,000 bp) was used as a reference for primer pairs Oe fw_1 / Oe rev_4 (Oe) (Grecken & Schmidt 2014), BO / BOAS (BO) (Cochennec et al. 2000) and C_F / C_R ($C_{F/R}$) (Carnegie et al. 2000), amplifications. All 'Oe' columns show positive results for *Ostrea edulis* COI gene amplification. Positive results were obtained for microcell Haplosporidian DNA in all BO columns, no positive results were obtained for *Bonamia ostreae* DNA in the $C_{F/R}$ columns for these five individuals.

(a)



(b)

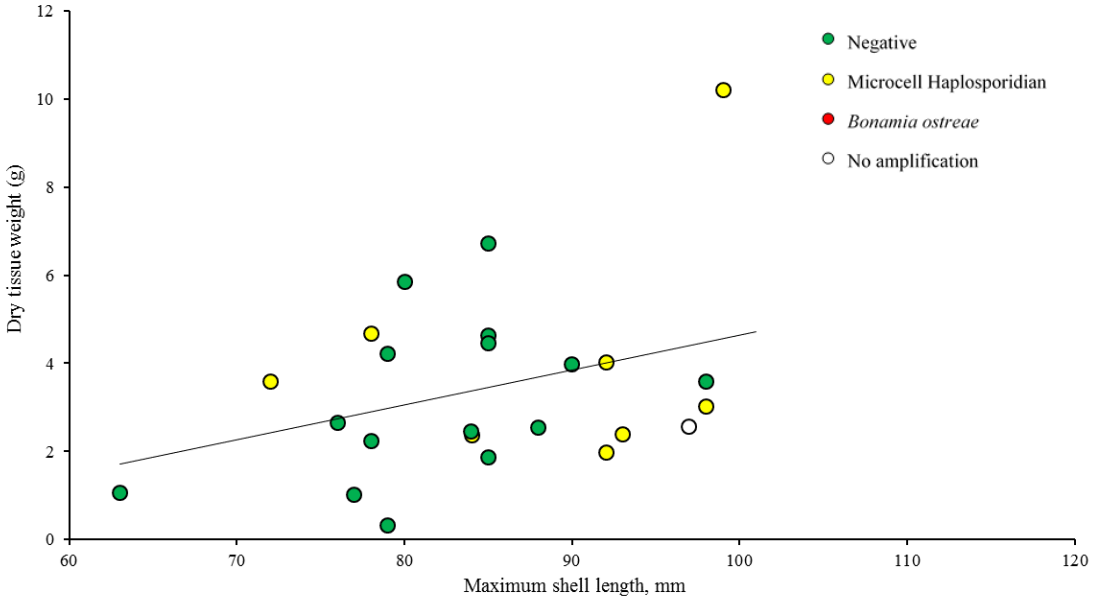
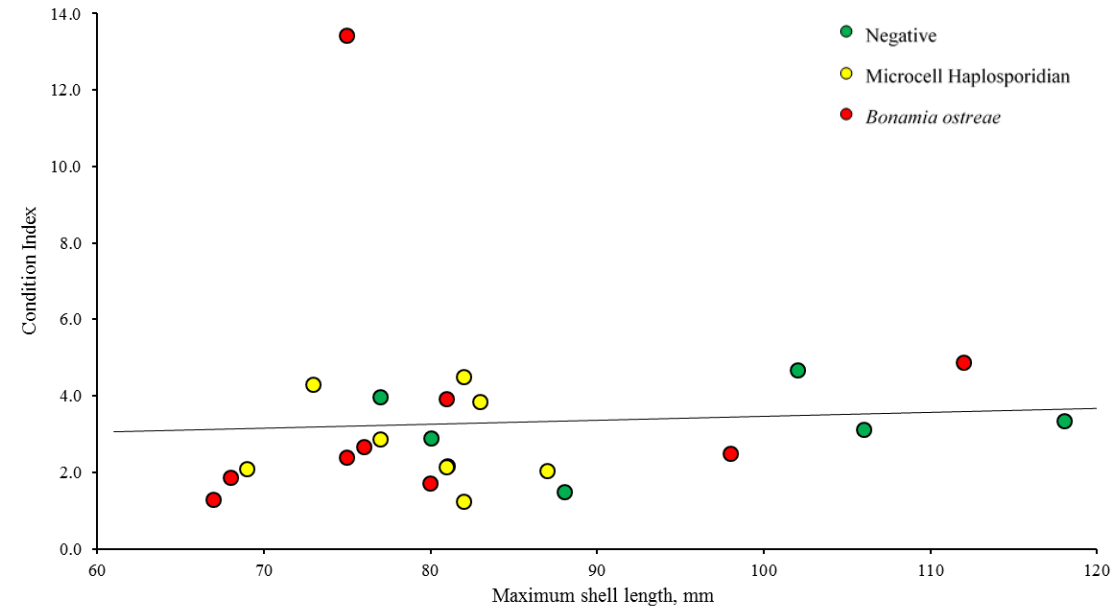


Figure 26. Occurrence of microcell Haplosporidians and *Bonamia ostreae*, specifically, within the (a) Chichester *Ostrea edulis* fishery sample population (n = 24) (b) Portsmouth *Ostrea edulis* fishery sample population (n= 24), in relation to maximum shell length and dry tissue weight. Each point represents an individual oyster, with the line of linear regression allowing comparison of the size - dry tissue weight relationship between uninfected and infected oysters.

(a)



(b)

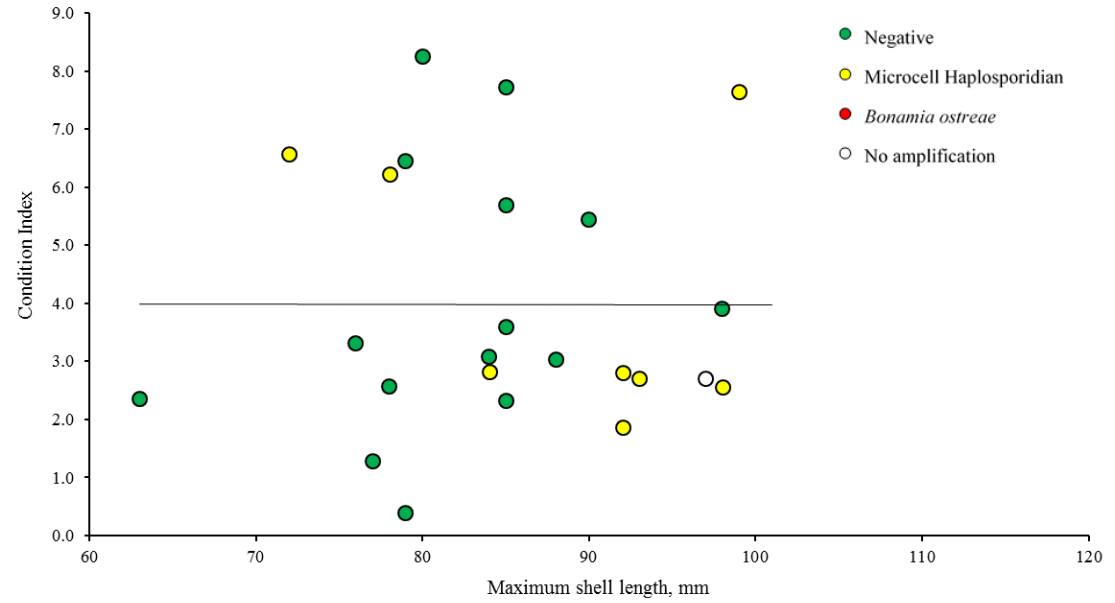


Figure 27. Occurrence of microcell Haplosporidians and *Bonamia ostreae*, specifically, within the (a) Chichester *Ostrea edulis* fishery sample population (n = 24) (b) Portsmouth *Ostrea edulis* fishery sample population (n = 24), in relation to maximum shell length and condition index. Each point represents an individual oyster, with the line of linear regression allowing comparison of the size - dry tissue weight relationship between uninfected and infected oysters.

3.2. Broodstock population study in Langstone and Portsmouth Harbours

3.2.1. Survivorship of broodstock oysters

The LRBAR pontoon population was maintained at 675 oysters throughout the eight-month period. The percentage mortality of the oyster population within all the cages was consistently low throughout this time, with a peak of 2.2 % (n = 15) in June 2016 (Figure 28a).

Percentage mortality within the LRBAR high density cages peaked in June 2016 at 2.7 % (n = 12), whilst the low density percentage mortality peaked in May 2016 at 1.8 % (n = 4) (Figure 29a). Similar cumulative percentage mortalities were experienced between the two populations with final values of 7.3 % (n = 33) and 7.1 % (n = 16) for the high and low densities, respectively (Figure 29a). When considering the number of individual mortalities, the high density population experienced 2.1 times more mortalities than the low density population.

Cumulative mortalities reached 10 % (n = 22), 4 % (n = 10) and 8 % (n = 17) for the LRBAR pontoon top middle and bottom cages, respectively, in July 2016 (Figure 30a). The top cage mortality peaked in June and July at 3.1 % (n = 7), the middle cage mortality peaked in February at 1.3 % (n = 3), with the bottom cage mortality peaking in June at 2.7 % (n = 6) (Figure 30b).

In April 2016 at the UoPRR site, one low density cage (n = 75) and three high density cages (n = 450) were lost, due to unforeseen circumstances involving extreme weather, and were replaced in June 2016. Total percentage mortalities remained low within the population until July 2016, with a peak of 46.2 % (n = 312) (Figure 28b).

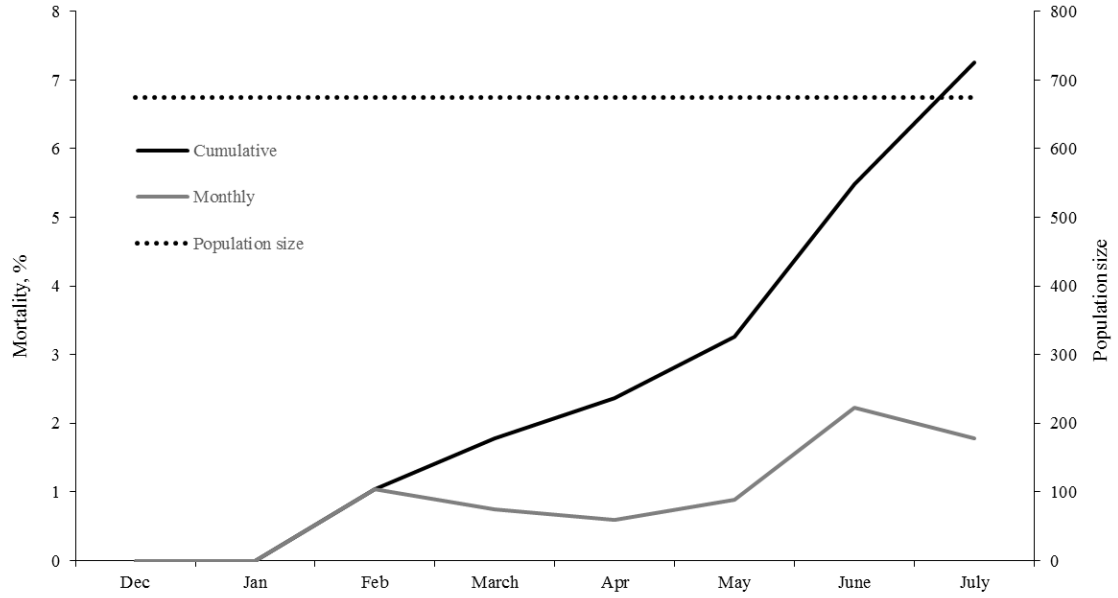
The loss of the UoPRR cages is shown in relation to percentage mortality which remained low within the two remaining low density cages throughout April and May (Figure 29b).

Once the cages were replaced the monthly mortalities peaked in July for both densities, with the high density cages experiencing 47.8 % (n = 215) mortality and the low density cages experiencing 52.9 % (n = 119) mortality (Figure 29b). When considering the number of individual mortalities, the high density population experienced 1.8 times more mortalities than the low density population.

Cumulative mortalities reached 53.8 % (n = 121), 41.3 % (n = 93) and 53.3 % (n = 120) for the UoPRR top middle and bottom cages, respectively, in July 2016 (Figure 30c). All cage mortalities peaked in July with the top cage mortality peaking at 52 % (n = 117), the middle cage mortality peaked at 38 % (n = 85), with the bottom cage mortality peaking at 49 % (n = 110) (Figure 30d).

When the overall mortalities are combined with the change in temperature, it can be seen that the LRBAR population cumulative mortality correlates well with an increase in temperature (Figure 31a). In comparison, the UoPRR cumulative mortality correlates less well until June and July (Figure 31b). All raw data for the abiotic water parameters can be seen in Appendix D.

(a)



(b)

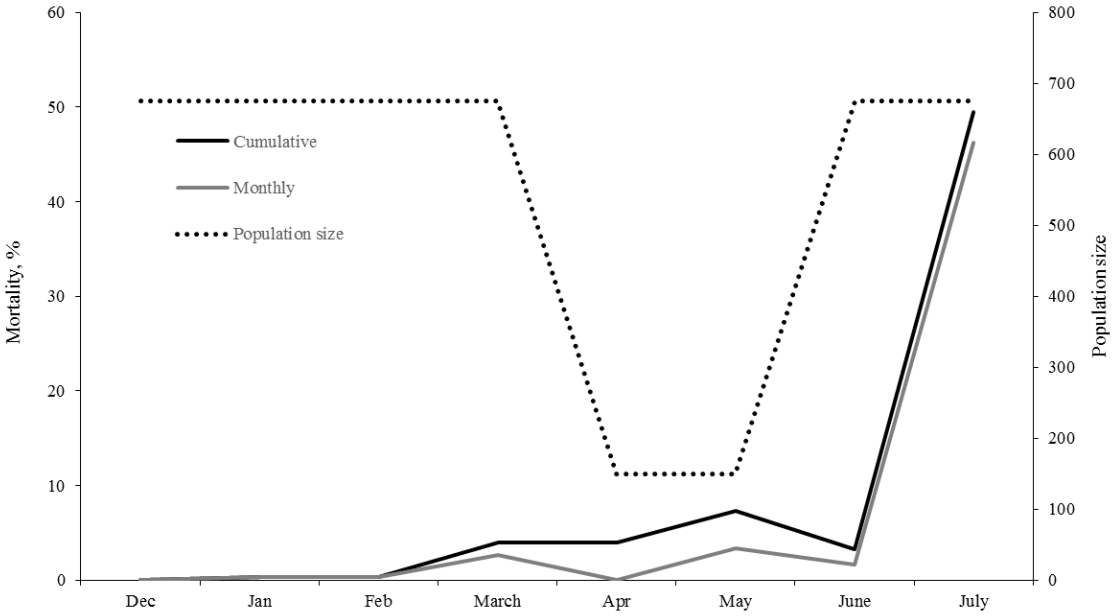
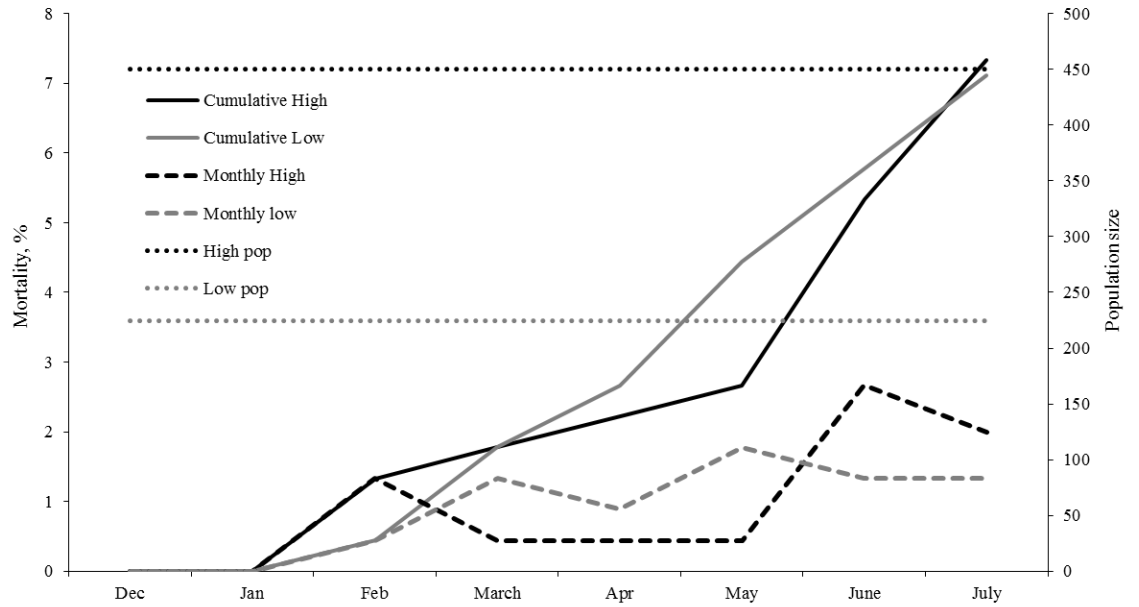


Figure 28. The cumulative and monthly percentage mortalities for the total broodstock population at (a) the Land Rover Ben Ainslie Racing pontoon (b) the University of Portsmouth Research Raft. Population size is shown by the corresponding dashed lines.

(a)



(b)

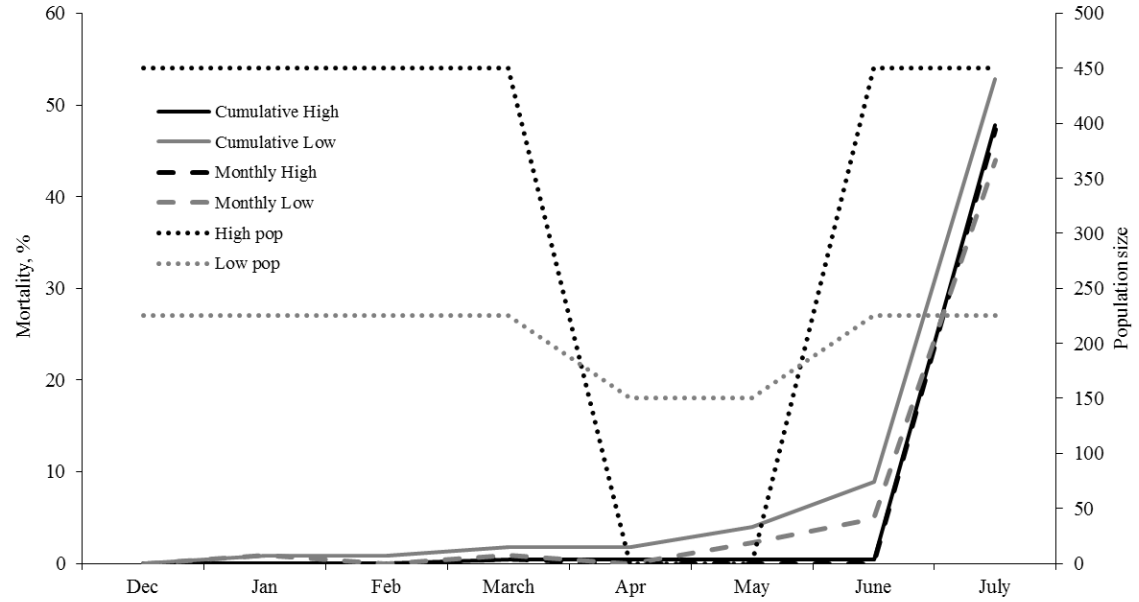
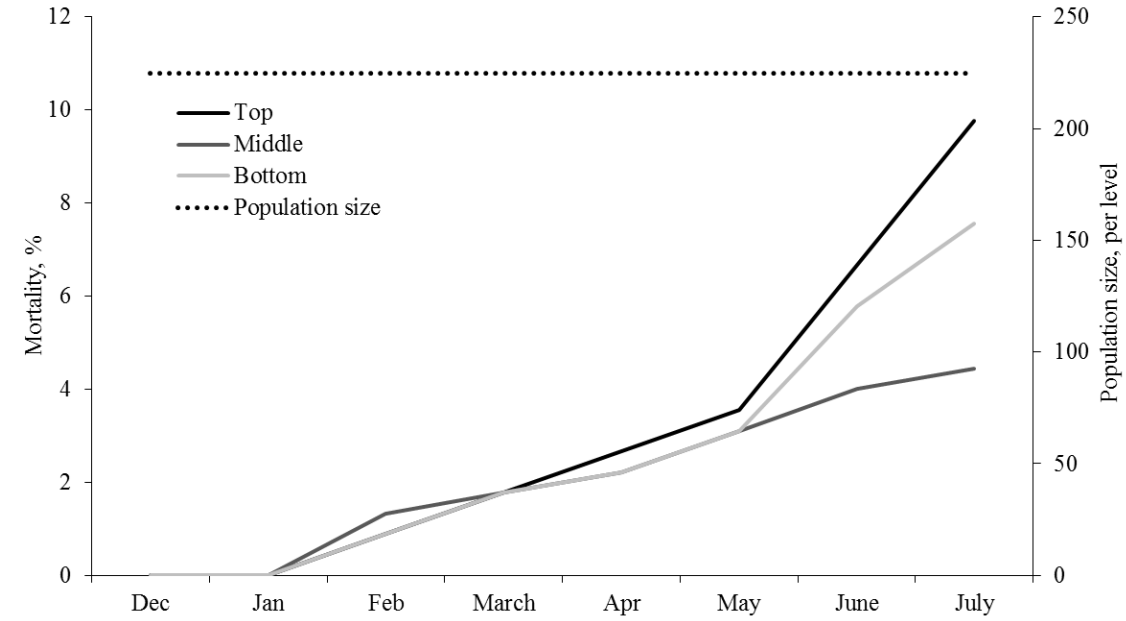
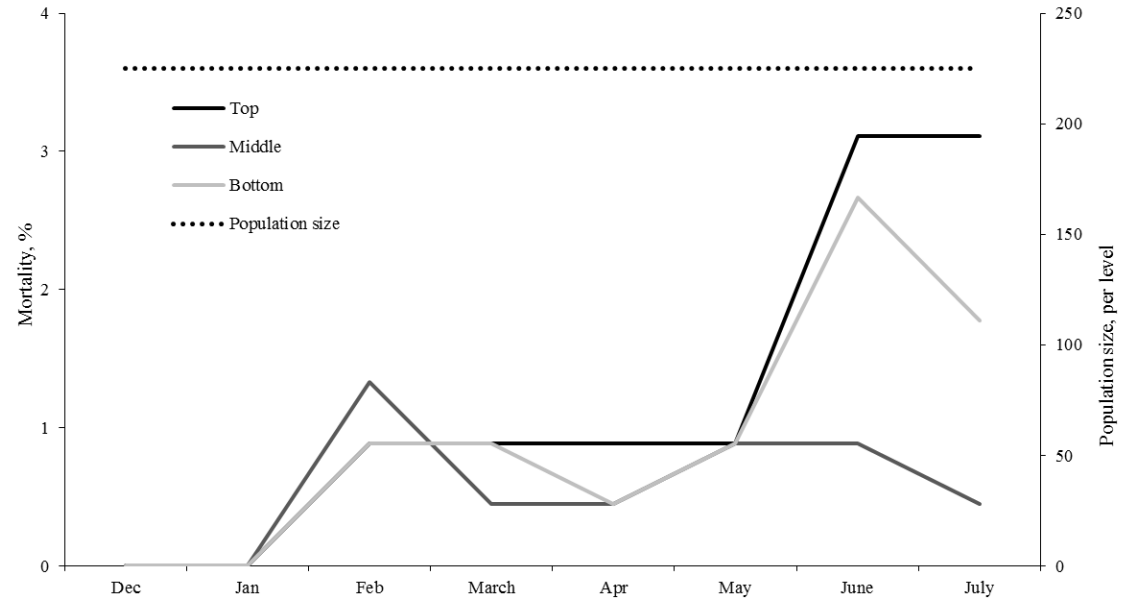


Figure 29. Cumulative and monthly percentage mortalities for the high and low density broodstock populations at (a) the Land Rover Ben Ainslie Racing pontoon (b) the University of Portsmouth Research Raft. Population sizes are shown by the corresponding dashed lines.

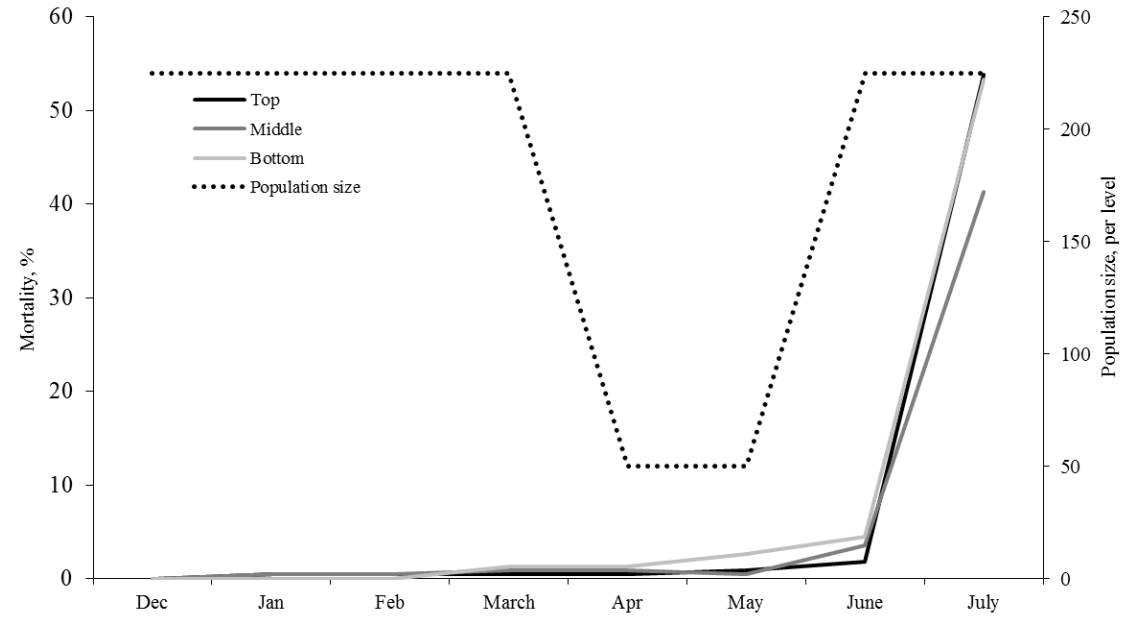
(a)


12
10
8
6
4
2
0
Mortality, %
250
200
150
100
50
0
Population size, per level
Top
Middle
Bottom
Population size
Dec
Jan
Feb
March
Apr
May
June
July

(b)


4
3
2
1
0
Mortality, %
250
200
150
100
50
0
Population size, per level
Top
Middle
Bottom
Population size
Dec
Jan
Feb
March
Apr
May
June
July

(c)



(d)

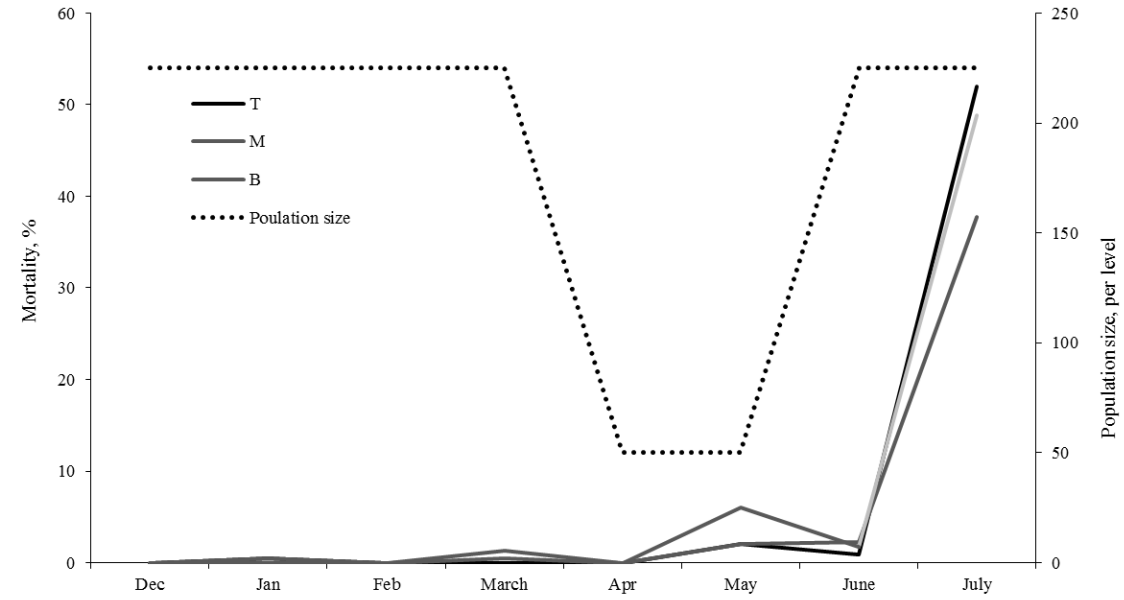
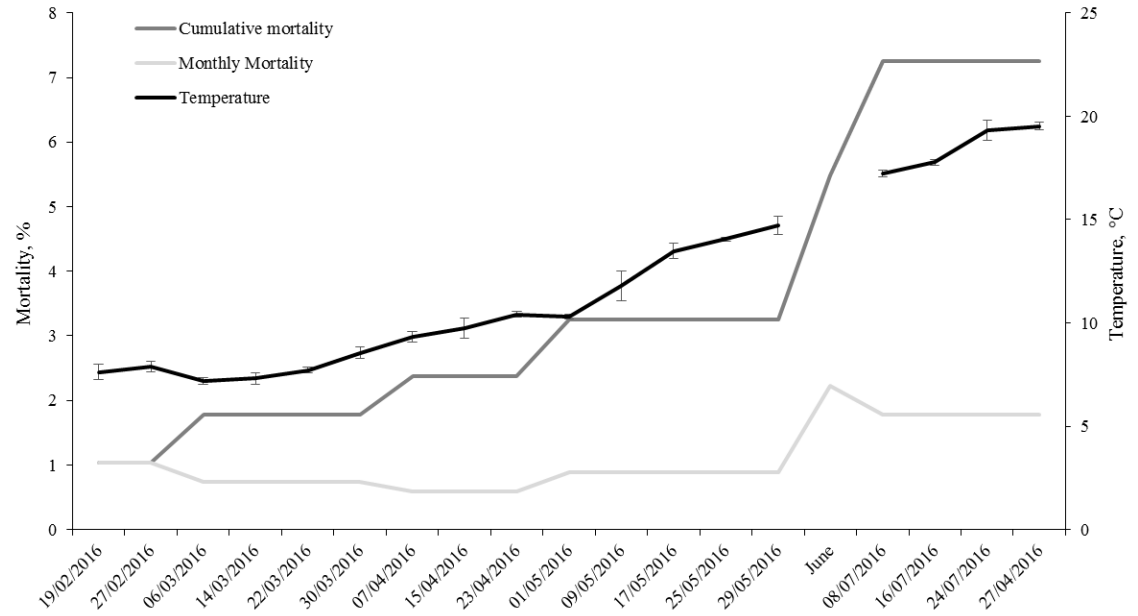


Figure 30. The Land Rover Ben Ainslie Racing pontoon (a) cumulative percentage mortalities for the top, middle and bottom broodstock cage populations (b) monthly percentage mortalities for the top, middle and bottom broodstock cage populations. The University of Portsmouth Research Raft (c) cumulative percentage mortalities for the top, middle and bottom broodstock cage populations (d) monthly percentage mortalities for the top, middle and bottom broodstock cage populations. Population sizes per level are shown by the corresponding dashed lines.

(a)



(b)

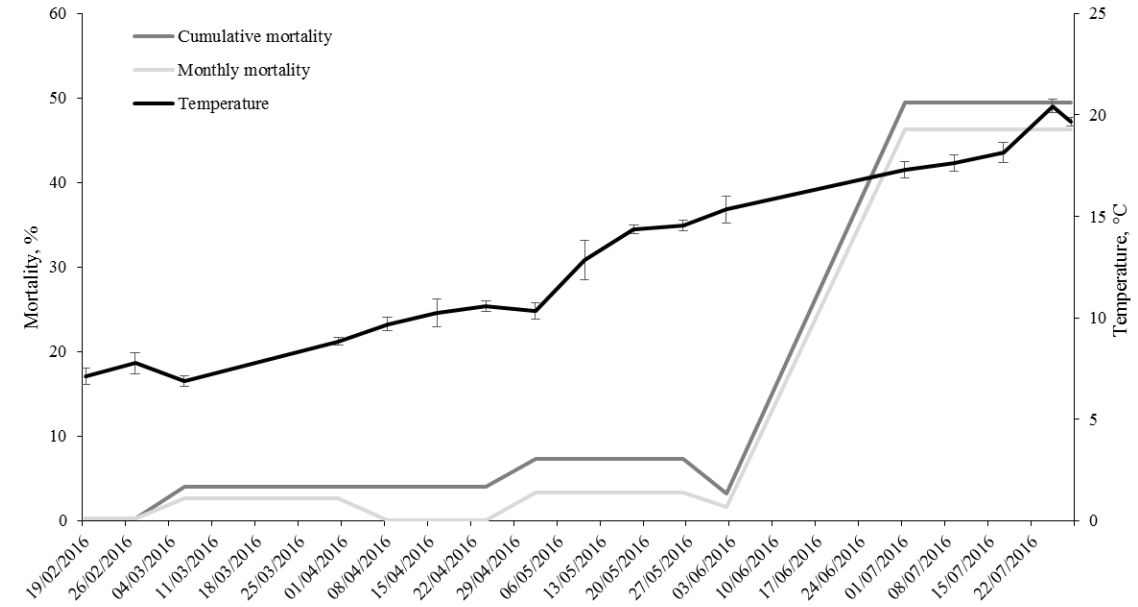


Figure 31. The correlation between cumulative and monthly mortalities with temperature (mean ± SD) for (a) the LRBAR broodstock population (b) the UoPRR broodstock population. Gaps in the temperature data indicate the removal of the sonde.

The total broodstock oyster population on the UoPRR had a significantly higher percentage mortality, than those exposed at the LRBAR pontoon, 49 % and 7%, respectively (PERMANOVA main test, mortality vs location, $F_{1, 142} = 54.8$, $p = < 0.001$). Two distinct groups were found, shown by the principle component ordination (Figure 32a).

Mortalities within the broodstock oyster population from the UoPRR were influenced by: average temperature fluctuation, average ODO fluctuations (% saturation and mg/L), average ODO (% saturation), average temperature, month, average TDS and average salinity fluctuations (Figure 32b). Mortalities within the broodstock oyster population from the LRBAR pontoon were influenced by: cage density, cage position, average ODO (mg/L) and average turbidity (Figure 32b).

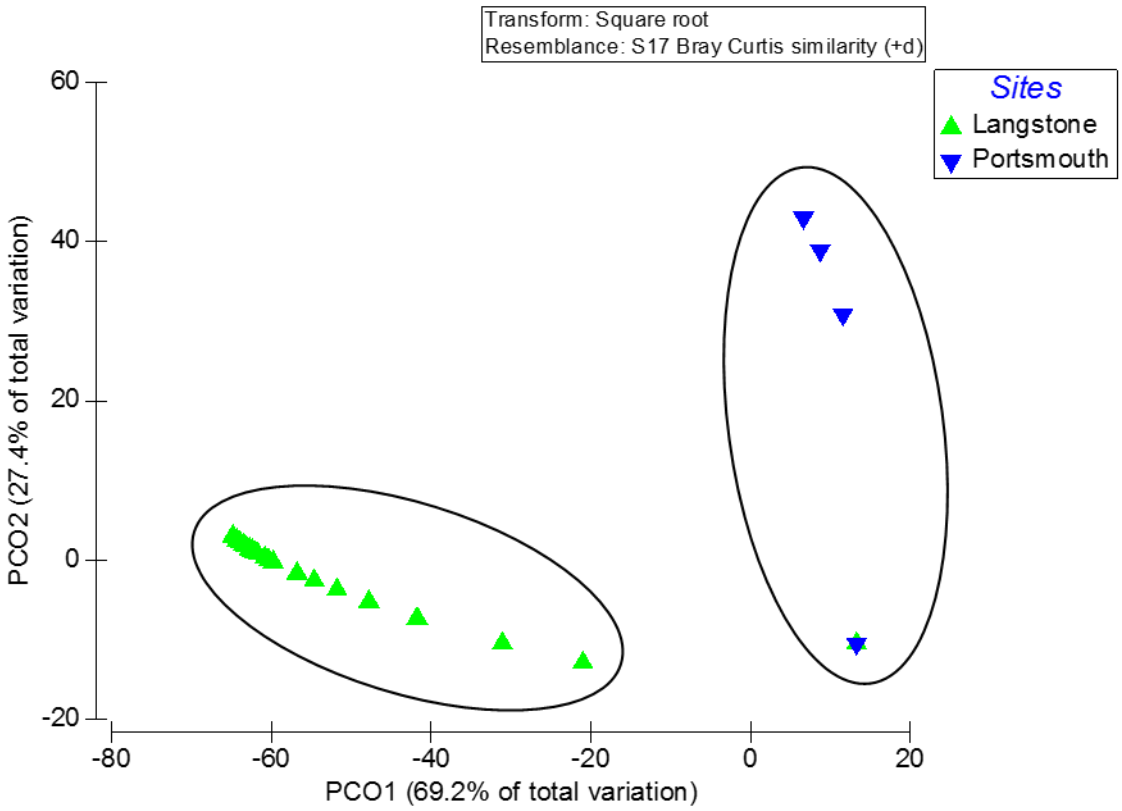
Difference of percentage mortalities from the high and low densities between the two locations were significant (PERMANOVA main test, mortality vs location vs density, $F_{3, 140} = 2.7$, $p = < 0.01$).

These differences were found from the high density broodstock oysters from the UoPRR (47.8 %), which was significantly higher than that of the LRBAR high (7.3 %) and low (7.1 %) density broodstock oysters (PERMANOVA pairwise $p = < 0.001$ and < 0.001, respectively). The percentage mortality of the UoPRR low density broodstock oysters (52.9 %) was also significantly higher than that of the LRBAR high (7.3 %) and low (7.1 %) density broodstock oysters (PERMANOVA pairwise $p = <0.01$ and < 0.001, respectively).

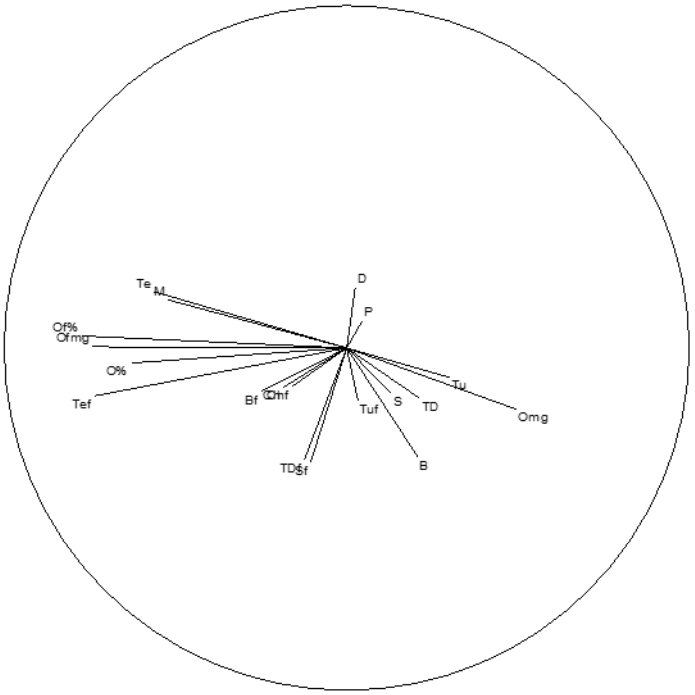
The difference between the percentage mortality of the UoPRR high (47.8 %) and low (52.9 %) density broodstock oysters was not significant (PERMANOVA pairwise $p = > 0.05$). The difference between the percentage mortality of the LRBAR high (7.3 %) and low (7.1 %) density broodstock oysters was not significant (PERMANOVA pairwise $p = > 0.05$).

Two distinct groups of low density population mortalities from both locations were found, shown by the PCO (Figure 32a). The UoPRR low density oyster mortalities were influenced by: average ODO (mg/L and % saturation) fluctuations, average temperature, average temperature fluctuation, month and average ODO (% saturation) influencing (Figure 32b). The LRBAR low density population mortalities were influenced by: average ODO (mg/L), average BGA-PC, average turbidity and average TDS (Figure 32b). Analysis of the effect of pH was removed from all tests due to technical issues experienced with the sensor.

(a)



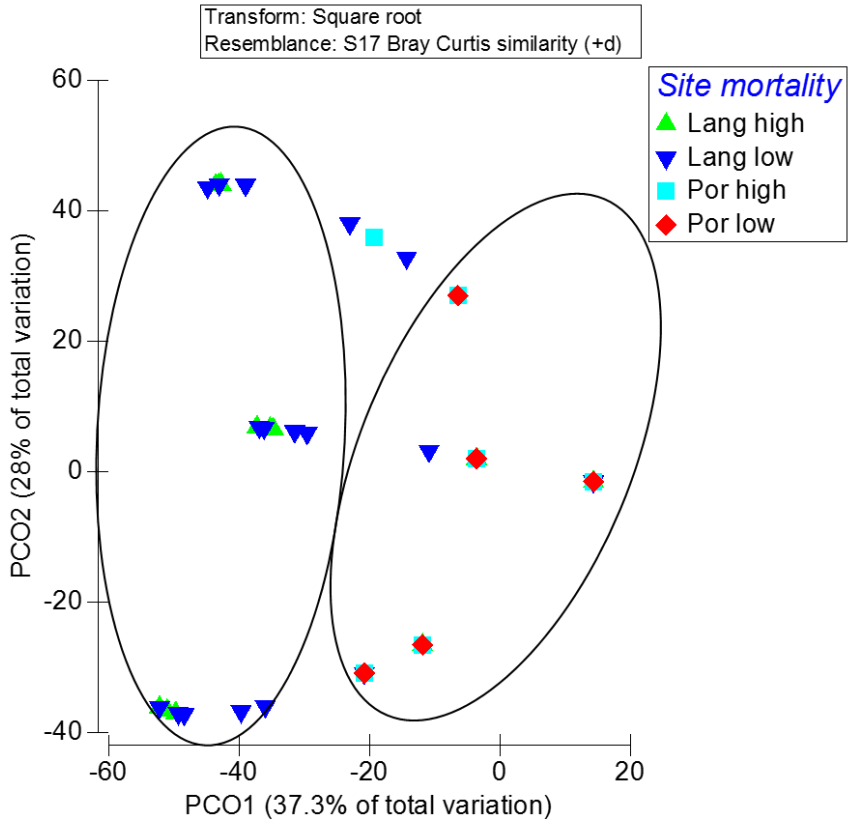
(b)



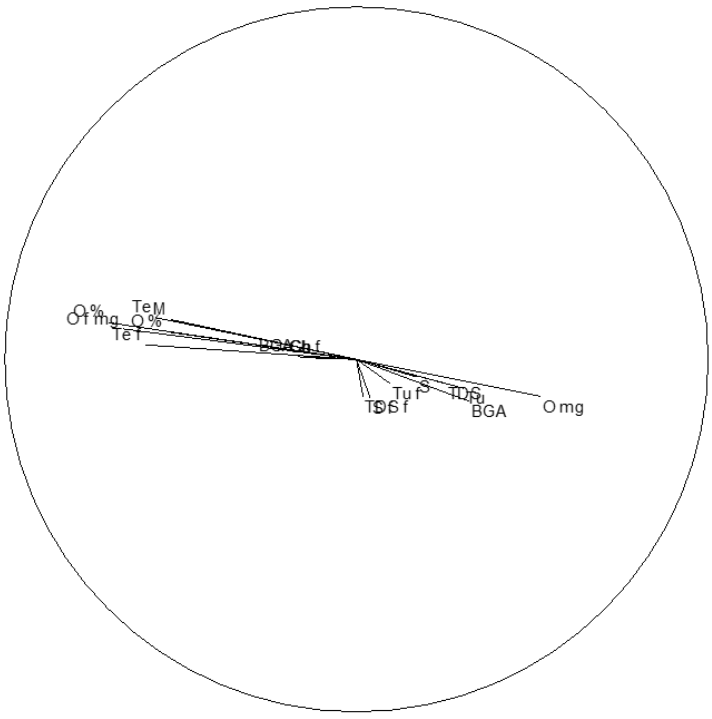
M: Month
D: Cage density
P: Cage position
Te: Temperature
Tef: Temperature fluctuation
S: Salinity
Sf: Salinity fluctuation
Omg: Optical dissolved oxygen, mg/L
Of mg: ODO fluctuation, mg/L
O%: ODO, % saturation
Of%: ODO fluctuation, % saturation
Tu: Turbidity
Tuf: Turbidity fluctuation
TD: Total dissolved solids
TDf: Total dissolved solids fluctuation
Ch: Chlorophyll *a*
Chf: Chlorophyll *a* fluctuation
B: Blue-Green algae
Bf: Blue-Green algae fluctuation

Figure 32. A Principle component ordination (PCO) illustrating the differences between (a) the two distinct groups (circled) of percentage mortalities of broodstock oysters on the University of Portsmouth Research Raft (UoPRR - Langstone) and the Land Rover Ben Ainslie Racing pontoon (LRBAR - Portsmouth). PCO1 and PCO2 account for 96.6 % of total variation. (b) The abiotic parameters that influence broodstock oyster mortality. All abiotic water parameters are monthly averages, taken from daily averages.

(a)



(b)



M: Month
D: Cage density
P: Cage position
Te: Temperature
Tef: Temperature fluctuation
S: Salinity
Sf: Salinity fluctuation
Omg: Optical dissolved oxygen, mg/L
Of mg: ODO fluctuation, mg/L
O%: ODO, % saturation
Of%: ODO fluctuation, % saturation
Tu: Turbidity
Tuf: Turbidity fluctuation
TD: Total dissolved solids
TDf: Total dissolved solids fluctuation
Ch: Chlorophyll *a*
Chf: Chlorophyll *a* fluctuation
B: Blue-Green algae
Bf: Blue-Green algae fluctuation

Figure 33. A Principle component ordination (PCO) illustrating the differences between (a) the two distinct groups (circled) of percentage mortalities of the different density broodstock oysters on the University of Portsmouth Research Raft (UoPRR - Lang) and the Land Rover Ben Ainslie Racing pontoon (LRBAR - Por). PCO1 and PCO2 account for 65.3 % of total variation. (b) The abiotic parameters that influence broodstock oyster mortality. All abiotic water parameters are monthly averages, taken from daily averages.

3.2.2. Growth of broodstock oysters

The average increase in weight for the LRBAR population remained between 1.5 and 3.0 g from March until May (n = 126, 124, 126), after which the average increase, from May to June, was 7.9 g (n = 124). A decrease in average weight was experienced between June and July, 3.1 g (n = 120) (Figure 34). In comparison, the average increase in weight for the UoPRR population was 6.9 g (March to April, n = 42) rising to 15.8 g for April to May (n = 35), after which is decreased to – 0.3 g (May to June, n = 32). An average increase in weight of 15.5 g followed this decrease from June to July (n = 53) (Figure 34). No significant difference between the growth in the different cage positions was observed (PERMANOVA main test, growth vs cage position, $F_{2, 117} = 1.26$, $p = > 0.05$) (Figure 35a), with minimal influence from cage density or cage position (Figure 35b). Growth fringes were observed on numerous individuals within cages at both locations throughout May, June and July (Figure 36).

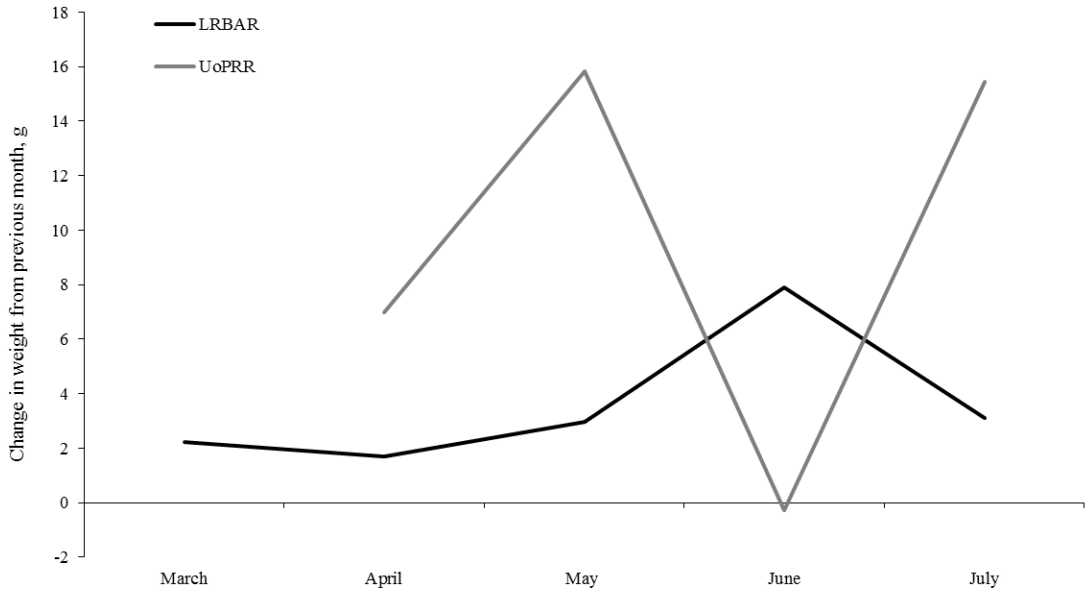
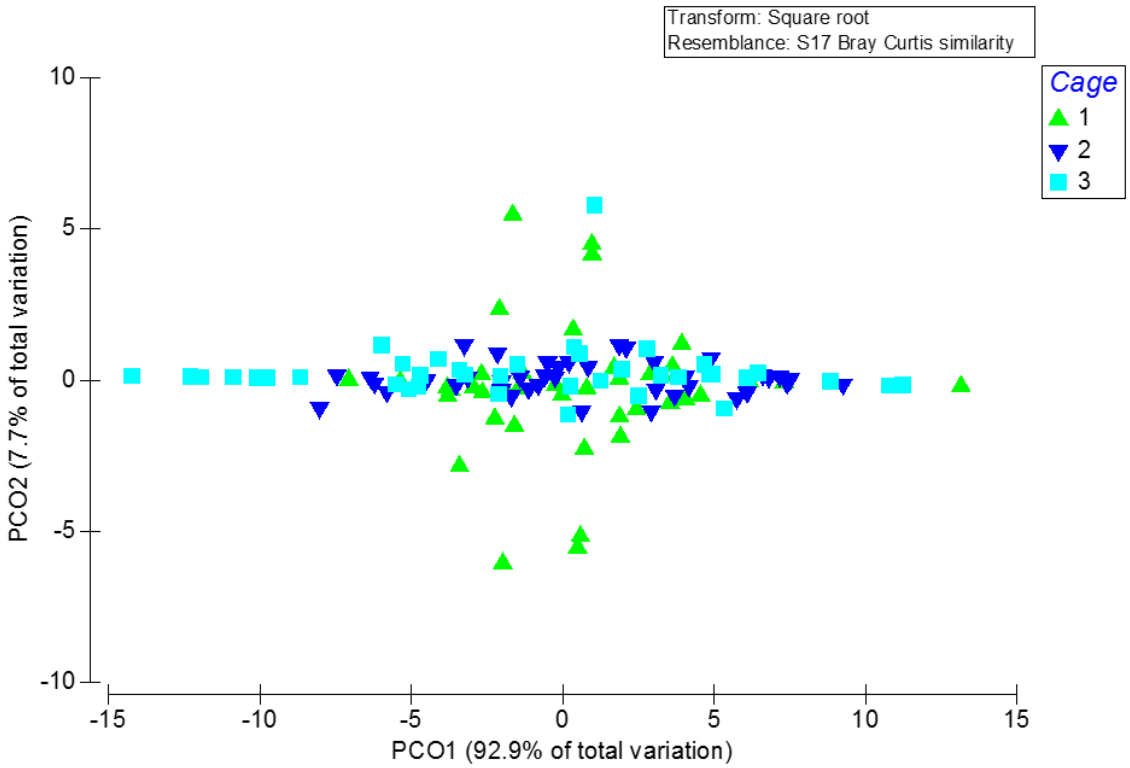


Figure 34. The average change in weight (g) per month for both the Land Rover Ben Ainslie Racing and University of Portsmouth Research Raft populations.

(a)



(b)

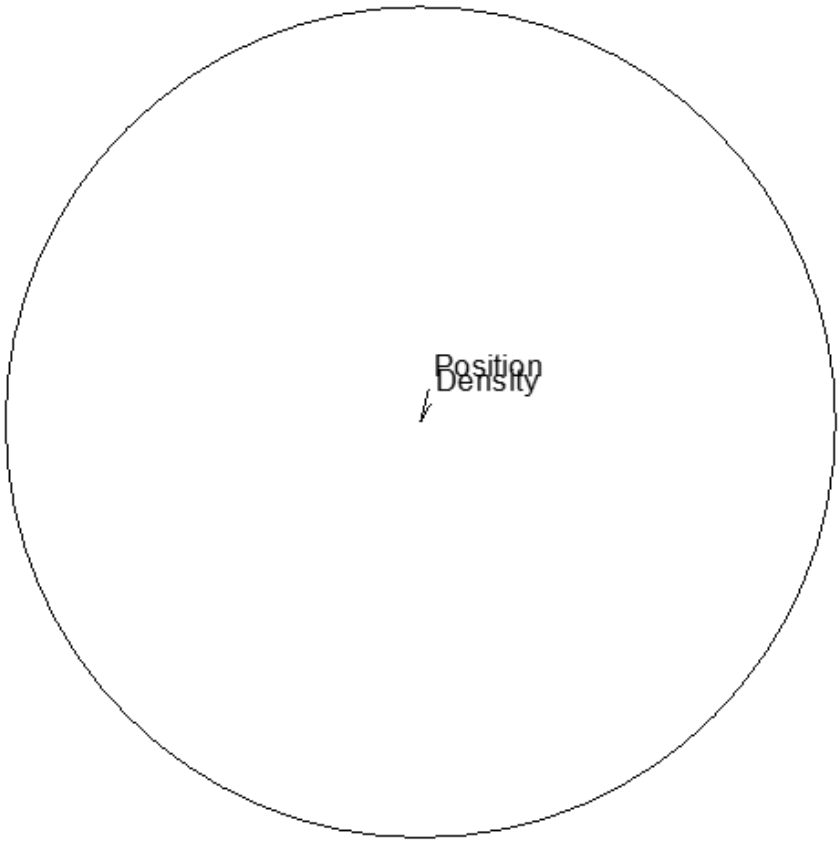


Figure 35. A Principle component ordination (PCO) illustrating the differences between (a) the monthly growth of the Land Rover Ben Ainslie Racing pontoon broodstock oysters in different cage positions (Top - 3, Middle – 2 and Bottom – 1). PCO1 and PCO2 account for 100.6 % of total variation. (b) The abiotic parameters of position and density that influence the growth.

Figure 36. Typical growth fringe (show with arrows) noted on numerous individuals within the broodstock cages, the individual in the image was from the Land Rover Ben Ainslie Racing pontoon. No scale bar available for actual growth.

3.2.3. Reproductive state of broodstock oysters

Broodstock oysters sampled, as described in Section 2.2.3., were observed for the presence of offspring at various stages of the larval cycle with multiple individuals containing either eggs, eggs surrounded by sperm, a ‘white sick’ or a ‘grey sick’. The ‘white sick’ was observed to be dense populations of developing zygotes and the ‘grey sick’ was observed to be dense populations of early stage veliger ‘D’ larvae (Figure 37). Samples of the pallial cavity contents and gonadal tissue have been preserved and comprehensive quantatative analysis will be conducted at the end of the breeding season in future studies.

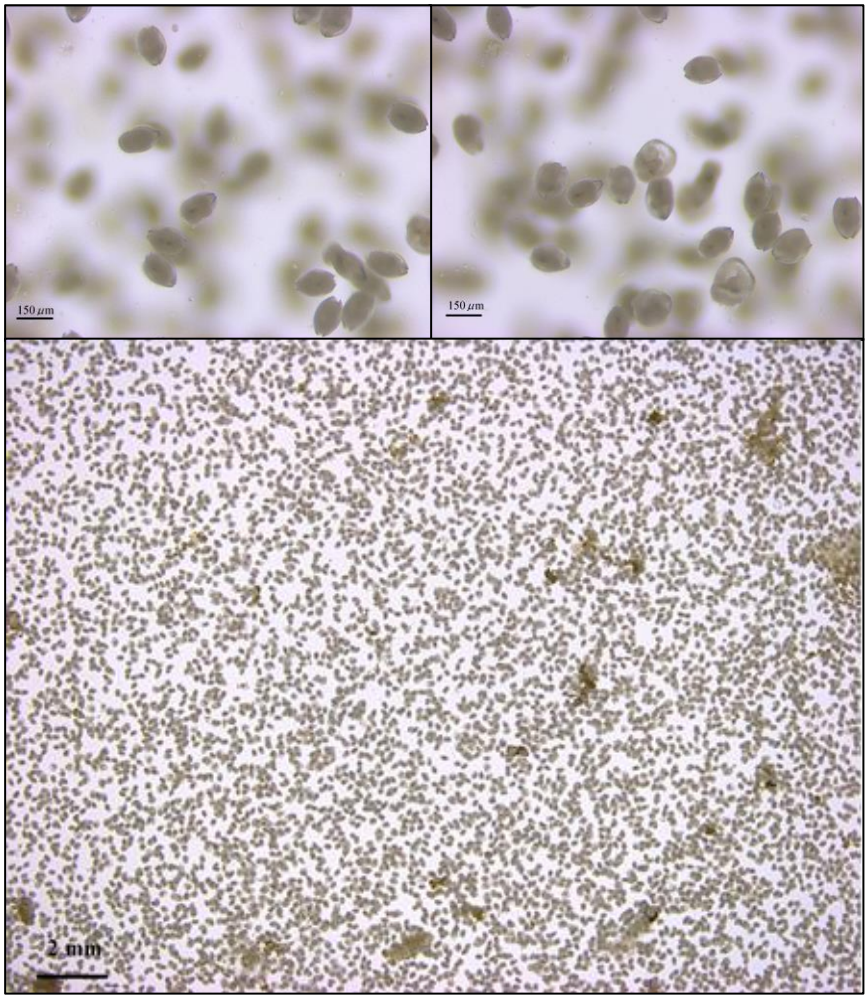


Figure 37. Light microscopy images of the numerous veliger ‘D’ larvae contained within the pallial cavity of a broodstock oyster collected from the Land Rover Ben Ainslie Racing pontoon on 27th July 2016.

3.3. Larval assessment within the water column

3.3.1. Plankton tows and larval abundances

Due to the time constraints, posed by the spawning season extending beyond the duration of this study, larval abundances within the surface waters could not be fully analysed and were reserved for future analysis. This being said, initial findings suggest high abundances of *C. fornicata* larvae during samples from May and June. In comparison *O. edulis* larval abundances appear to be low during May but increase dramatically during June (Fabra, pers. Comm.).

3.3.2. Settlement plates

Due to the time constraints, posed by the spawning season and settlement extending beyond the duration of this study, the number of spat per disk and number of spat per cm^2 could not be calculated. Therefore, any spat that would have been present, but too small to be seen amongst the large amount of fouling (Figure 38), were allowed to remain and on-grow for future analysis.

Figure 38. Fouling organisms, such as *Ciona intestinalis*, present upon the settlement disk arrangement on (a) the University of Portsmouth research raft in June 2016. (b) The Land Rover Ben Ainslie Racing pontoon in July 2017.

4. Discussion

The conduction of a comprehensive stock assessment was essential to determine the current baseline status of the two fisheries of Chichester and Portsmouth Harbours. The data collected provides necessary information that will be used to determine the success of restoration attempts to be made in the coming years (Harding et al. 2016). A plethora of observations can be made with respect to all aspects of the assessment, the first of which is the 29.48 mm difference between the smallest size class cohorts from Chichester and Portsmouth Harbours.

This smaller cohort present in Chichester could be attributed to the re-stocking, as part of the restoration project, that took place in November 2010 (Vause 2010, Eagling 2012, cited in Gravestock et al. 2014, MEDIN 2016). Despite the mean condition index of the Portsmouth population being 0.67 higher than that of the Chichester population, there was no statistical significant difference. Indicating that the stark contrast in size classes, average shell depth and average wet weight, did not necessarily reflect a decrease in overall health of the oysters within the Chichester population. However, the condition index sample sizes were much smaller than the size class sample sizes and this should be taken into account when analysing the results.

There was also a distinct lack of oysters < 50 mm, in diameter (length or width), from both populations, which could potentially be associated with the fishing gear used, although personal communications with the fishermen indicate this is unlikely to be the case as the gear used was able to catch small stones < 50 mm (Legg pers. comms., Breeze pers. comms.). Since the same gear was used for both harbours, the 29.48 mm difference in the smallest cohort size is highly indicative of an aging population within the Portsmouth population. The large size of both smallest cohorts indicates continued

recruitment failure in Chichester Harbour, Portsmouth Harbour and possibly the wider Solent.

Another noticeable difference between the two populations was the contrast in the prevalence of *B. ostreae*. The result which raised cause for concern was the relatively high infection within the Chichester population, which in previous years, from 1993 - 2007, was at an average of 12.1 % (Laing et al. 2014), increasing by 9.18 % to 21.28 % in 2015. The results obtain in this study are in agreement with the findings of Eagling (2012), citied in Gravestock et al. (2014), who reported prevalence of between 25 - 35 %. This rise in prevalence of *B. ostreae* could be attributable to the increase in deaths of many of the re-laid oysters within the harbour observed by Jensen (pers. comm. with Gravestock et al. 2014).

In contrast to this, the prevalence within the Portsmouth population had decreased from an average of 5.6%, from 1993 - 2007 (Laing et al. 2014), to 0.0% in 2015. This result is encouraging with the apparent absence of *B. ostreae* from this population allowing for the mature individuals, remaining within the fishery, to be considered as a source of 'bonamiosis free' broodstock for deployment within the cage systems across the Solent wide restoration project. Flannery et al. (2014) found that prevalence of *B. ostreae* was seasonal in wild populations, peaking during April within various populations in Ireland. With Laing et al. (2014), observing peaks during October within cultivated stocks in the UK. Therefore, collecting, preserving and analysing samples during April and October would be likely to reveal if the Portsmouth population is truly free of the parasite within Spit Bank and Hamilton Bank areas.

Although not included in the current study, the prevalence within the Langstone Harbour population was on average 9.1 % from 1993 - 2007 (Laing et al. 2014). Molecular analysis of this population for current prevalence, both with the native population and those introduced within the broodstock cages on the UoPRR, would allow a comparison of the

three interlinked harbours to gain a better understanding of the overall infection fluctuations within the East section of the Solent.

Those individuals within the Chichester population that were infected, were shown to have a lower dry tissue weight, indicating either selection by the parasites for individuals in poor physical state or, a reduction in tissue weight due to the presence of the parasite.

There was a lack of correlation between condition index, length and the presence or absence of the parasite in the Chichester population. As with the dry tissue weight comparison, the results obtained only describe the presence or absence of *B. ostreae*, not the abundance of parasites within infected individuals. If obtained, this information could provide a greater understanding of the relationship between infection density and dry tissue weight, or condition index. This may provide future indications of *B. ostreae* presence or absence from these measurements prior to molecular analysis.

Sanger sequencing of the PCR products, followed by the use of the GenBank® Basic Local Alignment Search Tool (BLAST®) would allow for species identification and complete conformation that all results were indeed *B. ostreae* and not species such as *B. exitiosa* that has been described in European waters (Abollo et al. 2008).

To the knowledge of the author this is the first study designed to trial suspended broodstock cages, attached to existing floating structures, in an attempt to contribute to the repopulation of an existing *O. edulis* fishery. The loss of cages from the UoPRR population, experienced during adverse weather conditions, highlights the requirement for appropriate structural integrity when designing and installing the broodstock cage systems. Another factor to incorporate into the design is the accessibility of the stacked cage systems as a whole, as well as the separate cages at the various levels (top, middle and bottom). Difficulties were experienced when removing the cages as a whole at the

UoPRR and when accessing the oysters from the top, middle and bottom levels at both the UoPRR and the LRBAR sites.

Despite the difficulties experienced, this concept, once modified and reformed, would appear to be the most logical, with regards to cage accessibility, effectiveness and cost-benefit. The only other known studies for comparison, are the elevated broodstock reef structures tested by (Sawusdee et al. 2015) and suspended bags used by (Kamphausen, 2012). Destruction and loss of the cage systems, by adverse weather conditions and fishing gear, were also experienced during the Sawusdee et al. (2105) study (Hauton pers. comm.). Alongside these issue experienced, there is also the issue of the use of vessels and divers to allow for cage accessibility and monitoring of populations on the sea floor. These issues are greatly reduced by suspending the systems from existing pontoons and rafts, as in this study.

The high survivorship experienced within the LRBAR population compared to the high mortality within the UoPRR population is likely to be attributed to a variety of factors. The first of which was evident from the multivariate analysis conducted on the environmental parameters for the two locations. The high mortality experienced at the UoPRR as a whole was attributed to average temperature fluctuation, average ODO fluctuations (% saturation and mg/L), average ODO (% saturation), average temperature, month, average TDS and average salinity fluctuations. All of these parameters are likely to be caused by the extreme tidal variation experienced at this site. In addition, it is possible that an extreme stress even, or events, took place in Langstone Harbour with the potential occurrence of harmful algal blooms, see graphs in Appendix D.

In comparison to this, the mortality experienced at the LRBAR population was influenced by cage density, cage position, average turbidity and average ODO (mg/L). Kamphausen (2012) also kept bags of oysters (n = 80) under a pontoon at the National Oceanography Centre in Southampton and experienced low mortalities (1.8 ± 0.75 (average ± S.D.))

between April and November 2009. Despite the fact that bags will increase competition for food and reduce flow rate, these survival rates indicate that these sheltered environments, that experience reduced photoperiod and minimal fluctuations in abiotic parameters, are suitable habitats for the expansion of the broodstock cage project around the Solent.

The contrast in mortality levels experienced at the two locations in this study may also be attributed to the level of fouling organisms present upon the cage structures. The UoPRR cages were subject to greater lengths of photoperiod and were located within a more ecologically productive environment than the LRBAR cages, allowing for rapid colonisation of organisms such as the invasive ascidian *Ciona intestinalis*. The LRBAR cages also experienced fouling of *C. intestinalis* but at a reduced density compared with the UoPRR cages. Not only will these filter feeding organisms directly compete with the oysters for food and space within the cages, but they are also likely to reduce the flow of water through the cage systems, further reducing food availability. Further studies would be required to determine the credibility of this statement, however, a reduced flow rate and competition for food are likely to be extremely detrimental for the oysters within these cages.

The monthly changes in weight experienced were likely to be attributed to the spawning season. Oysters at both locations were, on average, seen to increase in weight between April and May and then decrease during June and July. These changes in growth are likely to be explained by the initial increase in gonadal development to produce gametes, followed by the release of larvae thus reducing the weight of the individuals.

Using estimations from Walne (1974) for the fecundity of oysters, the 675 individuals within the broodstock cages attached to the LRBAR pontoon, with an average diameter of 80 mm, have the potential to produce approximately 742,500,000 veliger larvae. With the low level of mortalities experienced within the high density populations, it could be

suggested that all low density cages have their densities increased to that of the high density cages. This increase would provide 900 broodstock oysters with the potential of producing 990,000,000 veliger larvae on an annual basis, at this site alone. Accounting for predation, disease, natural mortalities and lack of settlement, a conservative estimation of 1 in 1,000,000 larvae surviving and settling, half that suggested by Guerra (2002) cited in Laing et al. (2005), would represent a contribution of 990 oysters to the Solent population. Theoretically, once mature and are combined with the original broodstock cage population, a total of 1,890 oysters would be able to produce 2,079,000,000 veliger larvae. Extrapolating these numbers over a ten year period would provide a total of 8,335 oysters capable of producing 9,168,500,000 larvae from one broodstock population. Despite these hypothetical figures and extrapolations being based on published larval densities (Walne 1974) and survival rates (Guerra 2002 cited in Laing et al. 2005), this study confirms that off bottom broodstock cages can provide a significant and continuing source of juvenile oysters to support the recovery of *O. edulis* within the Solent.

Preliminary findings, yet to be fully analysed to produce quantitative data, from the plankton community samples correlate with the data of Richard et al. (2006). Indicating high abundances of *C. fornicata* larvae during May and June, compared with this lower abundances of *O. edulis* larvae were observed in May, which increased dramatically in June. These initial findings show that *C. fornicata* spawns earlier than *O. edulis*, allowing the larvae of *C. fornicata* to settle in advance of *O. edulis*. The extremely large numbers of *C. fornicata* present within the Solent area (Barnes et al. 1973, Blanchard 1997) and a peak in spawning prior to that of *O. edulis* is likely to provide overwhelming competition for the oyster veliger larvae when they come to settle on the seabed.

The broodstock cages, at a raised temperature from that on the seafloor, may inadvertently induce the earlier spawning of the individuals within them. This would allow for much greater competition between the two species and, with additional management plans that

are being considered such as spat collectors, cultch bags and harrowing of selected *C. fornicata* beds (Harding et al. 2016), the larvae produced from the broodstock populations will have an increased chance of survival.

With the knowledge that larval densities show significant spatial variation and are greater in proximity to natural, commercial, broodstock populations (Kennedy & Roberts 2006), the addition of spat collectors in the vicinity of the broodstock cages and in other locations based on future hydrodynamic models is recommended. This would inevitably increase the survivorship of the larval population released from the broodstock oysters. In addition to this, the use of a variety of carefully selected source populations from multiple locations, will allow for the prevention of reduced genetic diversity within the spawned cohorts that occurs naturally (Hedgecock et al. 2007).

5. Conclusion

It is apparent that the natural population of *O. edulis* within the Solent is in need of anthropogenic assistance to return it to its once thriving fishery status. Multiple, if not all, aspects of the reproductive lifecycle of the species require further investigation to inform management and intervention. This study focused on the viability of off-bottom protected broodstock populations as a potential means to increase larval input. It can be concluded that the concept is feasible, however there is the requirement for minor adjustments to cage design and the careful selection of suitable marina environments. The lack of significant difference in the percentage mortalities of the high and low density populations allow for the suggested use of 50 oysters per 0.5 m^2 for the projects expansion. The low mortality alongside successful reproduction and larval development within the broodstock oysters at the LRBAR pontoon suggests that sheltered, industrial ports are suitable environments and was an extremely encouraging outcome. Further work is required to determine if the high mortality in the more variable, high energy site at Langstone was due to its inherent abiotic and hydrodynamic characteristics or one off, or multiple, stress events.

6. Appendix A

"*Evaluation of threats and impacts: An assessment of the sensitivity of O. edulis based on a literature review can be found on the Marine Life Information Network for Britain & Ireland (MarLIN) (Jackson 2007). The species is listed as being highly sensitive to substrate loss, smothering, synthetic compound contamination, introduction of microbial pathogens/parasites, introduction of non-native species and direct extraction. Summary of key threats and impacts to O. edulis*." OSPAR Commission (2009, p 11 - 13).

Type of impact	Cause of threat	Comment	Scale of threat
Habitat Degradation through smothering & siltation	Aggregate extraction industry; navigational dredging; dredge spoil dumping	Operations leading to significant siltation or smothering of the seabed might be expected to have a significant effect on both flat oyster beds and their associated communities, particularly in low energy environments where the silt is unlikely to be dispersed easily. Adequate EIA before such developments begin should identify any risks to the habitat.	Medium
Habitat degradation through physical damage	Anchoring	Anchors dragging through *O. edulis* beds can cause significant localised damage, breaking up clumps of oysters and damaging the overall structure of the habitat. Dislodging of oysters may make the beds more susceptible to subsequent damage by wave action or predation. Potential for such damage is highest in sheltered inlets (sea lochs, estuaries, fjords) where anchoring is more likely.	Low
Habitat loss or degradation through physical damage	Bottom trawling	In the past *O. edulis* beds were dredged, directly targeting *O. edulis.* Now the main threat to *O. edulis* beds is bottom fishing, using trawls and dredges, targeting bottom fish and bivalve molluscs. Trawling damages both *O. edulis* and associated epibenthic species and impacts the abiotic environment. Obvious effects include the loss of epifaunal and the alteration/degradation of the habitat.	High
Habitat loss or alteration	Infrastructure development (wind farms, oil & gas, cables)	*O. edulis* beds can be damaged by any infrastructure development which disturbs or alters the seabed habitat: offshore industry developments such as wind farms, oil and gas rigs, trenching and pipe/cable-laying. Provided proper EIAs are undertaken before such developments begin, and sensitive areas of this habitat avoided, the risks can be kept to a minimum.	Low
Overfishing	Target fishery	*O. edulis* has been over harvested during the 19th century and the former beds have not recovered in most cases. Long-term harvesting of particular	Medium

		populations could be unsustainable, due to slow growth rates and poor recruitment success.	
Change in tidal current regimes	Tidal power schemes; causeway building	*Ostrea* beds tend to occur in areas of moderate to strong tidal currents, particularly in tidal channels between islands and offshore sandbanks, narrow entrances to lochs and basins. Any constructions which alter the tidal flow rates through such channels could affect the viability of *O. edulis* beds (either in the channels or down stream of them), alter the associated communities and potentially lead to loss of the beds.	Low
Changes in sea temperature affecting reproduction	Climate change	*O. edulis* cannot tolerate low temperatures. Thus, *O. edulis* may profit from future increases in water temperature. This is likely to be most important in areas towards the northern edge of its range. The influence of water temperature on the prevalence of diseases is not clear. On the other hand, higher temperatures combined with eutrophication and algal blooms can lead to lower oxygen levels causing direct mortality.	?
Pollution	Land-based and marine industrial or commercial sources	*O.edulis* is known to accumulate contaminants, such as heavy metals, in spoil disposal areas. The effects on condition, reproduction and mortality rates are unknown (UKBAP 1999). *O.edulis* is highly sensitive to synthetic compound contamination (*e.g.* Rees et al. 2001) and tributyl tin. Eutrophication may lead to excessive algal blooms, leading to low oxygen levels.	Medium
Diseases	Introduction of microbial pathogens/parasites	The protozoan *Bonamia ostreae* has caused massive mortalities of *O.edulis* in France, the Netherlands, Spain, Iceland and England (Edwards 1997, da Silva et al. 2005, Culloty & Mulcahy 2007). Another protist, *Marteilia refringens* can cause 75 - 100% mortality in *O.edulis*. There is evidence for interspecies transmission of diseases. The protozoan *Mikrocytos mackini* also affects *O.edulis* and the oyster herpesvirus infects *O.edulis* and results in larval and seed mortality. The bacterium *Nocardia crassostreae* can infect *O.edulis* and make it heat sensitive (Ruesink et al. 2005).	High
Changes in Genetic integrity	Importation and relaying of (seed) oysters of foreign origin on commercial beds	Mixing of potentially genetically different strains, from a different geographical origin, could result in problems with physiological adaptation; it can affect the genetic diversity of the species, and introduce diseases to non-resistant populations.	Medium

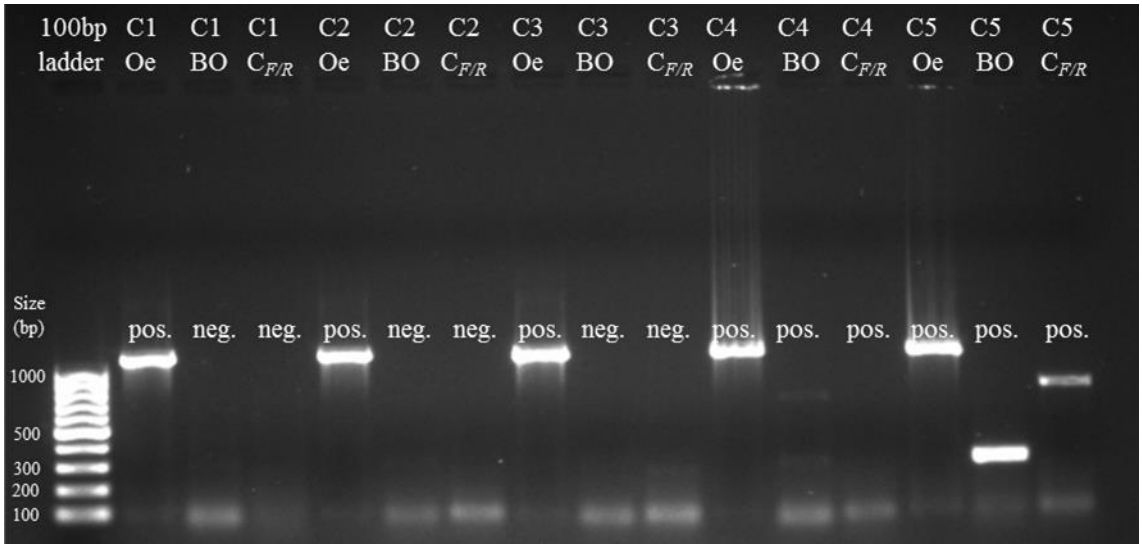
7. Appendix B

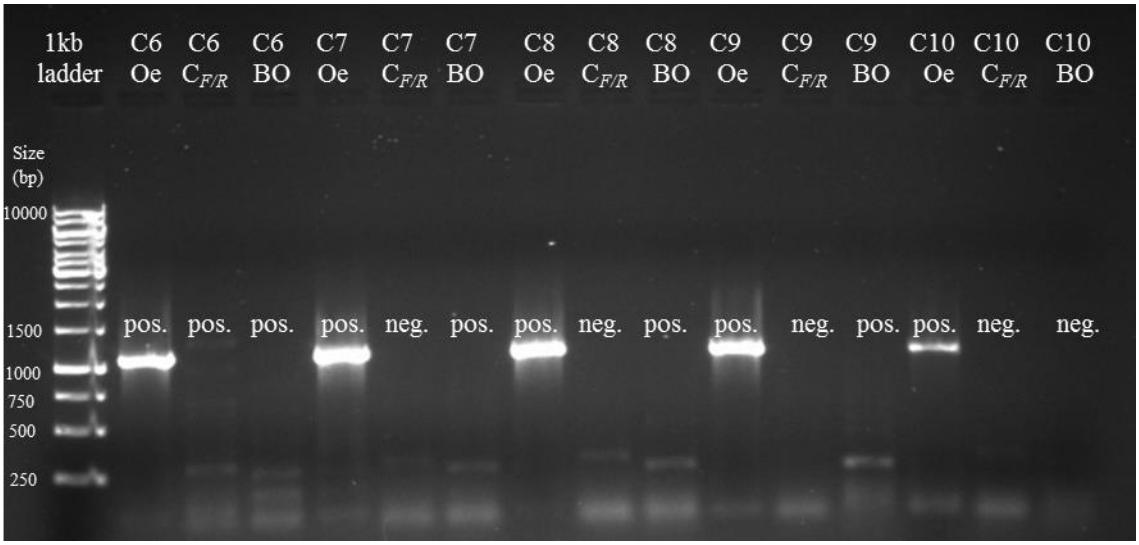
The quantities of DNA (ng/μl), and the 260/280 260/230 ratios, obtained from the oyster gill tissue in section 2.1.2.3.

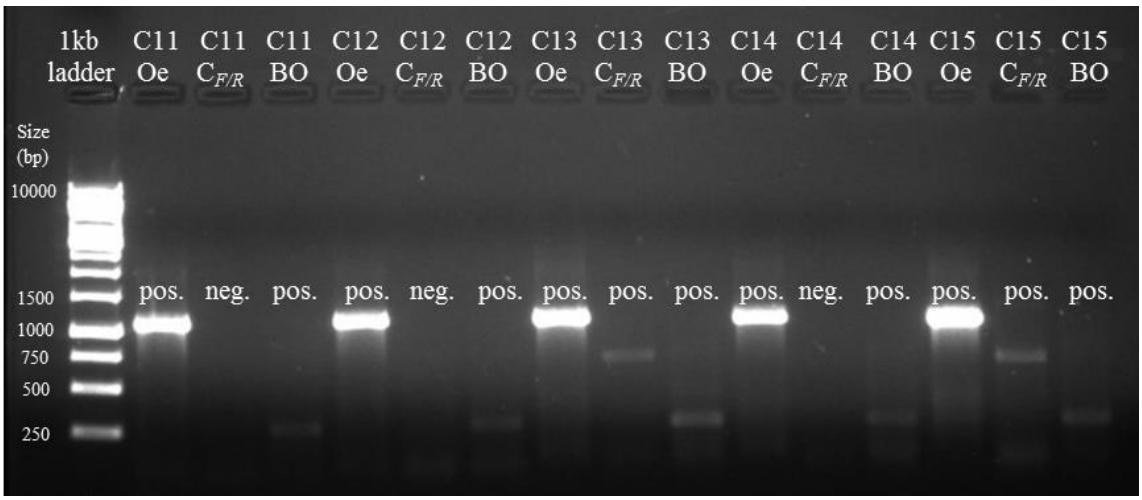
Code	DNA (ng/μl)	260/280	260/230	Code	DNA (ng/µl)	260/280	260/230
C1	164.3	1.99	1.54	**P1**	250.5	2.07	1.68
C2	73.2	2.09	1.42	**P2**	225.3	2.12	2.10
C3	93.2	2.13	1.76	**P3**	105.9	2.11	1.72
C4	114.0	1.99	1.43	**P4**	85.3	2.14	1.62
C5	130.2	1.29	1.74	**P5**	231.5	2.11	1.80
C6	205.6	2.12	2.01	**P6**	68.6	2.14	1.76
C7	185.6	2.06	2.23	**P7**	27.3	2.07	1.26
C8	186.1	2.11	1.99	**P8**	160.4	2.07	1.61
C9	120.3	2.00	1.86	**P9**	297.8	2.21	2.05
C10	150.9	2.06	1.89	**P10**	329.6	2.17	1.89
C11	197.4	2.11	2.12	**P11**	292.3	2.18	1.95
C12	116.3	1.08	2.01	**P12**	261.4	2.16	1.83
C13	133.6	2.13	2.16	**P13**	49.6	2.09	1.48
C14	200.1	2.08	2.32	**P14**	135.4	2.19	1.83
C15	159.1	2.06	2.29	**P15**	219.5	2.16	1.94
C16	210.0	2.09	2.12	**P16**	266.5	2.19	2.03
C17	269.9	2.10	2.08	**P17**	133.4	2.15	1.79
C18	159.3	2.08	2.19	**P18**	276.5	2.19	2.07
C19	144.3	2.13	2.21	**P19**	151.1	2.16	1.86
C20	159.8	2.10	2.07	**P20**	104.4	2.17	1.90
C21	200.6	2.01	1.74	**P21**	49.2	2.10	1.98
C22	144.3	2.07	2.28	**P22**	387.6	2.19	2.01
C23	171.5	2.04	1.82	**P23**	288.8	2.25	2.15
C24	78.3	2.04	1.82	**P24**	82.7	2.14	1.62
C25	104.1	2.07	2.00	**P25**	116.5	2.12	1.98
C26	30.9	2.21	1.39	**P26**	195.0	2.11	1.91
C27	151.0	2.13	2.18	**P27**	283.9	2.10	1.88
C28	173.5	2.10	2.19	**P28**	76.3	2.12	1.90
C29	101.1	1.92	1.42	**P29**	138.6	2.13	1.76
C30	205.7	2.20	1.92	**P30**	92.6	2.10	1.63
C31	252.0	2.16	1.91	**P31**	139.9	2.08	1.79
C32	250.9	2.17	1.91	**P32**	110.6	2.10	1.75
C33	202.6	2.18	2.00	**P33**	86.2	2.07	1.53
C34	303.1	2.18	1.90	**P34**	62.7	1.97	1.73
C35	26.6	2.09	1.06	**P35**	45.6	2.07	1.79
C36	74.8	2.13	1.87	**P36**	79.6	2.14	1.66
C37	191.2	2.21	1.88	**P37**	48.0	2.16	1.37
C38	152.8	2.12	1.99	**P38**	39.1	1.98	1.56
C39	53.3	2.14	1.70	**P39**	58.7	2.07	1.77
C40	238.1	2.09	1.80	**P40**	186.7	2.09	1.90
C41	376.2	2.17	2.00	**P41**	40.4	1.97	1.46
C42	363.9	2.10	2.08	**P42**	24.5	1.94	1.20
C43	190.9	2.08	1.83	**P43**	63.5	2.06	1.72
C44	130.5	2.10	1.81	**P44**	16.1	2.01	1.07
C45	47.3	2.13	1.95	**P45**	101.3	2.08	1.74
C46	233.3	2.15	2.17	**P46**	99.1	2.08	1.75
C47	142.2	2.12	1.91	**P47**	22.9	1.96	1.43
C48	90.7	2.10	1.66	**P48**	117.4	1.98	1.47

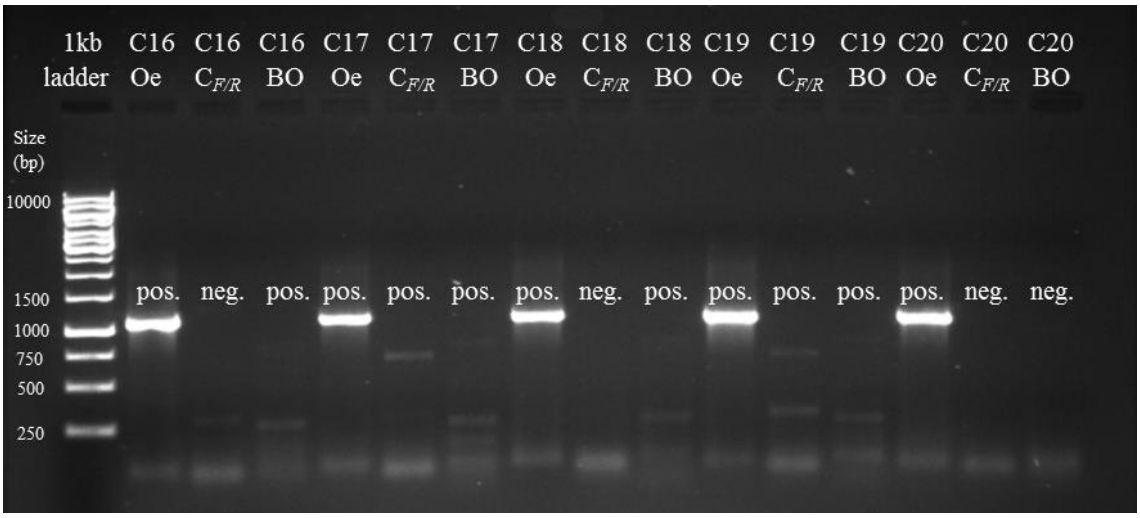
8. Appendix C

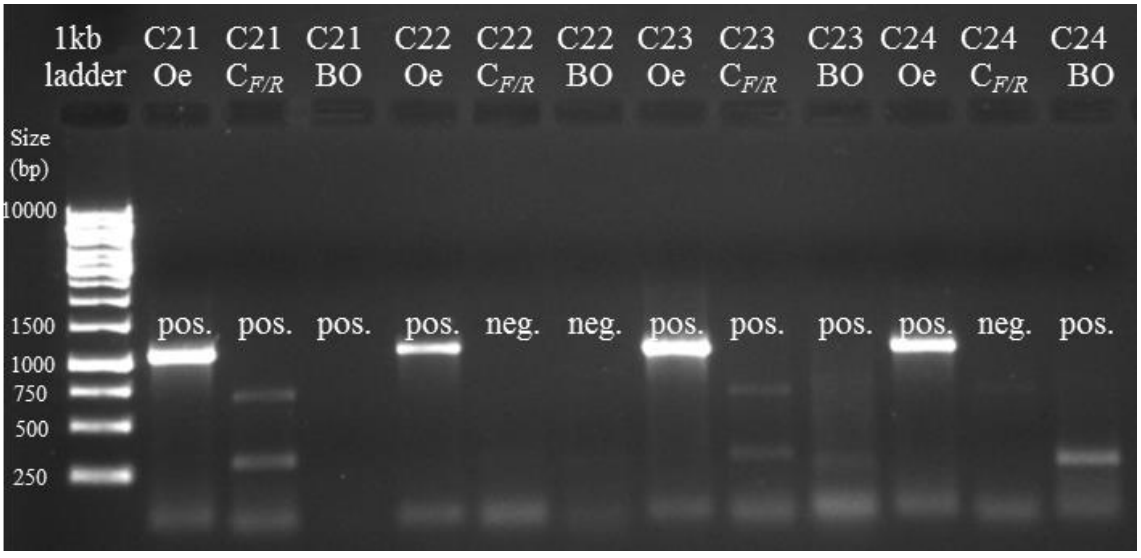
All UV transilluminated electrophoresis gel samples for the amplifications of *Ostrea edulis* COI gene, the nearly complete SSU microcell Haplosporidian DNA and the nearly complete SSU of *Bonamia ostreae* 18S rDNA from Chichester Harbour and Portsmouth Harbour oysters. A 1 kilobase (kb) ladder (250 - 10,000 bp) was used as a reference for primer pairs Oe fw_1 / Oe rev_4 (Oe) (Grecken & Schmidt 2014), BO / BOAS (BO) (Cochennec et al. 2000) and C_F / C_R ($C_{F/R}$) (Carnegie et al. 2000), amplifications in all but the first gel where a 100 bp (100 – 1000 bp's) ladder was used.

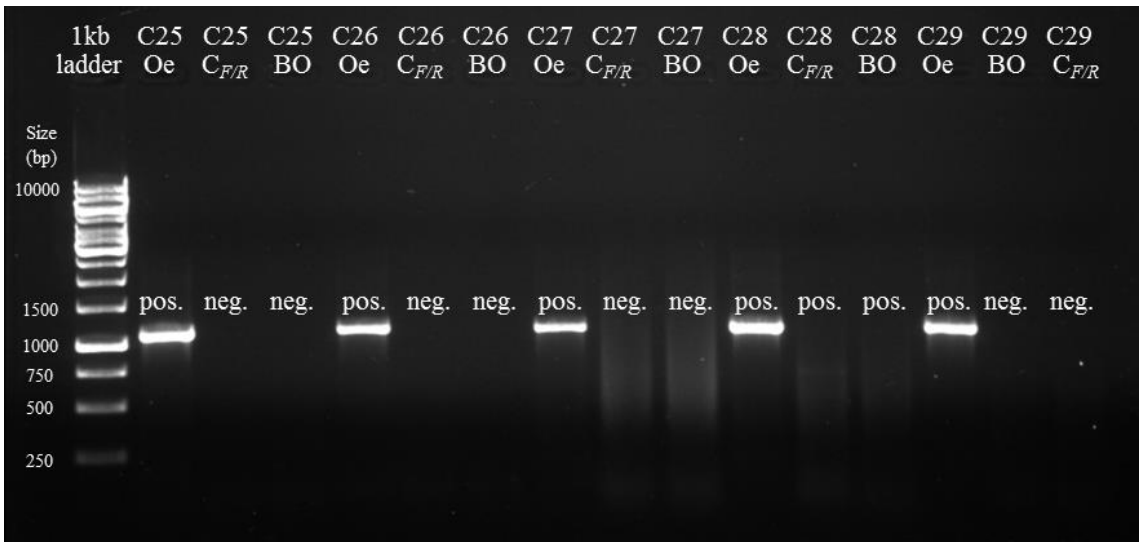


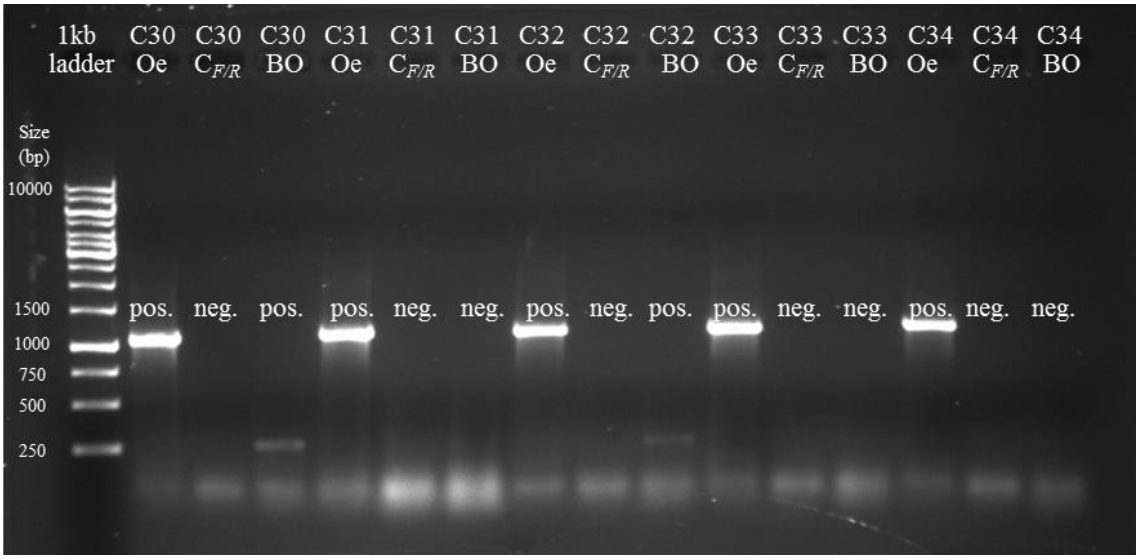


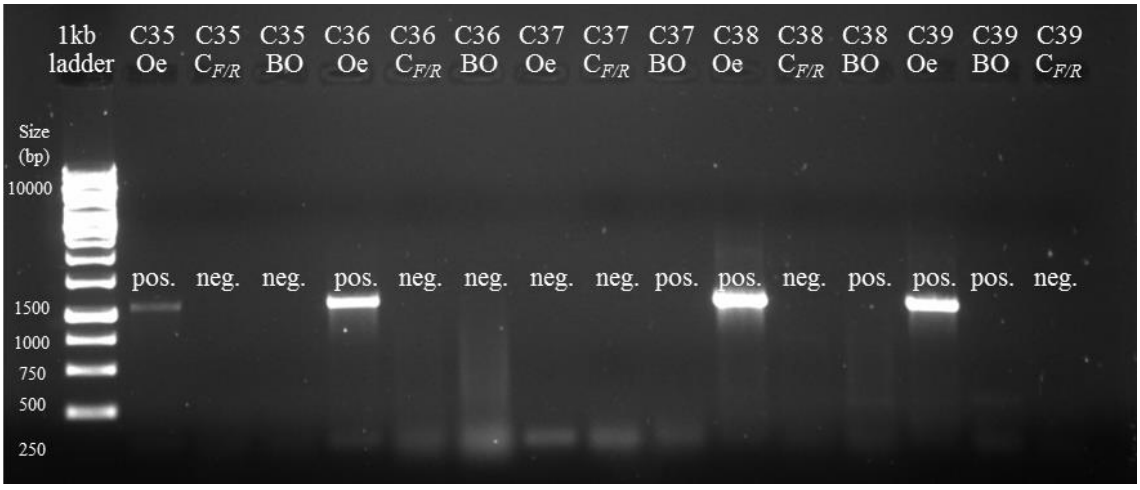

1kb ladder
C11 Oe
C11 $C_{F/R}$
C11 BO
C12 Oe
C12 $C_{F/R}$
C12 BO
C13 Oe
C13 $C_{F/R}$
C13 BO
C14 Oe
C14 $C_{F/R}$
C14 BO
C15 Oe
C15 $C_{F/R}$
C15 BO
Size (bp)
10000
1500
1000
750
500
250
pos. neg. pos. pos. neg. pos. pos. pos. pos. pos. neg. pos. pos. pos. pos.

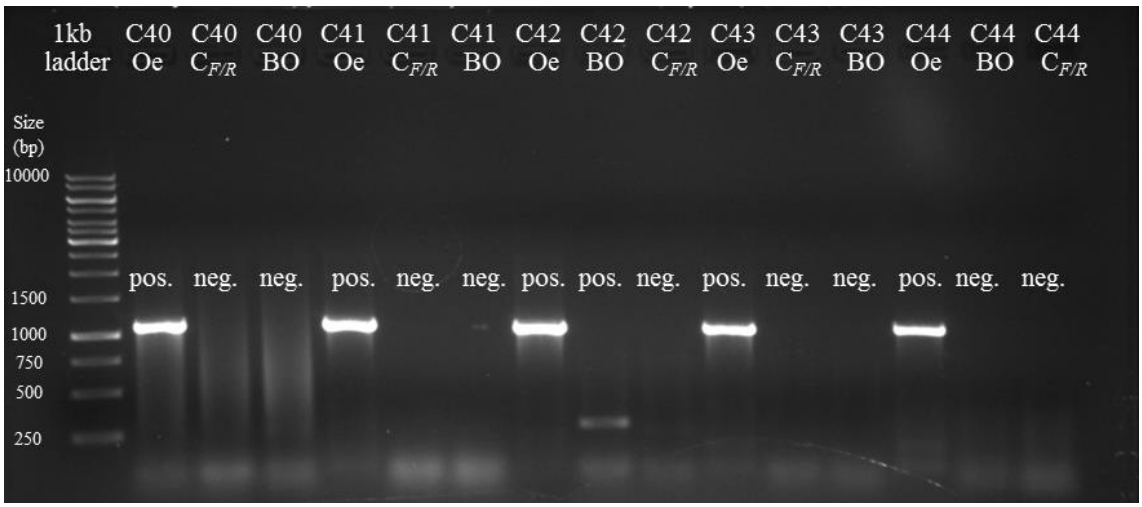

1kb ladder
C16 Oe
C16 $C_{F/R}$
C16 BO
C17 Oe
C17 $C_{F/R}$
C17 BO
C18 Oe
C18 $C_{F/R}$
C18 BO
C19 Oe
C19 $C_{F/R}$
C19 BO
C20 Oe
C20 $C_{F/R}$
C20 BO
Size (bp)
10000
1500
1000
750
500
250
pos. neg. pos. pos. pos. pos. pos. neg. pos. pos. pos. pos. pos. neg. neg.

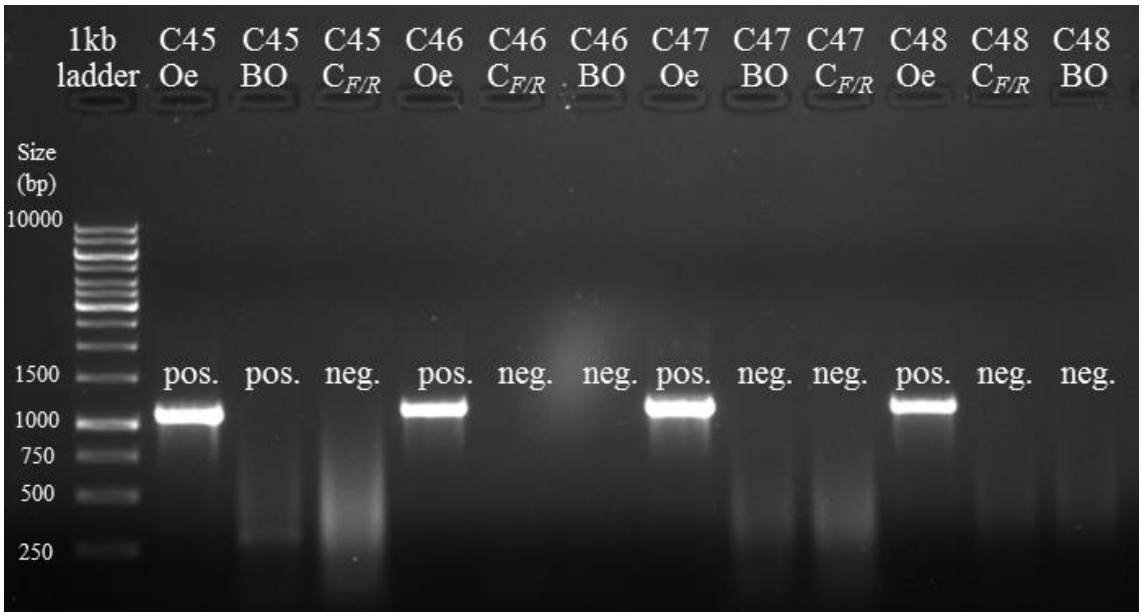

1kb ladder
C21 Oe
C21 $C_{F/R}$
C21 BO
C22 Oe
C22 $C_{F/R}$
C22 BO
C23 Oe
C23 $C_{F/R}$
C23 BO
C24 Oe
C24 $C_{F/R}$
C24 BO
Size (bp)
10000
1500
1000
750
500
250
pos. pos. pos. pos. neg. neg. pos. pos. pos. pos. neg. pos.

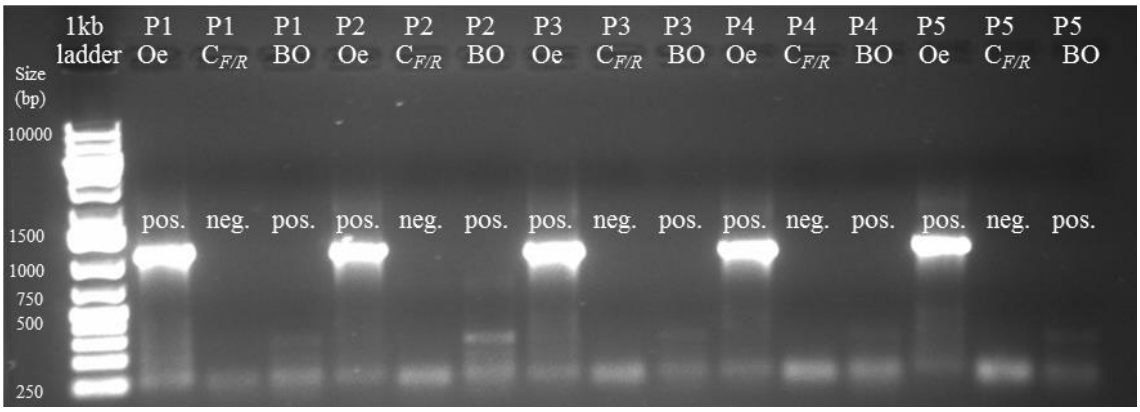

1kb ladder
C25 Oe
C25 $C_{F/R}$
C25 BO
C26 Oe
C26 $C_{F/R}$
C26 BO
C27 Oe
C27 $C_{F/R}$
C27 BO
C28 Oe
C28 $C_{F/R}$
C28 BO
C29 Oe
C29 BO
C29 $C_{F/R}$
Size (bp)
10000
1500
1000
750
500
250
pos. neg. neg. pos. neg. neg. pos. neg. neg. pos. pos. pos. pos. neg. neg.

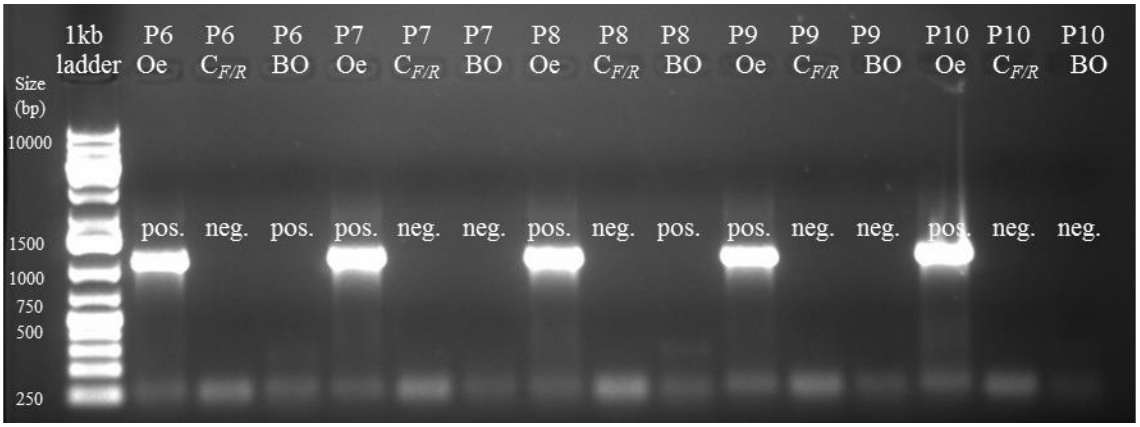

1kb ladder
C30 Oe
C30 $C_{F/R}$
C30 BO
C31 Oe
C31 $C_{F/R}$
C31 BO
C32 Oe
C32 $C_{F/R}$
C32 BO
C33 Oe
C33 $C_{F/R}$
C33 BO
C34 Oe
C34 $C_{F/R}$
C34 BO
Size (bp)
10000
1500
1000
750
500
250
pos. neg. pos. pos. neg. neg. pos. neg. pos. pos. neg. neg. pos. neg. neg.

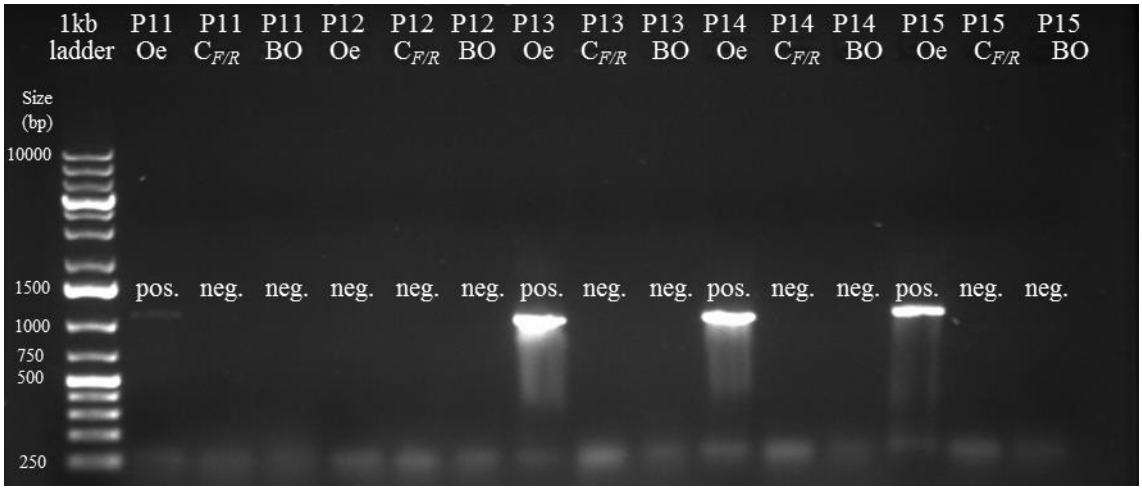

1kb ladder
C35 Oe
C35 $C_{F/R}$
C35 BO
C36 Oe
C36 $C_{F/R}$
C36 BO
C37 Oe
C37 $C_{F/R}$
C37 BO
C38 Oe
C38 $C_{F/R}$
C38 BO
C39 Oe
C39 BO
C39 $C_{F/R}$
Size (bp)
10000
1500
1000
750
500
250
pos. neg. neg. pos. neg. neg. neg. neg. pos. pos. neg. pos. pos. pos. neg.

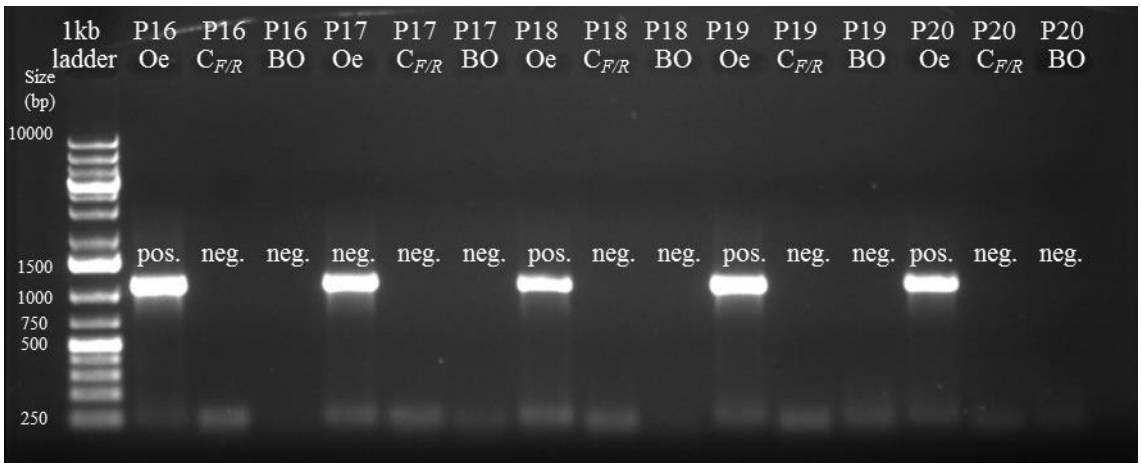

1kb ladder
C40 Oe
C40 $C_{F/R}$
C40 BO
C41 Oe
C41 $C_{F/R}$
C41 BO
C42 Oe
C42 BO
C42 $C_{F/R}$
C43 Oe
C43 $C_{F/R}$
C43 BO
C44 Oe
C44 BO
C44 $C_{F/R}$
Size (bp)
10000
1500
1000
750
500
250
pos. neg. neg. pos. neg. neg. pos. pos. neg. pos. neg. neg. pos. neg. neg.

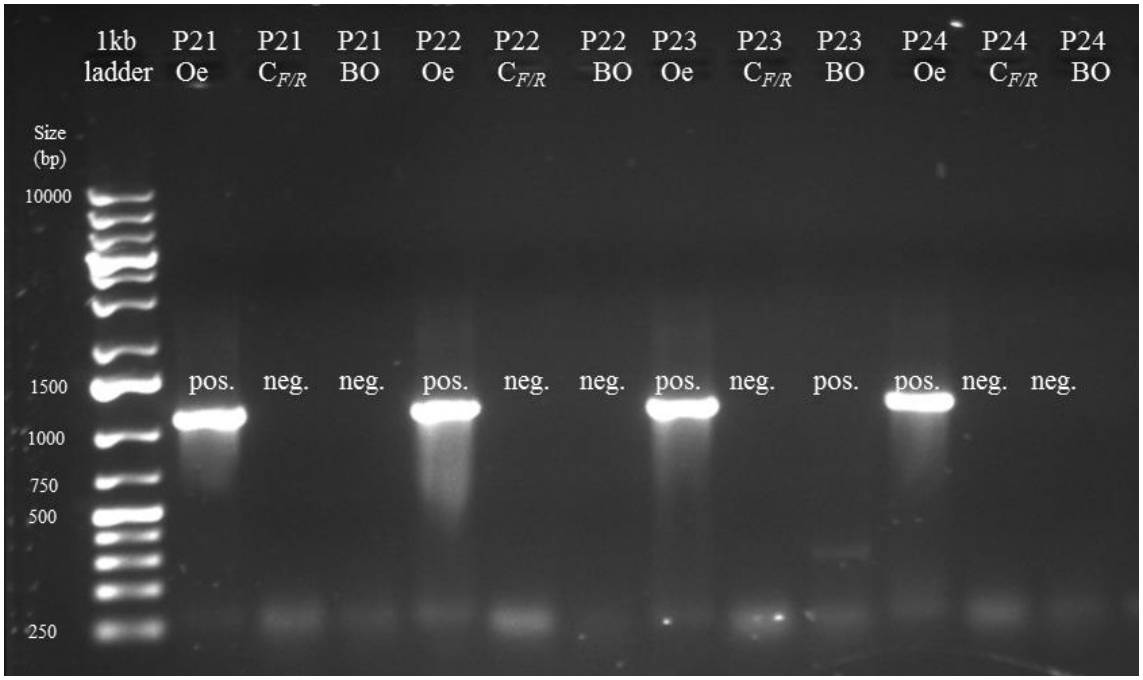

1kb ladder
C45 Oe
C45 BO
C45 $C_{F/R}$
C46 Oe
C46 $C_{F/R}$
C46 BO
C47 Oe
C47 BO
C47 $C_{F/R}$
C48 Oe
C48 $C_{F/R}$
C48 BO
Size (bp)
10000
1500
1000
750
500
250
pos. pos. neg. pos. neg. neg. pos. neg. neg. pos. neg. neg.

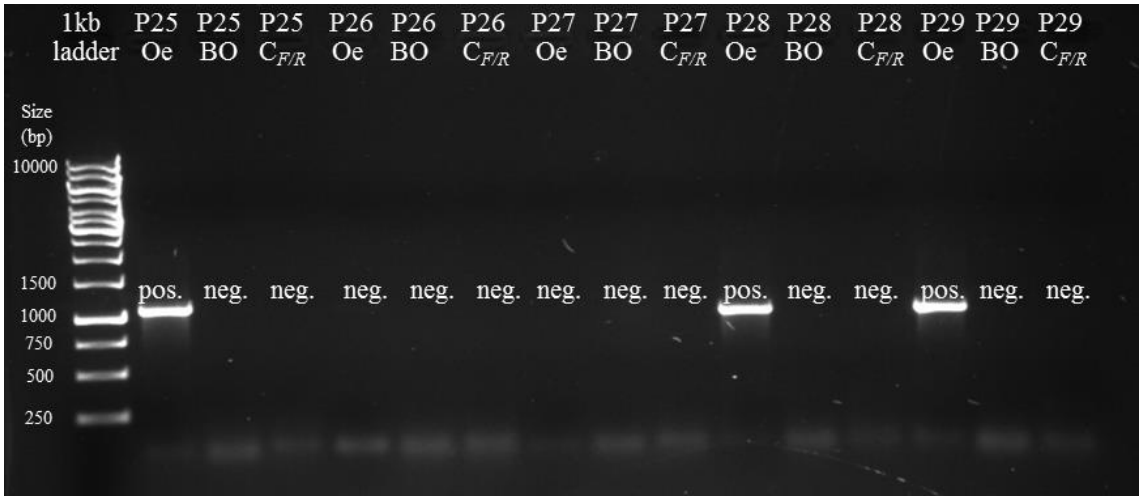

1kb ladder
P1 Oe
P1 $C_{F/R}$
P1 BO
P2 Oe
P2 $C_{F/R}$
P2 BO
P3 Oe
P3 $C_{F/R}$
P3 BO
P4 Oe
P4 $C_{F/R}$
P4 BO
P5 Oe
P5 $C_{F/R}$
P5 BO
Size (bp)
10000
1500
1000
750
500
250
pos. neg. pos. pos. neg. pos. pos. neg. pos. pos. neg. pos. pos. neg. pos.

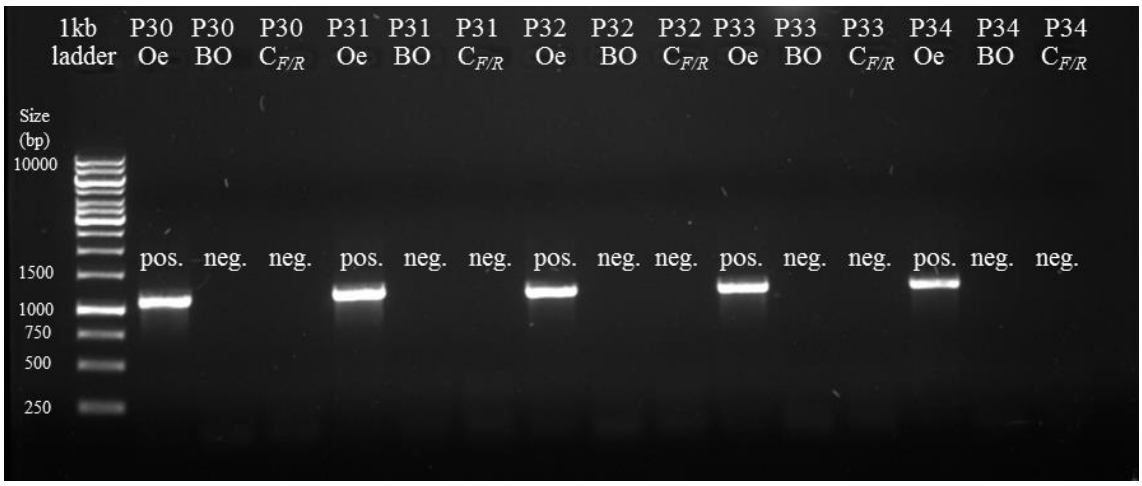

1kb ladder
P6 Oe
P6 $C_{F/R}$
P6 BO
P7 Oe
P7 $C_{F/R}$
P7 BO
P8 Oe
P8 $C_{F/R}$
P8 BO
P9 Oe
P9 $C_{F/R}$
P9 BO
P10 Oe
P10 $C_{F/R}$
P10 BO
Size (bp)
10000
1500
1000
750
500
250
pos. neg. pos. pos. neg. neg. pos. neg. pos. pos. neg. neg. pos. neg. neg.

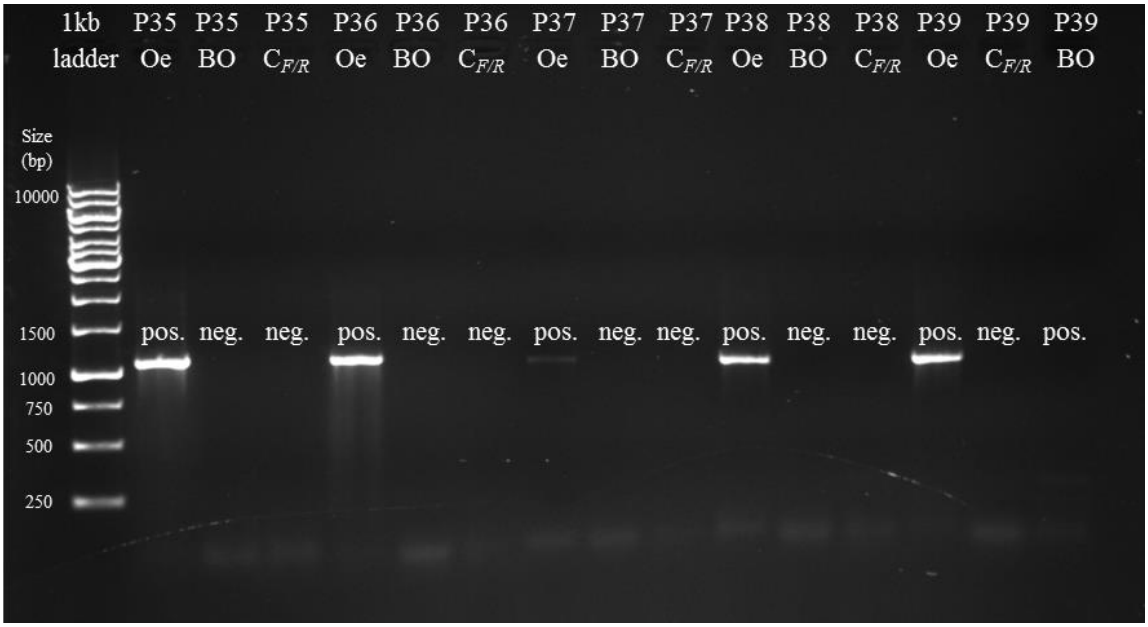

1kb ladder
P11 Oe
P11 $C_{F/R}$
P11 BO
P12 Oe
P12 $C_{F/R}$
P12 BO
P13 Oe
P13 $C_{F/R}$
P13 BO
P14 Oe
P14 $C_{F/R}$
P14 BO
P15 Oe
P15 $C_{F/R}$
P15 BO
Size (bp)
10000
1500
1000
750
500
250
pos. neg. neg. neg. neg. neg. pos. neg. neg. pos. neg. neg. pos. neg. neg.

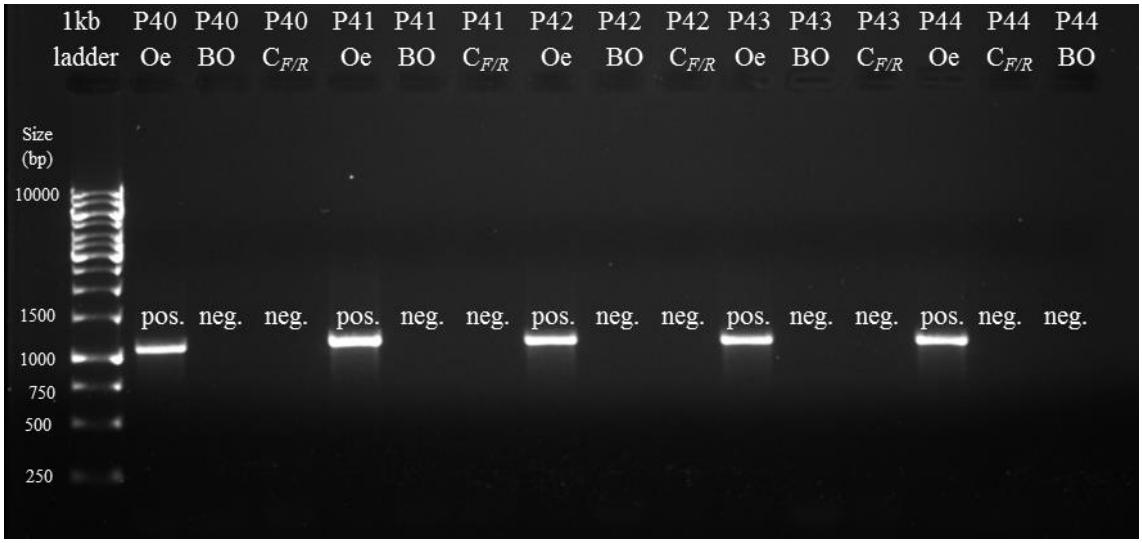

1kb ladder
P16 Oe
P16 $C_{F/R}$
P16 BO
P17 Oe
P17 $C_{F/R}$
P17 BO
P18 Oe
P18 $C_{F/R}$
P18 BO
P19 Oe
P19 $C_{F/R}$
P19 BO
P20 Oe
P20 $C_{F/R}$
P20 BO
Size (bp)
10000
1500
1000
750
500
250
pos. neg. neg. neg. neg. neg. pos. neg. neg. pos. neg. neg. pos. neg. neg.

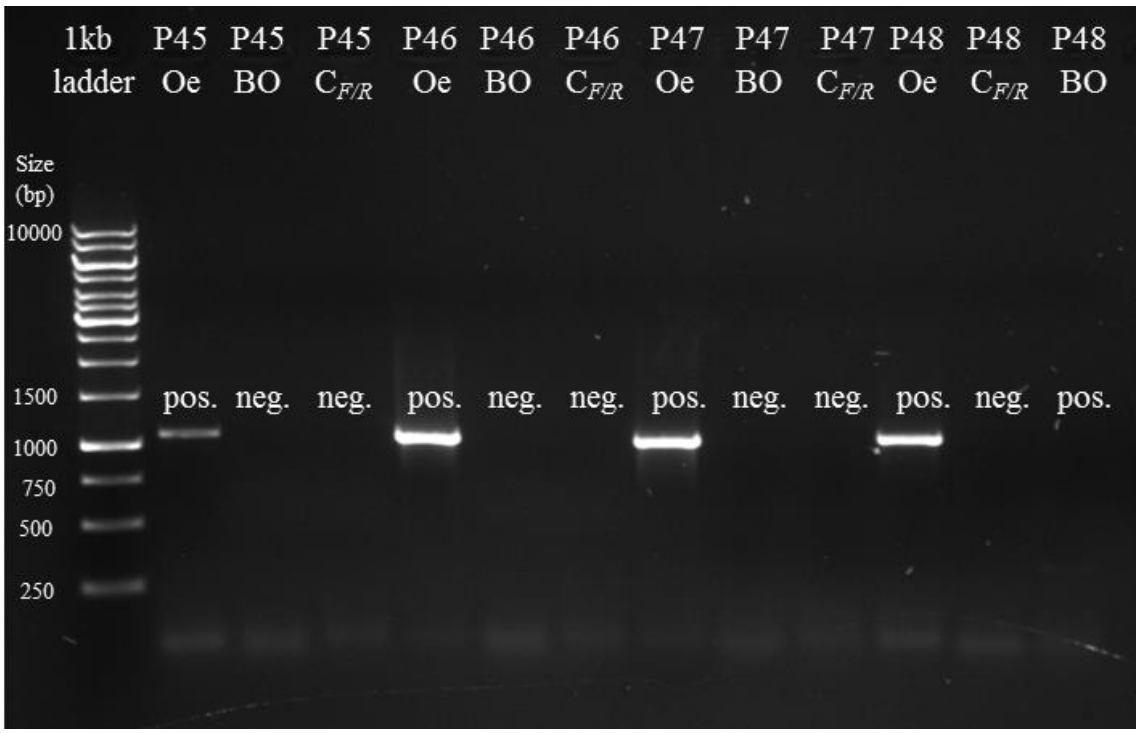

1kb ladder
P21 Oe
P21 $C_{F/R}$
P21 BO
P22 Oe
P22 $C_{F/R}$
P22 BO
P23 Oe
P23 $C_{F/R}$
P23 BO
P24 Oe
P24 $C_{F/R}$
P24 BO
Size (bp)
10000
1500
1000
750
500
250
pos. neg. neg. pos. neg. neg. pos. neg. pos. pos. neg. neg.


1kb ladder
P25 Oe
P25 BO
P25 $C_{F/R}$
P26 Oe
P26 BO
P26 $C_{F/R}$
P27 Oe
P27 BO
P27 $C_{F/R}$
P28 Oe
P28 BO
P28 $C_{F/R}$
P29 Oe
P29 BO
P29 $C_{F/R}$
Size (bp)
10000
1500
1000
750
500
250
pos. neg. neg. neg. neg. neg. neg. neg. neg. pos. neg. neg. pos. neg. neg.


1kb ladder
P30 Oe
P30 BO
P30 $C_{F/R}$
P31 Oe
P31 BO
P31 $C_{F/R}$
P32 Oe
P32 BO
P32 $C_{F/R}$
P33 Oe
P33 BO
P33 $C_{F/R}$
P34 Oe
P34 BO
P34 $C_{F/R}$
Size (bp)
10000
1500
1000
750
500
250
pos. neg. neg. pos. neg. neg. pos. neg. neg. pos. neg. neg. pos. neg. neg.


1kb ladder
P35 Oe
P35 BO
P35 $C_{F/R}$
P36 Oe
P36 BO
P36 $C_{F/R}$
P37 Oe
P37 BO
P37 $C_{F/R}$
P38 Oe
P38 BO
P38 $C_{F/R}$
P39 Oe
P39 $C_{F/R}$
P39 BO
Size (bp)
10000
1500
1000
750
500
250
pos. neg. neg. pos. neg. neg. pos. neg. neg. pos. neg. neg. pos. neg. pos.

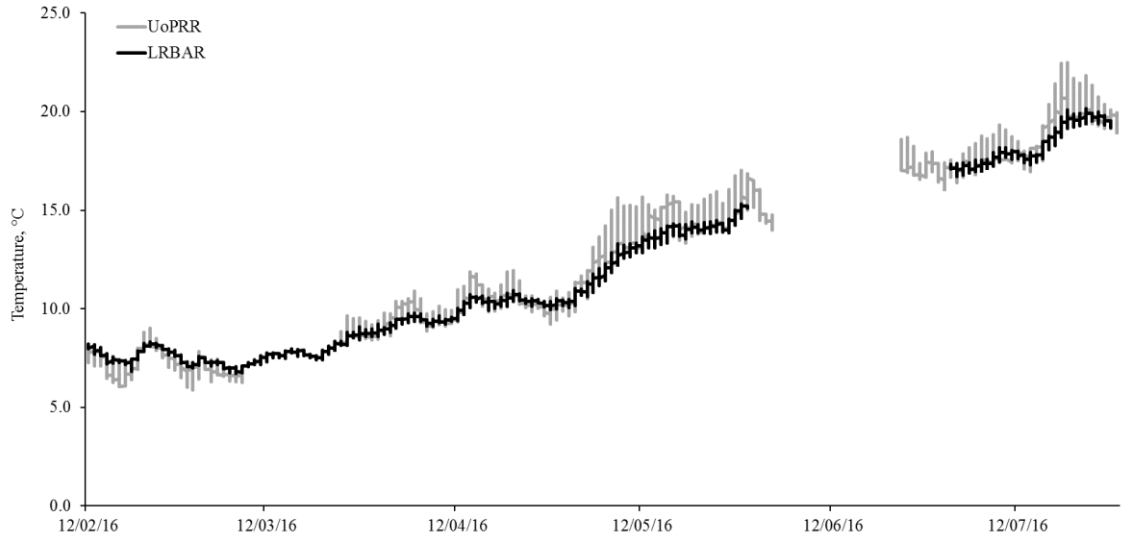

1kb ladder
P40 Oe
P40 BO
P40 $C_{F/R}$
P41 Oe
P41 BO
P41 $C_{F/R}$
P42 Oe
P42 BO
P42 $C_{F/R}$
P43 Oe
P43 BO
P43 $C_{F/R}$
P44 Oe
P44 $C_{F/R}$
P44 BO
Size (bp)
10000
1500
1000
750
500
250
pos. neg. neg. pos. neg. neg. pos. neg. neg. pos. neg. neg. pos. neg. neg.


1kb ladder
P45 Oe
P45 BO
P45 $C_{F/R}$
P46 Oe
P46 BO
P46 $C_{F/R}$
P47 Oe
P47 BO
P47 $C_{F/R}$
P48 Oe
P48 $C_{F/R}$
P48 BO
Size (bp)
10000
1500
1000
750
500
250
pos.
neg.
neg.
pos.
neg.
neg.
pos.
neg.
neg.
pos.
neg.
pos.

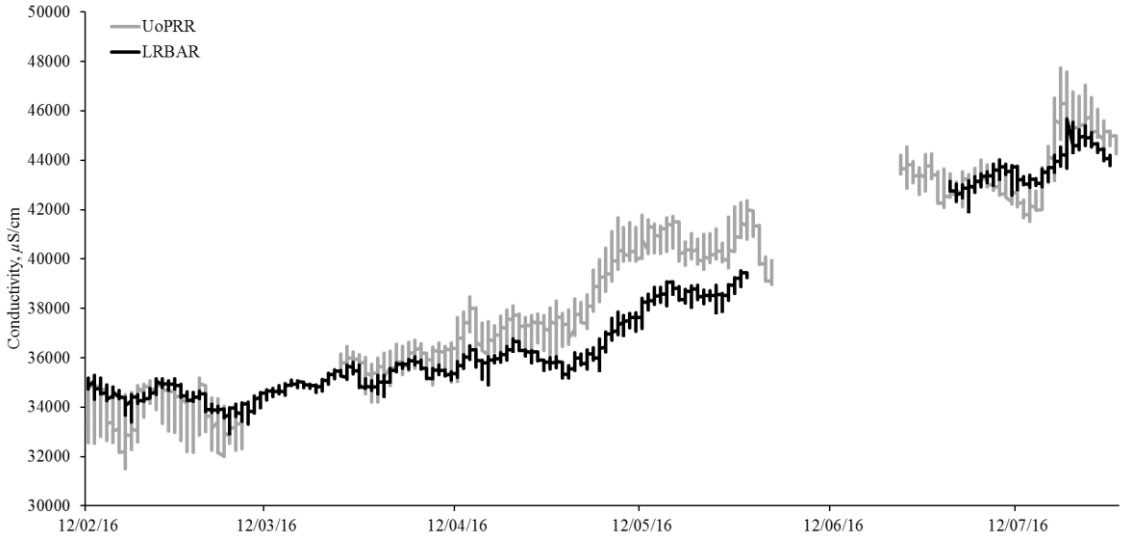
9. Appendix D

EXO2® Sonde recordings of (a) Temperature (°C) (b) Conductivity (µS/cm) (c) Specific conductivity (µS/cm) (d) Salinity (psu) (e) Non-linear function (nFL) conductivity (µS/cm) (f) Total dissolved solids (mg/L) (g) Chlorophyll *a* (RFU) (h) Chlorophyll *a* (µg/L) (i) Blue-Green algae - phycocyanin (BGA-PC) (RFU) (j) Blue-Green algae - Phycocyanin (µg/L) (k) Optical dissolved oxygen (% saturation) (l) Optical dissolved oxygen (mg/L) (m) Turbidity (FNU) (n) pH (o) Depth (cm) at both the Land Rover Ben Ainslie Racing pontoon and the University of Portsmouth Research Raft between February and July. Gaps in the data indicate removal of the sonde for maintenance or unforeseen use elsewhere.

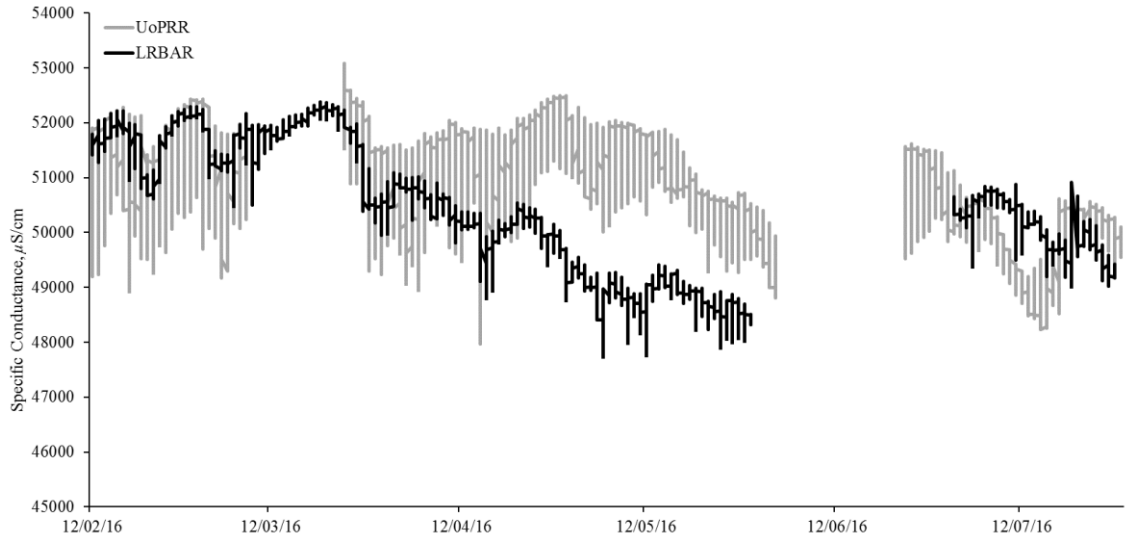
(a)



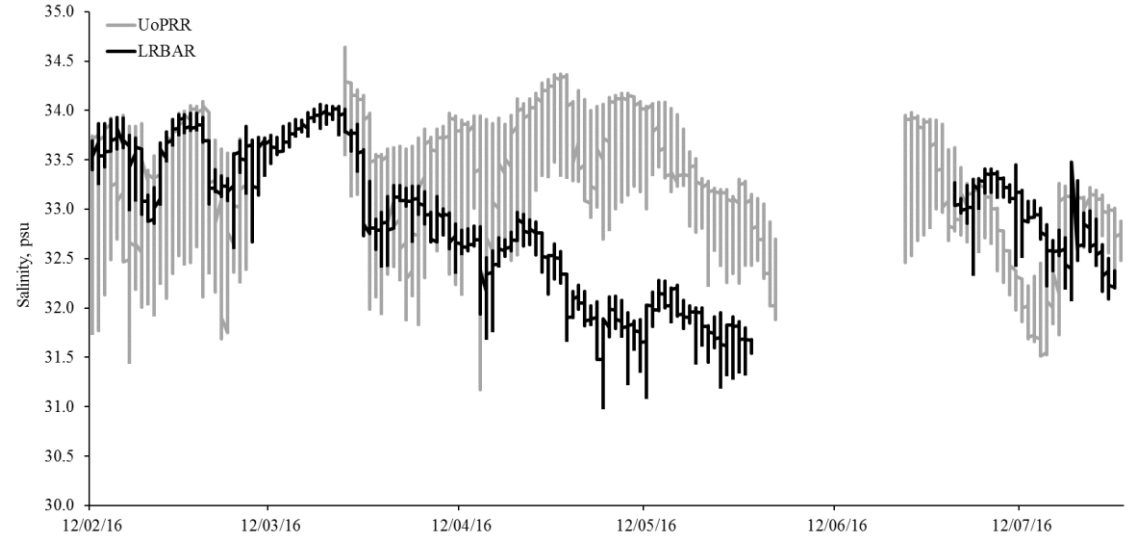
(b)



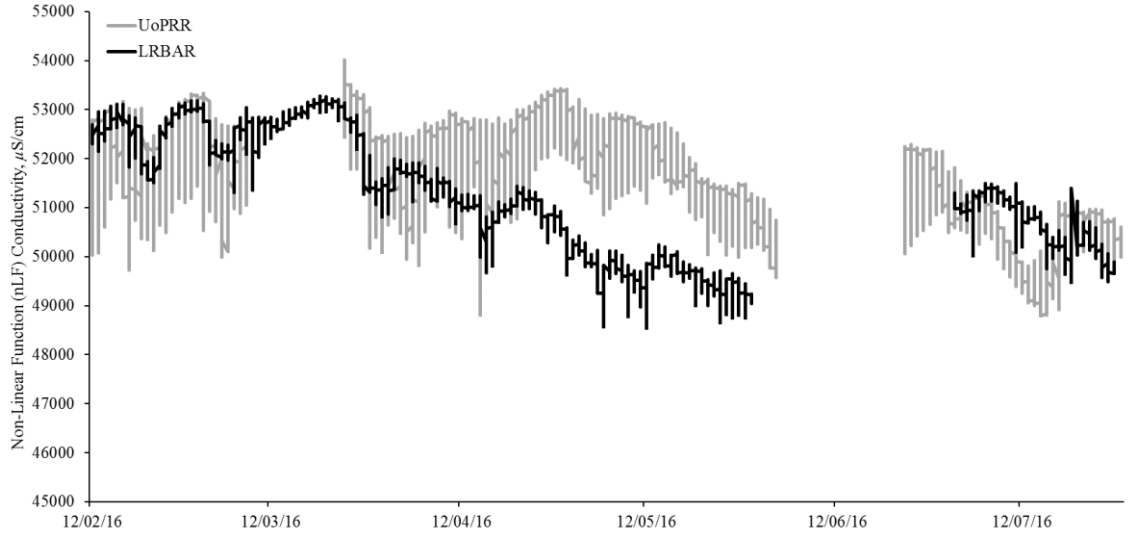
(c)


UoPRR
LRBAR
Specific Conductance, µS/cm
54000
53000
52000
51000
50000
49000
48000
47000
46000
45000
12/02/16
12/03/16
12/04/16
12/05/16
12/06/16
12/07/16

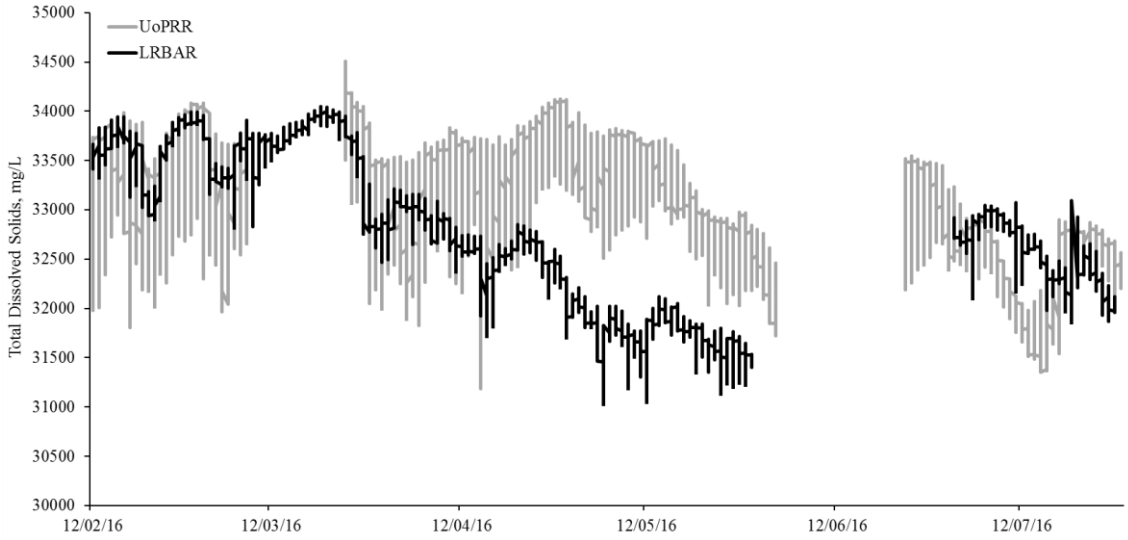
(d)


UoPRR
LRBAR
Salinity, psu
35.0
34.5
34.0
33.5
33.0
32.5
32.0
31.5
31.0
30.5
30.0
12/02/16
12/03/16
12/04/16
12/05/16
12/06/16
12/07/16

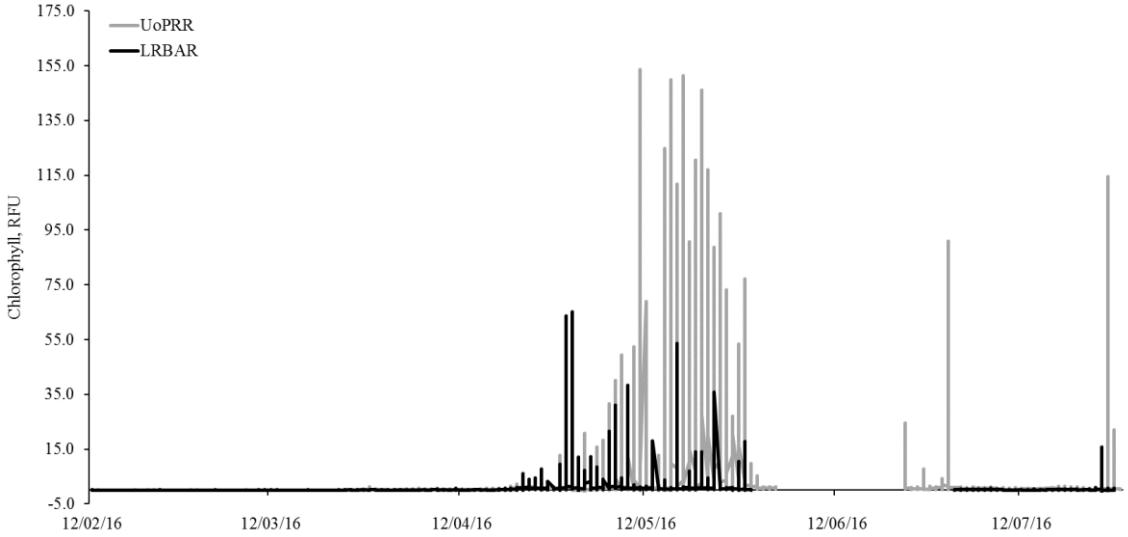
(e)


UoPRR
LRBAR
Non-Linear Function (nLF) Conductivity, µS/cm
55000
54000
53000
52000
51000
50000
49000
48000
47000
46000
45000
12/02/16
12/03/16
12/04/16
12/05/16
12/06/16
12/07/16

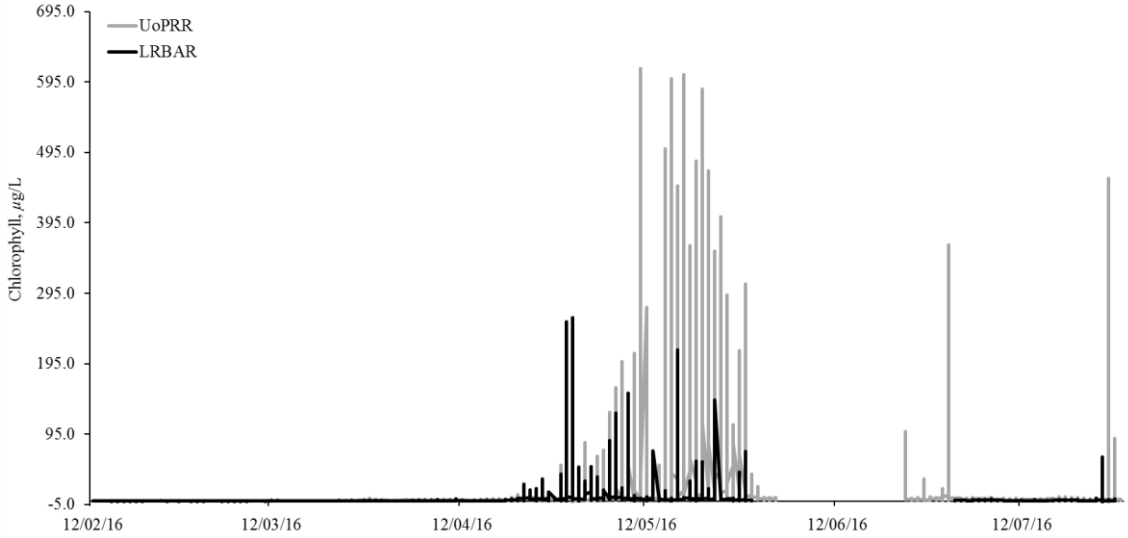
(f)


UoPRR
LRBAR
Total Dissolved Solids, mg/L
35000
34500
34000
33500
33000
32500
32000
31500
31000
30500
30000
12/02/16
12/03/16
12/04/16
12/05/16
12/06/16
12/07/16

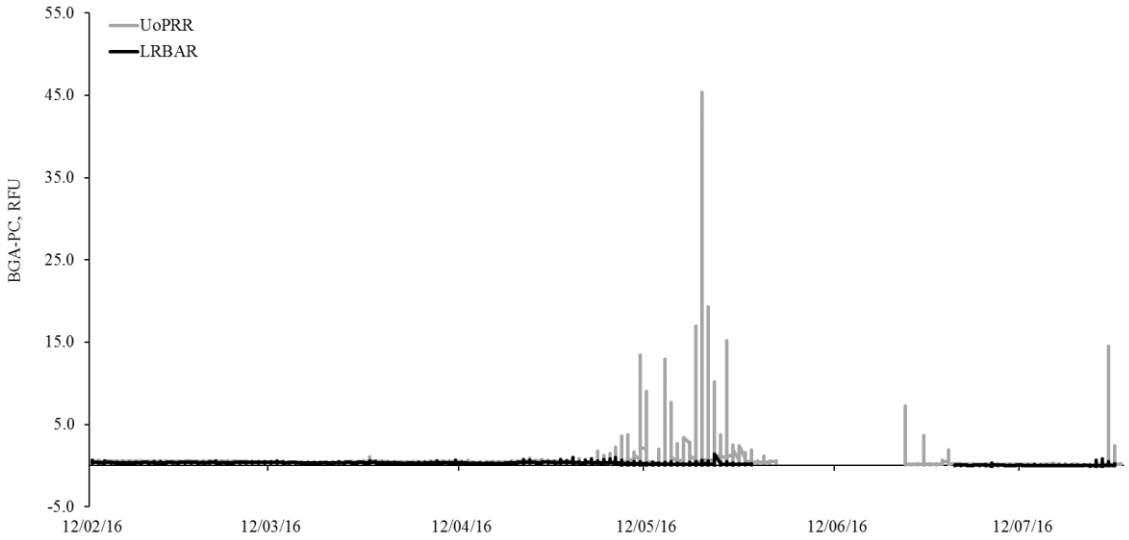
(g)


UoPRR
LRBAR
Chlorophyll, RFU
175.0
155.0
135.0
115.0
95.0
75.0
55.0
35.0
15.0
-5.0
12/02/16
12/03/16
12/04/16
12/05/16
12/06/16
12/07/16

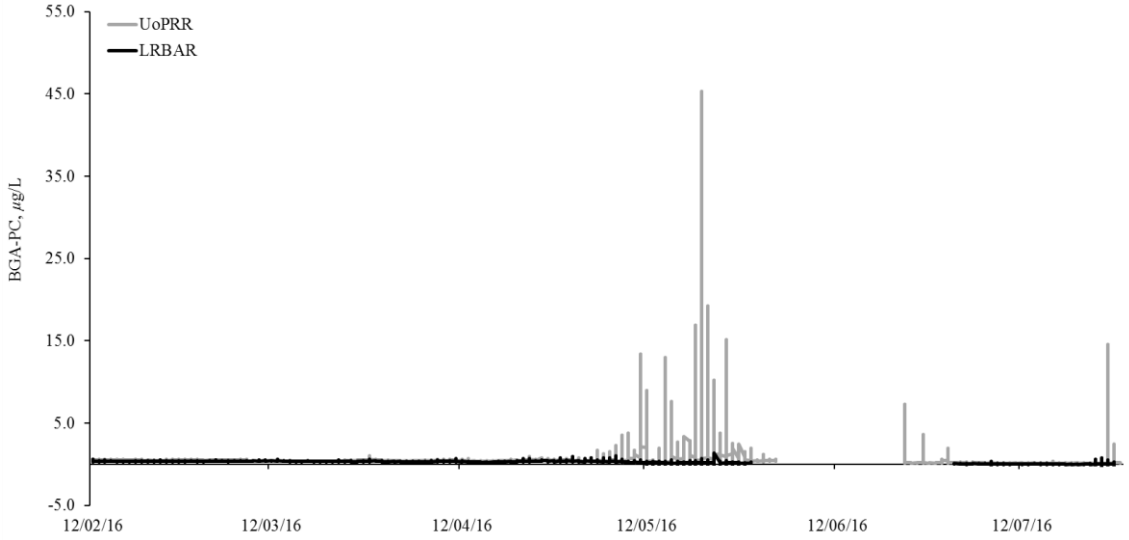
(h)


UoPRR
LRBAR
Chlorophyll, µg/L
695.0
595.0
495.0
395.0
295.0
195.0
95.0
-5.0
12/02/16
12/03/16
12/04/16
12/05/16
12/06/16
12/07/16

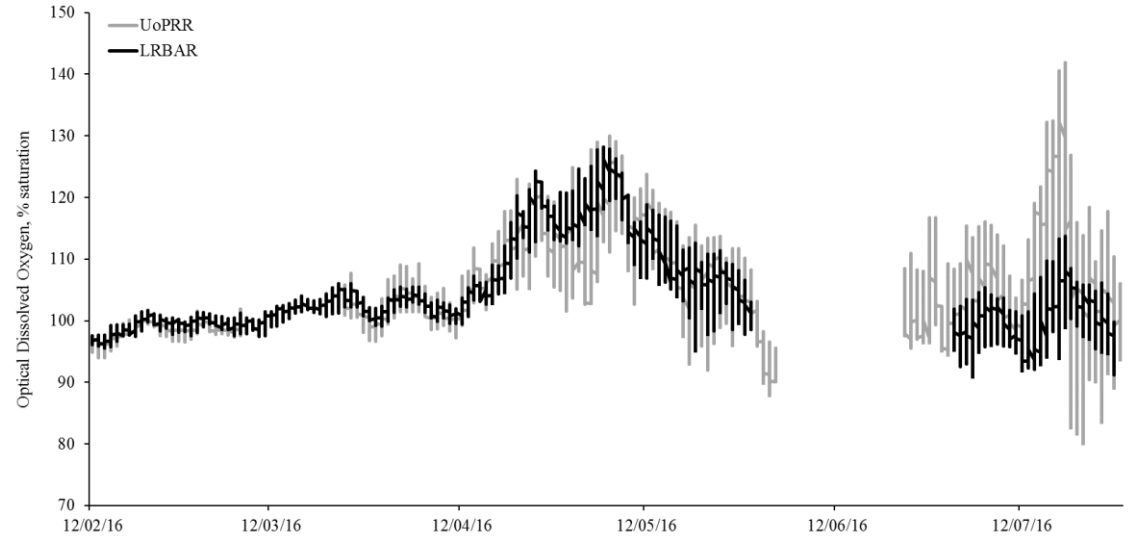
(i)


UoPRR
LRBAR
BGA-PC, RFU
55.0
45.0
35.0
25.0
15.0
5.0
-5.0
12/02/16
12/03/16
12/04/16
12/05/16
12/06/16
12/07/16

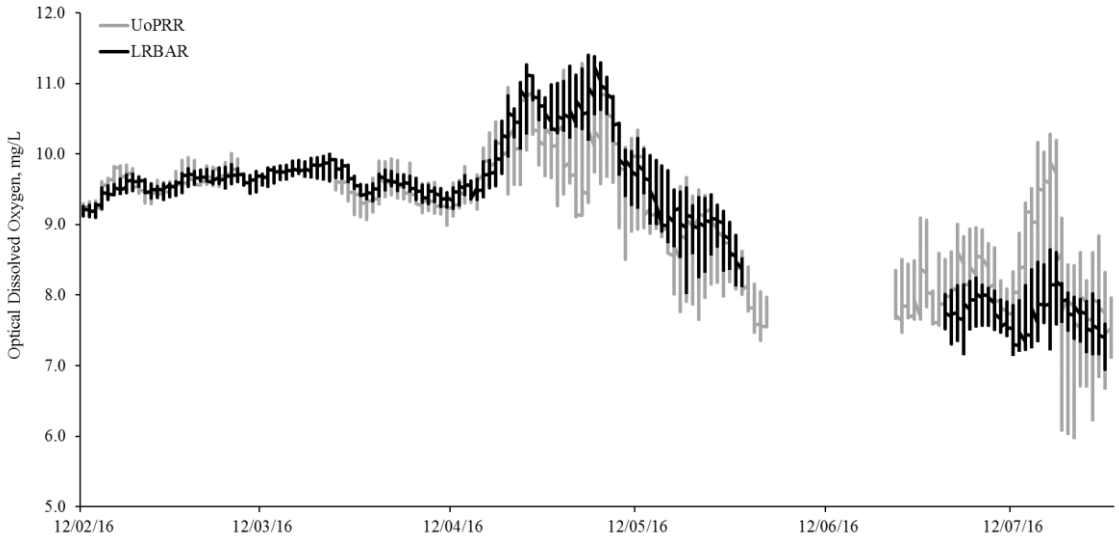
(j)


UoPRR
LRBAR
BGA-PC, µg/L
55.0
45.0
35.0
25.0
15.0
5.0
-5.0
12/02/16
12/03/16
12/04/16
12/05/16
12/06/16
12/07/16

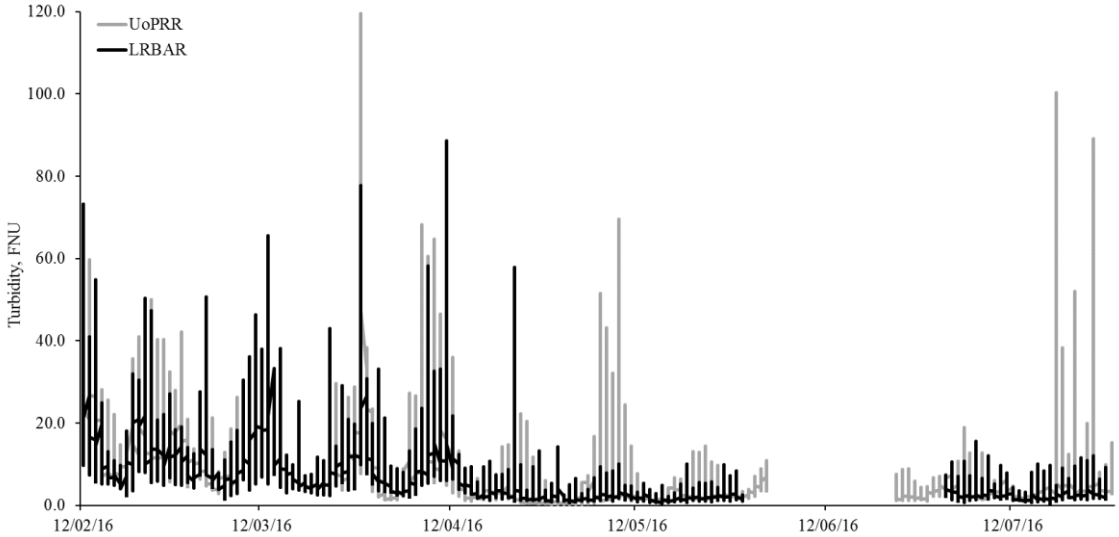
(k)


UoPRR
LRBAR
Optical Dissolved Oxygen, % saturation
150
140
130
120
110
100
90
80
70
12/02/16
12/03/16
12/04/16
12/05/16
12/06/16
12/07/16

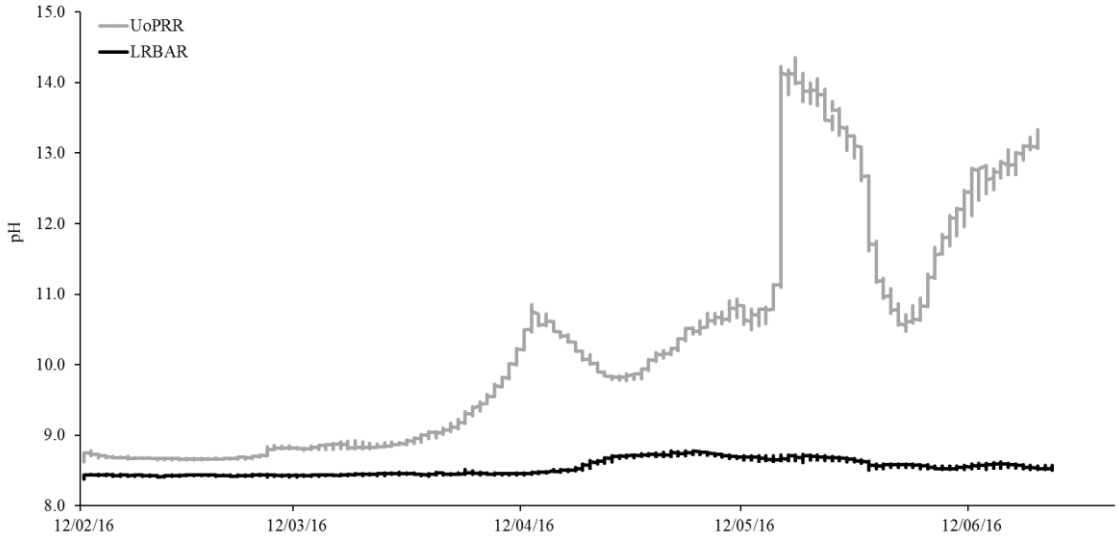
(l)


UoPRR
LRBAR
Optical Dissolved Oxygen, mg/L
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
12/02/16
12/03/16
12/04/16
12/05/16
12/06/16
12/07/16

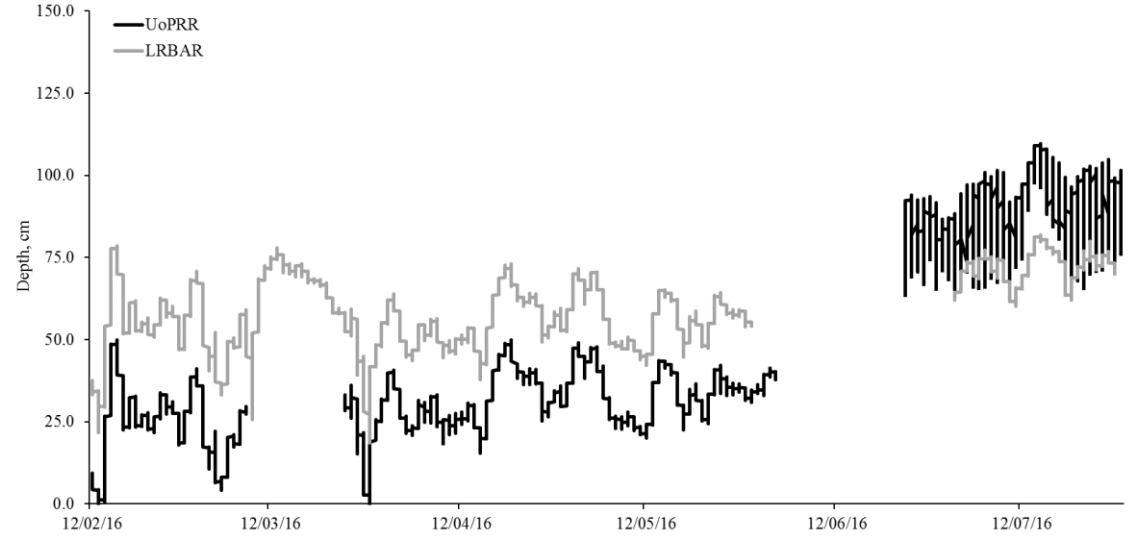
(m)


UoPRR
LRBAR
Turbidity, FNU
120.0
100.0
80.0
60.0
40.0
20.0
0.0
12/02/16
12/03/16
12/04/16
12/05/16
12/06/16
12/07/16

(n)


UoPRR
LRBAR
pH
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
12/02/16
12/03/16
12/04/16
12/05/16
12/06/16

(o)


UoPRR
LRBAR
150.0
125.0
100.0
75.0
50.0
25.0
0.0
Depth, cm
12/02/16
12/03/16
12/04/16
12/05/16
12/06/16
12/07/16

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