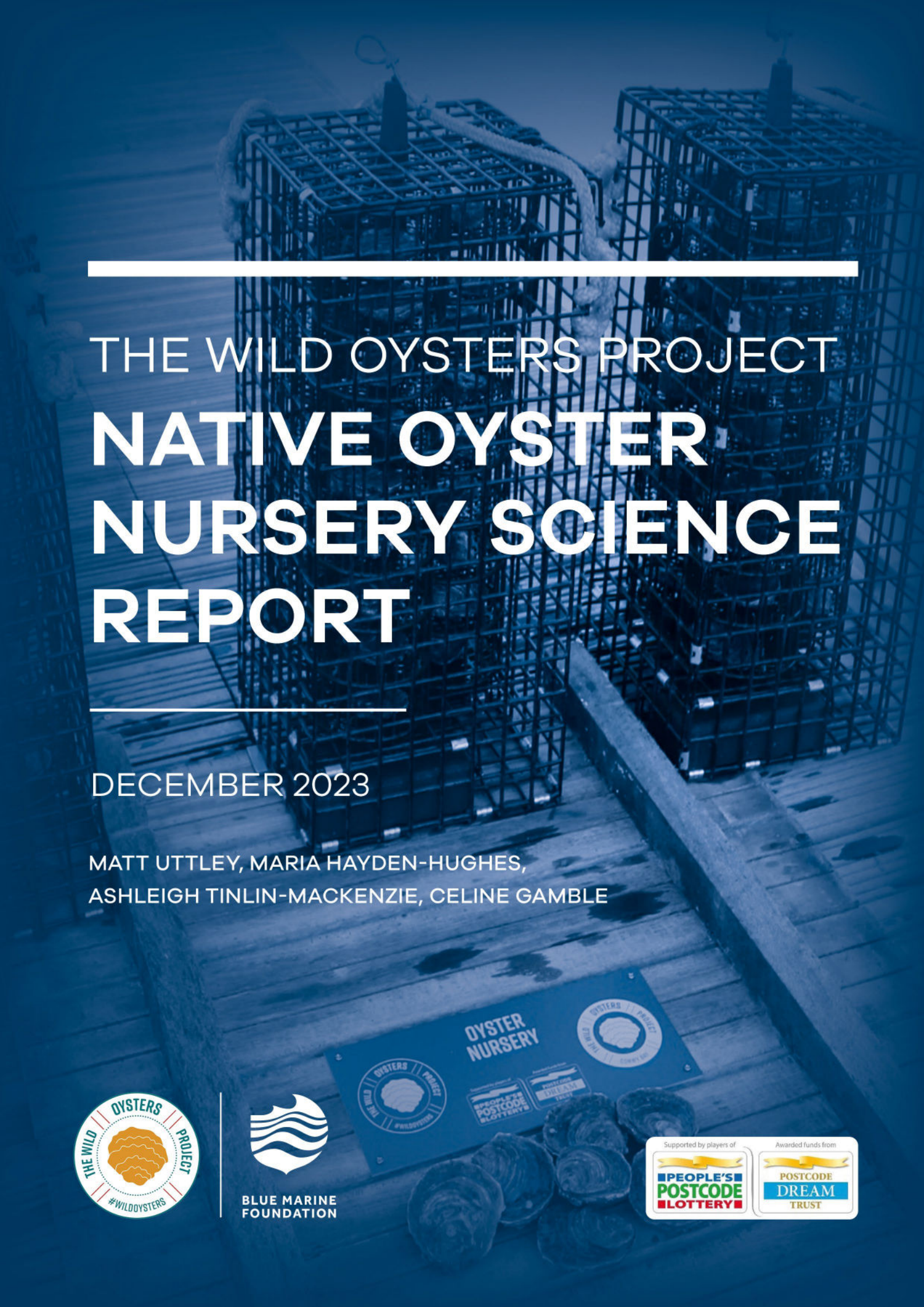

THE WILD OYSTERS PROJECT

NATIVE OYSTER NURSERY SCIENCE REPORT

DECEMBER 2023

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THE WILD OYSTERS PROJECT #WILDOYSTERS

BLUE MARINE FOUNDATION

Supported by players of PEOPLE'S POSTCODE LOTTERY

Awarded funds from POSTCODE DREAM TRUST

BLUE MARINE FOUNDATION

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FUNDER

The Wild Oysters Project received £1.18m in funding that was raised by players of People's Postcode Lottery through the annual Dream Fund award. The Dream Fund receives player raised funds through Postcode Innovation Trust and exists to give organisations the opportunity to bring ambitious, innovative and collaborative projects to life.

Cover photo: Native oyster nurseries at Conwy Marina © *Celine Gamble, ZSL*

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EXECUTIVE SUMMARY

Across the UK, wild native oysters (*Ostrea edulis*) have declined by over 95 per cent since the 1800s, as a result of a combination of factors including habitat loss, over-harvesting, pollution and disease. Europe-wide efforts to restore native oysters focus on addressing the factors limiting recovery, firstly the removal of pressures and then focusing on increasing recruitment potential and settlement substrate.

This report provides a detailed overview of the logistical requirements, methodology and monitoring results with regards to the oyster nurseries installed by the Wild Oysters Project in Conwy Bay restoration hub in North Wales and the Tyne and Wear restoration hub in North East England. Native oyster nurseries are a system specifically designed to house adult breeding oysters and provide a larval input into an area with no or very few wild native oysters. The methods of site selection, cage design and nursery monitoring protocols are also presented within this report.

The oyster nurseries installed in the marina sites were monitored regularly throughout the duration of the project from April 2021 to October 2023, along with the associated biodiversity of marine animals interacting with the oyster nurseries. We found that the growth, survival and reproductive potential of the oysters was good across both restoration hubs. There was clear correlation between growth and time, and clear spikes in mortality that coincided with the increased water temperatures and spawning activity.

Associated mobile diversity in the nurseries was found to be high, with over 86 different species found within the cages across both sites. Peaks in diversity were seen in the Conwy Bay restoration hub in July each year and in late summer in the Tyne and Wear hub. There was a significant separation in community composition between the two restoration hubs, largely caused by the difference in dominant species between the hubs, with *Palaemon* spp. featuring heavily in the Conwy Bay restoration hub and Amphipoda presented as the dominant taxa at the Tyne and Wear restoration hub. There were also species significantly associated with each hub independently, and species that were only present in a single hub. Environmental parameters were found to have a limited impact on mobile diversity, with only the state of the tide found to have a significant effect.

Finally, the abundance of oyster shell epibiota was monitored and found to be unimpacted by marina site or time. A significant difference was found in the oyster shell epibiota community composition within each site due to season and year of monitoring, but there was no overall apparent trend in seasonal changes in community composition for most marinas. Differences were likely just natural variation being detected. Acorn barnacles (*Balanus glandula*) and *Spirobranchus* spp. were highly abundant and in all months were driving similarity between months tested. The dorsal and ventral sides of the shells were compared for differences in relative abundance, with the dorsal side found to support a greater abundance and richness of epifauna than the ventral side.

The installation of native oyster nurseries in the Conwy Bay and Tyne and Wear restoration hubs was the first step towards native oyster restoration by beginning to address recruitment limitation at each site. The installation and regular monitoring of the nurseries is an extremely time-consuming and labour-intensive task. However, since the installation of the nurseries, hundreds of millions of native oyster larvae have been released into the surrounding waters. In addition, the nurseries have provided an excellent outreach and engagement tool, with thousands of school children and local volunteers actively engaging with the project. Through this active engagement, they have undertaken hands-on citizen science and explored the marine environment and discovered the benefits of native oysters.

We found that the growth, survival and reproductive potential of the oysters was good across both restoration hubs.

Photo: Native oyster © *Celine Gamble, ZSL*

1. BACKGROUND AND INTRODUCTION

1.1 Native Oyster restoration

Across the UK, wild native oysters (*Ostrea edulis*) have declined by over 95 per cent since the 1800s, as a result of a combination of factors including habitat loss, over-harvesting, pollution and disease (Lown *et al.*, 2021; Beck *et al.,* 2011). With this decline, the many environmental and social benefits known as ecosystem services that native oysters provide (Figure 1) have also been lost. These benefits include improved water clarity and quality, increased biodiversity, sediment stabilisation and denitrification (Lown *et al.*, 2021; Fariñas-Franco *et al.*, 2018; Helmer *et al.*, 2019; Pogoda, 2019; Thomas *et al.*, 2022). The presence of native oysters in the environment and the complex three-dimensional habitat they provide is essential to other marine life, as it provides vital nursery and feeding grounds. In addition, the water filtration capacity of native oysters is vast, with each adult oyster capable of filtering over 200 litres of water per day (Thomas *et al.*, 2022).

Efforts to restore native oysters around the UK and Europe continue to grow momentum with the Native Oyster Network – UK and Ireland (NON - UK) and Native Oyster Restoration Alliance (NORA) bringing together restoration practitioners, scientists, industry, government, environmental non-governmental organisations (eNGOs) and other stakeholders to share best practices and improve chances of restoration success.

Restoration of native oysters is not a quick or simple process. The life cycle of the native oyster is complex, with many stages needing to occur for the next generation to establish (Figure 2). The process is reliant on a wide range of environmental and biological factors and is therefore sporadic in nature (Lown *et al.*, 2020).

There are two key limiting factors to many restoration sites – substrate, recruitment, or both. Substrate-limited refers to an environment with a lack of suitable habitat for larvae to settle upon. Recruitment-limited refers to a system with a lack of broodstock to supply enough larvae to the area to enable settlement and further recruitment (Lown *et al.*, 2020; Colsoul, *et al.*, 2020). Therefore, restoration efforts generally focus on addressing these limitations, through active seabed restoration for substrate-limited areas and the addition of mature, broodstock oysters into the system for recruitment-limited areas.

200 LITRES

EACH ADULT OYSTER IS CAPABLE OF FILTERING OVER 200 LITRES OF WATER PER DAY (THOMAS *ET AL.*, 2022).

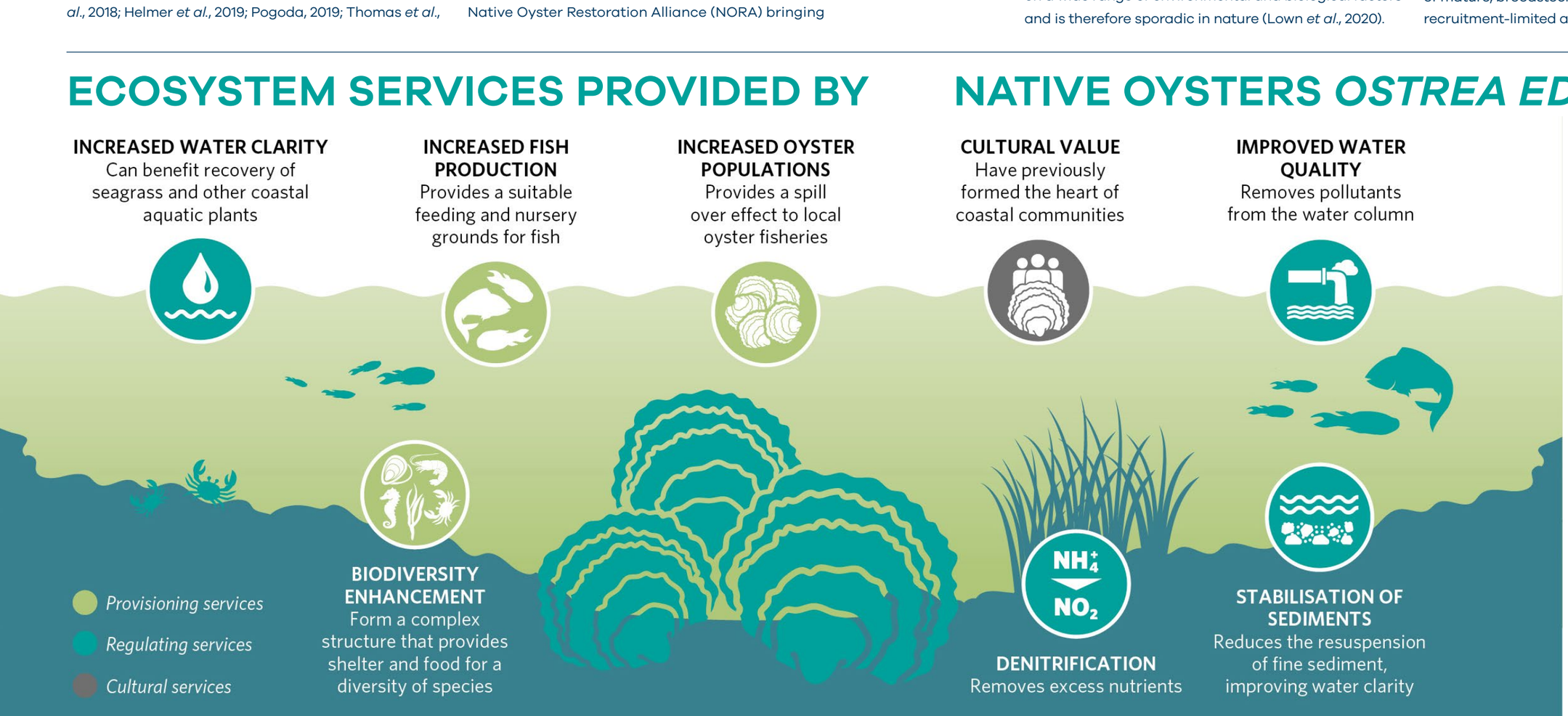


Figure 1, *Ecosystem services provided by native oysters (Ostrea edulis). Source: Preston et al. (2020a). © Matt Uttley, NORA*

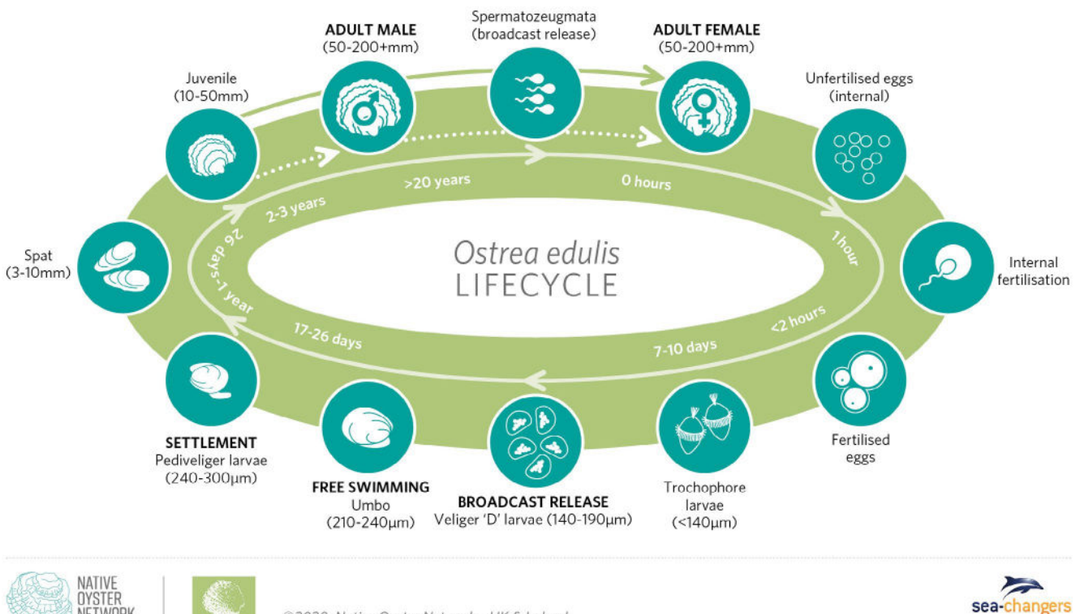


Figure 2, *Lifecycle of the native oyster (Ostrea edulis). Source: Preston et al. (2020a), modified from Helmer et al. (2019).*

Photo: Volunteer Daniel Lear monitoring during nursery monitoring work at Deganwy Marina © Maria Hayden-Hughes

1.2 The Wild Oysters Project

The Wild Oysters Project is an initiative aiming to recover self-sustaining populations of native oysters to UK seas. It is a partnership between the Zoological Society of London (ZSL), Blue Marine Foundation (Blue Marine), and British Marine, and local project partners; the School of Ocean Sciences at Bangor University and Groundwork North East and Cumbria.

The Wild Oysters Project has established native oyster restoration hubs in the North East of England, the Tyne and Wear restoration hub, and North Wales, the Conwy Bay restoration hub. These hubs are undertaking native oyster restoration through active seabed restoration involving native oyster reef creation and have seen the installation of native oyster nurseries. The Tyne and Wear restoration hub has native oyster nurseries situated in Sunderland Marina and the Port of Blyth. The Conwy Bay restoration hub has native oyster nurseries situated in Conwy Marina and Deganwy Marina. This report details the process of nursery installation, monitoring and results, and provides a comprehensive overview of this work.

> "The Wild Oysters Project has established hubs in the North East of England and North Wales."

1.3 Native Oyster Nurseries

The Wild Oysters Project established the system of broodstock nurseries using the lessons learned from the Solent Oyster Restoration Project, where adult oysters are placed in cages and suspended below marina pontoons. The nurseries assist with local oyster population recovery as they act as 'larval pumps', overcoming some of the issues in recruitment-limited sites. Once mature, native oysters have an increased likelihood of successfully reproducing when they are in higher densities (Colsoul, *et al.*, 2020; Preston *et al.*, 2020b). The design of the oyster nurseries are based on this to improve the chances of successful reproduction.

These nurseries also act to test a site's suitability for further restoration activity, based on mortality rates observed.

Broodstock nurseries are also intended to be used as an initial outreach and education tool to kick-start the discussion in the local community around restoration. The design also enables outreach and engagement by making the marine environment accessible without the need for SCUBA diving experience or a vessel. The ability to remove the cages from the water by hand allows school, community and other groups to easily engage with the project.

2. METHODS

The native oyster nurseries were monitored for oyster survival, growth and reproductive potential. Associated biodiversity was also monitored in the nurseries.
The methods section of this report sets out nursery cage design, how the marina sites for the oyster nurseries were selected, how the nurseries were installed in the marinas, and then how the oysters and associated biology were monitored.

Photo: Native oyster nurseries at Sunderland Marina © Celine Gamble, ZSL

Photo: Volunteers monitoring biodiversity and oyster survival at Conwy Marina © Luke Helmer

2.1 Oyster Nurseries

2.1.1 Oyster Nursery Design

Native oyster nurseries comprise of a solid exterior rectangular frame and an inner plastic scallop tray that holds oysters individually in separate sections (Figure 3).

The exterior frame is an Aquamesh® housing. Aquamesh® is PVC Coated Welded Wire Mesh. It is commonly used in aquaculture and is a solid material that does not break down or corrode in the marine environment. The outer housing provides a degree of protection from predation and prevents damage to the oysters from knocks or compression of the nursery between marina pontoons. The exterior frame also ensures no oysters are lost if they are washed out of the scallop trays during rough conditions. The inner scallop trays are a plastic set of interlocking chambers that each fits a single mature oyster. These inner scallop trays can be raised from the outer housing and individually opened. Each nursery for this project was designed with space for 27 adult oysters.

Oyster nurseries are situated in marinas. This provides both excellent access and facilities for outreach and education as well as security and controlled access, protecting the nurseries from the risk of illicit harvesting or damage to property. The nurseries are suspended on metal bars secured beneath pontoons in marinas through removable hatches. The existing pontoon deck boards are modified to form a lifting hatch whilst maintaining appearance and structural integrity of the surrounding pontoon. Nurseries are suspended in the water rather than resting on the marina floor as this reduces sedimentation in the nurseries and limits access to some predatory species such as echinoderms and large crabs. Hatches slide into metal brackets and, where necessary, have a locking mechanism to ensure that they are secure and cannot come loose or be removed by members of the public. During monitoring, hatches are unlocked and removed, and an individual nursery can then be unclipped and removed from the water (Figure 4). The design can be modified to suit site-specific infrastructure. For example, at the Port of Blyth site, nurseries were unable to be suspended in the traditional method. As a result, the design was modified to hold the nurseries alongside the pontoon (described in Section 2.1.5).

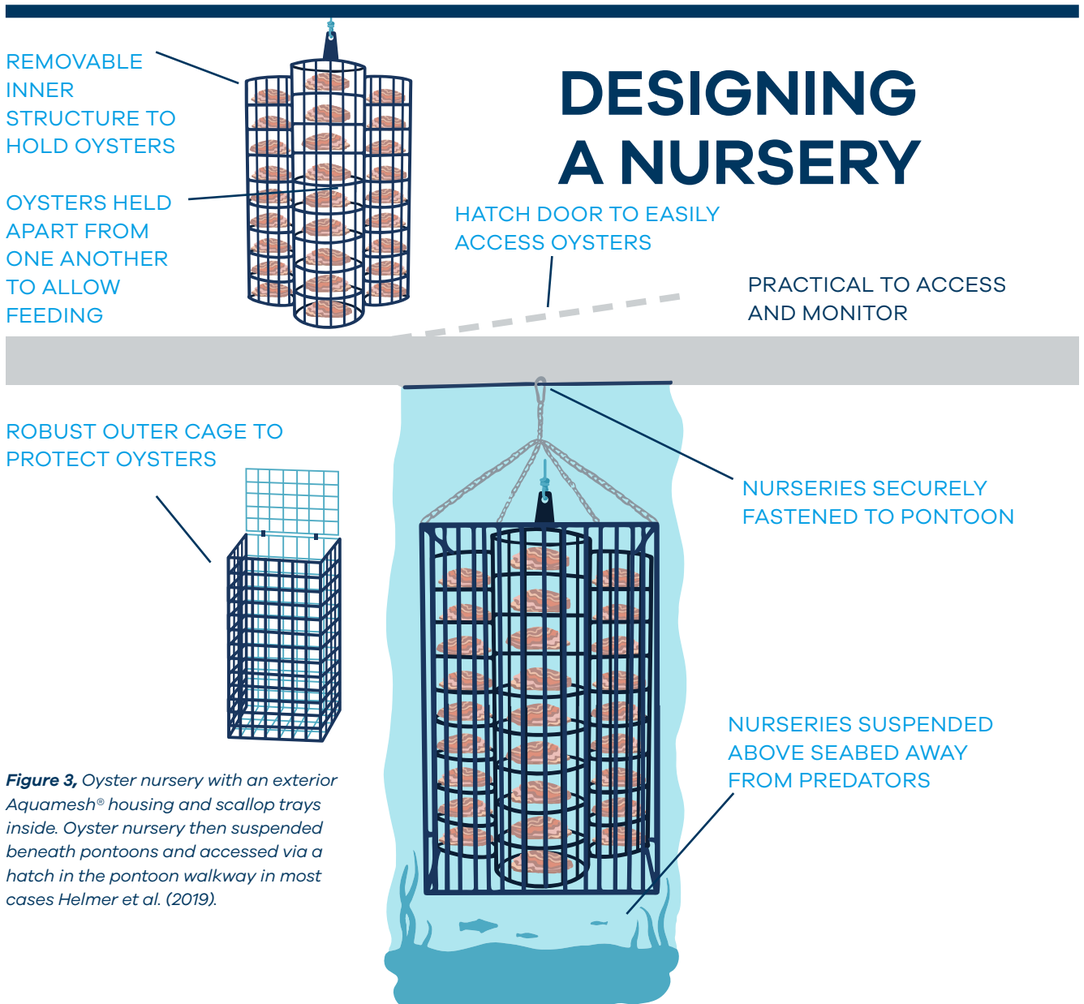


Figure 3, *Oyster nursery with an exterior Aquamesh® housing and scallop trays inside. Oyster nursery then suspended beneath pontoons and accessed via a hatch in the pontoon walkway in most cases Helmer et al. (2019).*

Figure 4, *, Local Project Officer, Maria Hayden-Hughes, with native oyster nurseries removed through the modified, open pontoon hatch at Conwy Bay restoration hub.* © Celine Gamble, ZSL

2.1.2 Site Selection

Following the site selection criteria in Preston *et al.*, (2020a) and Hughes and zu Ermgassen, (2021), historic evidence of the presence of native oysters in the region was assessed for both restoration hubs. Comparison with Environment Agency native oyster potential maps (native-oyster-bed-potential-maps) was used in the Tyne and Wear restoration hub. In the Conwy Bay restoration hub, Natural Resources Wales (NRW) maps of the seabed habitat type to assess the presence of subtidal mixed sediment and also considered the Special Area of Conservation (SAC) features were used (naturalresources.wales). For each potential marina location, logistical requirements and a range of environmental parameters were assessed for their suitability, detailed in Helmer *et al.*, (2021). This typically included water temperature, salinity, flow rate, and freshwater input. This data was captured both through *in situ* monitoring and where national lockdowns and restrictions prevented this, archived water quality data (Environment Agency, NRW and Bangor University). These initial parameter checks eliminated several potential sites and Royal Quays Marina due to low salinity, below the tolerance of native oysters.

The potential for public engagement with monitoring and other restoration work was also assessed during site selection. British Marine membership of scoped marinas was a preference, as was the presence of existing local project partner organisations and ongoing networks that would allow for successful community-focused activities.

Photo: Local Project Officer Ashleigh Tinlin-Mackenzie deploying native oyster nurseries at Sunderland Marina © Celine Gamble, ZSL

> For each potential marina location, logistical requirements and a range of environmental parameters were assessed for their suitability.

2.1.3 Conwy Bay Restoration Hub

The Conwy Bay restoration hub (Figure 5) consists of two marina sites; Conwy Marina (Figure 6A) and Deganwy Marina (Figure 6B). The marina sites are located on the river Conwy in North Wales and are situated almost directly opposite one another along the same longitude. Deganwy Marina sits on the eastern side of the river and hosts 165 berths and is operated by Lakeland Leisure Estates, Conwy Marina on the Western side of the river hosts 510 berths and is operated by Boatfolk. Both locations are well protected by breakwaters and marina walls with relatively narrow entrances, with marina gates that close and open approximately three hours either side of low water. This is to avoid them drying out so the direct flow from the river is buffered and nurseries are immersed throughout the tidal cycle.

Figure 5, *map of the location of the Conwy Bay restoration hub, showing Conwy Marina and Deganwy Marina. ArcGIS software by Esri was used to create the map.*

> The marina sites are located on the river Conwy in North Wales and are situated almost directly opposite one another along the same longitude.

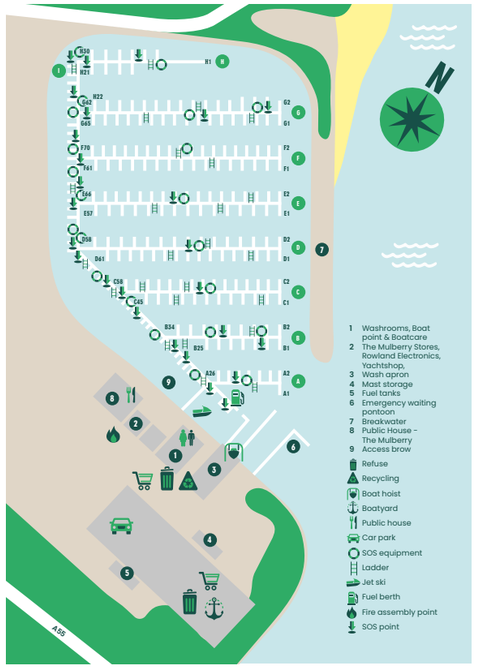


Figure 6A

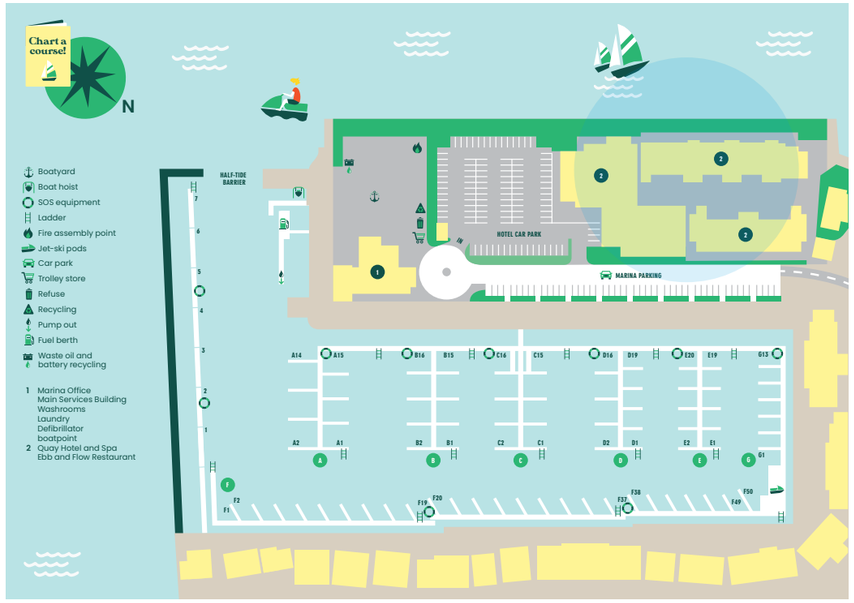


Figure 6B

Figure 6A and 6B, (A) *Conwy Marina layout - The native oyster nurseries are situated on Pontoon A, nearest the marina entrance.* ***(B)*** *Deganwy Marina - The native oyster nurseries are situated on Pontoon A, nearest the marina entrance.*

2.1.4 Tyne and Wear

The Tyne and Wear restoration hub (Figure 7) consists of two marina sites; Sunderland Marina (Figure 8A) and the Port of Blyth (Figure 8B). Sunderland Marina is situated on the North East coast of England at the mouth of the River Wear. The marina operates alongside a charitable trust which also provides outdoor education through its centre 'Adventure Sunderland' based on the seafront. The marina hosts 132 pontoon moorings and 95 fore and aft moorings and has a relatively narrow entrance resulting in no direct flow through the area from the river.

The Port of Blyth site (Figure 8) is also located on the North East coast of England, 15 miles north of Sunderland Marina. The nurseries are suspended from an access pontoon on the river Blyth used by Newcastle University that is central within the port area, located on Commissioners Quay. The pontoon is directly within the river and so experiences a good rate of flow and is directly impacted by the tidal regime.

Figure 7, *map of the location of the Tyne and Wear restoration hub, showing Sunderland Marina and the Port of Blyth site. ArcGIS software by Esri was used to create the map.*

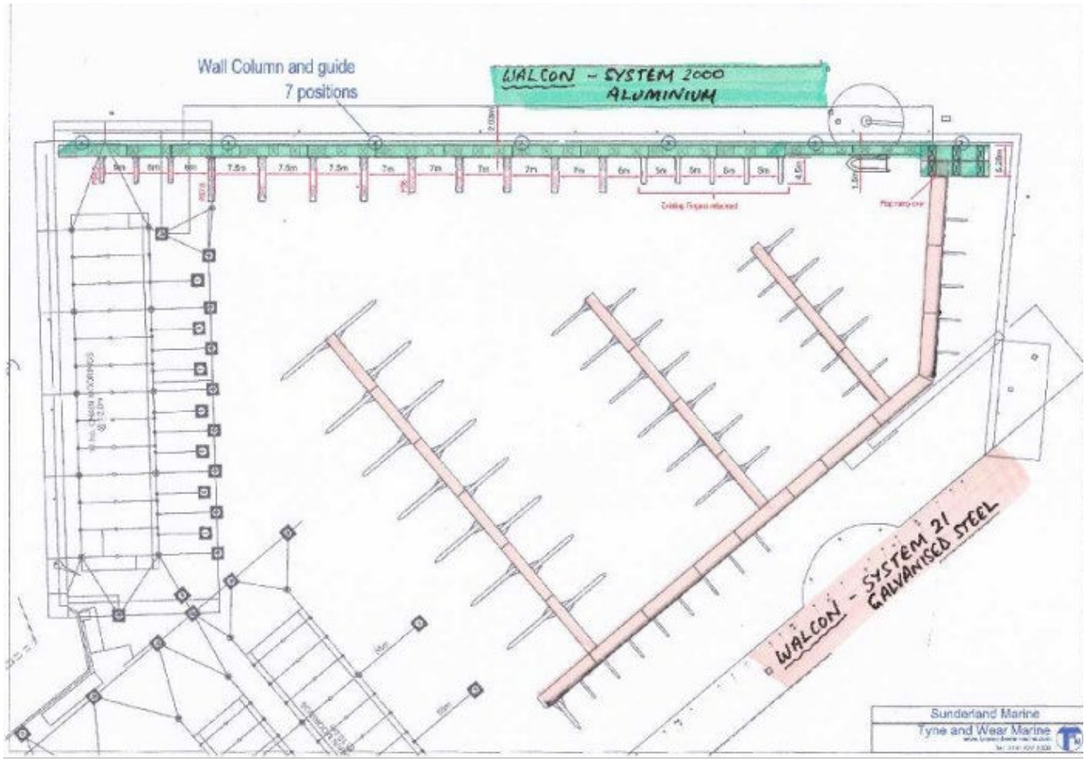


Figure 8A, *shows the Sunderland Marina layout, the nurseries are situated on the central pontoon.*

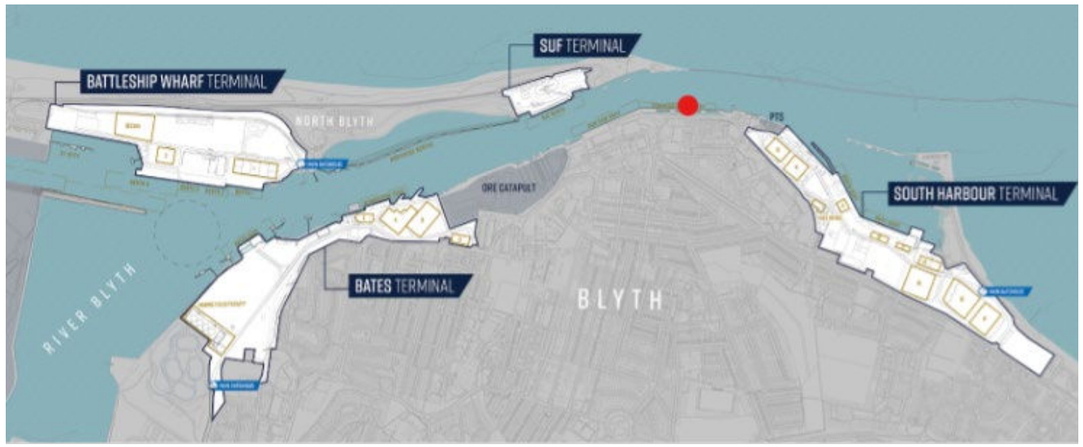


Figure 8B, *8B shows the Port of Blyth layout (red dot shows the location of the pontoon at Commissioners Quay).*

2.1.5 Location of nurseries at marinas

The nurseries at Sunderland, Conwy and Deganwy marinas are accessed through a hatch system in modified pontoons (described in section 2.1 and Helmer *et al*., 2021).

In the Conwy Bay restoration hub, at both Conwy Marina and Deganwy Marina the hatches were located on pontoon A, nearest the marina gate (Figure 6A and 6B). In the Tyne and Wear restoration hub, at Sunderland Marina the nurseries were located on the pontoon closest to the sea (Figure 8A). The access hatch method was not suitable for the area selected at the Port of Blyth site, instead nurseries were suspended from metal support frames attached to the side of the pontoon (Figure 9A-C).

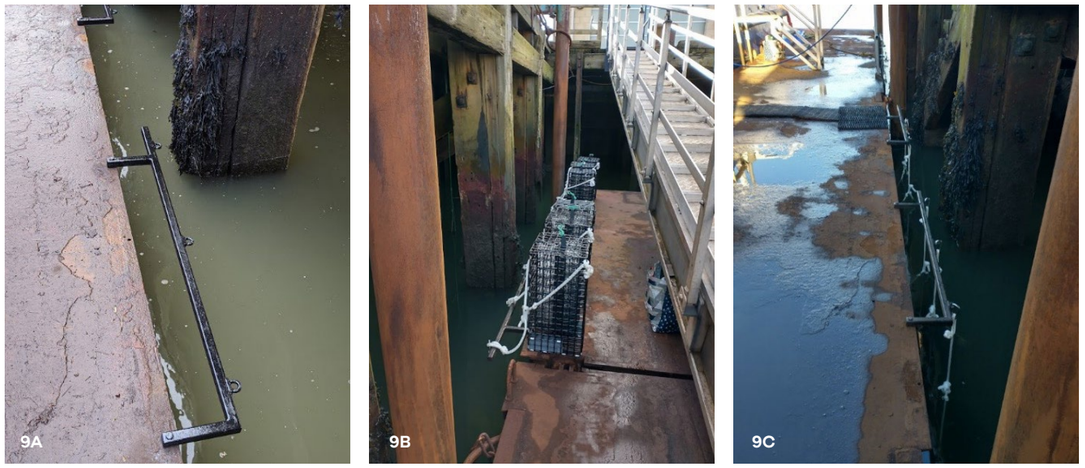


Figure 9A, 9B and 9C, *Metal frames on the pontoon structure at the Port of Blyth site that extend outward rather than beneath the pontoon as was the case at most of the other sites. A) showing the bar attachments with eye holes that the nurseries are clipped to. B) showing the nurseries during installation but also how they are later positioned during monitoring, and C) showing the nurseries suspended from the bars.* © Ashleigh Tinlin-Mackenzie

Photo: Native oysters © Matt Uttley, Blue Marine Foundation

2.2 Oyster Survival

Any spikes in mortality could be an indication of post-spawning mortality, disease outbreaks or extreme weather events. Therefore, mortality information was important to monitor over time to assess the suitability of marina locations and potential inferences for wider restoration efforts in the area. The monitoring of mortality within the nurseries is a condition of the Centre for Environment, Fisheries and Aquaculture Science (Cefas) aquaculture licence in place and an integral part of the project's biosecurity measures plan. Oyster mortality monitoring was conducted monthly where possible (zu Ermgassen *et al*., 2021). In some months, adverse weather conditions made monitoring unfeasible. During mortality monitoring, every nursery at each site was checked, and oyster mortalities in each cage recorded and removed.

To monitor oyster survival, individual oysters were removed from the nursery system and inspected. Live oysters will close their shell tightly when disturbed. If an oyster was clearly open with no flesh inside, they were immediately declared deceased. If there was flesh within but the valves did not close after three attempts at squeezing the valves together then the individual was declared deceased. If a response was evident during the squeezing process or the individual was clearly shut with the force of the adductor muscle, then it was declared alive. Care was taken to squeeze and slide the two valves gently to assess live oysters as occasionally the valves can appear 'shut' when they are in fact held together with sediment or a vacuum within.

During mortality monitoring, every nursery at each site was checked, and any observed oyster mortalities recorded and removed.

We noted environmental conditions to understand the potential cause of short-term spikes in mortality (i.e. during heatwaves, freezing events or post-spawning). If unexplained mass mortalities were to be observed (which did not occur during monitoring), The Fish Health Inspectorate (FHI) would have been informed, and an investigation undertaken to establish whether caused by disease *Bonamia ostreae* parasite for example. As part of the monitoring procedure, when restockings of the nurseries took place, oysters of similar age groups and deliveries of oysters were kept together in the same nurseries where possible. This allowed for any mortality in newly delivered oysters to be monitored while acclimatising to the environment in the marinas. In addition, this allowed the nurseries under biodiversity monitoring (see Section 2.5) to consistently contain oysters that had been in the marinas since the start of the project. New oysters were scrubbed prior to deployment in the nurseries, and so putting new oysters into the nurseries would impact upon recorded species presence or richness.

Photo: Native oyster nurseries at Conwy Marina © Celine Gamble, ZSL

2.3 Oyster Spawning Activity

Monitoring for oyster spawning activity took place at both restoration hubs to assess if the broodstock were successfully reproducing from the nurseries. Spawning was monitored through observations of how many oysters were brooding larvae within the pallial cavity during each monitoring session. Larval samples were collected and later analysed in the laboratory to estimate total fecundity and the number of larvae produced per oyster. Spawning was monitored in one marina site per hub, Conwy Marina and Sunderland Marina, in both years 2022 and 2023. Forty oysters from each marina were taken during each monitoring session and underwent non-lethal spawning monitoring using five per cent magnesium chloride ($MgCl_2$) solution to anaesthetise each oyster. When anaesthetised, the oysters relax and the shell remains open, allowing for inspection for spawning condition. During inspection, visual checks of gonad stage were conducted to identify the stage of larval development, clearly differentiated by colour and referred to as white, grey or black sick (Figure 10).

Full protocol for $MgCl_2$ sampling can be found in Appendix 1.

40 **OYSTERS FROM EACH MARINA WERE TAKEN DURING EACH MONITORING SESSION AND UNDERWENT NON-LETHAL SPAWNING MONITORING**

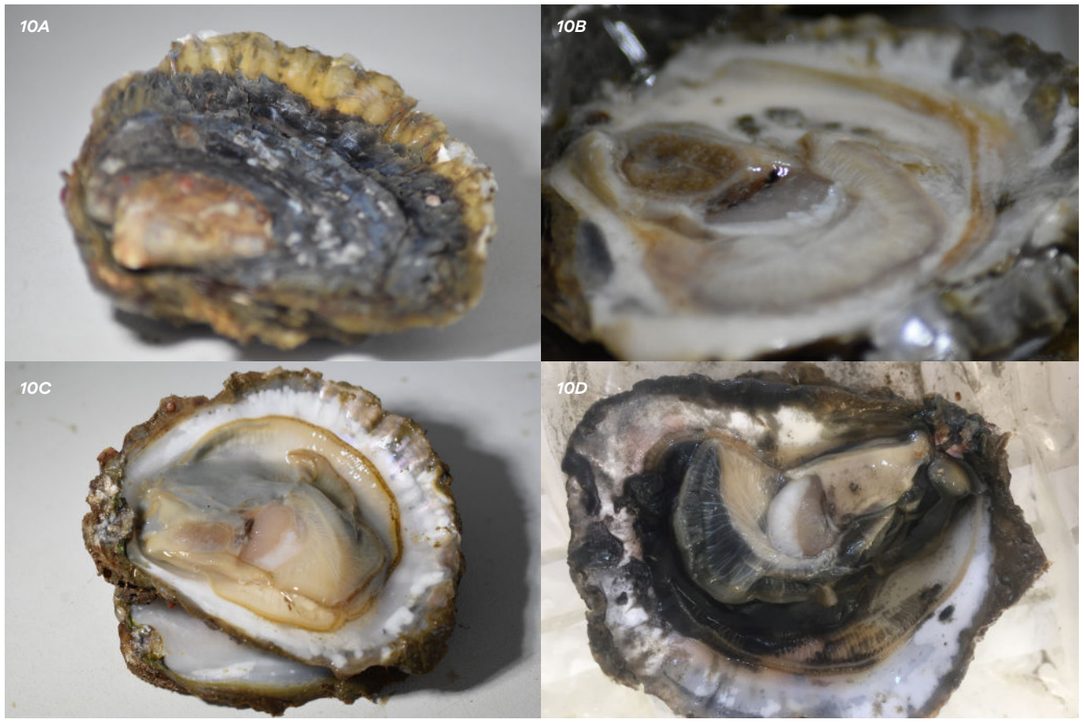


Figure 10, (A) *Brooding native oyster (Ostrea edulis) prior to shucking; brooding O. edulis containing larvae referred to as* ***(B)*** *white 'sick',* ***(C)*** *grey 'sick' and* ***(D)*** *black 'sick' stages of development. Larvae within the pallial cavity in and around the gill and mantle structures, indicated by arrows. Source: © Luke Helmer, Helmer et al. (2020).*

2.3.1 Larval sample laboratory analysis

In 2022, samples were sent to the Institute of Marine Sciences, University of Portsmouth. In 2023, samples were sent to the School of Ocean Sciences, Bangor University for processing. During processing, excess fluid was removed and larvae were preserved using ethanol in 2022 by the University of Portsmouth. In 2023, larval samples were fixed using Lugol's iodine prior to being processed at Bangor University. Once preserved, 1ml was extracted and diluted in 999ml of water. A 1ml aliquot of this 1000ml solution was then placed on a Sedgewick rafter counting slide and all larvae were counted (Figures 11 and 12).

In 2022, it was found that this was not possible for the white sick stages in 2022 due to clumping, so the fixing process was adjusted in 2023. 2023 larval samples were fixed in Lugol's iodine prior to being processed at Bangor University.

The counting of larvae informed estimations of the number of larvae released from each marina over the duration of the project.

Full protocol for oyster larval density estimation can be found in Appendix 1.

Figure 11, *Leica DM1000 compound microscope and digital image capture system, plus associated sample processing kit*

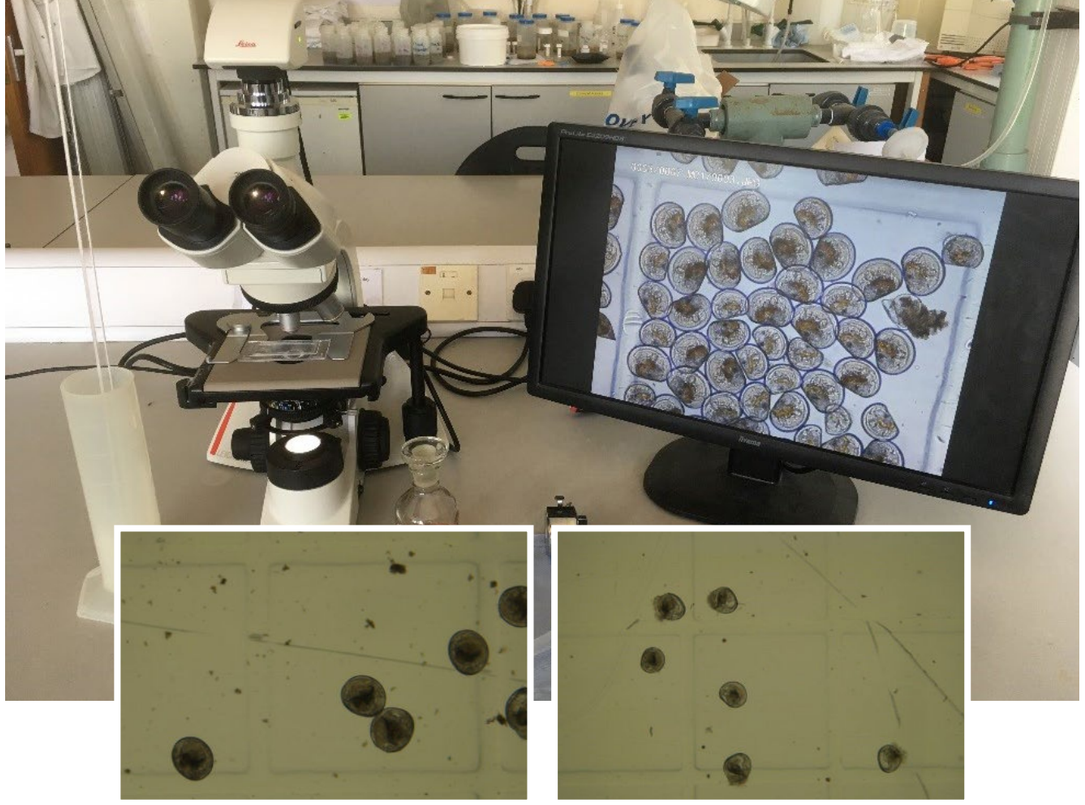


Figure 12, *Digital images of native oyster (Ostrea edulis) larvae from larval sample taken using the Leica DM1000* © Maria Hayden-Hughes.

2.4 Water Filtration

Water filtration values were estimated for the total number of oysters held in nurseries at marina sites across the two restoration hubs. The filtration values were based on the number of live oysters in the marinas during the given month filtering at a rate of 3l/h/oyster (Haure *et al*., 1998) over a 24-hour period for the duration of the project. This estimate is used with the understanding that several factors can influence this value, including season, temperature, time of day, size of oyster, condition of oyster, food availability, water flow rate, and disturbance.

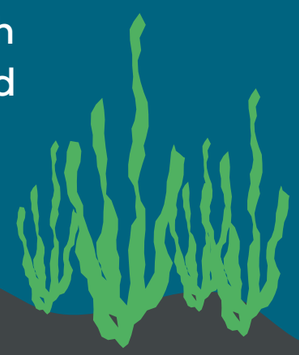
2.5 Biodiversity of Fauna

2.5.1 Mobile Fauna

The mobile fauna interacting with the oyster nurseries was recorded within dedicated biodiversity nurseries. There were six dedicated biodiversity nurseries in each of the marinas at the Conwy Bay and Tyne and Wear restoration hubs. The biodiversity nurseries were monitored prior to the removal of other nurseries in close proximity, to minimise disturbance and reduce the likelihood of mobile species dispersing away during sampling.

During the monitoring of a biodiversity nursery, a custom made 1mm mesh net was passed beneath the entire nursery, before being pulled around the unit and removing it from the water. A bucket was then used to wash off any mobile species from within the nursery into the bottom of the net, this rinsing process was repeated three times. The contents of the net were rinsed into a 1mm mesh sieve, and transferred into a white tray containing seawater for identification. Each individual oyster within the biodiversity nursery was removed from the scallop tray system and rinsed in a bucket of seawater, the contents of which were then passed over a 1mm mesh sieve to collect any additional mobile species clinging to the oyster shells. All mobile species were placed into containers or white trays for on-site identification.

> **The mobile fauna interacting with the oyster nurseries was recorded within dedicated biodiversity nurseries.**



2.5.2 Oyster Shell Epibiota (Sessile Fauna)

Within each biodiversity nursery, a sub-sample of oysters were rinsed and photographed on the dorsal and ventral sides. Photographs were taken with the oyster set on a pre-marked and labelled background to provide image scaling points and clear annotation of dates, location, nursery number and oyster number. A subset of these photographs were then analysed for oyster shell sessile biota analysis. Species that settled on the nurseries were not recorded but were monitoring for Invasive Non-Native Species (INNS). The structure of nurseries supports settlement of a variety of sea squirts, bivalves, ascidians, hydroids, peacock fan worms and others but as the nurseries were periodically cleaned to ensure they were free of excessive fouling, this settlement was not recorded.

The following image analysis protocol was then followed:

1. Images were uploaded to BIIGLE, the sampling period covered two full years (May 2021 to May 2023) and images were analysed for all sites from bimonthly monitoring sessions.
2. A custom label tree was created in BIIGLE based on expected taxa and recording method, allowing for the user to quickly and simply label identified species and for the label tree to be updated as and when required if new taxa were found.

Image Analysis:

1. Each image was assessed for quality, with only images of good or excellent quality analysed further:
    - Excellent – Image is clear and fully focussed with excellent colour and exposure. All levels of analysis possible;
    - Good – Image is in focus but may be slightly over or under exposed. Small and cryptic taxa still visible;
    - Poor – Some elements in focus but exposure or camera angle not ideal. Small and cryptic taxa likely to be missed;
    - Very Poor – Image predominantly blurred. Organisms unlikely to be distinguished.
2. Scaling point labels were added to each image using the pre-drawn box around the oyster, or other scalable items if box missing.
3. Each image was tagged with the corresponding metadata:
    - Month
    - Year
    - Site Name
    - Cage/Nursery Number
    - Oyster Replicate Number (1–9)
    - Shell side – dorsal (with hinge – curved) or ventral (flat)
4. Oyster measurement labels were added:
    - Shell height (line drawn from the point of hinge to the top edge of shell)
    - Shell width (line drawn along the widest point, perpendicular to the shell height)
    - Shell area (a freehand polygon was drawn around the edge of the oyster shell).
5. Each image was annotated with sessile taxa – identified to highest taxonomic rank possible. Encrusting species were given as percentage cover (a polygon drawn for area calculations). Solitary species and colonies were separate counts.
6. The appropriate reports were downloaded from BIIGLE for processing and data analysis.
7. Data analysis carried out on taxa data from a total of 862 analysed images (631 oysters).

2.6 Statistical Analysis

2.6.1 Growth and Survival

Oyster growth in the nurseries was monitored through two years of shell measurement data taken from 862 oyster images and analysed in BIIGLE. The shell height (line drawn from the point of hinge to the top edge of shell), shell width (line drawn along the widest point, perpendicular to the shell height), and shell area (a freehand polygon was drawn around the edge of the oyster shell). The shell area was plotted over time to demonstrate growth through the monitoring period. In addition, the relationship between the shell measurements and time (sample date) were tested using Pearson Correlations.

To compare mortality rates across marina sites and assess the impact of seasonality on mortality rates, all mortality data was plotted against time. Average percentage mortality rates were then calculated for each of the restoration hubs and plotted to provide an indication of how mortality varied seasonally between restoration hubs.

To test the effect of spawning on mortality in each marina, the percentage of oyster mortality over time and number of oysters spawning per month during the same period were plotted.

Photo: Native oysters in the Tyne and Wear restoration hub © ZSL, Celine Gamble

2.6.2 Spawning

Spawning was sampled following the methods given in Section 2.3. This data was analysed with respect to the following key research questions:

- How does temperature impact spawning within each marina?
- How does spawning vary within each marina?
- How does spawning vary between the Tyne and Wear and Conwy Bay sites?
- What percentage of oysters spawned at each marina?
- What is the total estimated larval production of the nurseries?

To test the impact of temperature and seasonality on spawning within each marina site, weekly spawning monitoring was completed at Conwy Marina and Sunderland Marina using a sub-sample of oysters. This data was plotted against the average weekly seawater temperature (°C) from June to September 2022 and 2023. A 15°C threshold line was added to charts to depict the threshold at which spawning could be expected. The results for Conwy Marina and Sunderland Marina were compared to understand variation in spawning time between the two restoration hubs. The breakdown of spawning condition was given through larval development stage, and the total number of oysters exhibiting each development stage was plotted for each marina. Finally, the average number of larvae produced per oyster was calculated.

2.6.3 Mobile Biodiversity

Mobile biodiversity was sampled following the methods given in Section 2.5.1. This information was then analysed examining the following key research questions:

- How does the mobile faunal community evolve over time at each restoration hub?
- What is the seasonal variation in mobile fauna at each marina?
- How does the mobile faunal community differ between each marina?
- How do environmental parameters impact community composition at each marina?
- How do environmental parameters impact species richness at each marina?
- How do environmental parameters impact mobile faunal abundance at each marina?

Differences in mobile faunal community diversity

Mobile faunal community diversity over time (including seasonal variation) was assessed using Shannon-Weiner Species Diversity Index. Shannon's Diversity Index is calculated by taking the number of each species, the proportion each species is of the total number of individuals, and sums the proportion multiplied by the natural log of each species proportion. It is an assessment of richness and abundance and the value given provides an indication of diversity of the community. A chart was created for each of the restoration hubs, to show change in species richness and species abundance against time at all four marinas between 2021 and 2023. These plots provide a clear visual interpretation of seasonal variation of mobile fauna and the change in mobile faunal diversity over time.

To understand how the mobile fauna differed between restoration hubs and marinas, a community composition chart was created in RStudios for each marina. These charts plotted all species occurring more than ten times in each monitoring session to draw out dominant species present in the nurseries

A chart was created for each of the restoration hubs, to show change in species richness and species abundance against time.

at each marina. In Deganwy Marina, a substantial number of species occurred over ten times throughout the monitoring period, so the minimum occurrence threshold was increased to 20. These charts show the change in community composition throughout the year, and between years.

Following the creation of these charts, a non-metric multidimensional scaling (nMDS) plot, using Bray-Curtis dissimilarity matrix, was created of total species abundance at the Port of Blyth, Sunderland Marina, Conwy Marina and Deganwy Marina using all survey data from all years (monthly data from 2021–2023). Additionally, the entire mobile fauna dataset was tested for the statistical significance of location and year on the community composition using ANalysis Of Similarities (ANOSIM) tests. An ANOSIM test is a non-parametric test of significant difference between two or more groups, based on any distance measure.

To understand which species were responsible for any statistically significant differences in community composition between the sites, an Indicator Species Analysis was completed using a Multilevel pattern analysis. This test set out the number of species that were significantly associated with a single or multiple marinas, and then listed out said species.

The three species that occurred in the highest abundance in each marina in each monitoring session were listed (species/taxa were only included in the count if more than 10 individuals of that species were recorded). The single most abundant species each month, in each marina, was then listed and charts were created that depicted this species dominance.

Testing the impact of environmental parameters

To investigate the effect of environmental parameters on both multivariate and univariate biological data, we employed Principal Component Analysis (PCA) and BEST analysis using PRIMER v6. PCA is a powerful and versatile method capable of providing an overview of complex multivariate data and to summarise patterns within and between biotic and abiotic samples. In our study, PCA aimed to extract the main orthogonal contributors (principal components) which explain most of the variance of the data matrix analysed. The components were the following environmental abiotic parameters: Salinity, Temperature and Tidal State.

The RELATE and BEST procedures in PRIMER finds the best match between the multivariate among-sample patterns of an assemblage and that from environmental variables associated with those samples. The extent to which these two patterns match reflects the degree to which the chosen environmental data 'explains' the biotic pattern. The response variables in the PCA were again used as part of the RELATE and BEST analyses.

To further demonstrate the effect of environmental parameters on total species abundance, vectors were added to the nMDS abundance plot created for all marina sites and an envfit test run to test the association of each individual environmental parameter with abundance across each marina and each year of monitoring.

Both multivariate (community composition) and univariate (species richness and abundance) response variables were tested against environmental variables. All survey data from all years (monthly data from 2021–2023). Within each month, several cages were sampled (cage = lowest level of replication (n = 600)). During each sampling regime, number of each species, identified to the lowest taxonomic level possible, were recorded and enumerated. All environmental variables were also recorded. Data where environmental variables were missing were excluded from the analysis. Sampling was undertaken by a range of personnel, so instances where species had been identified to differing levels of taxonomic resolution were grouped to the highest level.

Environmental variables range

Salinity recordings during sampling across the three years ranged from 8–35 ppt. Air temperature ranged from -1.1°C to 23°C. Sea state and time of sampling were recorded, as were daily high and low tide times. Sampling at times ± two hours of high or low tide were considered as 'Low' or 'High' tidal state samples. Sampling times more than two hours from a high or low tide were considered as 'Mid' tidal state samples.

Photo: Volunteers and Wild Oysters team members monitoring mobile biodiversity at Deganwy Marina © Luke Helmer

2.6.4 Oyster Shell Epibiota

Sessile biodiversity on the oyster shells was sampled following the methods given in Section 2.5.2. This information was analysed examining the following key research questions:

- Does the sessile community evolve within each marina over time?
- Do the sessile communities vary between the Tyne and Wear and Conwy Bay restoration hubs?
- What is the seasonal variation in sessile fauna at each restoration hub?
- How does biodiversity (species abundance/ richness) differ between the dorsal or ventral sides of the oysters?

Temporal and Spatial Sessile Diversity

To investigate the evolution of the sessile community over time at each marina site, and to test for variation in the sessile communities between the marina sites a non-metric multidimensional scaling (nMDS) plot, using Bray-Curtis dissimilarity matrix, was created using survey data from all marinas and across all years (monthly monitoring data from 2021–2023). This nMDS plot provides a visual indication of how dissimilar data is for each marina site within each year of monitoring. To test for any statistical significance between sessile communities at each site across years, two ANOSIM tests were run in R. Firstly with a grouping by year and secondly by site.

Seasonal variation

The first tests on sessile community grouping by year were inconclusive. To investigate the sessile community compositional changes over time at each marina and assess variation in the sessile communities between 'season' nMDS plot, using Bray-Curtis dissimilarity matrix, was created using survey data from all marinas and across all years (monthly monitoring data from 2021–2023) and season was expressed as month.

This nMDS provides a visual indication of how dissimilar data is for each marina within each year of monitoring. To test for statistical significance between sessile communities at each site across years, PERMANOVA testing was undertaken using the response variables of Year (2021–2023) and Season (months: January, February, March, May, June, August, September, October, November) and an interaction effect (Year x Season) between the two responses. The sampling design was unbalanced, with a varying number of samples collected in each month, within each year, for each marina. For this reason, PERMANOVA analyses were selected as a non-parametric test robust to community zero inflated data, and suitable for testing unbalanced designs. Data were square root transformed prior to statistical testing.

Following this, univariate testing of species richness and abundance response variables was also undertaken. Abundance data were expressed as relative abundance per m^2 calculated from known shell area. Counts for dorsal and ventral sides of the shells were combined for each replicate. Abundance data were first square root transformed before Euclidean dissimilarity matrices were calculated (for each marina). Replicate data were then averaged per month.

Dorsal vs Ventral shell colonisation

The total number of species present on the dorsal and ventral surfaces of each individual oyster was calculated. From this an average was calculated and plotted showing the percentage of the total abundance of each taxa observed living on either the dorsal or ventral shell surface. A Two-sample T-test was run to assess significant difference in the total abundance of sessile organisms depending on whether organisms were on the dorsal or ventral surface and a second Two-sample T-test run based on taxa richness depending on whether organisms were on the dorsal or ventral surface. Interval plots of abundance and richness using individual standard deviations were also created to demonstrate the difference between the dorsal and ventral surfaces.

3. RESULTS

3.1 Growth

Shell measurement data indicated obvious oyster growth over the two-year monitoring period. Average shell area showed the strongest positive correlation with sample date, followed by shell height and lastly shell width, all of which were statistically significant ($r = 0.361, 0.346, 0.281$, $p < 0.005$) (Figure 13). Within monitoring sites, the growth of oysters appears to be stronger in the Tyne and Wear restoration hub than the Conwy Bay restoration hub, with a stronger positive correlation observed for shell area over time, although both are statistically significant ($r = 0.448, 0.275$, $p = <0.005$). In Tyne and Wear, the growth rate was highest in the first year (May 2021 to May 2022) compared to the second (May 2022 to May 2023) where growth appeared to stagnate. The mean shell height increased by 13 per cent (1.025 cm), shell width by 10 per cent (0.717 cm), and shell area by 25 per cent (12.34 cm^2) in year one, with no further increase observed between May 2022 and May 2023 (although some increases in earlier months which are not carried through into May averages). In Conwy Bay, the growth was slower in year one when compared to Tyne & Wear but continued to show a high growth rate into the second year. The mean shell height increased by 8 per cent (0.676 cm) in year one and 7 per cent (0.617 cm) in year two, shell width by 7 per cent (0.526 cm) in year one and 9 per cent (0.732 cm) in year two, and shell area by 20 per cent (9.99 cm^2) in year one and 15 per cent (8.85 cm^2) in year two.

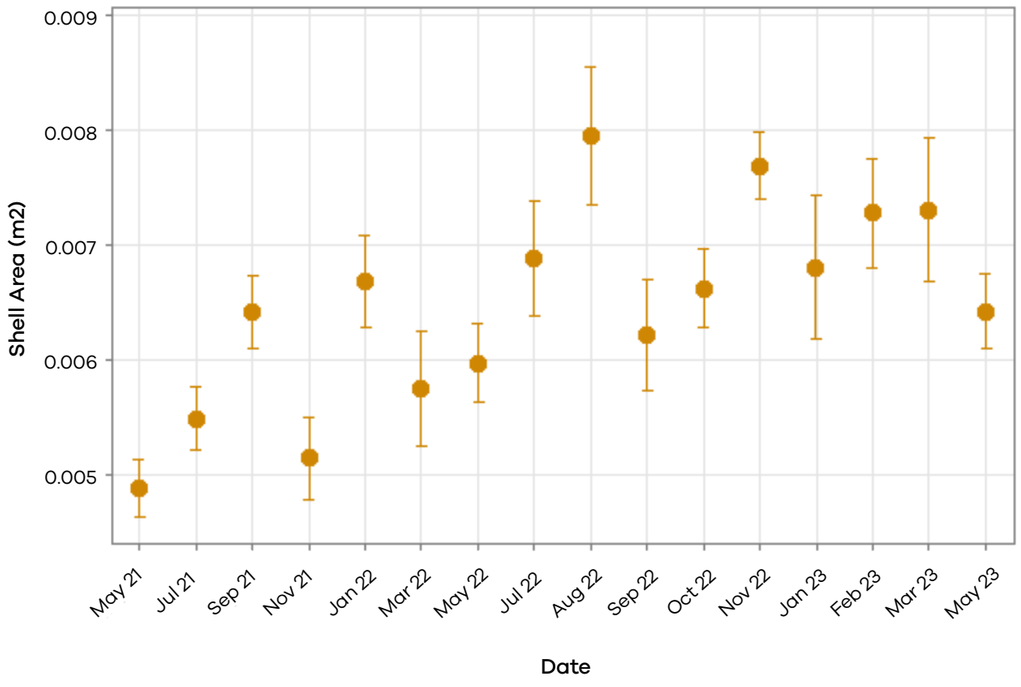


Figure 13, *Mean shell area (m^2) +/- 95 per cent confidence interval (calculated using individual standard deviations) per sample date from May 2021 to May 2023. All sites combined, n = 862 images.* © Ashleigh Tinlin-Mackenzie

3.2 Mortality

Monthly mortality percentages were plotted over the full lifetime of the nurseries until October 2023 (Figure 14). Survival rates were high at all sites (average annual oyster survival was 78% for both sites), with initial spikes in mortality seen when the nurseries were first installed (Figure 14). Following this, mortality rates reduced and remained low, with average monthly survival of 97.6 per cent at Sunderland Marina, 98.4 per cent at the Port of Blyth (Figure 15), 98.3 per cent at Deganwy Marina, and 96.9 per cent at Conwy Marina (Figure 16), over the 12-month period of May 2022 to April 2023.

> **Average annual oyster survival was 78% for both sites**

Both restoration hubs experienced a similar trend with a substantial mortality peak in July 2021 and then subsequent smaller spikes of 5–12 per cent in the months surrounding July 2022 and 2023. For the remaining months of the year the mortality rate has been low at both locations (less than 5 per cent throughout the year).

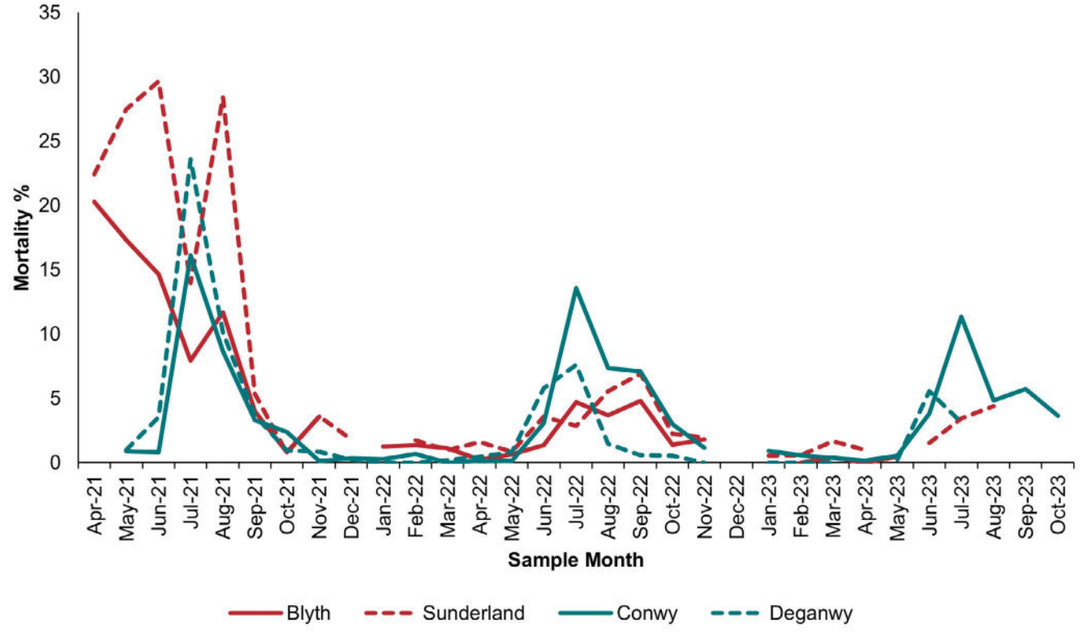


Figure 14, *Monthly mortality percentages for each marina site plotted over the full lifetime of the nurseries from April 2021 to October 2023 (gaps in the data are due to adverse weather conditions preventing data collection)*

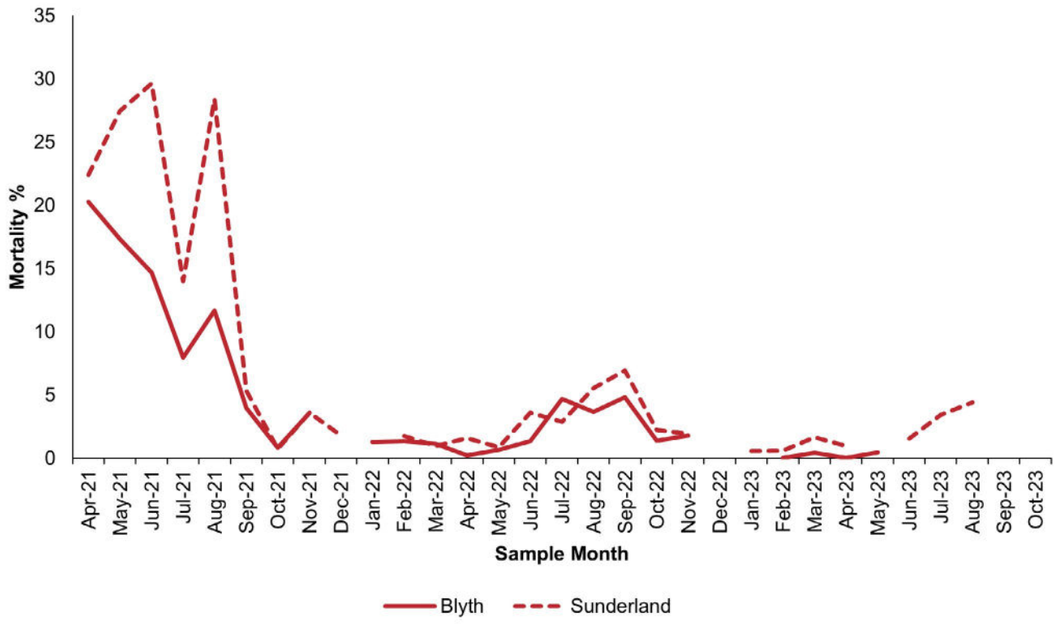


Figure 15, *Monthly mortality percentages for the Tyne and Wear restoration hub marina sites plotted over the full lifetime of the nurseries from April 2021 to August 2023.*

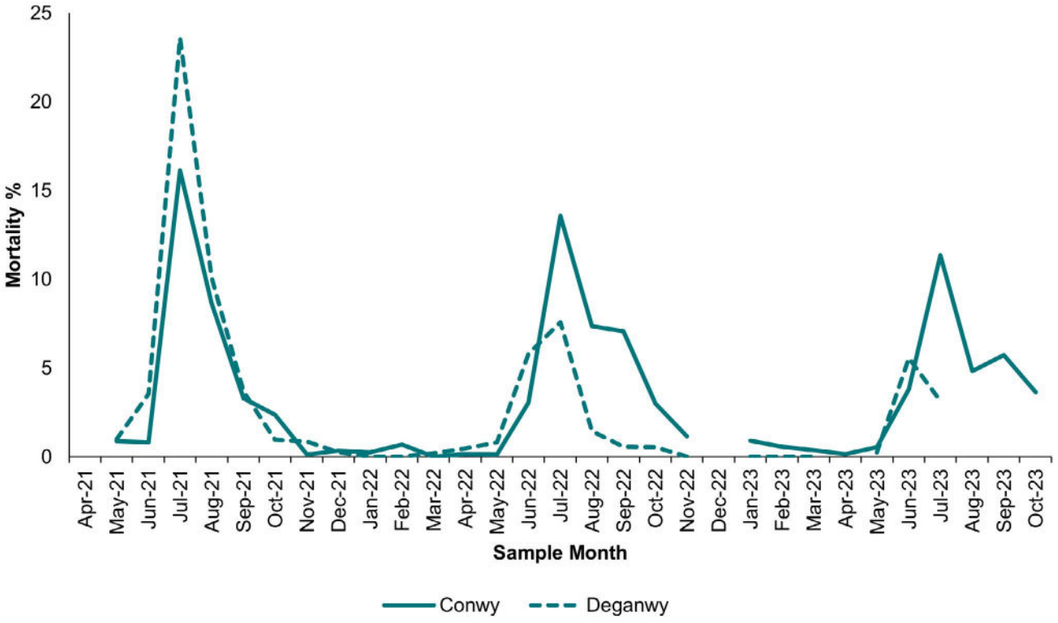


Figure 16, *Monthly mortality percentages for the Conwy Bay restoration hub marina sites plotted over the full lifetime of the nurseries from May 2021 to October 2023.*

3.3 Spawning

Oysters were observed to brood larvae at both sites when larval monitoring was taking place (Conwy Marina and Sunderland Marina). In 2022, oysters were brooding larvae from the beginning of June to the end of August in Conwy Marina and from mid-July to the beginning of August in Sunderland Marina (Figure 17). In Conwy Marina, larvae were observed in 47 and 40 oysters in 2022 and 2023, respectively. In Sunderland Marina, larvae were observed in 20 and 9 oysters in 2022 and 2023, respectively (Figure 18).

In 2022, over 70 per cent of larvae samples collected across both sites were white 'sick' (Figure 19). In 2023, rates between marinas differed, with 100 per cent of samples from Sunderland Marina being white 'sick' compared to 60 per cent in Conwy Marina (Figure 20). Results were extrapolated from this sub-sampling, and it was estimated that during the spawning season in 2022, 14 per cent of oysters in Conwy Marina spawned and 8.2 per cent in Sunderland Marina. In 2023, it is estimated that 9.7 per cent of oysters spawned in Conwy Marina and 3.8 per cent in Sunderland Marina. Of those spawning oysters, samples were analysed to calculate the average number of larvae produced by spawning oysters at each of the marina sites. In 2022, brooding oysters in Conwy Marina produced approximately 2.2 million larvae per oyster, compared to approximately 1.7 million larvae per oyster in Sunderland Marina (Figure 21). A Kruskal-Wallis H test showed no significant difference in the number of larvae brooded between the two marinas, $\chi2(1) = 0.048$, $p = 0.827$. In 2023, brooding oysters in Conwy Marina and Sunderland Marina both produced approximately 1.8 million larvae per oyster (Figure 22). Again, a Kruskal-Wallis H test showed no significant difference in the number of larvae brooded between the two marinas, $\chi2(1) = 0.017$, $p = 0.897$. The data was extrapolated to estimate the total number of larvae released from the oyster populations at each marina site. During the spawning season in 2022, the oysters at Conwy Marina released 172 million larvae, and 93 million larvae at Sunderland Marina. In 2023, the oysters released 177 million larvae at Conwy Marina and 56 million larvae at Sunderland Marina into the surrounding waters.

Photo: Matt Uttley, Blue Marine Foundation, monitoring oyster survival at Conwy Marina © Lucie Machin

Oysters were observed to brood larvae at both sites where larval monitoring was taking place (Conwy Marina and Sunderland Marina).

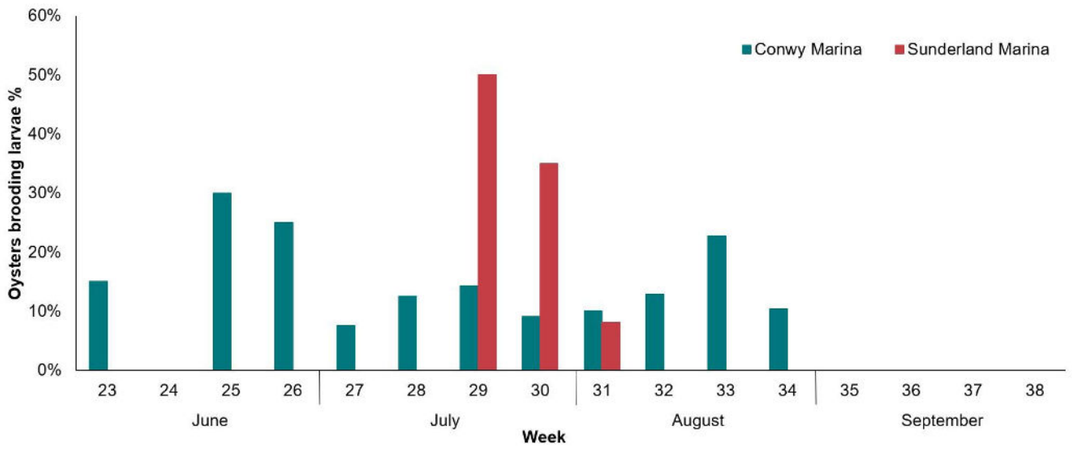


Figure 17, *Weekly observations between June and September 2022 of the percentage of oysters brooding larvae subsampled at each site; Conwy Marina, North Wales, Sunderland Marina, North East England.* © Maria Hayden-Hughes

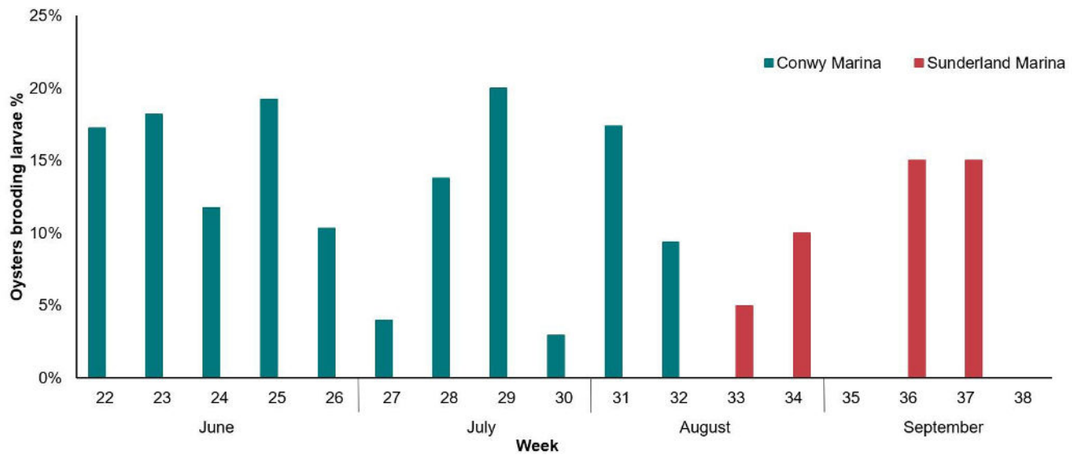


Figure 18, *Weekly observations between June and September 2023 of the percentage of oysters brooding larvae subsampled at each site; Conwy Marina, North Wales, Sunderland Marina, North East England.* © Maria Hayden-Hughes

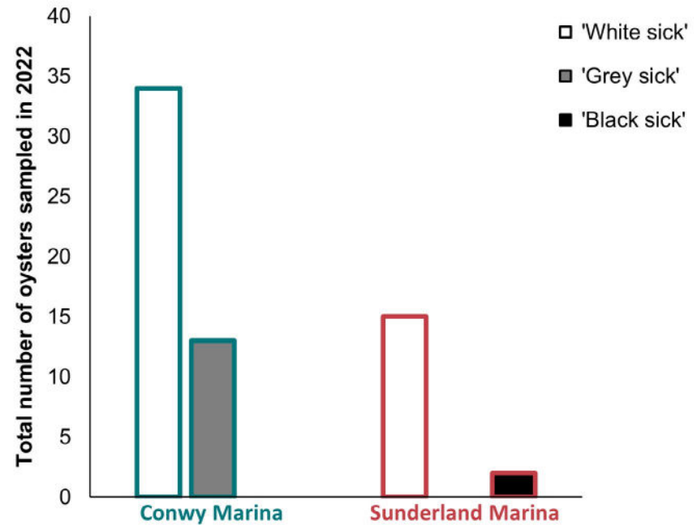


Figure 19, *Total number of larval samples at native oyster (larval development stages; 'white sick', 'grey sick', 'black sick', recorded at sampling locations; Conwy Marina, North Wales, Sunderland Marina, North East England. Samples collected during the spawning season in 2022.*

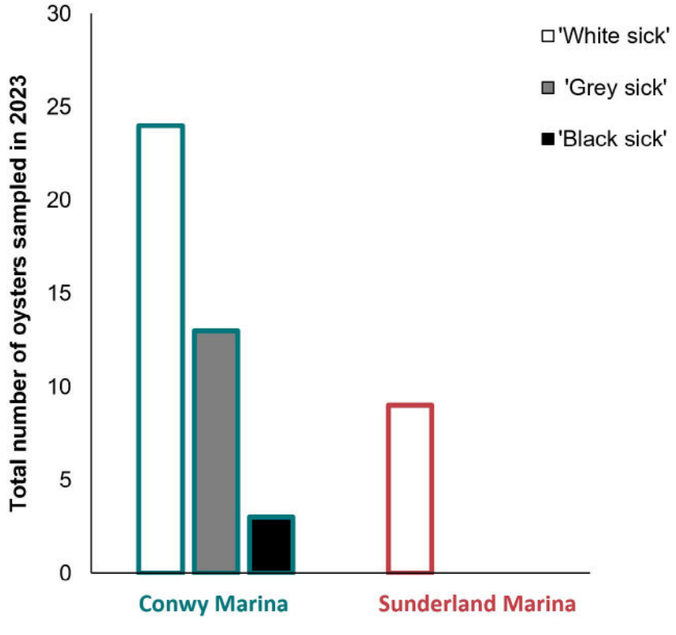


Figure 20, *Total number of larval samples at native oyster larval development stages; 'white sick', 'grey sick', 'black sick', recorded at sampling locations; Conwy Marina, North Wales, Sunderland Marina, North East England. Samples collected during the spawning season in 2023.*

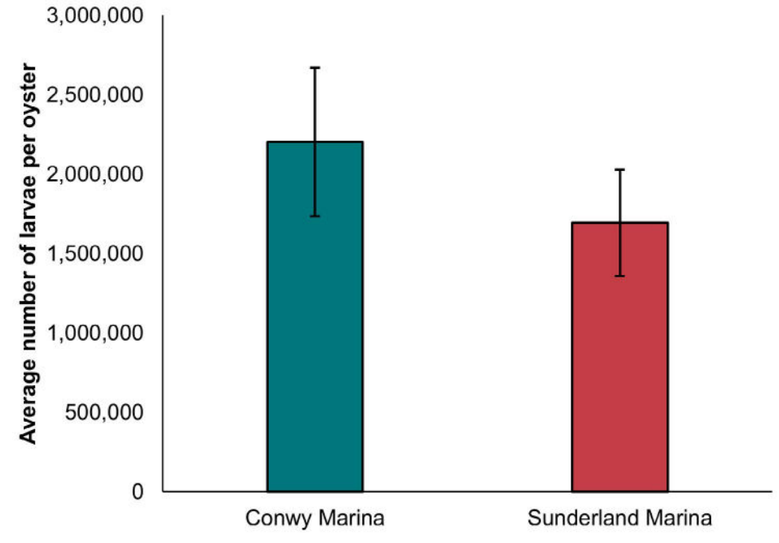


Figure 21, *Average number of oyster larvae per oyster ± 1SE recorded in 2022 at each sampling location; Conwy Marina, North Wales, Sunderland Marina, North East England.*

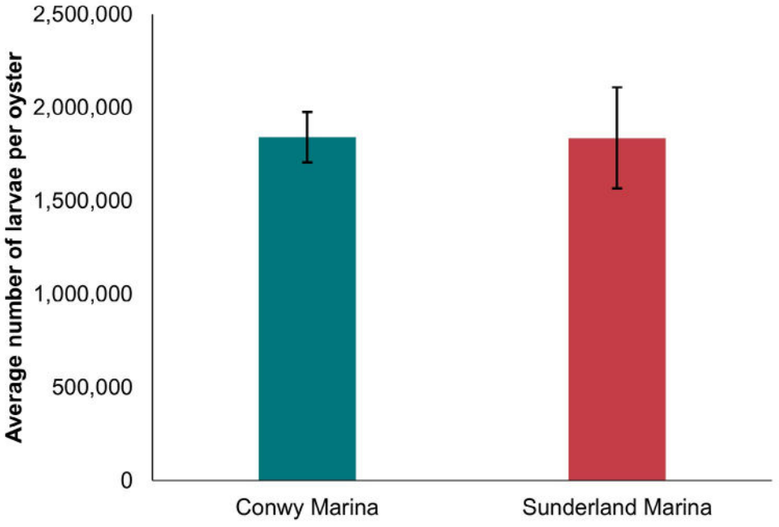


Figure 22, *Average number of oyster larvae per oyster ± 1SE recorded in 2023 at each sampling location; Conwy Marina, North Wales, Sunderland Marina, North East England.*

Mortality spikes appeared to coincide with spawning events. To understand the relationship between mortality and spawning, the mortality rates were plotted against the number of oysters spawning per month for all marinas (Figure 23), and independently for each restoration hub (Figures 24 and 25). There is no spawning data in 2021 during the initial mortality spikes during the acclimation period after oysters were first introduced, but the spawning events in 2022 and 2023 were observed to align directly with the summer mortality spikes.

Temperature impact on spawning

Spawning data were plotted against recorded water temperature (Figures 26 and 27). There is a clear relationship between temperature and commencement of larvae brooding. Larvae were observed in the pallial fluid when seawater temperatures with the marina sites reached approximately 15°C. In Conwy Marina, the water temperature reached 15°C in the first week of June in 2022 and the second week of June in 2023. Brooding oysters were found in the same weeks.

In Sunderland Marina, water temperatures did not reach 15°C until the third week of July in 2022, when a high number of oysters were also found in brooding condition. In 2023, temperatures were cooler and did not reach 15°C until the third week of September. However, in the third week of August 2023 the temperature reached 14°C and low levels of brooding oysters were found (one oyster in the third week and two in the fourth week). No further spawning was seen in Sunderland Marina until the water temperature reached 14–15°C in mid-September.

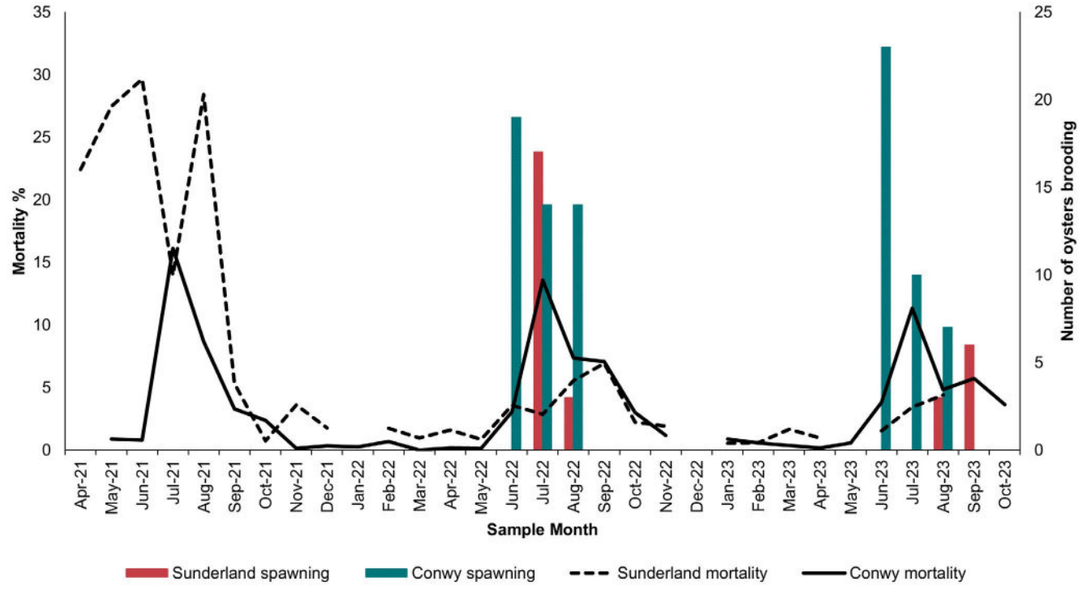


Figure 23, *Percentage of oyster mortality over time, across all marina sites and number of oysters spawning per month during the same period.*

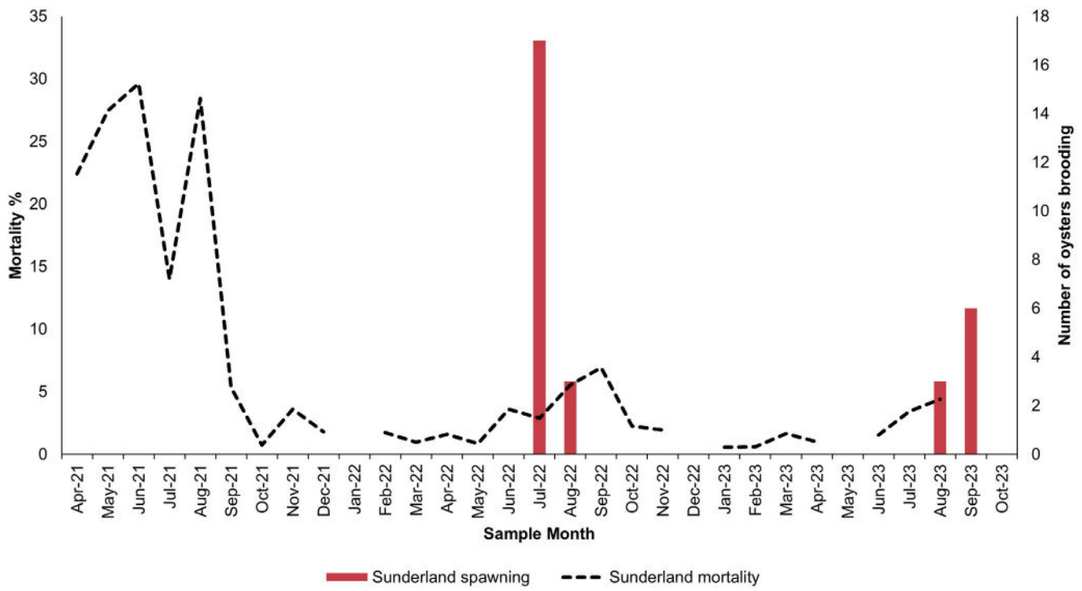


Figure 24, *Percentage of oyster mortality over time at Sunderland Marina, and number of oysters spawning per month during the same period.*

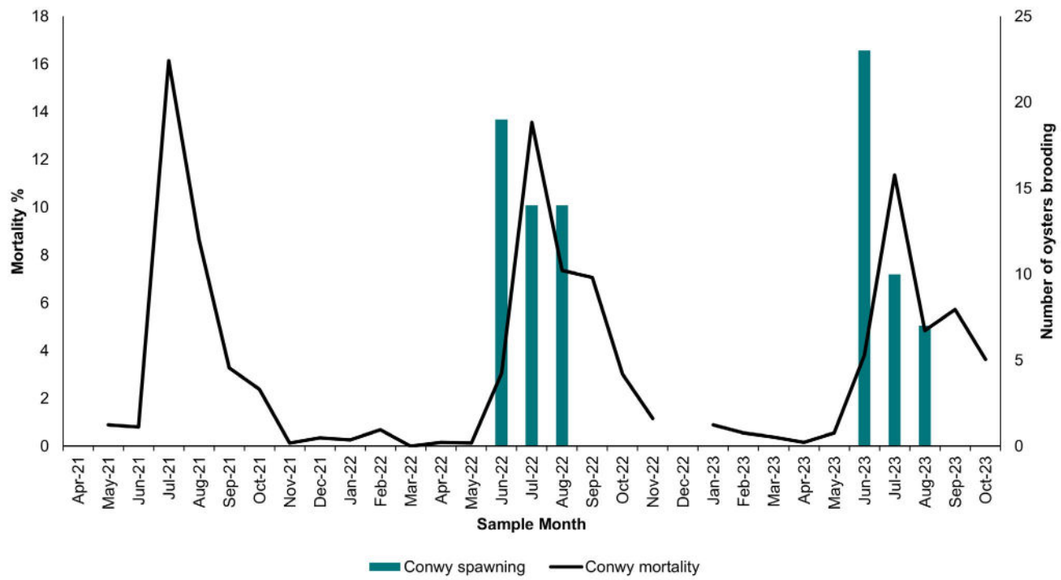


Figure 25, *Percentage of oyster mortality over time at Conwy Marina, and number of oysters spawning per month during the same period.*

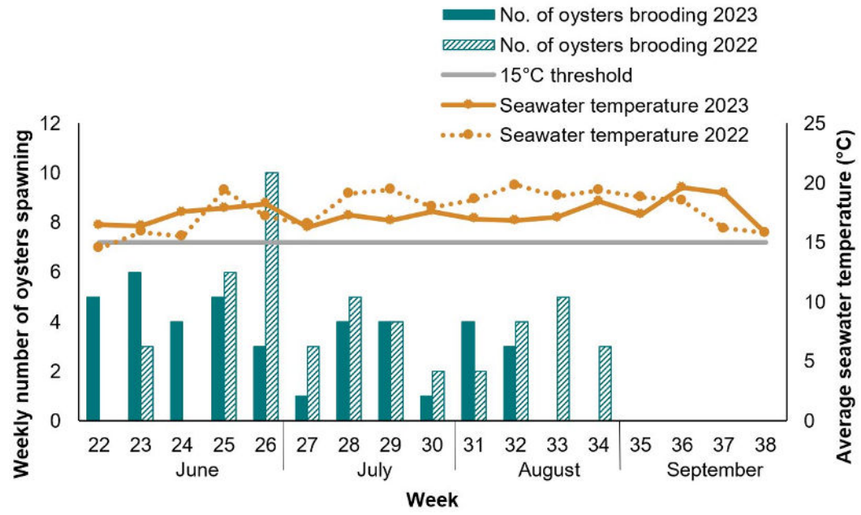


Figure 26, *Weekly observations during the spawning season in 2022 and 2023, between June and September, of the number of oysters brooding larvae recorded at Conwy Marina, North Wales. Average weekly seawater temperature (°C) in 2022 and 2023, with line denoting 15°C, which when exceeded, spawning is initiated.*

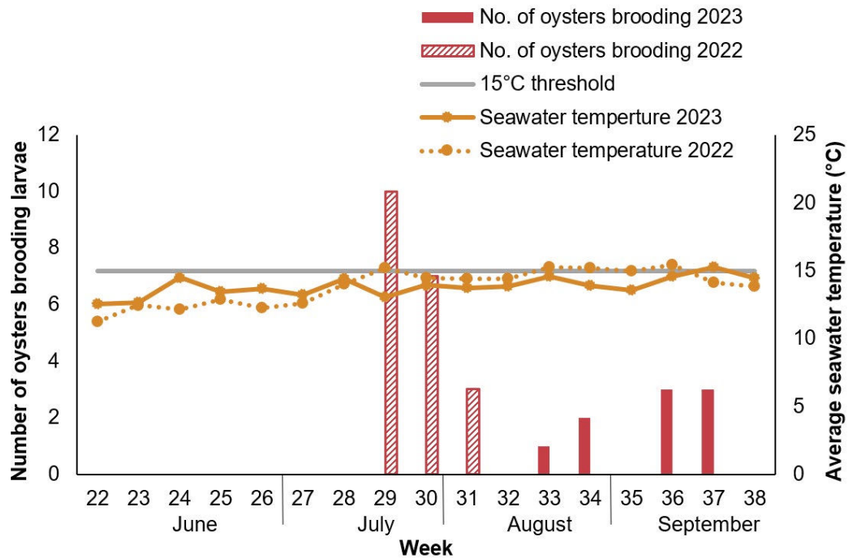


Figure 27, *Weekly observations during the spawning season in 2022 and 2023, between June and September, of the number of oysters brooding larvae recorded at Sunderland Marina, Tyne and Wear. Average weekly seawater temperature (°C) in 2022 and 2023, with line denoting 15°C, which when exceeded, spawning is initiated.*

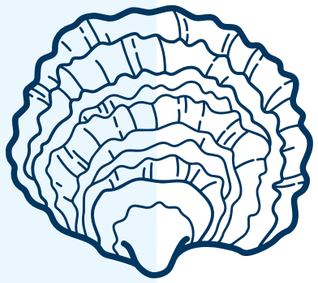
3.4 Filtration

From April 2021 to October 2023, Conwy Marina had an average of 648 oysters in the nurseries in each month. After accounting for mortality and restocking figures across the year, these oysters will have filtered approximately of ~44,100,000 litres of water in this time. From April 2021 to July 2023 Deganwy Marina had an average of 626 oysters. These are estimated to have filtered approximately ~38,400,000 litres of water. Therefore, total oyster water filtration for the Conwy Bay restoration hub for the project duration is approximately ~82,500,000 litres of water.

From March 2021 to August 2023, in Sunderland Marina it is estimated that the average monthly total of 656 oysters in Sunderland Marina will have filtered approximately ~43,200,000 litres of water. Between March 2021 and May 2023, the average monthly total of 388 oysters in the Port of Blyth are estimated to have filtered approximately ~23,000,000 litres of water. Therefore, the total oyster water filtration for the Tyne and Wear restoration hub since the start of the project is approximately ~66,200,000 litres of water.

Photo: Native oysters for a nursery restocking event at Conwy Marina © Celine Gamble, ZSL

3.5 Biodiversity associated with oyster nurseries



3.5.1 Mobile biodiversity

Diversity

The mobile community present at each of the marinas was assessed for species abundance and richness change over time. These metrics were combined and presented in a Shannon's Diversity Index for each restoration hub (Figure 28). There are apparent spikes in diversity around July each year, but a severe decline in diversity over winter in 2022 and no obvious overall increase in diversity throughout the duration of monitoring.

The diversity index for the Tyne and Wear restoration hub (Figure 29) differs greatly throughout the years of monitoring but with clear late summer spikes. In contrast to the sites in the Conwy Bay restoration hub, there is an increased diversity index rating at the end of the monitoring period relative to the value at the start of monitoring.

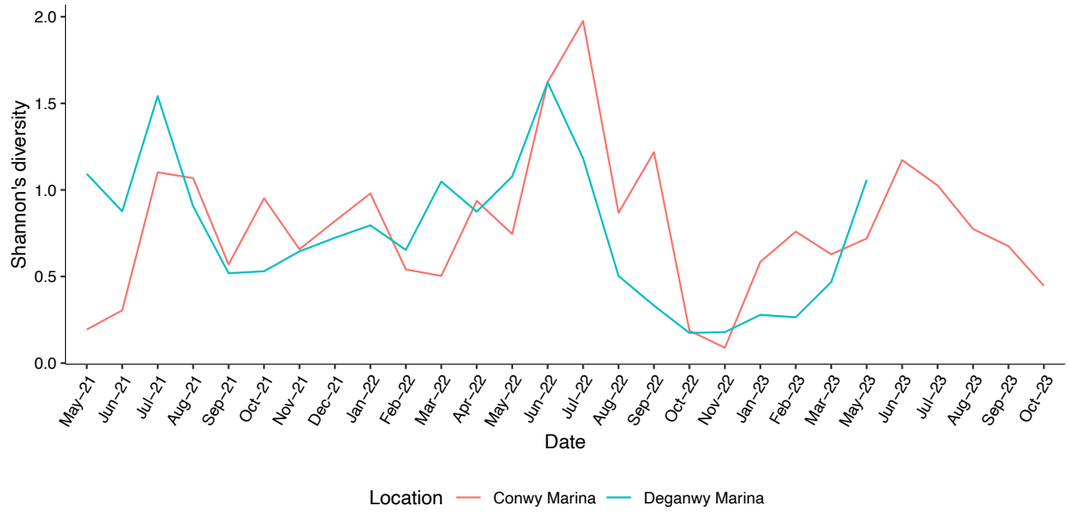


Figure 28, *Shannon's Diversity Index for the Conwy Bay restoration hub. Showing both Conwy Marina and Deganwy Marina. The diversity index combines species abundance and richness over time.*

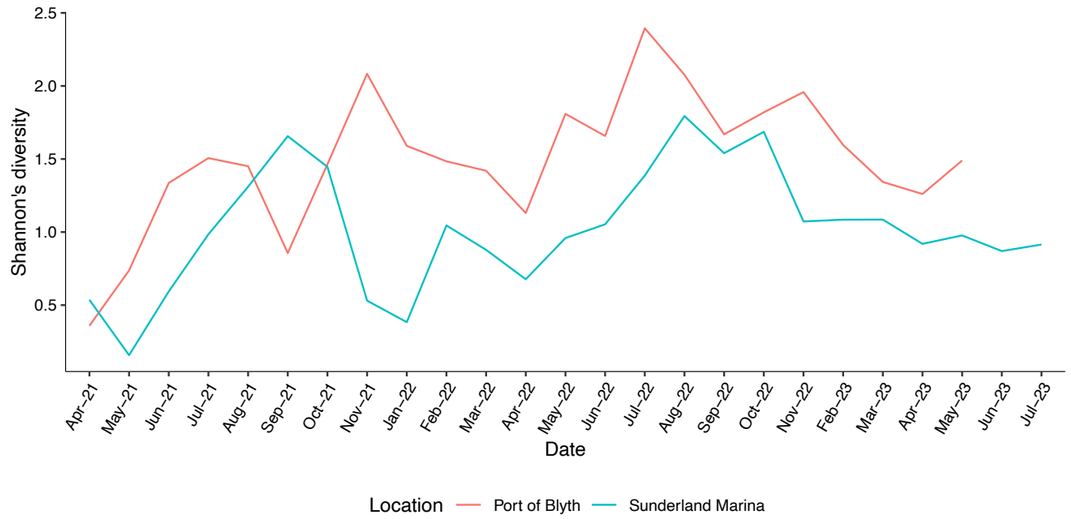


Figure 29, *Shannon's Diversity Index for the Tyne and Wear restoration hub. Showing both Sunderland Marina and the Port of Blyth sites. The diversity index combines species abundance and richness over time.*

Photo: Volunteers monitoring during nursery monitoring work at Conwy Marina © Luke Helmer

Community Composition

To investigate the apparent differences shown between restoration hubs in the Shannon's Diversity Indexes, a community composition chart was created for each marina site (Figures 30A and 30B for Tyne and Wear restoration hub and Figures 31A and 31B for Conwy Bay restoration hub), displaying the abundance of each of the dominant species for each month throughout the project timeline. These charts show the change in composition throughout the year and between years. As the only key similarity between the charts is a strong presence of crustaceans at all sites, particularly *Eumalacostraca* with the composition of all sites consisting of a high percentage of Amphipoda, Isopoda or *Palaemon* spp. The community composition charts highlight more differences than similarities, both within and between restoration hub sites.

Within the Tyne and Wear restoration hub, Sunderland Marina (Figure 30A) does not show a clear seasonality trend and has a high Amphipoda presence throughout the year. In contrast, the Port of Blyth only had high Amphipoda presence in spring each year, has very clear trends of higher total community abundance in late summer and a very varied composition throughout the year.

Within the Conwy Bay restoration hub, again there are clear differences in both species present and community trends. Conwy Marina retains a consistent total abundance throughout the monitoring period, except from a large spike in Amphipoda upon initial installation of the nurseries. Amphipoda presence is then similar to the Port of Blyth with only high abundance in the spring and summer months. Deganwy Marina also only has high numbers of Amphipoda in the spring, but in contrast to Conwy Marina is always in relatively low numbers and the composition chart is almost entirely dominated by *Palaemon* spp.

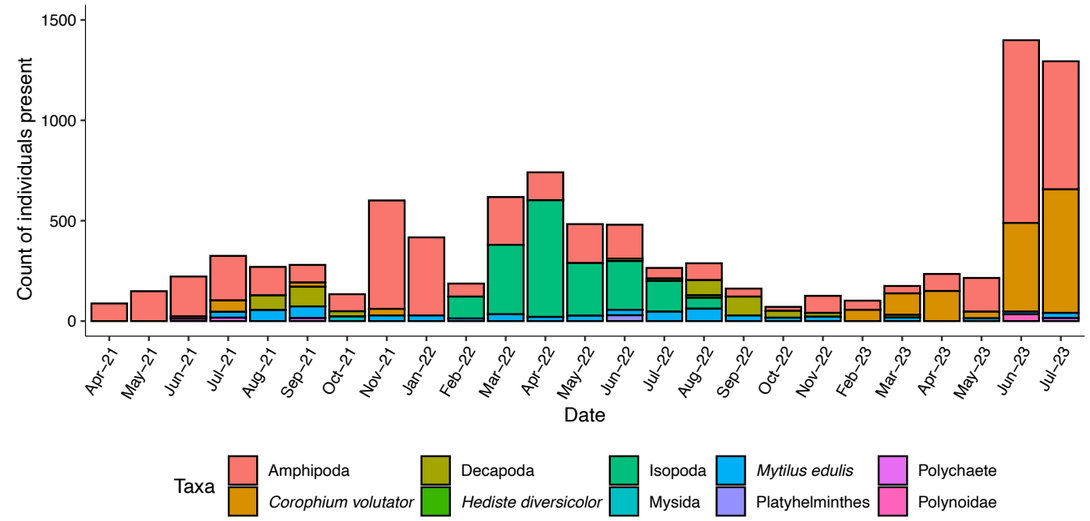


Figure 30A

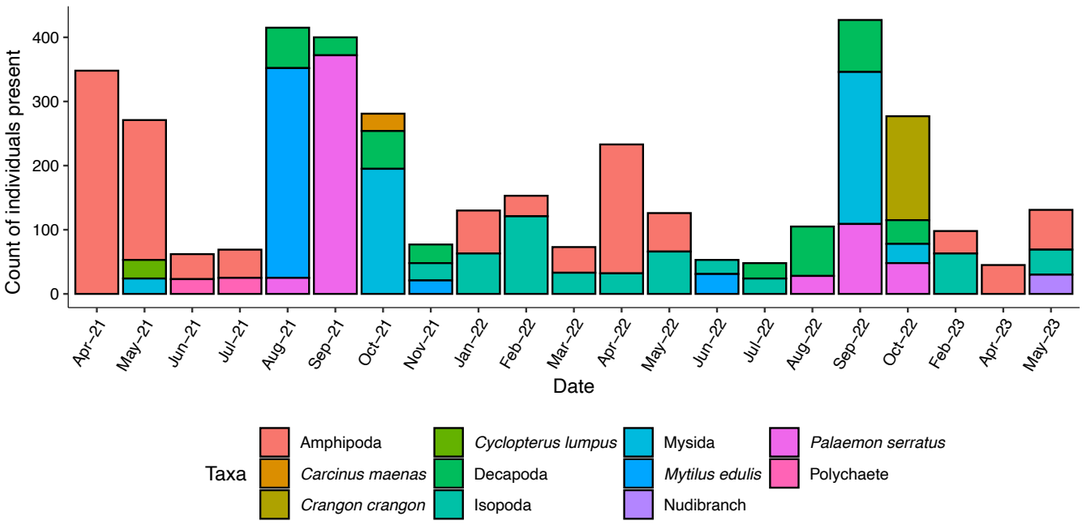


Figure 30B

Figure 30A and 30B, *Community composition chart for each site in the Tyne and Wear restoration hub over time. Species occurring more than 10 times in a monitoring session recorded in both sites.*

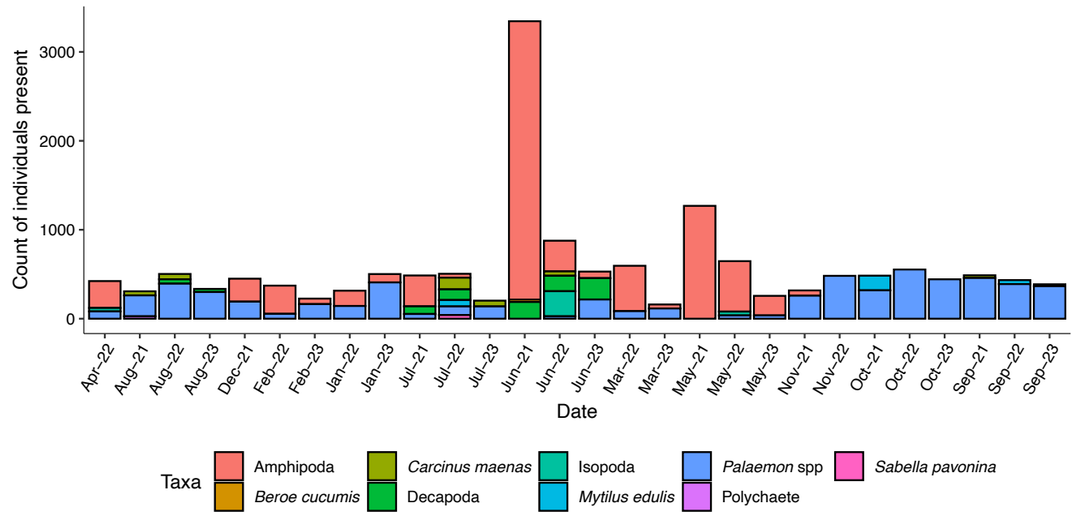


Figure 31A

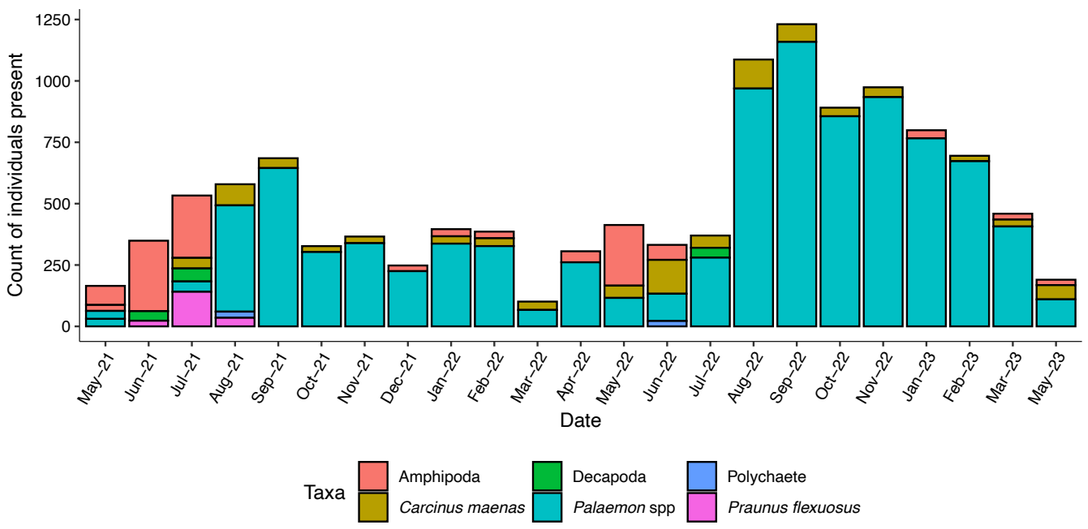


Figure 31B

Figures 31A and 31B, *Community composition chat for each site in the Conwy Bay restoration hub over time. Species occurring more than 10 times in a monitoring session recorded in Conwy Marina and more than 20 times per session in Deganwy Marina.*

The single highest occurring species at each site from March 2021 to November 2023 for each monitoring session were listed (Table A in Appendix 2) then plotted (Figure 32) to show the difference in dominant species.

These plots clearly showed a strong dominance of Amphipoda at the Port of Blyth site (Figure 32A) and Sunderland Marina (Figure 32B), with Amphipoda recorded as dominant in 44 per cent of monitoring sessions in the Port of Blyth site and 52 per cent of sessions at the Sunderland Marina site. Other dominant species at these two sites were mixed with lower percentage dominance, these species included Isopoda at Sunderland Marina (22 per cent), Decapoda and Isopoda at the Port of Blyth site (16 per cent and 12 per cent respectively).

A different pattern of species dominance was seen in the Conwy Bay restoration hub sites. *Palaemon* spp., was recorded as dominant in 48 per cent of monitoring session in Conwy Marina (Figure 32C) and 72 per cent of sessions at Deganwy Marina (Figure 32D). There were far fewer other species recorded as dominant in the monitoring sessions in both marinas. The only other key species that occurred regularly as the most abundant species was Amphipoda, being the dominant species in 39 per cent of monitoring sessions at Conwy Marina, and 16 per cent of sessions at Deganwy Marina.

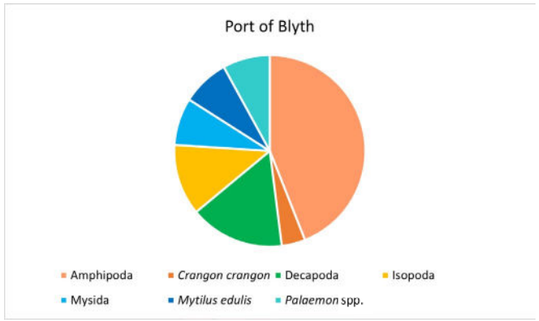


Figure 32A

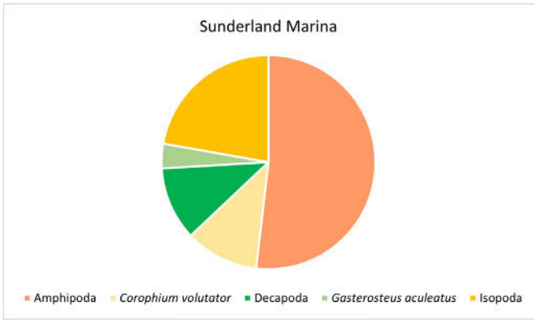


Figure 32B

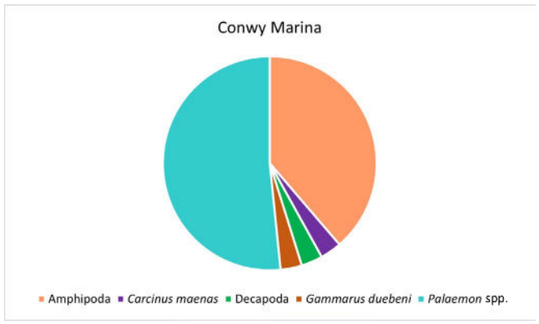


Figure 32C

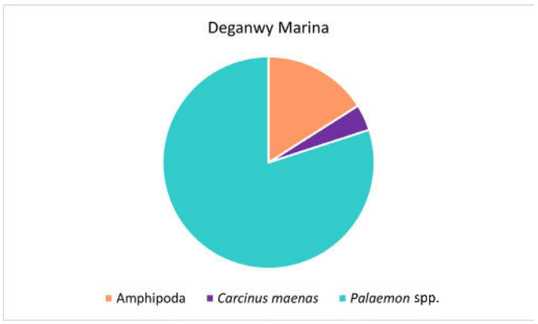


Figure 32D

Figure 32A-D, *Plots of the most dominant species in each monitoring session between March 2021 and October 2023 for all marina sites (32A, Port of Blyth site; 32B, Sunderland Marina site; 32C, Conwy Marina site, & 32D, Deganwy Marina site). The dominant species in a monitoring session was considered the species with the greatest abundance in that singular session. If no species had a count greater than 10 individuals in a session, then no species was recorded for that month.*

NMDS plot – Visualisation of data

Following the creation of the community composition charts and dominant species plots, a non-metric multidimensional scaling (nMDS) plot (Figure 33), using Bray-Curtis dissimilarity matrix, was plotted with all abundance data for the four marinas through the three years of the project and provided a visual representation of how dissimilar the species abundance is depending on the functions Date and Site. A 0.1414 stress value indicates 2D location is representative of actual differences, with larger distances between treatments indicating greater dissimilarities. The nMDS shows clear separation of the Conwy Bay restoration hub and the Tyne and Wear restoration hub, which is a statistically significant difference when all sites are compared (ANOSIM (9999 permutations), R = 0.7809, p < 0.001). Despite some suggestion that the 2023 points are distinct in the nMDS, there is no significant difference depending on the function of Date (ANOSIM (9999 permutations, R = -0.01157, p > 0.05).

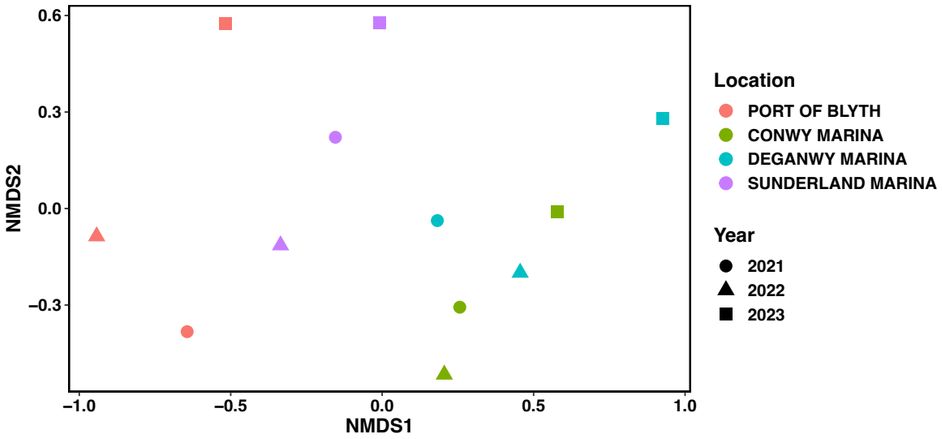


Figure 33, *A non-metric multidimensional scaling (nMDS) plot, using Bray-Curtis dissimilarity matrix, of total species abundance at Port of Blyth, Sunderland Marina, Conwy Marina and Deganwy Marina in 2021–2023. Stress = 0.14*

The nMDS shows clear separation of the Conwy Bay restoration hub and the Tyne and Wear restoration hub, which is a statistically significant difference when all sites are compared.

Indicator Species Analysis

To understand the cause of the significant difference in species abundance between the marina sites and the dissimilarity shown between restoration hubs in the nMDS plot, an indicator species analysis (Multilevel pattern analysis) was used to identify which species are significantly associated with specific marinas.

This test flagged that there are three species associated significantly with a single marina site, and one species significantly associated with two marina sites (Conwy and Deganwy marinas). These species associations are likely what caused the significant differences seen in the ANOSIM and likely a large cause of the clusters between the Conwy Bay restoration hub sites shown in the nMDS.

Table 1, *results of the multilevel pattern analysis testing association of species abundance with presence in each marina site. The table shows the species that were significantly associated with one or more marina sites and the strength of the association (stat value).*

Taxon	Associated site	Stat value	P value
Taurulus bubalis	Port of Blyth only	0.811	0.0365
Mysida	Port of Blyth only	0.790	0.0379
Decapoda	Conwy Marina	0.761	0.0422
Palaemon spp.	Conwy and Deganwy marinas	0.840	0.0041

As a result, of the 99 species in the dataset, only four were significantly associated with one or more sites. *Taurulus bubalis* (long-spined scorpion fish) (p = 0.0365), and Mysida (mysid shrimps) (p = 0.0379) were strongly associated to the Port of Blyth as show by the high statistical values (0.811 and 0.790 respectively).

The Decapoda family were strongly (Stat = 0.761) and significantly associated to Conwy Bay restoration hub (p = 0.0422). Both Conwy Marina and Deganwy Marina were strongly (Stat = 0.840) and significantly (p = 0.0041) associated with *Palaemon* spp. in comparison to the sites in the Tyne and Wear restoration hub.

Photo: The Wild Oysters Project nursery © Georgie Bull, ZSL

Effect of Environmental Parameters

To demonstrate the effect of environmental parameters on total species abundance, vectors were added to the nMDS plot created for all marina sites (Figure 34) and an envfit test run to test the association of each individual environmental parameter with abundance across each marina and each year of monitoring. The vectors in this nMDS are visual indicators of the correlation of each environmental parameter with the species abundance data for each site across the three monitoring years. There are some clear implied correlations in this plot, such as the wind speed being most correlated with the mobile community at Deganwy Marina in 2023, and likewise humidity most correlated with the community at the Port of Blyth in 2023. To test the statistical significance of the environmental parameters, each parameter was subject to an envfit test. Full results of this test are displayed in Table B Appendix 2, but the only parameter that displayed a significant correlation with the mobile communities was sea state (p = 0.04167).

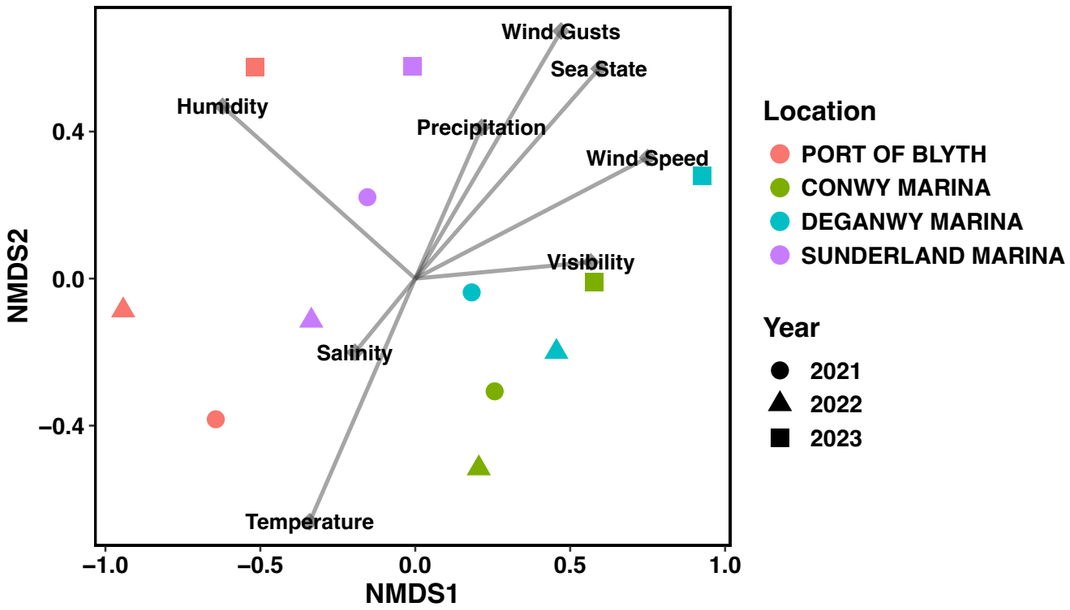


Figure 34, A non-metric multidimensional scaling (nMDS) plot, using Bray-Curtis dissimilarity matrix, of total species abundance at Port of Blyth, Sunderland Marina, Conwy Marina and Deganwy Marina in 2021, 2022, 2023 with environmental parameters added as vectors, direction and length of the vector lines indicates correlation with the nearer data points.

Effect of Environmental Parameters on Community Composition

The PCA did not reveal significant patterns in our dataset. The first principal component, salinity (PC1) explained 38.3 per cent of the total variance, while the second principal component, air temperature (PC2) accounted for 33.2 per cent. This indicates that the two components collectively explain 71.5 per cent of the total variance in our data with PC3, tidal state, explaining the remaining (28.5 per cent) (Full PCA results presented in Table C, Appendix 2). These results suggest each of the environmental variables tested were contributing to a similar degree when explaining community composition suggesting that none of these variables were driving any differences. This was consistent across all years as seen in the PCA biplot (Figure 35) which plots the distribution of samples and the contribution of variables to PC1 and PC2.

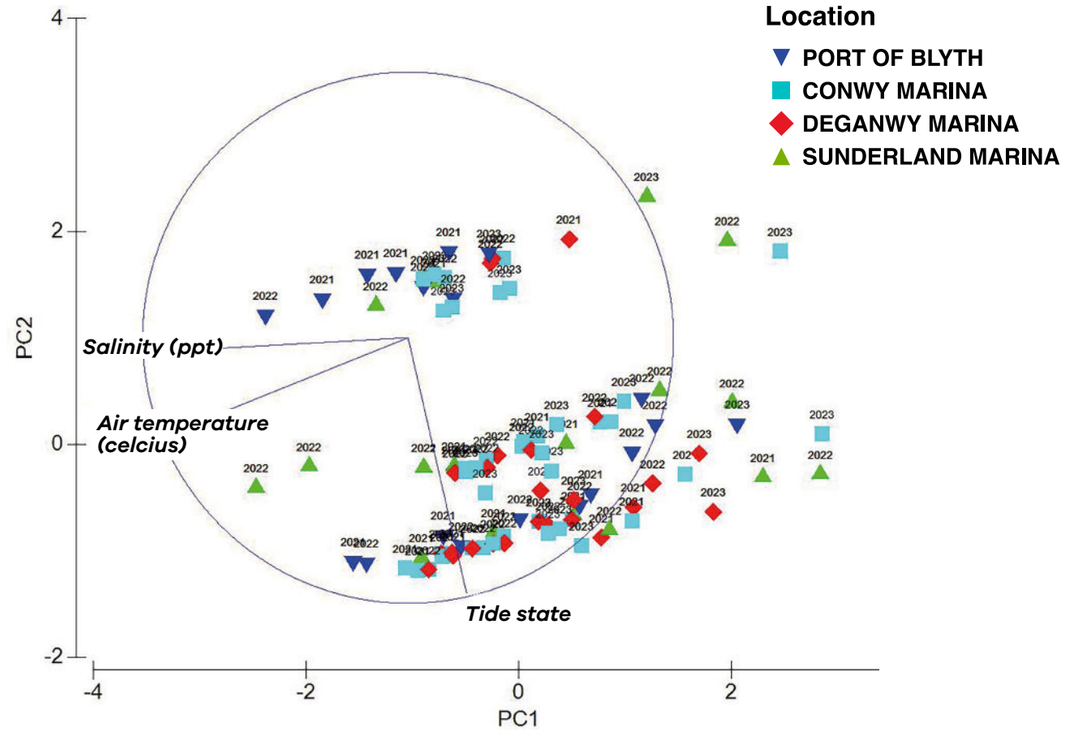


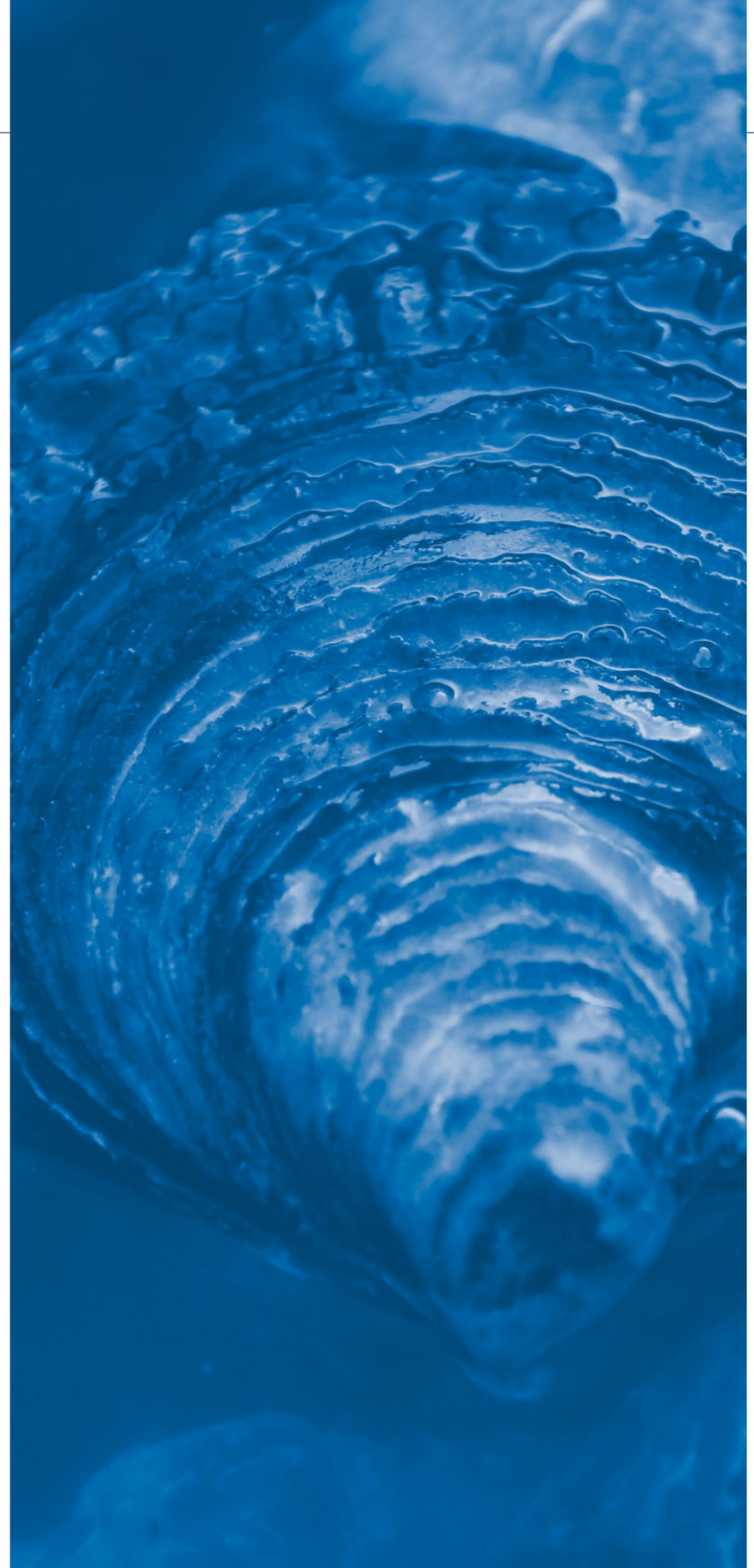
Figure 35, PCA ordination plot (PCA1 and 2) of environmental variables for all sites in all years (2021-2023).

In addition to the PCA, the RELATE and BEST analysis was performed using Primer to identify the environmental variables that best explain patterns observed on community composition. The analysis supported the PCA outputs in that all three environmental variables (salinity, air temperature and tide state) were equal contributors to the observed biological patterns. RELATE results (Spearman Rank Correlation, 999 permutations, Rho 0.113) suggested that patterns in environmental data did not influence patterns seen in the community composition.
The BEST rank correlation coefficient (Table 2) indicated strongest correlation between the biological and environmental data when all variables were included (Correlation = 1) suggesting no single variable was contributing to any patterns greater than the next. Air temperature and tidal state were the next strongest predictor of any patterns but together only explain 0.8 per cent of the variation in the biological data.

Table 2, *showing the BEST rank correlation coefficient impact on community composition (999 permutations, Sample Statistic Rho 1) for each of the environmental parameters singularly, combined in pairs and all three parameters combined.*

Variables	Correlation
Salinity (ppt)	0.486
Air Temperature (°C)	0.476
Tide state	0.559
Salinity (ppt) and Air Temperature (°C)	0.771
Salinity (ppt) and Tide state	0.792
Air Temperature (°C) and Tide state	0.805
All variables	1.000

The analysis supported the PCA outputs in that all three environmental variables (salinity, air temperature and tide state) were equal contributors to the observed biological patterns.



Effect of Environmental Parameters on species richness and abundance

Abundance

Overall abundance was tested against the environmental response variables using the BEST analysis. BEST rank correlation coefficient indicated no correlation between the biological and environmental data. The best model results explained only 0.035 per cent of variation in overall abundance in response to environmental variables (Table 3).

Table 3, *showing the BEST rank correlation coefficient impact on abundance (999 permutations, Sample Statistic Rho 0.035, Number of permuted statistics greater than or equal to Rho 107) for each of the environmental parameters singularly, combined in pairs and all three parameters combined.*

Variables	Correlation
Salinity (ppt)	-0.007
Air Temperature (°C)	0.032
Tide state	0.014
Salinity (ppt) and Air Temperature (°C)	0.008
Salinity (ppt) and Tide state	-0.007
Air Temperature (°C) and Tide state	0.035
All variables	0.013

Species richness

Total species richness was tested against the environmental response variables using the BEST analysis. BEST rank correlation coefficient indicated no correlation between the biological and environmental data. The best model results explained only 0.03 per cent of variation in overall abundance in response to environmental variables (Table 4).

Table 4, *showing the BEST rank correlation coefficient impact on species richness (999 permutations, Sample Statistic Rho 0.065, Number of permuted statistics greater than or equal to Rho 2) for each of the environmental parameters singularly, combined in pairs and all three parameters combined.*

Variables	Correlation
Salinity (ppt)	0.064
Air Temperature (°C)	0.058
Tide state	0.010
Salinity (ppt) and Air Temperature (°C)	0.065
Salinity (ppt) and Tide state	0.048
Air Temperature (°C) and Tide state	-0.045
All variables	0.060

3.5.2 Oyster Shell Epibiota

Temporal and Spatial Sessile Diversity

To investigate the evolution of the sessile community over time at each marina site, and to test for variation in the sessile communities between the marina sites, a nMDS plot, using Bray-Curtis dissimilarity matrix, was created using survey data from all marinas across all years (Figure 36). The nMDS plot shows that Conwy Marina 2021, 2022 and Deganwy Marina 2021 are dissimilar to all other sites and dates. To test for any significant difference in the sessile community based on marina site or date, an ANOSIM test was undertaken.

Both ANOSIM tests to assess sessile communities across by site (ANOSIM (9999 permutations), R = 0.1821, p > 0.05) and year groupings (ANOSIM (9999 permutations), R = -0.02083, p > 0.05) were statistically insignificant.

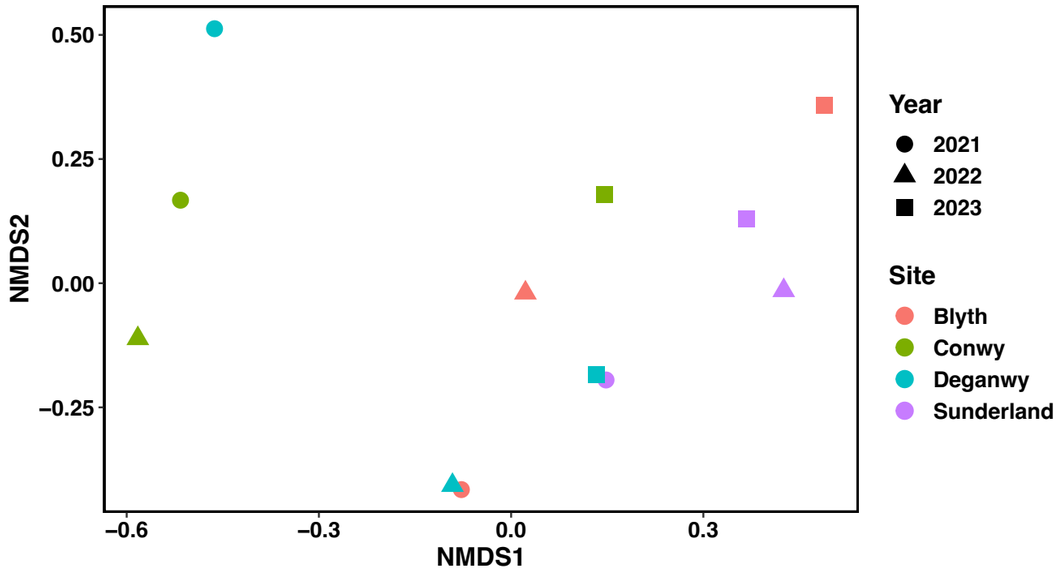
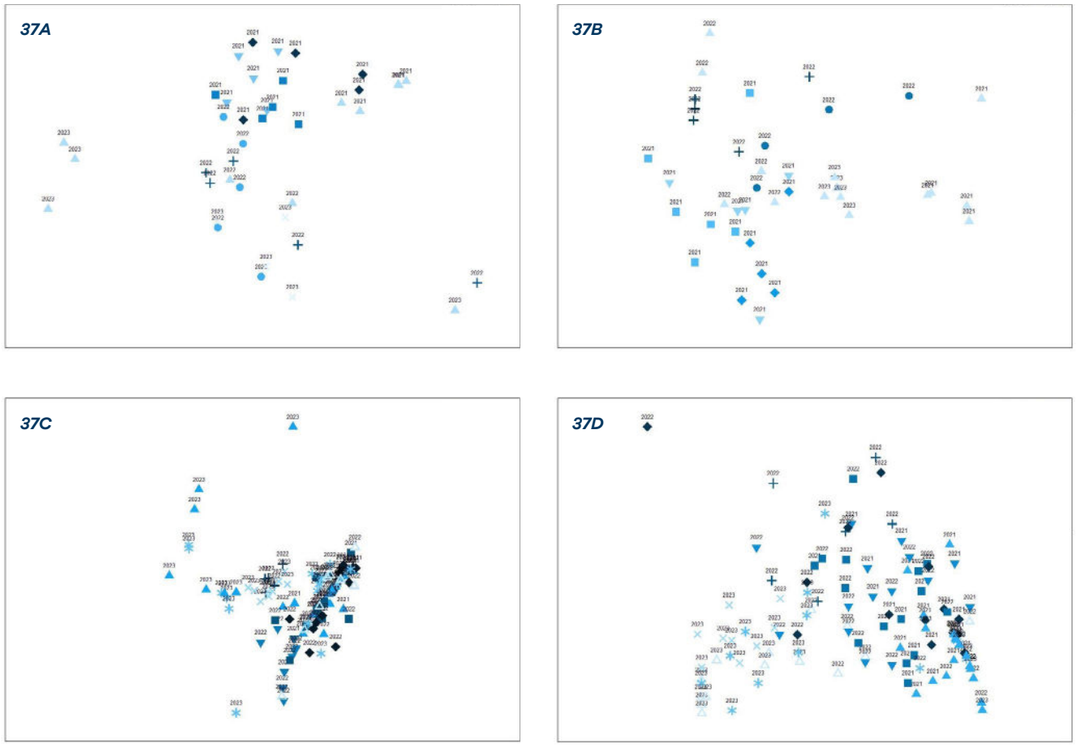


Figure 36, *nMDS plot, using Bray-Curtis dissimilarity matrix showing the sessile community composition differences between all marina sites site across all years of survey data (2021–2023) Stress = 0.083.*

Community composition

Sessile community composition within each marina showed some variation between season and year, which in most cases was significant (Port of Blyth, Sunderland Marina, Conwy Marina and Deganwy Marina; against Year = <0.001, against Season <0.001). The nMDS plots (Figures 37A-D) for each marina shows a level of dissimilarity between seasons and years in all sites. However, most marinas show no apparent trend in seasonality changes in community composition, and differences are likely to be natural variation. The exception to this is Deganwy Marina where there appears to be a difference in community composition in winter/early spring (months 1–3). However, not all marinas were sampled in January so this should be interpreted with caution. SIMPER testing showed that Acorn barnacles and *Spirobranchus* spp. were highly abundant and in all months were driving similarity between months tested (Table D, Appendix 2).



Figures 37A-D, *nMDS plots (followed by PERMANOVA) testing all sites using the response variables of Year (2021-2023) and Season (months: January, February, March, May, June, August, September, October, November) and an interaction effect (Year x Season) between the two responses.*

Univariate testing

Seasonal variation in species richness and relative abundance of oyster shell epibiota were compared within each marina and presented in Figures 38 and 39. Species richness was highest in November for both the Port of Blyth (5 ± 0.72 species per shell) and Sunderland Marina (3.8 ± 0.72 species per shell) whereas species richness was highest in July for Deganwy Marina (4.06 ± 0.41 species per shell) and September for Conwy Marina (3.2 ± 0.44 species per shell). Species richness was significantly different between seasons, and this was consistent across sites (PERMANOVA, Season x Site, p = 0.001). In general, Port of Blyth and Sunderland Marina (Tyne and Wear restoration hub) showed an increase in species richness throughout the year, while Conwy and Deganwy marinas were more variable between seasons with no clear pattern shown. Conwy Marina had the lowest species richness of all marina sites, irrespective of season.

For relative abundance, similar patterns were observed, with the highest abundances of sessile taxa recorded in November for Sunderland Marina (264 individuals per m^2 ± 35.4) and the Port of Blyth (233 individuals per m^2 ± 35.4). An increasing trend in relative abundance for the sites in Tyne and Wear restoration hub was seen throughout the year. The Conwy and Deganwy marinas showed no clear trends in abundance. Relative abundance was significantly different between seasons, and this was consistent between sites (PERMANOVA, Season x Site, p = 0.001). Full PERMANOVA results given in Table E, Appendix 2.

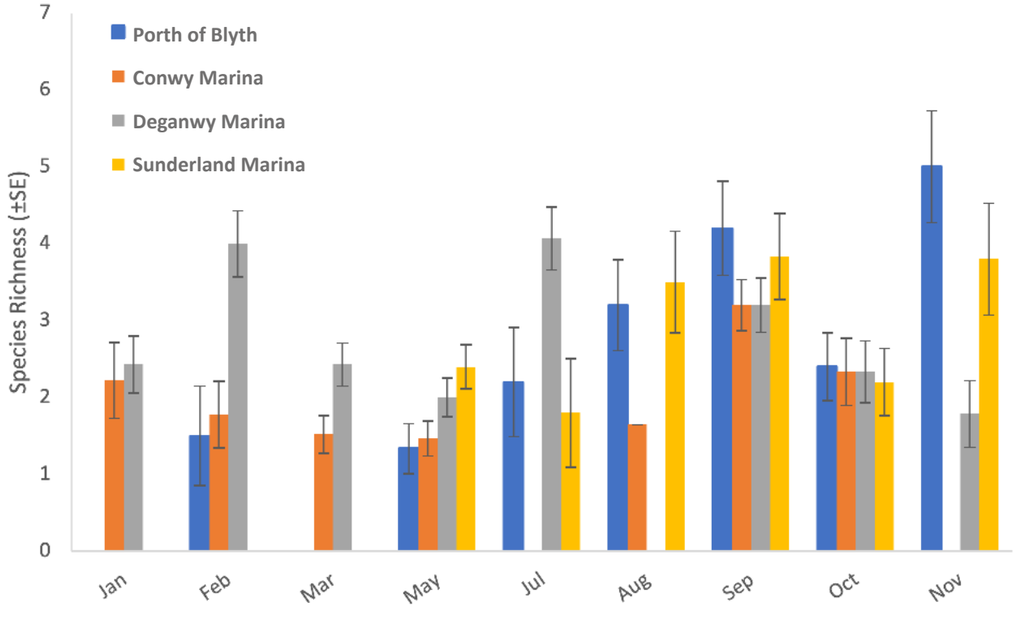


Figure 38, *Average oyster shell epibiota species richness across the year for all marinas (data combined from all years of monitoring: 2021, 2022 and 2023).*

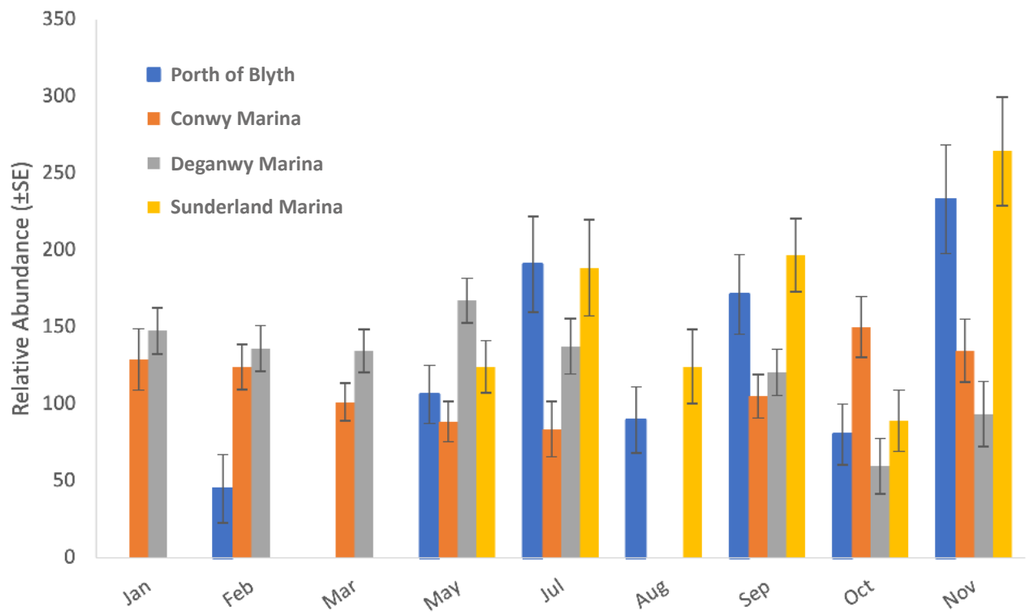


Figure 39, *Average oyster shell epibiota species abundance across the year for all marinas (data combined from all years of monitoring: 2021, 2022 and 2023).*

Species richness:

Table 5, *PERMANOVA examining oyster shell epibiota species richness against year, season and site, singularly and combined.*

PERMANOVA table of results						
Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Unique perms**
Year	2	4.7086	2.3543	2.2561	0.1061	9961
Season	8	75.572	9.4465	9.0523	**0.0001**	9951
Site	3	57.902	19.301	18.495	**0.0001**	9958
Year x Season	5	26.497	5.2995	5.0783	**0.0005**	9946
Year x Site	6	32.58	5.43	5.2034	**0.0001**	9948
SexSi	17	119.87	7.0513	6.757	**0.0001**	9918
Year x Season x Site	4	8.0674	2.0169	1.9327	0.1154	9955
Res	254	265.06	1.0435	-	-	-
Total	299	561.59	-	-	-	-

Abundance:

Table 6, *PERMANOVA examining oyster shell epibiota relative abundance against year, season and site, singularly and combined.*

PERMANOVA table of results						
Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Unique perms**
Year	2	64189	32094	3.3129	0.1467	9963
Season	8	59644	7455.5	0.75586	0.627	9959
Site	3	95176	31725	17.218	**0.0001**	9950
Year x Season	5	19790	3958.1	0.832	0.5956	9961
Year x Site	6	1.18E+05	19634	10.656	**0.0001**	9959
Season x Site	17	1.89E+05	11111	6.0299	**0.0001**	9909
Year x Season x Site	4	19282	4820.5	2.6162	0.0389	9942
Res	254	4.68E+05	1842.6	-	-	-
Total	299	1.19E+06	-	-	-	-

Dorsal vs Ventral shell colonisation:

During monitoring of the nurseries, it was noted anecdotally that there appeared to be a difference in the number of organisms on the dorsal and ventral surfaces of the oysters. This was tested using a 2 Sample T-test. The dorsal side of the shells housed a greater abundance (Figure 40) and richness (Figure 41) of epifauna than the ventral shells. Dorsal shells were occupied by an average of 5527 individuals per m^2 (+/- 5995 SD), compared to 2829 individuals per m^2 (+/- 3895 SD) on ventral shells, a statistically significant difference ($t_{(745)}$ = 7.84, p = <0.005). The taxonomic richness was also statistically significantly higher on dorsal (mean = 1.69 +/- 1.04 SD) than ventral sides (mean = 1.33 +/- 0.97 SD) ($t_{(855)}$ = 5.28, p = <0.005). Most taxa appeared to prefer the dorsal shell surface (91 per cent) (Figure 42). Some taxa appear to show the opposite preference, including records of 'white encrusting Bryozoan' and 'red faunal crust'. However, these taxa were too rare to assess (red faunal crust) or are not statistically significant differences (white encrusting Bryozoan) ($t_{(808)}$ = -0.63, p = >0.5).

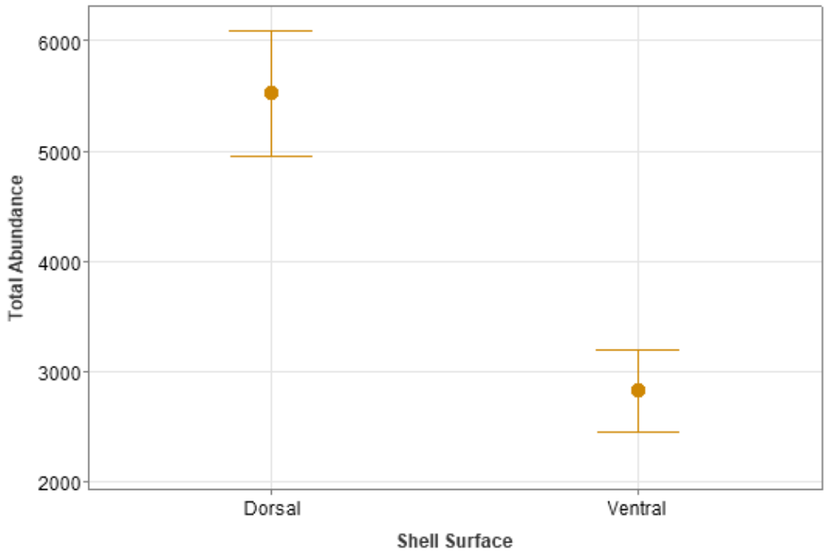


Figure 40, *Mean total abundance +/- 95 per cent confidence interval (calculated using individual standard deviations) of organisms on the dorsal and ventral surfaces of oysters across all years and all marinas, monitored during sessile biota analysis, n = 862 images.*

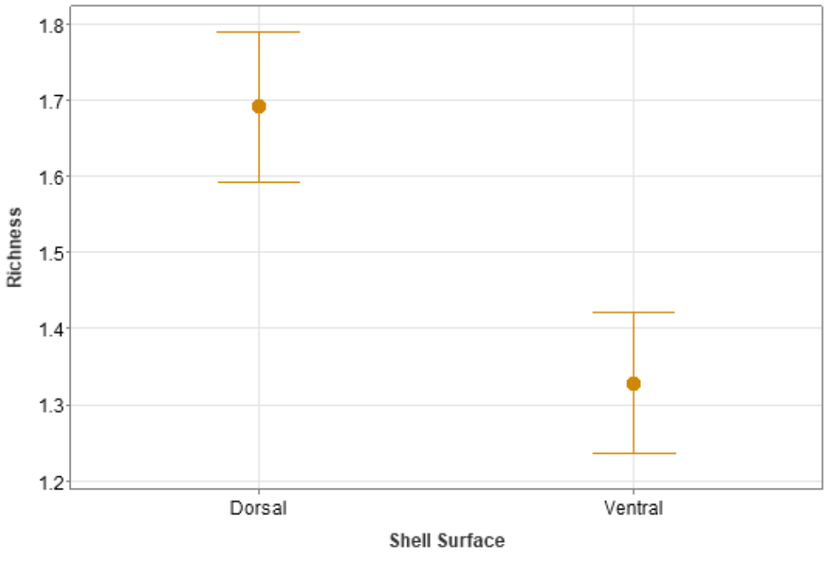


Figure 41, *Mean taxonomic richness +/- 95 per cent confidence interval (calculated using individual standard deviations) of organisms on the dorsal and ventral surfaces of oysters across all years and all marinas, monitored during sessile biota analysis, n = 862 images.* © Ashleigh Tinlin-Mackenzie

Taxa level detail:

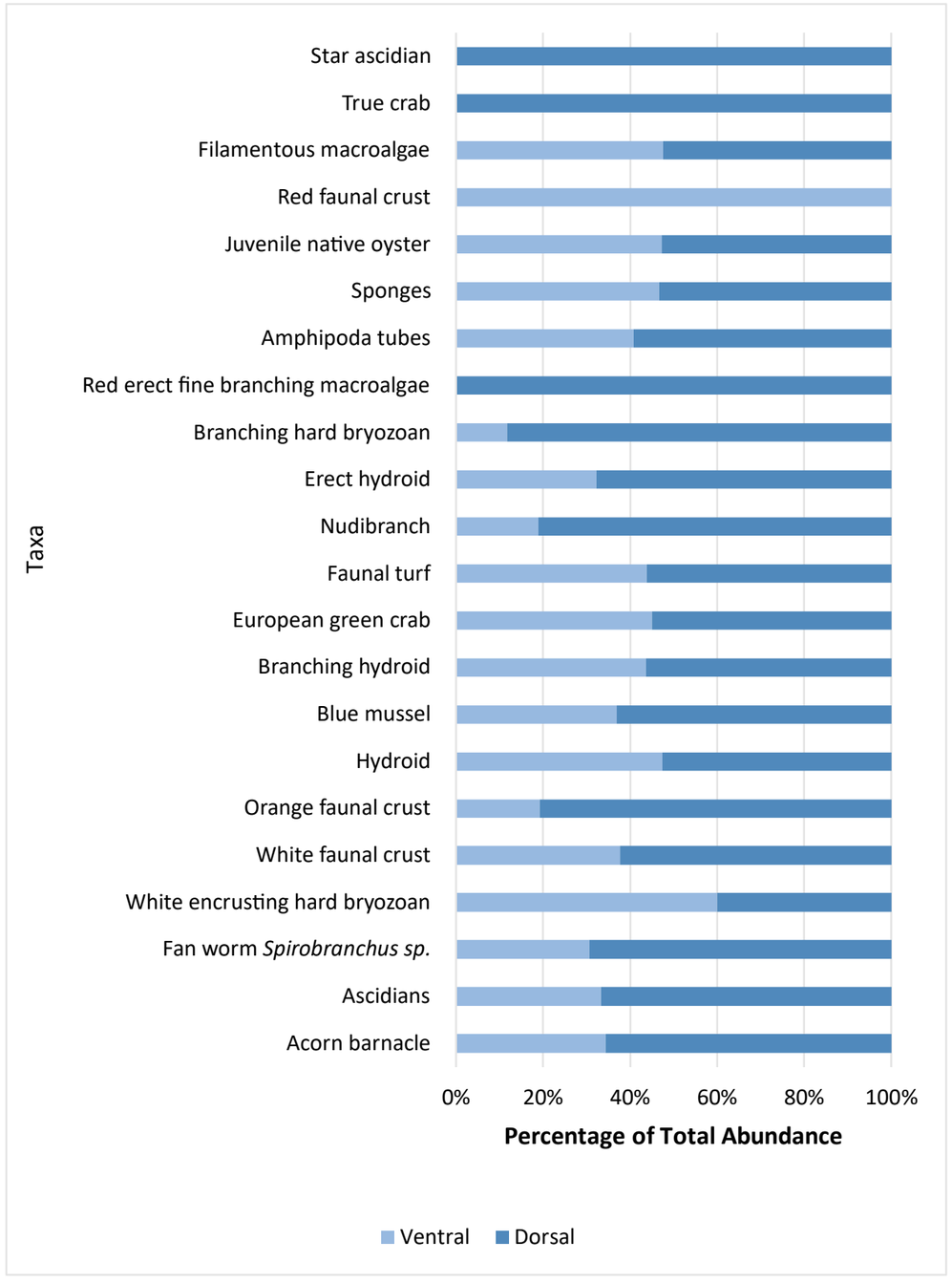


Figure 42, *Percentage of the total abundance of each taxa observed living on the oyster shells occupying either the dorsal or ventral shell surface salini(n=862).*

4. DISCUSSION

The success and long-term viability of the use of oyster nurseries for restoration purposes can be assessed by reviewing oyster survival, growth and reproductive success.

4.1 Survival, Growth and Filtration

Overall, the survival rates of the oysters in nurseries were high. This resulted in consistently high numbers of oysters in the nurseries, meaning significant volumes of water were filtered at all marinas throughout the project. It is estimated that over ~139 million litres of water have been filtered across the four nursery sites since March 2021. This marks a substantial ecosystem service provided by the nurseries.

Survival was initially poor at the Sunderland Marina and Port of Blyth site. Both locations experienced a similar trend with mortality initially high between April 2021 and June 2021, peaking in April 2021 with 142 mortalities in Sunderland Marina and 81 in the Port of Blyth. This level of mortality is not standard in native oyster nurseries (Woods, F. currently unpublished results). Following discussions with the supplier, it was suggested that some individuals within this batch of oysters may have been in poor condition. In addition, the oysters were transported during a spring heatwave, potentially explaining this trend in mortality.

This hypothesis was supported by the higher survival rates and no significant mortality spikes following subsequent restocking events that took place (Figure 14) (Restocking events: October 2021 in the Port of Blyth and Sunderland Marina; May 2023 in Sunderland Marina; April 2022 in Conwy and Deganwy marinas; and April 2023 in Conwy and Deganwy marinas).

Observed mortality similar trend across both restoration hubs, with mortality peaking in July each year, coinciding with spawning events (Figures 23, 24, 25) and the increase in water temperature which triggered spawning (Figures 26 & 27). These spikes were likely a factor of the physiological stress of increased water temperatures (Rybovich *et al.,* 2016; Samain *et al.,* 2007) and post-spawning energy expenditure (Zorita *et al.,* 2021; Eymann *et al.,* 2020). For the remaining months of each year, the mortality rate was low at both restoration hubs (on average less than 15 per month from September to May at each hub and less than 5 per month at each hub from October to May).

The low mortality rates in the marinas were an encouraging sign for wider restoration efforts in both hubs. It is an early indication that the conditions in both marina sites were suitable for oyster nurseries, and potentially therefore the wider marine environment will also be suitable for deployment of native oysters. The correlation between growth of oysters and time in the oyster nurseries (Figure 13) is further indication that marina conditions were suitable for the oysters.

Photo: *Scallop tray from inside an oyster nursery at Sunderland Marina. © Celine Gamble, ZSL*

4.2 Spawning

Monitoring spawning using a non-destructive method was a time consuming and labour-intensive activity. However, it was essential to understanding and quantifying the role of the nurseries as a larval pump. The effect of temperature is known to impact reproduction directly by determining the start and end of the breeding period and gametogenic progression. Oysters coming into condition for larval development is dependent upon a thermal constant commonly referred to as 'Degree Days' (described in Wilson & Simons, 1985). The effect of temperature also has a secondary impact on spawning potential of native oysters by regulating sex ratio (Eagling *et al.*, 2017; Joyce, *et al.*, 2013). Native oysters are sequential hermaphrodites (Orton, 1937), which first reproduce as a male on reaching sexual maturity and thereafter alternate between sexes. Eagling *et al.*, (2017) identified a positive correlation with male phase oysters and temperature. Sex ratio was not examined as part of this study but could have been a factor that contributed to the differences seen in brooding numbers between restoration hubs.

The dominant feature of spawning monitoring results is the relationship between oyster brooding larvae and temperature. Larval brooding only took place once the temperature reached approximately 15°C. This was seen in both restoration hubs and was an expected result, as temperature is known to have a major effect on reproduction of native oysters (Maneiro *et al.*, 2016; Cano *et al.*, 1997). The relationship between temperature and spawning resulted in spawning occurring later in the year and over a shorter timeframe in the Tyne and Wear restoration hub than in the Conwy Bay restoration hub.

Results also indicated a difference in the percentage of oysters brooding larvae at any one time between restoration hubs. In 2022, the Conwy Marina oysters were brooding for a period spanning over twelve weeks. However, the Sunderland Marina oysters were brooding for only three weeks. In that shorter window of time, 50 per cent of oysters sampled were recorded as brooding, compared to a peak of approximately 30 per cent in Conwy Marina.

This difference could have been due to environmental factors such as food availability, or the colder temperature in Sunderland Marina resulting in oysters putting more energy into shell growth than spawning. It could also have been a result of the shortened brooding period concentrating brooding activity.

Photo: Volunteers monitoring during nursery monitoring work at Conwy Marina © Luke Helmer

4.3 Diversity

4.3.1 Mobile Species Community Variation

The mobile diversity supported by the oyster nurseries was one of the key monitoring outputs of the project. One of the primary ecosystem services provided by native oysters is their habitat-forming ability and the subsequent increase in biodiversity. Understanding which species are found inside the oyster nurseries gives an insight into the species that could be found on the restored native oyster reefs in the restoration hubs. We acknowledge that without a control of an empty nursery in the marinas, it cannot be stated definitively that any species found within the nurseries are due to the presence of the oysters. However, (Woods, F. currently unpublished) studies from the Solent have shown that it is the presence of living native oysters in the nurseries (as opposed to dead oyster shell or empty cages) that cause a significant difference in the species abundance and richness. We can therefore infer a similar impact in this case.

A total of 29,248 (Conwy Bay restoration hub) and 14,862 (Tyne and Wear restoration hub) individual mobile organisms have been counted in the biodiversity nurseries (biodiversity nurseries make up 20 per cent of cages in Sunderland Marina. 33 per cent in the Port of Blyth, and 25 per cent in the Conwy Bay restoration hub sites) from the inception date at each hub.

To date, 67 species have been identified to species level and approximately 86 to higher taxonomic levels (order, family or genus level). The presence of such diversity and abundance of organisms is important, particularly as many of those present (such as small crustaceans) are known to be primary or secondary consumers and provide a link between lower and higher trophic levels (Arfianti & Costello, 2020; Stål *et al.*, 2007). This highlights the oyster's role in providing refuge for many prey species at the base of the food web that in turn support predatory (more mobile) species higher up the food web.

The presence of such diversity and abundance of organisms is important.

The most abundant organisms in the native oyster nurseries were Crustacea, particularly Amphipoda and *Palaemon* spp. which accounted for 34.5 per cent and 36.5 per cent of all organisms found, respectively. This distribution was not similar across all sites. In Conwy Bay restoration hub, the most dominant species was *Palaemon* spp. in Deganwy Marina, which was recorded as the most abundant species on 80 per cent of the recorded monitoring sessions. In Conwy Marina, *Palaemon* spp. occurred as the dominant, most abundant species in 52 per cent of monitoring sessions, with Amphipoda making up a further 39 per cent. In the Tyne and Wear restoration hub, *Palaemon* spp. did not feature as a dominant species in any of the 27 monitoring sessions in Sunderland Marina. Rather, this site was dominated by Amphipoda which occurred as the species with the greatest abundance on 14 sessions. Similarly, in the Port of Blyth site, Amphipoda was the most abundant species on 11 of the 25 sessions.

This difference in dominant species was a large cause of the statistically significant difference in community composition between marina sites (ANOSIM and Multilevel Pattern Analysis), with several species being associated with particular marinas or restoration hubs. The difference in species composition across marina sites is clearly observed. There is variation between marinas with regards to the species present, the spikes in species abundance and seasonal trends.

In all marinas, the presence of any Amphipoda occurs in highest numbers in the spring, but is otherwise in very low numbers or not present in other months. Amphipoda contributed to the majority of the total abundance for all marina sites when the cages were first installed in 2021. This is because Amphipoda are one of the most ubiquitous and abundant invertebrate groups in marine habitats (Vázquez-Luis *et al.*, 2008) and have a broad capability to colonise new habitat (Vázquez-Luis *et al.,* 2012). Following this, other highly mobile species began to move into the nurseries, mainly Crustacea, namely Decapoda, Isopoda, *Palaemon* spp. but also various fishes, likely to feed on the initial colonisation species (Stål *et al*., 2007) and to use the nurseries as a shelter (Patranella *et al*., 2017; Bradley *et al.,* 2019).

Fish recorded in the nurseries include; blenny (*Blennioidei*), butterfish (*Pholis gunnellus*), common goby (*Pomatoschistus microps*), corkwing wrasse (*Symphodus melops*), fifteen-spined stickleback (*Spinachia spinachia*), five bearded rockling (*Ciliata mustela*), lumpsucker (*Cyclopteridae*), pollock (*Pollachius*), rock cook wrasse (*Centrolabrus exoletus*), rock goby (*Gobius paganellus*), rockpool blenny (*Parablennius parvicornis*), shanny (*Lipophrys pholis*), shore rockling (*Gaidropsarus mediterraneus*), worm pipefish (*Nerophis lumbriciformis*), longspined bullhead (*Taurulus bubalis*) and Critically Endangered European eels (*Anguilla anguilla*) again highlighting the importance of the nursery structure for highly mobile species.

Corkwing wrasse, Symphodus melops © Maria Hayden-Hughes

Five-bearded rockling, *Ciliata mustela* © Maria Hayden-Hughes

Shrimp, *Palaemon spp* © Georgie Bull

Mysid shrimp, *Praunus flexuosus* © Maria Hayden-Hughes

Photo: Organisms found during native oyster nursery biodiversity monitoring

Once the nurseries were established, patterns in dominant species for each marina began to form and distinction could be made between the communities. The difference in the mobile community groups is particularly distinct between the two restoration hubs. This is likely due to environmental and physical differences between the marina sites. For example, Conwy and Deganwy marinas contain a large amount of muddy substrate, and the marinas are boarded by large rocks rather than solid walls, providing a highly complex, three-dimensional surrounding. This is more suitable for species such as *Palaemon* spp. than the solid wall sides of Sunderland Marina (Figure 43A and 43B).

There is also likely a difference in species presence between the sites due to the difference in species that occur widely at the two restoration hubs. For example, hooded shrimp (*Athanas nitescens*) was recorded in the Conwy Bay restoration hub but not in the Tyne and Wear restoration hub. Hooded shrimps are widely recorded on the south and west coasts of Britain, but has only been recorded once in the North East (Rowley, 2008). Likewise, the Japanese skeleton shrimp (*Caprella spp*) was recorded in the Conwy Bay restoration hub but is not common on the east coast of England and was not found in the Tyne and Wear restoration hub sites (Oakley, 2006). Conversely, there were species recorded in the Tyne and Wear restoration hub but not the Conwy Bay restoration hub. These included the Bristly crab (*Pilumnus hirtellus*), a species found on all British coasts but mostly frequent on the south and east coasts (Skewes, 2008).

Figure 43A and 43B, *Conwy Marina showing the rock surrounding rather than a solid marina wall* © Conwy Marina

4.3.2 Mobile Species Community evolution over time

There was no significant difference between the mobile community groups across marinas based on the year that monitoring took place. However, at the time of analysis, the first and third year of sampling did not contain data for the complete year, therefore do not give a full representation of a change in community across a whole year. The non-significance is likely to come from the seasonal variation that has already been highlighted above outweighing any difference seen due to long term change in community. There is some suggestion of a change over time in the nMDS plot (Figure 33) by the beginning of separation of the 2023 years in the higher parts of the plot, but a complete dataset over several more years of monitoring would be required to determine this.

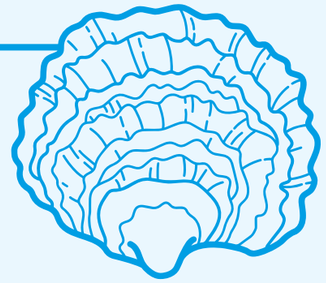
4.3.3 Impact of environmental parameters on mobile faunal

There were some apparent correlations between environmental parameters and mobile fauna, but only sea state had a significant effect on the abundance of mobile data. Anecdotally, a difference in the number of mobile species had been noted based on whether the monitoring session was taking place at high tide or low tide. We hypothesise that there is a lower abundance of species in the marinas at low tide, and that species move in and out of marinas with the tide. Tide-based cyclic patterns have been observed in species common in the nurseries, particularly the dominant species of Amphipoda (De Backer *et al.,* 2010) and *Palaemon* spp. (Rodriguez & Naylor, 1972). There is a tidal sill at Conwy Marina only. There are marina gates on both marinas which are lowered and raised 3 hours either side of low water. This could have the effect of trapping animals in the marina and boosting numbers over low tide, while also preventing others from coming back into the marina relative to the two sites in the Tyne and Wear restoration hub. The impact of environmental variables was further investigated using BEST analysis, but this indicated no correlation between the biological and environmental data existed for either species abundance or richness.

It was surprising that there was no impact of environmental parameters on species abundance or richness. This was particularly the case of temperature, given the Shannon's diversity index showed a substantial change in diversity over the course of each year, with clear spikes in spring. We attributed this to the substantial range and inconsistency in the seasonal diversity between years. Additional data may help to level out some of these inconsistencies. However, there are many other factors that could explain the variation and abundance of species throughout the year, such as food availability, lifecycles of dominant species (Dauvin, 1989) or migration of species (Emmerson *et al.*, 2017; Grenfell, 2013).

There was no significant pattern on community composition based on these environmental factors. Figure 35 does show two distinct groupings within the complete dataset, a large amount of which appear correlated with tidal state, despite this not being a significant result. This grouping is likely a result of the aforementioned significance of the relationship between tidal state and abundance. The environmental parameters were also tested in combination against the community composition, but again, no significant results were found.

The impact of environmental variables was further investigated using BEST analysis, but this indicated no correlation between the biological and environmental data existed for either species abundance or richness.



4.3.4 Oyster Shell Epibiota

There was evidence of seasonal variation in the sessile communities on oyster shells throughout the duration of this study. It is clear across all marinas that composition changes throughout year, and this occurred in all years. However, there was no real trend detected for this. While seasonal variation was high, this differed within and between years and the differences observed are just a factor of a highly variable system that responds to environmental cues. The differences were probably caused by the contribution of a few species such as Acorn barnacles and *Spirobranchus spp.,* rather than a significant shift in community composition. These two species were contributing greatest to community composition (sometimes around 75 per cent of total composition) and so fluctuations in the abundances of these two species could explain the differences observed. Patterns were not repeatable across years. The abundance and species richness did show an increasing trend for the Port of Blyth and Sunderland Marina throughout the year and this pattern seemed to be consistent. This is expected, as increases in both the abundance and species richness of sessile species is likely influenced by warming water throughout the year, with autumn and winter months showing highest for both these response variables. This is related to summer spawning events for many of the sessile species observed (White, 2008; Gibson-Hall, 2018). Furthermore, warmer waters increase settlement rates, growth and survival of sessile species before a slowing, or even die-off of established and planktonic biomass in late-winter and spring months. A similar pattern was not observed in the two other marinas in the Conwy Bay restoration hub.

Photo: Native oyster, *Ostrea edulis*, © Celine Gamble, ZSL

CONCLUSION

This report provides a case study of the use of native oyster nurseries in the Wild Oysters Project and how native oyster nurseries played a significant role in the restoration efforts of the project and in the project's outreach and engagement work. Monitoring of the native oyster nurseries found that the growth survival and reproduction of oysters was good across both restoration hubs. Observations of note included a clear correlation between growth and time and mortality spikes that coincided with increased water temperatures and spawning activity.

Mobile diversity was high in the oyster nurseries, with spikes in the Conwy Bay restoration hub sites in July each year, and in late summer in the Tyne and Wear restoration hub. There was a significant dissimilarity in community composition between the two restoration hubs, largely caused by species that were only present in a single restoration hub, and the difference in dominant species. For example, *Palaemon* spp. featured heavily in the Conwy Bay restoration hub, while Amphipoda was the dominant taxa at the Tyne and Wear restoration hub. There were also species, that although not dominant in each restoration hub, were significantly associated with an independent restoration hub. This means that although they may have been present in both restoration hubs, the distribution of these species was heavily weighted towards a particular restoration hub. It was shown that environmental parameters had a limited impact on mobile diversity, and only the state of the tide was found to have a significant effect.

Finally, the oyster shell epibiota community composition showed a significant difference within each site, when accounting for season and year of monitoring. However, there was no overall trend in seasonal changes in community composition. Differences are believed to be natural variation. Acorn barnacles (*Balanus glandula*) and *Spirobranchus* spp. were highly abundant and were driving similarity between months tested. The dorsal and ventral sides of the shells were compared for differences in relative abundance. The dorsal side supported a greater abundance and richness of epifauna than the ventral side. The oyster shell epibiota community composition showed a significant difference within each site, when accounting for season and year of monitoring. However, there was no overall trend in seasonal changes in community composition for most marinas.

Native oyster nurseries are an effective method in recruitment-limited restoration sites to increase larval supply and support associated species assemblages. Although a significant number of oyster nurseries would be required to provide a larval supply comparable to a healthy native oyster reef. Additional native oyster habitat restoration is recommended to provide suitable substrate to encourage larval settlement. Although the accessibility of the oyster nurseries facilitates regular scientific monitoring providing useful indictors, such as a better understanding of larval production and oyster survival, to inform native oyster habitat restoration efforts within the area. The nurseries also act as a practical engagement tool for citizen science and educational activities, increasing ocean literacy and promoting marine stewardship for long-term protection and further marine restoration initiatives to achieve UK targets of restoring 30 per cent of our land and sea by 2030.

Photo: Native oyster nurseries ©ZSL

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Photo: Peacock Fan Worms on oyster nursery © Georgie Bull, ZSL

APPENDIX 1.

Sampling Methodologies

Magnesium chloride ($MgCl_2$) Oyster Sampling Protocol

The protocol followed during spawning monitoring was:

1. 40 oysters were randomly removed from nurseries that were not subject to biodiversity monitoring (see Section 2.5) (nurseries were cycled to avoid anesthetising oysters on consecutive weeks and spawning oysters marked to avoid repeat sampling) to be monitored for spawning. From the 40 oysters, the aim was for a sample size of 20 oysters to open to analyse. If this number could not be reached, sufficient oysters would be opened (shucked) in order to fulfil the quota of 20. However, in almost all cases, at least 20 oysters opened, and shucking was not necessary.
2. Both valves of the oyster were thoroughly cleaned with a wire brush and rinsed to remove as much sediment and as many soft organisms as physically possible.
3. Each oyster was placed into a container of $MgCl_2$ solution. Only one oyster was placed in each container to avoid risk of cross-contamination of larvae samples. Magnesium chloride reacts exothermically with water therefore the solution was made up the day before monitoring took place and allowed to cool before use. It was important to keep this solution cool prior to, and during, the monitoring process. The containers were also stored under shade or in cool boxes while the $MgCl_2$ took effect. The salinity of the solution was monitored using a salinometer to ensure the solution was the same as measured in the nurseries.

Note: $MgCl_2$ solution was created with the following ratios:

1L $MgCl_2$ solution at 5 per cent conc. = 500ml seawater + 500ml fresh water + 50g $MgCl_2$

To sedate 40 oysters 20l $MgCl_2$ solution was used.

4. Once in the solution, the oysters took up to three hours to become fully anesthetised, and the valves open. During this time, the containers were stored under shade to keep the oysters cool and out of the way of other marina users.
5. For successfully anaesthetised oysters, the following steps were taken:
   a. Once open, visual checks of gonad stage were conducted to identify the stage of development, clearly differentiated by colour and referred to as white, grey or black sick (Figure 10).
   b. Oysters were gently rinsed in the $MgCl_2$ solution within their individual containers and observed for pallial fluid containing larvae washing out of the oyster into the container. Any oysters that were not spawning were returned to the nurseries. Observations were noted in the data sheet.
   c. After larvae was rinsed from the oyster into the container, the container was carefully emptied over a 40-micron mesh sieve to collect the larvae from the spawning oyster.
6. Seawater in a squeeze bottle was used to wash any remaining larvae from the sieve and the entirety of the larvae sample was collected into a labelled 30ml universal container:

Note: The equipment was rinsed between samples, to ensure no cross-contamination. The waste $MgCl_2$ was collected into a bucket to dispose of through municipal water treatment works with ample amounts of fresh water. No $MgCl_2$ solution was disposed of into the marine environment.

7. The length, width and depth of the spawning oyster was measured and recorded.
8. The spawning oysters were revived by immersion in seawater before being returned to the oyster nursery. Where oysters did not self-close, when putting the oyster back into the nursery, a rubber band was attached, tight enough to keep the two valves together but not to force the shell shut to allow the oyster to feed after recovery. The rubber bands were removed during the next sampling at the pontoon.
9. If less than 20 oysters opened, the remaining number of oysters were shucked and sampled to secure a sample size of 20 oysters for observations (shucking was largely not required if enough time was given for the oysters to open in the MgCl2).

Larval Density Calculation Protocol

Oyster larval density was estimated using the following protocol:

1. Fix larval samples by adding 0.05ml of concentrated Lugol's Iodine directly to the 30ml larvae sample container.
2. Agitate the larval sample to ensure suspension of all collected material, before pouring into a 50ml measuring cylinder. Record the total volume of the larval sample.
3. Place 1L of filtered seawater into beaker
4. Using a plunger or stirring rod, gently agitate the larval sample in the measuring cylinder, before extracting a 1ml sub-sample using a variable pipette.
5. Pipette 1ml of larvae and place into beaker with water and stir well.
6. Using the plunger or stirring rod, continuously agitate the larvae solution while drawing up 1ml of solution into a pipette.
7. Place the 1ml sub-sample on a Sedgewick Rafter counting slide and gently apply a cover slip.
8. Using a Leica DM1000 compound microscope and digital image capture system, count the larvae in each of the 5 x 1ml sub-samples, using a click counter to keep track. Separate counts into live, dead and shell (empty) (Figure 11).
9. Take digital images of the first 10 bivalve larvae counted (Figure 12). When taking digital images, ensure that a complete grid square is present in at least one image, and all images are taken at the same magnification (make a note of the magnification). Use the attached monitor to help guarantee a clear image is captured.
10. If processing several sub-samples, replicates or a number of different sites, take blank photos to delineate between them (1 blank photo = different subsample, 2 blanks = different oyster sample, 3 blanks = different site). Record the order the photos are taken in along with their corresponding sample.
11. Repeat the process for each replicate and each collection site, recording the number of larvae and the number of photos taken for each sub-sample.
12. Use the following formula to calculate the total number of larvae in the entire sample. The multiplication factor is the number of times the sub-sample volume needs to be multiplied to reach 1ml volume.
13. $$\text{Total number of larvae} = \left[\frac{\text{Sum of larvae}}{\text{5 subsamples}} \times 1000\right] \times \text{Total volume}$$
14. Calculate percent survival using the following formula

$$\text{Survival rate (\%)} = \frac{\text{Number of dead}}{\text{Total number of larvae}} \times 100$$

APPENDIX 2.

Single most dominant (abundant) Species/Taxa at each marina per month (>10)

Date	Port of Blyth	Conwy Marina	Deganwy Marina	Sunderland Marina
Mar-21	Mysida	*Gammarus duebeni*	-	*Gasterosteus aculeatus*
Apr-21	Amphipoda	-	-	Amphipoda
May-21	Amphipoda	Amphipoda	Amphipoda	Amphipoda
Jun-21	Amphipoda	Amphipoda	Amphipoda	Amphipoda
Jul-21	Amphipoda	Amphipoda	Amphipoda	Amphipoda
Aug-21	*Mytilus edulis*	*Palaemon* spp.	*Palaemon* spp.	Amphipoda
Sep-21	*Palaemon* spp.	*Palaemon* spp.	*Palaemon* spp.	Decapoda
Oct-21	Mysida	*Palaemon* spp.	*Palaemon* spp.	Amphipoda
Nov-21	Decapoda	*Palaemon* spp.	*Palaemon* spp.	Amphipoda
Dec-21	-	Amphipoda	*Palaemon* spp.	-
Jan-22	Amphipoda	Amphipoda	*Palaemon* spp.	Amphipoda
Feb-22	Isopoda	Amphipoda	*Palaemon* spp.	Isopoda
Mar-22	Amphipoda	Amphipoda	*Palaemon* spp.	Isopoda
Apr-22	Amphipoda	Amphipoda	*Palaemon* spp.	Isopoda
May-22	Isopoda	Amphipoda	Amphipoda	Isopoda
Jun-22	Mytilus edulis	Amphipoda	*Carcinus maenas*	Isopoda
Jul-22	Decapoda	*Carcinus maenas*	*Palaemon* spp.	Isopoda
Aug-22	Decapoda	*Palaemon* spp.	*Palaemon* spp.	Amphipoda
Sep-22	*Palaemon* spp.	*Palaemon* spp.	*Palaemon* spp.	Decapoda
Oct-22	*Crangon crangon*	*Palaemon* spp.	*Palaemon* spp.	Decapoda
Nov-22	Amphipoda	*Palaemon* spp.	*Palaemon* spp.	Amphipoda
Dec-22	-	Amphipoda	*Palaemon* spp.	-
Jan-23	-	*Palaemon* spp.	*Palaemon* spp.	-
Feb-23	Isopoda	*Palaemon* spp.	*Palaemon* spp.	*Corophium volutator*
Mar-23	Amphipoda	*Palaemon* spp.	*Palaemon* spp.	*Corophium volutator*
Apr-23	Amphipoda	-	-	*Corophium volutator*
May-23	Amphipoda	Amphipoda	*Palaemon* spp.	Amphipoda
Jun-23	-	Decapoda	-	Amphipoda
Jul-23	-	*Palaemon* spp.	-	Amphipoda
Aug-23	-	*Palaemon* spp.	-	-
Sep-23	-	*Palaemon* spp.	-	-
Oct-23	-	*Palaemon* spp.	-	-
Nov-23	Decapoda	*Palaemon* spp.	*Palaemon* spp.	Amphipodaw

Table A, *Single highest occurring species or taxa in each monthly monitoring session. Species were only included if they occur more than ten times in a session, blanks denote no monitoring session or not species occurred more than ten times.*

Envfit test of environmental parameters

VECTORS	NMDS1	NMDS2	r2	Pr(>r)
Date	0.99731	-0.07327	0.8183	0.33333
Temperature	-0.45765	-0.88913	0.8159	0.50000
Sea State	0.72045	0.69351	0.9996	**0.04167**
Precipitation	0.46201	0.88687	0.3168	0.79167
Wind Speed	0.91566	0.40195	0.9886	0.12500
Wind Gusts	0.57272	0.81975	0.9947	0.12500
Visibility	0.99687	0.07904	0.4766	0.75000
Humidity	-0.79856	0.60191	0.8931	0.41667
Salinity	-0.69632	-0.71773	0.1150	0.87500

Permutation:	free			
Number of permutations:	23			

Table B, *envfit test examining the significance of environmental parameters on total species abundance in the native oyster nurseries across both restoration hubs. Only sea state showed any significant effect.*

Eigenvalues			
PC	**Eigenvalues**	**%Variation**	**Cum.%Variation**
1	1.15	38.3	38.3
2	0.996	33.2	71.5
3	0.854	28.5	100.0
Eigenvectors (Coefficients in the linear combinations of variables making up PC's)			
Variable	**PC1**	**PC2**	**PC3**
Salinity (ppt)	-0.702	-0.039	0.711
Air Temperature (celcius)	-0.677	-0.272	-0.684
Tide state	0.220	-0.962	0.164

Table C, *envfit test examining the significance of environmental parameters on total species abundance in the native oyster nurseries across both restoration hubs. Only sea state showed any significant effect.*

SIMPER testing

Examines Month groups (across all Year groups)					
Group 5 (May)					
Average similarity: 74.54					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Acorn Barnacle	147.17	71.03	3.51	95.28	95.28
Group 7 (July)					
Average similarity: 57.22					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Acorn Barnacle	64.72	36.41	2.58	63.63	63.63
Spirobranchus sp.	24.48	9.82	1.01	17.17	80.8
Ascidians	8.21	3.05	0.46	5.32	86.12
Amphipoda Tubes	10.03	2.78	0.48	4.86	90.98
Group 9 (September)					
Average similarity: 60.43					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Acorn Barnacle	70.65	45.94	3.48	76.02	76.02
Spirobranchus sp.	19.34	7.82	0.88	12.95	88.96
Ascidians	6.08	3.16	0.47	5.23	94.2
Group 11 (November)					
Average similarity: 65.81					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Acorn Barnacle	75.06	62.2	2.28	94.52	94.52
Group 3 (March)					
Average similarity: 64.80					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Spirobranchus sp.	58.42	32.61	1.27	50.32	50.32
Acorn Barnacle	65.25	29.85	0.92	46.06	96.38
Group 1 (January)					
Average similarity: 76.25					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Spirobranchus sp.	91.01	64.27	2.37	84.29	84.29
Acorn Barnacle	39.28	10.33	0.62	13.55	97.85
Group 10 (October)					
Average similarity: 52.27					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Acorn Barnacle	29.34	37.4	2.51	71.55	71.55
Spirobranchus sp.	18.12	9.86	0.7	18.86	90.41
Group 2 (February)					
Average similarity: 73.57					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Spirobranchus sp.	77.75	48.25	4.84	65.58	65.58
Ascidians	21.14	12.53	3.17	17.03	82.61
Acorn Barnacle	16.95	6.69	0.99	9.09	91.7

Table D, *SIMPER test showing the species contributing to similarity across the response variables of Year (2021-2023) and Season (months: January, February, March, May, June, August, September, October, November). Average abundance is given alongside the percentage contribution to similarity and cumulative percentage similarity.*

Port of Blyth						
						Unique
Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**per**
Year	2	20316	10158	12.091	**0.0001**	9939
Season	6	25650	4275.1	5.0887	**0.0001**	9908
YexSe	0	0		No test		
Res	31	26043	840.11			
Total	39	72454				
Sunderland Marina						
						Unique
Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**perms**
Year	2	12057	6028.6	12.608	**0.0001**	9945
Season	5	24849	4969.8	10.394	**0.0001**	9930
YexSe	0	0		No test		
Res	32	15301	478.16			
Total	39	48946				
Conwy Marina						
						Unique
Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**perms**
Year	2	48552	24276	36.364	**0.0001**	9934
Season	7	22783	3254.8	4.8755	**0.0001**	9916
YexSe	4	10411	2602.6	3.8986	**0.0002**	9929
Res	96	64088	667.58			
Total	109	1.52E+05				
Deganwy Marina						
						Unique
Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**perms**
Year	2	18626	9313.2	12.91	**0.0001**	9922
Season	7	51314	7330.6	10.162	**0.0001**	9920
YexSe	5	27271	5454.2	7.5606	**0.0001**	9923
Res	92	66369	721.41			
Total	106	1.94E+05				

Table E, *PERMANOVA testing all sites using the response variables of Year (2021-2023) and Season (months: January, February, March, May, June, August, September, October, November) and an interaction effect (Year x Season) between the two responses when examining sessile community composition (Oyster shell epibiota).*

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