

## Ten priority questions for increasing the consistency and success in hatchery production of the European flat oyster for habitat restoration

zu Ermgassen, Sophus O.S.E.; Albentosa, Marina; Bakker, Nienke; Blanco, Ainhoa; Bonacic, Krano; Carboni, Stefano; Brundu, Gianni; Colsohl, Berenger; Pineiro, Nicolas Araujo; da Costa, Fiz; Dubbeldam, Marco; Fabra, Monica ; Galley, Tom; Gowland, Dennis; Jones, Nick; Hernandez, Angel; Hernandis, Sebastian; Laugen, Ane T.; Magnesen, Thorolf; Malham, Shelagh; Pogoda, Bernadette; Preston, Joanne; Sas, Hein; Saurel, Camille; Barja, Juan L.; Kamermans, Pauline

### Aquatic Living Resources

DOI:

[10.1051/alr/2023025](https://doi.org/10.1051/alr/2023025)

Published: 20/10/2023

Publisher's PDF, also known as Version of record

[Cyswllt i'r cyhoeddiad / Link to publication](#)

*Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):*

zu Ermgassen, S. O. S. E., Albentosa, M., Bakker, N., Blanco, A., Bonacic, K., Carboni, S., Brundu, G., Colsohl, B., Pineiro, N. A., da Costa, F., Dubbeldam, M., Fabra, M., Galley, T., Gowland, D., Jones, N., Hernandez, A., Hernandis, S., Laugen, A. T., Magnesen, T., ... Kamermans, P. (2023). Ten priority questions for increasing the consistency and success in hatchery production of the European flat oyster for habitat restoration. *Aquatic Living Resources*, 36, Article 29. <https://doi.org/10.1051/alr/2023025>

#### Hawliau Cyffredinol / General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

#### Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

# Ten priority questions for increasing the consistency and success in hatchery production of the European flat oyster for habitat restoration

Philine S.E. zu Ermgassen<sup>1,\*</sup>, Marina Albentosa<sup>2</sup>, Nienke Bakker<sup>3</sup>, Ainhoa Blanco<sup>4</sup>, Krno Bonačić<sup>5</sup>, Stefano Carboni<sup>6</sup>, Gianni Brundu<sup>6</sup>, Béranger Colsoul<sup>7</sup>, Nicolás Araujo Piñeiro<sup>7</sup>, Fiz da Costa<sup>8</sup>, Marco Dubbeldam<sup>9</sup>, Monica Fabra<sup>10</sup>, Thomas Galley<sup>11</sup>, Dennis Gowland<sup>12</sup>, Nicholas Jones<sup>13</sup>, Ángel Hernández<sup>14</sup>, Sebastián Hernandis<sup>2</sup>, Ane T. Laugen<sup>13</sup>, Thorolf Magnesen<sup>14</sup>, Shelagh Malham<sup>11</sup>, Bernadette Pogoda<sup>7</sup>, Joanne Preston<sup>10</sup>, Hein Sas<sup>15</sup>, Camille Saurel<sup>16</sup>, Juan L. Barja<sup>17</sup> and Pauline Kamermans<sup>4</sup>

<sup>1</sup> Changing Oceans Group, School of Geosciences, University of Edinburgh, James Hutton Rd, King's Buildings, Edinburgh EH9 3FE, UK

<sup>2</sup> Instituto Español de Oceanografía (IEO, CSIC). Centro Oceanográfico de Murcia. Varadero, 1, 30740, San Pedro del Pinatar (Murcia), Spain

<sup>3</sup> Roem van Yerseke BV, Yerseke, The Netherlands

<sup>4</sup> Wageningen Marine Research, Wageningen University and Research, Yerseke, The Netherlands

<sup>5</sup> Department of Applied Ecology, University of Dubrovnik, Dubrovnik, Croatia

<sup>6</sup> International Marine Centre – IMC, Loc. Sa Mardini, 09170 Torre Grande, Italy

<sup>7</sup> Biological Institute Helgoland, Alfred-Wegener-Institut Helmholtz-Zentrum für Polar-und Meeresforschung, Helgoland, Germany

<sup>8</sup> Instituto Español de Oceanografía (IEO, CSIC), Centro Oceanográfico de Vigo, Subida a Radio Faro, 50, 36390, Vigo, Spain

<sup>9</sup> Stichting Zeeschelp, Kamperland, The Netherlands

<sup>10</sup> Institute of Marine Sciences, School of Biological Sciences, University of Portsmouth, Portsmouth, UK

<sup>11</sup> Centre for Applied Marine Sciences, School of Ocean Science, Bangor University, Menai Bridge, LL59 5AB, UK

<sup>12</sup> Northbay Innovations Ltd, New Horries, Deerness, Orkney UK. KW17 2QL

<sup>13</sup> Centre for Coastal Research (CCR), Department of Natural Sciences, University of Agder, Postboks 422, NO-4604 Kristiansand, Norway

<sup>14</sup> Department of Biological Sciences, University of Bergen, Norway

<sup>15</sup> Sas Consultancy, Amsterdam, The Netherlands

<sup>16</sup> Coastal Ecology, Danish Shellfish Centre, National Institute of Aquatic Resources, Technical University of Denmark, Ørøddevej 80, 7900 Nykøbing Mors, Denmark

<sup>17</sup> University of Santiago de Compostela, Department of Microbiology & Institute of Aquaculture. 15782, Coruña, Spain

Received 28 February 2023 / Accepted 9 August 2023

Handling Editor: Tom C. Cameron

**Abstract** – The European flat oyster, *Ostrea edulis*, once formed extensive reef habitats throughout European seas and estuaries. These reefs are now largely functionally extinct, yet interest and support for their restoration is rapidly growing. A major bottleneck to scaling up oyster reef restoration is the lack of available oysters to supply the growing demand. This study aimed to identify the ten questions which, if answered, would increase the consistency and success in hatchery production of *O. edulis* for habitat restoration. Candidate questions were submitted by representatives from twelve commercial and research hatcheries across Europe. The list of 98 candidate questions were collaboratively discussed by experts from nine research hatcheries across eight countries in Europe, to identify the top ten questions via an iterative and open process. Questions were grouped into the following themes: conditioning and feeding, larval rearing, disease and water quality, hatchery protocol, genetics, and hatchery management. There were several overarching topics spanning these themes, including diet optimisation, maximising the effective population size, and developing the technical skillbase in order to increase hatchery production to meet the projected

\*Corresponding author: [philine.zuermgassen@cantab.net](mailto:philine.zuermgassen@cantab.net)

increase in demand for oyster seed for habitat restoration efforts. We anticipate this list will provide a starting point for collaborative research efforts across Europe, as well as assisting policy makers and funders in identifying key knowledge gaps.

**Keywords:** *Ostrea edulis* / aquaculture / larvae / ecological restoration / bivalve / marine

## 1 Introduction

The European flat oyster (*Ostrea edulis*) was once found in abundance throughout European coasts and seas (Möbius 1877, Olsen 1883, Joubin 1908), but has suffered catastrophic declines due to overfishing, habitat degradation, pollution and disease (Thurstan et al., 2013; Pogoda, 2019). As a result, the habitat is largely functionally extinct (Beck et al., 2011) and the species and habitat it forms is now listed as threatened and/or declining by OSPAR (OSPAR Commission, 2009). Increased recognition of the value of the species as a biogenic keystone species along with growing knowledge on the practice of marine habitat restoration (Fitzsimons et al., 2020), has resulted in the recent and rapid growth of *O. edulis* restoration efforts throughout Europe (Preston et al., 2020). There is also an increasing impetus to restore habitats in Europe to meet a number of obligations set out by the European Union (e.g., the Habitats Directive and Marine Strategy Framework Directive) and the International Convention on Biological Diversity (CBD, 2022). This demand is set to grow further given the recent proposal for a regulation on nature restoration by the European Commission ([https://environment.ec.europa.eu/publications/nature-restoration-law\\_en](https://environment.ec.europa.eu/publications/nature-restoration-law_en)).

A major barrier to the expansion and scaling up of *O. edulis* restoration efforts in Europe is the lack of reliable production of *O. edulis* seed (Pogoda et al., 2019; zu Ermgassen et al., 2020; zu Ermgassen et al., 2023). Oysters can be sourced from various means of production, including from wild fisheries and oyster farming for adults; and wild seed collection, spatting ponds and hatcheries for seed (Colsoul et al., 2021; Strand et al., 2021). Wild “sea-based” seed collection relies on natural populations stimulating a sufficient spat fall. These conditions are currently met in only a handful of locations across Europe, such as the bays of Brest and Quiberon in France (Robert et al., 2003), and the Mali Ston Bay in Croatia (Zrnčić et al., 2007). Spatting ponds, which involve the stocking of shallow man-made ponds with settlement substrate and broodstock oysters, are a viable option for restoration efforts if seeking to move oysters locally, but require substantial amounts of space, permissions associated with their construction and access to plentiful clean seawater. Hatcheries are less constrained in terms of their location, as they can be established also where *O. edulis* is rare or absent. They also allow for better management of broodstock provenance, disease control, and can be designed to meet high biosecurity standards, which in turn may allow for young oysters to be moved over greater distances with a low biosecurity risk. As the movement of oysters is known to be a significant vector of invasive species and diseases (Mineur et al., 2014), reducing the biosecurity risk of the movement of oysters is important to restoration efforts for both reputational and practical reasons. Hatchery produced larvae can also be transported for remote setting onto cultch (settlement substrate) at restoration sites, although this

has yet to be demonstrated for *O. edulis*. Furthermore, hatcheries can be set up to produce oysters out of season (González-Araya et al., 2013; Maneiro et al., 2020), thereby extending the breeding period.

Across the methods of oyster production, but particularly for hatcheries, knowledge and skill transfer related to *O. edulis* breeding and rearing diminished during 1970–2010 (Colsoul et al., 2021). This was primarily a result of *O. edulis* largely being replaced in aquaculture by the Pacific oyster (*Crassostrea gigas*), following its introduction to Europe in the 1970s in response to the collapse of *O. edulis* and Portuguese oyster (*Crassostrea angulata*) (Grizel and Héral 1991; Martínez-García et al., 2022). Research into oyster production subsequently focused on *C. gigas* (Buestel et al., 2009; Martínez-García et al., 2022), and knowledge of the breeding and rearing of *O. edulis* stagnated (Robert et al., 2003). Interest in developing hatchery production of *O. edulis* was re-awakened in the late 2000s, as exemplified by the SETTLE project, which ran from 2008–2010 (e.g., González-Araya et al., 2011; González-Araya et al., 2012). Nevertheless, knowledge gaps relating to the species biology and hatchery culture still prevent the reliable production of *O. edulis* seed (Roberts et al., 2017).

Existing knowledge gaps are greatest in the production of oysters for habitat restoration purposes, as the needs of projects seeking to place oysters in the water for habitat restoration purposes differ to those required for aquaculture. For example, habitat restoration efforts may place more emphasis on the genetics of the broodstock and on maintaining genetic diversity in the resulting offspring (Lallias et al., 2010; Alves Monteiro et al., 2022). There is also commonly a strong emphasis on disease resistance/tolerance and/or resilience, in particular to the disease caused by *Bonamia ostreae* (Egerton et al., 2020; Colsoul et al., 2021; Holbrook et al., 2021). Finally, restoration efforts are increasingly seeking to source spat-on-shell as opposed to the single seed oysters (i.e., those grown on microcultch within hatcheries) which are typically produced for the table (Pogoda et al., 2020). The specific demands of restoration raises additional challenges for the production of *O. edulis* spat in a hatchery setting.

It is not only scientific challenges that contribute to the lack of reliable supply of *O. edulis* spat for habitat restoration. Despite the anticipated growth and support for restoration efforts, current financial support for projects is still largely financed by short term grants to conservation NGOs or research organisations and is therefore often limited to a few years in duration. This makes it challenging to develop restoration project plans with appropriate timelines and to provide commercial growers with consistent demand (zu Ermgassen et al., 2023). There is therefore a need for market research to allow existing producers to better understand the anticipated market growth and give them confidence in diverting resources to flat oyster production for the habitat restoration market. In contrast, the scientific

challenges to ensure consistency and success are primarily related to the optimisation of larval production, settlement and survival (Colsou et al., 2020; Colsou et al., 2021; Gray et al., 2022). A key element in increasing the reliability in hatchery production is to address mass mortality events (crashes), which are widely identified as being a perpetual problem in hatchery production (Barbosa Solomieu et al., 2015; Gray et al., 2022). These crashes remain largely unexplained (Gray et al., 2022).

Here we present the results of a priority setting exercise to identify the top ten questions which need to be addressed to increase consistency and success in hatchery production of *O. edulis*, for habitat restoration. The focus of the process was on factors within the hatchery setting, with questions submitted from both commercial and research hatcheries. Factors relating to financing and market research were not considered, although the group recognised the critical role these factors play in the scaling up of oyster production for restoration purposes. The resulting questions are intended to provide a focus for researchers and funding bodies, by highlighting the key knowledge gaps which need to be addressed to ensure that *O. edulis* production can meet the growing number of restoration efforts throughout the species native range.

## 2 Materials and methods

To identify the most important questions that, if answered, will have the greatest impact on increasing the consistency and success in hatchery production of the European flat oyster, *O. edulis*, for habitat restoration, we undertook a priority setting exercise based on the approach established in Sutherland et al. (2009) and Ockendon et al. (2018). This approach involves a wide range of experts coming together to propose and discuss relevant questions through a multistage, iterative, collaborative and transparent process, to reach a democratically agreed list of priority questions.

A pool of potential participants was drafted from the comprehensive review of *O. edulis* producers in Europe undertaken by Kamermans et al. (2020) and updated to include hatcheries participating in the Native Oyster Restoration Alliance (NORA) production working group in 2022 (Pogoda et al., 2020). All identified organisations were approached independently in 2022 and asked whether they would be interested in participating in an international collaboration to identify and overcome current bottlenecks in hatchery production. Those who replied positively were invited to participate in this exercise. The call for candidate questions was sent to all participating hatcheries on 24th August 2022. Participants were encouraged to engage within their hatchery and their wider network and submit questions. Submitted questions were requested to be specific and answerable within the next c. 5 yr.

A total of 98 questions were proposed by experts from five commercial and seven research hatcheries across nine European countries. Questions were then grouped into broad themes: conditioning and feeding, disease and water quality, hatchery protocol, genetics, and hatchery management. All experts that submitted questions were invited to participate in the workshop during which the priority questions would be agreed. The workshop took place online on the 1st and 2nd September 2022. The workshop was attended by nine

participants from seven organisations on the first day, and eleven participants from nine organisations on the second day. All eleven participants involved in selecting the questions identified as working primarily for research organisations. The two participants who had been unable to attend the first day, had followed the development of the questions list and the results of rounds of voting through frequent email updates. Institutions from eight countries were represented including: Croatia, Denmark, England, Germany, Italy, The Netherlands, Spain and Wales. Participants had a mean of 6.3 (s.e., 4.4) years and a median of four years experience with *O. edulis* hatchery production, although many participants had several further years experience within hatcheries with other species. The majority of hatcheries only began producing *O. edulis* recently (median year in which the hatchery started to produce *O. edulis* was 2021). The expert panel represented a broad spectrum of expertise with hatcheries and production of *O. edulis*. Participants were asked to declare their primary area of expertise and 46% ( $n=5$ ) self-identified as experts in hatchery management, 18% ( $n=2$ ) identified as experts in oyster disease, a further 18% ( $n=2$ ) as experts in oyster nutrition, 9% ( $n=1$ ) of experts identified as oyster culture based on wild spat and 9% ( $n=1$ ) on oyster physiology.

All participants were asked to review the submitted questions in advance of the workshop, and to consider whether questions were duplicates. Participants were also asked to indicate their top 15% questions within each theme. As there were differing numbers of questions submitted to each theme, assigning a percentage of questions to be highlighted, ensured that all themes were given equal consideration at this early stage in the process. These votes were compiled and a summary was shared with participants in advance of the workshop. The first day of the workshop included a plenary session in which the relevance of the broad themes to the overarching question was discussed. Furthermore, identified potential duplicate questions were examined and consensus reached as to whether they were duplicates, or could be merged. The group was then divided into two subgroups with four or five expert participants, with experts self selecting to discuss the themes most closely aligned with their expertise. Within the subgroups participants were asked to assess whether each question directly addressed the overarching issue of increasing consistency and success in hatchery production of *O. edulis* for habitat restoration. The relative importance of each question, and its potential to be answered through research was also discussed. Participants were encouraged to note the number of votes that had been previously assigned to a question during the discussion. A number of questions were identified as not addressing the overarching issue or as being duplicates. Questions were also reworded and refined as necessary. Following these refinements, there were 20 questions remaining in the subgroup examining conditioning and feeding, disease and water quality, and hatchery management, and 19 questions remaining in the subgroup examining hatchery protocols and genetics. At the end of the subgroup discussion, participants were asked to vote anonymously online for their top 20% of questions across all themes within their own subgroup. The result of this vote was compiled and shared with all participants in advance of the second day of the workshop.



During the second day of the workshop, the chair of each subgroup presented to all participants, the questions which received votes within the subgroup. A brief explanation of the rationale for the importance of questions which received votes was provided and an open and frank discussion on the relevance and scope of the question was held. Questions receiving no votes within the subgroup were also reviewed in the plenary and all experts were given the opportunity to “rescue” questions to take them forward to the next round of voting. The remaining questions which received no votes were discarded. The experts were then asked to anonymously vote for their top ten questions across the 32 remaining questions (one vote per organisation). Nineteen questions received two or more expert votes. Each of these questions was in turn discussed and consensus was reached through group discussion to determine the ten most important questions which should be retained. For clarity, questions categorised as “conditioning and feeding” were subdivided into “conditioning and feeding”, and “larval rearing” for reporting in the results section.

### 3 Results

#### 3.1 The questions

##### 3.1.1 Conditioning and feeding

Conditioning refers to the husbandry and preparation of broodstock oysters to facilitate and induce gonad development, spawning and subsequent larval swarming within the hatchery. While the effect on gonad development and larval production of some aspects of *O. edulis* conditioning, such as water temperature, water flow rate, feed and photoperiod have been researched (González-Araya et al., 2011; González-Araya et al., 2012; Maneiro et al., 2016; Maneiro et al., 2017a, Maneiro et al., 2017b; Maneiro et al., 2020), the resulting impact on larval quality, survival and successful recruitment remains poorly understood. Yet it is known that broodstock conditioning can significantly influence larval growth and resulting larval settlement (Helm et al., 1973; Berntsson et al., 1997; González-Araya et al., 2012; da Costa et al., 2023).

Growing sufficient quantities of high-quality algae to supply the nutritional needs of the broodstock and larvae is a major and costly challenge, with algal production estimated to make up about 30% of seed production costs (Coutteau and Sorgeloos 1992). Broodstock oysters are filter feeders which require large volumes of algae. The use of alternative diets, such as algal pastes, to supplement live algae in hatchery diets is therefore of interest to save time and money (Coutteau and Sorgeloos 1992). The use of algal pastes in supplementary feeding for broodstock and juvenile *Crassostrea* species is commonplace (Gibbons et al., 1992; McCausland et al., 1999), but not yet successfully utilised for *O. edulis*. Given the importance of broodstock condition on larval growth, survival and metamorphosis, the impact of using algal pastes, and optimising the mix of microalgae used in the diet was identified as a key research need, in particular with regards to the impact on larval indicators, as opposed to directly on broodstock condition.

*O. edulis* is an asynchronous hermaphrodite capable of changing sex several times within a single breeding season, depending on temperature and food availability (Orton 1924;

Eagling et al., 2018). This presents a challenge in the hatchery setting, in particular when a key focus of reef restoration is to maintain genetic diversity, as skewed sex ratios and disproportionate parental contributions can lead to loss of genetic diversity and inbreeding depression within hatchery populations (Joyce et al., 2013). The role of conditioning in determining the sex-ratio of broodstock has received only limited attention to date, with the primary focus being on temperature (González-Araya et al., 2013; Joyce et al., 2013; Zapata-Restrepo et al., 2019). An understanding of the factors influencing broodstock sex differentiation in hatchery settings has the potential to increase both the number of larvae produced, and their genetic fitness for the reef restoration market, by increasing the parental contribution and effective population size.

1. How does broodstock diet impact larval growth, survival, metamorphosis and settlement under a range of conditioning scenarios?
2. What factors are responsible for broodstock sex differentiation at gametogenesis in hatchery settings?

##### 3.2 Larval rearing

Participants placed a strong emphasis on the need to better understand the optimal diet to promote growth and metamorphosis of larvae. Despite early research efforts to understand larval dietary needs (Walne 1963; Helm 1977; Wilson 1979; Wilson 1980), a better understanding of the nutritional needs of each larval stage is needed to improve growth and survival within hatchery settings.

The expert participants emphasised the importance not only of optimising protocols to improve larval growth and survival, but also to increase the synchronicity at which metamorphosis and settlement occur. Increased growth rate and synchronicity of settlement should reduce the risk of mortality by reducing the duration time spent in the sensitive larval stages. While the influence of temperature and salinity on larval growth and survival is relatively well understood (Davis and Ansell 1962; Davis and Calabrese 1969; Robert et al., 2017), their effect on the synchronicity and rate of metamorphosis and settlement remain a research gap, in addition to other potential factors such as larval density or diet (Colsoul et al., 2021). A negative phototropism has been observed at the pediveliger stage, suggesting that intense light might act as a catalyst, promoting both speed and intensity of larval settlement (Cole and Knight-Jones 1949).

While most mass mortality events occur primarily during the pelagic larval stage, poor settlement rates may also affect production (Gray et al., 2022). A number of factors are known to catalyse settlement and metamorphosis of larvae, including light and increased temperature (Carbonnier et al., 1990), and settlement substrate (Rodríguez-Pérez et al., 2020), whereas contamination by bacteria is a serious threat to survival during this sensitive stage (Colsoul et al., 2021). Further research into settlement cues and other factors to increase successful metamorphosis and settlement of larvae are therefore another area of research interest to increase the consistency and success of *O. edulis* hatchery production.

3. What is the most suitable diet in terms of algal species composition and ratio for each developmental stage from larvae to spat?
4. What factors (e.g., food, genetics, larval density, temperature) increase synchronicity of larval competency for settlement (eye-spot and foot fully-developed)?
5. What factors (e.g., diet composition and quality, genetics, larval density, and settlement cues) increase successful metamorphosis and settlement?

### 3.3 Disease and water quality

While the cause of hatchery crashes often remains unconfirmed (Gray et al., 2022), poor water quality and pathogens such as those in the genus *Vibrio* are recognized as significant drivers of mortality in hatchery settings (Richards et al., 2015). Water quality is a catch-all term, which includes a range of factors from pollution by heavy metals and detergents or persistent organic pollutants, to high pathogen or high sediment loads (Colsou et al., 2021; Walne 1970; Calabrese et al., 1977; Waugh 1964; Renzoni 1973). The impact of both organic and inorganic pollutants on some bivalves is well known (e.g., *C. gigas*, *Crassostrea virginica*, *Mytilus edulis* and *Mercenaria mercenaria*), but the threshold levels of most pollutants at which impacts are observed on *O. edulis* is lacking (His et al., 1999). Yet studies have identified that *O. edulis* may respond differently to pollutants than other well studied bivalve species (His et al., 1999; Perić et al., 2020). This knowledge gap regarding which pollutants *O. edulis* is most impacted by presents a challenge to hatcheries as it is not logistically or financially possible to test for all known pollutants. Even where testing is possible, measurements are of limited use without knowledge of the corresponding threshold levels at which pollutants result in chronic or acute impacts. Knowledge of thresholds would allow hatcheries to optimise which potential factors they screen for when investigating the cause of crashes.

In the case of *Vibrio*, hatcheries primarily seek to prevent infections through preventative actions, such as such as regular cleaning of the broodstock and the identification and control of potential vibrio sources (e.g., microalgae food, seawater, air, freshwater, staff and visitors) (Colsou et al., 2020). Vibriosis within the hatchery can be controlled by reducing the bacterial load in the water, usually (in flow-through systems) by increasing the water renewal rate of the tanks to prevent outbreaks. In the case of massive infection, when curative action is required, antibiotics can be applied. Antibiotics are generally viewed as an action of last resort due to their potential to negatively affect the environment and the risk of long term resistance developing (Dubert et al., 2017). The indiscriminate action of the antibiotics can also leave the remaining larval culture more susceptible to further outbreaks, as the antibiotics decrease the bacterial diversity and interspecies competition, creating conditions which may be exploited by opportunistic or resistant bacteria (Dubert et al., 2016).

Few curative solutions exist aside from antibiotics. The application of phages as an alternative to antibiotics, are currently being investigated with promising results (e.g., Kim et al., 2020). Additionally, the use of phages in preventing

contamination of algal cultures (Le et al., 2020) or probiotics in increasing resistance to vibrio (Karim et al., 2013; Sohn et al., 2016), can potentially contribute to enhanced preventative measures. As bacterial contamination is a leading identified cause of hatchery crashes, these developments were highlighted as key to improving the consistency and success of *O. edulis* hatchery production.

6. What are the thresholds for common organic and inorganic pollutants at which *O. edulis* reproduction and larval production are significantly negatively impacted?
7. Are there ethical and environmentally friendly standard controls that are effective to control pathogens (e.g., vibriosis) across the entire hatchery process (e.g., phages, probiotics, prebiotics)?

### 3.4 Hatchery protocol

Hatchery protocols outline in detail the production steps that should be taken from the point at which oysters are sourced, to when they leave the hatchery (e.g., quarantine, food production, broodstock conditioning, larval and spat cultivation). Several setups are possible, including static systems, Flow Through Systems (FTS) and Recirculating Aquaculture Systems (RAS). The static system is one of the main technologies used for bivalves, and specifically flat oysters since the development of large-scale hatchery production in the 1940s (Mann, 1984), and as such is supported by a substantial body of work (e.g., Walne 1966; Utting and Spencer 1991). FTS were developed more recently, and there is a moderate body of literature outlining protocols for production of *O. edulis* in these systems, including factors such as phytoplankton density and temperature (González-Araya et al., 2012; Robert et al., 2017). In contrast, the use of RAS for larval culture is in the early stages of development and work specifically related to *O. edulis* has yet to be published. Which system is most appropriate will depend on the location and setting of the hatchery, in particular the degree of access to suitable water sources and the external water quality, as well as the level of training of the staff operating the respective culture systems. Although a good understanding of the requirements and potential protocols across all three systems are needed to inform hatchery design and to facilitate decision-making, the largest knowledge gap is in RAS systems. Improved hatchery protocols are required to increase the consistency of *O. edulis* spat production as restoration efforts scale up.

8. Which conditions are required within each culture system (static, FTS, RAS) to maximise larval growth, survival and settlement?

### 3.5 Genetics

Despite a history of trading oysters throughout Europe since antiquity, significant genetic structures have been identified between extant populations at a regional scale (Launey et al., 2002; Beaumont et al., 2006; Vera et al., 2016). There is also evidence of more localised genetic structures, which may impact the adaptation to local abiotic conditions (Šegvić-Bubić et al., 2020; Hayer et al., 2021). A more

detailed understanding of the genetic structure across Europe will support decision making on the suitability of broodstock (Preston et al., 2020), particularly when restoring sites where local populations are extinct (Vera et al., 2016). The preservation of high genetic diversity can be a particular challenge in hatchery populations, where breeding effort is commonly dominated by few individuals (Launey et al., 2001; Alves Monteiro et al., 2022). In particular where wild genetic variability is low, efforts must be made to increase the effective population size within the hatchery to limit the degree to which variability is lost through the hatchery produced seed.

Recent evidence of the existence of a genetic basis for an immune response to the haplosporidian parasite *Bonamia ostreae* is also relevant for oyster restoration (Vera et al., 2019; Sambade et al., 2022). In disease-free areas, or areas considered disease free due to local extinction and absence of oysters as hosts, there is interest in introducing oysters which are genetically more predisposed to be resistant or resilient to Bonamiasis (Pogoda et al., 2019; Sas et al., 2020; Kamermans et al., 2023).

A method to determine both the variability in wild source populations, and the impact on the offspring in hatchery production has been developed (Alves Monteiro et al., 2022), and advances in understanding the genetics of disease resistance are rapidly developing (Sambade et al., 2022). Nevertheless, a Europe-wide overview of the genetic variability of existing populations, and the finer scale genetic structure, in particular in the Mediterranean, would facilitate decision-making regarding appropriate broodstock sources and the quantity of broodstock oysters required for hatchery production and restoration.

9. How should our understanding of pan-European and local genetic structure, and susceptibility to diseases, be accounted for when determining the source and quantity of broodstock required to produce oysters that are genetically suitable for the receiving environment?

### 3.6 Hatchery management

A hatchery protocol is only as good as the technicians who follow it. Unfortunately there is a recognized lack of staff with technical knowledge in hatchery production of *O. edulis*. Across all workshop participants, the challenge of finding and maintaining trained technicians was noted to be a crucial limiting factor in production. In particular during the breeding season, a large number of activities must be undertaken to maintain the algae, broodstock and larvae. Techniques such as the collection of larvae are challenging to master, and make a substantial difference in larval survival. All participants noted that in their experience *O. edulis* was substantially more sensitive to rough handling than other commonly reared bivalve species such as *C. gigas*, with poor handling contributing to excess mortalities. Additionally, there is a significant amount of knowledge that can currently only be gained “on the job”; by observing others executing particular husbandry techniques. Experienced technicians are noted to be more responsive and aware of when trouble shooting may be necessary. For example, they may recognise abnormal

behaviour in larvae or algal cultures, that may indicate the presence of pathogens and which are challenging and costly to screen for, but can significantly impact larval growth and survival. Addressing this training gap was seen as a critical step to sustain and increase hatchery capacity, but also to prevent crashes (Webster, 2011).

10. Is the current technical skill base sufficient for scale up of *O. edulis* hatchery production? If not, how can the capacity of qualified and experienced technicians be increased (e.g., opening online courses)?

## 4 Discussion

The questions presented here were identified through a priority setting exercise involving most European based hatcheries seeking to rear *O. edulis* for restoration purposes. The agreed ten most important questions cover all aspects of hatchery production, from the selection and handling of broodstock through to the settlement and metamorphosis of larvae, as well as relevant management aspects.

Mass mortality events are a universal problem across cultured oyster species (Barbosa Solomieu et al., 2015; Gray et al., 2022). Two of the priority questions sought specifically to address the causes of mass mortality events, with one focussing on developing alternatives to using antibiotics in response to outbreaks of vibrio (Question 6), and the other seeking to elucidate what thresholds of known pollutants are likely to cause mortalities in *O. edulis* larvae (Question 7). Although not explicitly addressing the causes of mass mortality events, a further two questions related to how to reduce the risk of mass mortality events occurring within a batch, by reducing the amount of time oysters spend at the sensitive larval stage (Questions 4 and 5). By increasing the synchronicity of larval competency for settlement, not only would the duration of the larval phase be reduced for some larvae, but each batch would also be subject to reduced amounts of handling which is also a source of mortality. An improved understanding of factors which result in increased success of larval metamorphosis and settlement, would have a similar impact.

All three questions pertaining to larvae contained reference to diet, despite the large body of research available on the topic (e.g., da Costa et al., 2023; González-Araya et al., 2011; González-Araya et al., 2012a; González-Araya et al., 2012b; González-Araya et al., 2013; Helm, 1977; Helm et al., 1973). In particular, Question 3 was focussed on examining the potentially differing requirements of each stage of larval development. Additionally, the impact of broodstock diet on larval fitness was identified as a knowledge gap (Question 1). In this case, however, the question focussed on optimising the broodstock diet to reduce reliance on live algae (e.g., by supplementing feeding with more easily managed and cheaper algal pastes), without negatively impacting larval fitness. It is notable that diet continues to be identified as a knowledge gap, both in this study and in others (Colsoul et al., 2021), despite the topic having been the subject of substantial research effort. The literature (Robert et al., 2013), as well as the participants noted that the results from conditioning protocols are inconsistent. It is not yet understood why protocols developed in one location appear to translate poorly to new settings, but



the role of nutrition was identified by participants as a potential factor.

A number of factors may be considered when selecting appropriate broodstock, including local genetic diversity, climate change predictions and disease status. How best to weigh up all factors, as well as which additional supporting information is required to support such decisions at each location, was identified as a current knowledge gap (Question 9). To some degree the demands of restoration practitioners for oysters capable of adapting to rapidly changing abiotic conditions and diseases status can be met through ensuring that seed oysters are representative of the full breadth of genetic diversity available for a given region. Restoration efforts are therefore concerned that the relatively small numbers of broodstock oysters used in hatchery production tend to result in low genetic variability in the offspring. This low effective population size is in part due to a biased sex ratio in hatchery settings which means that few females tend to contribute to individual batches (Lallias et al. 2007; Alves Monteiro et al., 2022). Improving the sex ratio of broodstock within hatcheries, thereby increasing the number of females making a parental contribution, would go some way to addressing this issue (Question 2). These questions relating to genetics were highlighted by participants as critically underpinning the work of hatcheries seeking to produce oysters for habitat restoration purposes.

The remaining questions focussed on how best to increase hatchery capacity at the European scale (Questions 8 and 10). Recent growth in the number of hatcheries focussed on production of *O. edulis* in Europe, alongside the lack of knowledge on some hatchery protocols for *O. edulis* specifically and the lack of trained and experienced technicians, has resulted in an urgent need for further trained staff. Developing clear, *O. edulis* specific guidelines for hatchery protocols, reduces the barriers to entry, thereby increasing the potential for growth or establishment of new hatcheries. Any increase in hatchery production must be matched by sufficient availability of trained and experienced personnel. Experience with the rearing of algae in a hatchery setting and *O. edulis* specific training for hatchery managers would be invaluable in reducing the associated costs of production and the risk posed by mass mortalities. Such experience is rapidly being gained, and shared, by the existing community of hatcheries. Many participants highlighted that they have undertaken visits to established hatcheries prior to launching their own efforts. The degree of organisation involved with hatchery visits, and the synchronicity of key activities within the hatchery calendar across Europe makes such exchanges difficult to undertake. Additionally, if visits cannot take place for logistical reasons during the busiest times of year, opportunities for shared learning of techniques are lost. The participants therefore identified open online learning courses as a potential mechanism for increasing the number and skill-level of qualified technicians (Question 10).

## 5 Conclusion

While hatchery production of bivalves has a long and successful history, the challenges associated with consistently

successfully rearing *O. edulis* raises specific questions. It was recognition that production remains inconsistent in many settings, that motivated several commercial and research hatcheries in Europe to collaborate to identify these priority research questions. The selected questions illustrate that substantial knowledge gaps remain in developing hatchery protocols for the selection of broodstock for restoration purposes, through to husbandry, diet for both broodstock and larvae, and hatchery management. These questions, while diverse, all have the potential to contribute to the scaling up of production; by improving the survival and growth of larvae, by reducing the risk of mass mortalities, improving the genetic suitability of seed produced, and by addressing the current lack of skilled technicians.

It is only once existing barriers in *O. edulis* production are overcome and production can take place at scale, that restoration efforts too will be able to meaningfully scale up. Given the functionally extinct status of native oyster reefs in Europe (Beck et al., 2011), and the density dependent fecundity of *O. edulis* (Guy et al., 2018), a great number of oysters will be needed to reverse the current threatened and declining status of this habitat building species (OSPAR Commission, 2009). It is our intention that these questions will be of use to researchers, funders and policy makers, as they seek to identify cost effective and collaborative ways to increase hatchery reliability in the face of rapidly growing demand for *O. edulis* seed for habitat restoration efforts, to meet this critical conservation challenge.

**Acknowledgements.** This research was sponsored by the Dutch Ministry of Agriculture, Nature and Food Quality (BO-43-116.01-012). The authors would like to thank Nik Sachlikidis and the many other colleagues who assisted by providing questions, bibliographic material, comments, suggestions and corrections. BP and BC acknowledge the support of the PROCEED project, funded by the German Federal Agency for Nature Conservation, BfN, with funds from the German Federal Ministry for the Environment, BMUV, as part of the Federal Program on Biological Diversity. The contribution by the Centre for Applied Marine Sciences (TG, NJ, SM) was funded by the European Maritime and Fisheries Fund 2014–2020 (Project reference: 82363). MA, FdC, AH and SH acknowledge the support of RemediOS Project, developed with the collaboration of the Biodiversity Foundation (Spanish Ministry for Ecological Transition and the Demographic Challenge), through the Pleamar Program, co-financed by the European Maritime and Fisheries Fund (EMFF).

## References

- Alves Monteiro H, Saurel C, Jacobsen MB, Hemmer-Hansen J, Bekkevold D. 2022. Genetic parentage reconstruction as a practical tool applied to aquaculture and restoration programs for the European flat oyster, *Ostrea edulis*. *Aquat Living Resour* 35: 18.
- Barbosa Solomieu V, Renault T, Travers MA. 2015. Mass mortality in bivalves and the intricate case of the Pacific oyster, *Crassostrea gigas*. *J Invertebr Pathol, Pathog Dis Process Marine Molluscs* 131: 2–10.



- Beaumont A, Truebano Garcia M, Hönig S, Low P. 2006. Genetics of Scottish populations of the native oyster, *Ostrea edulis*: gene flow, human intervention and conservation. *Aquat Living Resour* 19: 389–402.
- Beck MW, Brumbaugh RD, Airoidi L, Carranza A, Coen LD, Crawford C, Defeo O, Edgar GJ, Hancock B, Kay M, Lenihan HS, Luckenbach MW, Toropova CL, Zhang G, Guo X. 2011. Oyster reefs at risk and recommendations for conservation, restoration and management. *Bioscience* 61: 107–116.
- Berntsson KM, Jonsson PR, Wängberg SA, Carlsson AS. 1997. Effects of broodstock diets on fatty acid composition, survival and growth rates in larvae of the European flat oyster, *Ostrea edulis* *Aquaculture* 154: 139–153.
- Buestel D, Ropert M, Prou J, Goulletquer P. 2009. History, status, and future of oyster culture in France. *J Shellfish Res* 28: 813–820.
- CBD. 2022. *Final text of Kunming-Montreal Global Biodiversity Framework* CBD/COP/15/L25 <https://prod.drupal.www.infra.cbd.int/sites/default/files/2022-12/221222-CBD-PressRelease-COP15-Final.pdf> Downloaded Feb 21st 2023.
- Calabrese A, MacInnes JR, Nelson DA, Miller JE. 1977. Survival and growth of bivalve larvae under heavy-metal stress. *Mar Biol* 41: 179–184.
- Carbonnier N, Martin AG, Mazurie J, Barthelemy G, Le Mouroux G, Martin AG. 1990. Télécaptage de l'huître plate: travaux réalisés en 1990 [Remote setting of the flat oyster: work carried out in 1990]. *Section Regionale de Bretagne Sud: Plan de Relance de l'Huître Plate 1990*, CIC-IFREMER, Auray, pp. 1–97.
- Cole HA, Knight Jones EW. 1949. The setting behaviour of larvae of the European flat oyster *Ostrea edulis* L., and its influence on methods of cultivation and spat collection. Anonymous, in: *Fishery Investigations Series II, Her Majesty's Stationery Office*, London, pp. 1–39.
- Colsoul B, Boudry P, Pérez-Parallé ML, Bratoš Cetinić A, Hugh-Jones T, Arzul I, Mérou N, Wegner KM, Peter C, Merk V, Pogoda B. 2021. Sustainable large-scale production of European flat oyster (*Ostrea edulis*) seed for ecological restoration and aquaculture: a review. *Rev Aquacult* 13: 1423–1468.
- Colsoul B, Pouvreau S, Di Poi C, Pouil S, Merk V, Peter C, Boersma M, Pogoda B. 2020. Addressing critical limitations of oyster (*Ostrea edulis*) restoration: Identification of nature-based substrates for hatchery production and recruitment in the field. *Aquat Conserv* 30: 2101–2115.
- Colsoul B, Fabra M, Cowing D, Hauton C, Pogoda B, Sanderson W, Strand Å, Thompson K, Raimund Weber R, Preston J. 2020. Biosecurity guidelines for European native oyster hatcheries. in: P. zu Ermgassen, C. Gamble, A. Debney, B. Colsoul, M. Fabra, W. Sanderson, Å. Strand, J. Preston (Eds.), *European guidelines on biosecurity in native oyster restoration*, The Zoological Society of London, UK.
- Coutteau P, Sorgeloos P. 1992. The use of algal substitutes and the requirement for live algae in the hatchery and nursery rearing of bivalve molluscs: an international survey. *J Shellfish Res* 11: 467–476.
- da Costa F, González-Araya R, Robert R. 2023. Using combinations of microalgae to condition European flat oyster (*Ostrea edulis*) broodstock and feed the larvae: Effects on reproduction, larval production and development. *Aquaculture* 568: 739302.
- Davis HC, Ansell AD. 1962. Survival and growth of larvae of the European oyster, *O. edulis*, at lowered salinities. *Biol Bull* 122: 33–39.
- Davis HC, Calabrese A. 1969. Survival and growth of larvae of the European oyster (*Ostrea edulis* L.) at different temperatures. *Biol Bull* 136: 193–199.
- Dubert J, Barja JL, Romalde JL. 2017. New insights into pathogenic Vibrios affecting bivalves in hatcheries: Present and future prospects. *Front Microbiol* 8: 762.
- Dubert J, Osorio CR, Prado S, Barja JL. 2016. Persistence of antibiotic resistant Vibrio spp. in shellfish hatchery environment. *Microb Ecol* 72: 851–860.
- Eagling LE, Ashton EC, Jensen AC, Sigwart JD, Murray D, Roberts D. 2018. Spatial and temporal differences in gonad development, sex ratios and reproductive output, influence the sustainability of exploited populations of the European oyster, *Ostrea edulis*. *Aquat Conserv* 28: 1–12.
- Egerton S, Lynch SA, Prado-Alvarez M, Flannery G, Brennan E, Hugh-Jones T, Hugh-Jones D, Culloty SC. 2020. A naïve population of European oyster *Ostrea edulis* with reduced susceptibility to the pathogen *Bonamia ostreae*: are S-strategy life traits providing protection? *Integr Comp Biol* 60: 249–260.
- Fitzsimons JA, Branigan S, Gillies CL, Brumbaugh RD, Cheng J, DeAngelis BM, Geselbracht L, Hancock B, Jeffs A, McDonald T, McLeod IM, Pogoda B, Theuerkauf SJ, Thomas M, Westby S, zu Ermgassen PSE. 2020. Restoring shellfish reefs: Global guidelines for practitioners and scientists. *Conserv Sci Pract* 2: e198.
- Gibbons M, Kurkowski K, Castagna M. 1992. VIMS Hatchery Operations Manual. Special Reports in Applied Marine Science and Ocean Engineering (SRAMSOE) No. 318. *Virginia Institute of Marine Science, William & Mary*. <https://doi.org/10.21220/V5475X>
- González-Araya R, Mingant C, Petton B, Robert R. 2012a. Influence of diet assemblage on *Ostrea edulis* broodstock conditioning and subsequent larval development. *Aquaculture* 364–365: 272–280.
- González-Araya R, Lebrun L, Quéré C, Robert R. 2012b. The selection of an ideal diet for *Ostrea edulis* (L.) broodstock conditioning (part B). *Aquaculture, Smolt 2009: Proceedings of the 8th International Workshop on Smoltification*. 362–363: 55–66.
- González-Araya R, Quéau I, Quéré C, Moal J, Robert R. 2011. A physiological and biochemical approach to selecting the ideal diet for *Ostrea edulis* (L.) broodstock conditioning (part A). *Aquac Res* 42: 710–726.
- González-Araya R, Quillien V, Robert R. 2013. The effects of eight single microalgal diets on sex-ratio and gonad development throughout European flat oyster (*Ostrea edulis* L.) conditioning. *Aquaculture* 400–401: 1–5.
- Gray MW, Alexander ST, Beal BF, Bliss T, Burge CA, Cram JA, Luca MD, Dumhart J, Glibert PM, Gonsior M, Heyes A, Huebert KB, Lyubchich V, McFarland K, Parker M, Plough LV, Schott EJ, Wainger LA, Wikfors GH, Wilbur AE. 2022. Hatchery crashes among shellfish research hatcheries along the Atlantic coast of the United States: A case study of production analysis at Horn Point Laboratory. *Aquaculture* 546: 737259.
- Grizel H, Héral M. 1991. Introduction into France of the Japanese oyster (*Crassostrea gigas*). *ICES J Mar Sci* 47: 399–403.
- Guy C, Smyth D, Roberts D. 2018. The importance of population density and inter-individual distance in conserving the European oyster *Ostrea edulis*. *J Mar Biol Assoc UK* 1–7.
- Hayer S, Brandis D, Immel A, Susat J, Torres-Oliva M, Ewers-Saucedo C, Krause-Kyora B. 2021. Phylogeography in an “oyster” shell provides first insights into the genetic structure of an extinct *Ostrea edulis* population. *Sci Rep* 11: 2307.
- Helm MM. 1977. Mixed algal feeding of *Ostrea edulis* larvae with *Isochrysis galbana* and *Tetraselmis suecica*. *J Mar Biol Assoc UK* 57: 1019–1029.
- Helm MM, Holland DL, Stephenson RR. 1973. The effect of supplementary algal feeding of a hatchery breeding stock of *Ostrea edulis* L. on larval vigour. *J Mar Biol Assoc UK* 53: 673–684.

- His E, Beiras R, Seaman MNL. 1999. The Assessment of Marine Pollution - Bioassays with bivalve embryos and larvae, in: A.J. Southward, P.A. Tyler, C.M. Young (Eds.), *Advances in Marine Biology*, Academic Press, pp. 1–178.
- Holbrook Z, Bean TP, Lynch SA, Hauton C. 2021. What do the terms resistance, tolerance, and resilience mean in the case of *Ostrea edulis* infected by the haplosporidian parasite *Bonamia ostreae*. *J Invertebr Pathol* 182: 107579.
- Joubin L. 1908. Carte des Gisements de Coquilles Comestibles de la partie des côtes de l'Ille et Vilaine comprise entre le Cap Fréhel et al Pointe du Grouin, *Préparateur au Muséum d'Histoire Naturelle de Paris, Paris*.
- Joyce A, Holthuis TD, Charrier G, Lindegarth S. 2013. Experimental effects of temperature and photoperiod on synchrony of gametogenesis and sex ratio in the European oyster *Ostrea edulis* (Linnaeus). *J Shellfish Res* 32: 447–458, 412.
- Kamermans P, Blanco A, van Dalen P. 2020. Sources of European flat oysters (*Ostrea edulis* L.) for restoration projects in the Dutch North Sea, *Wageningen University and Research*, p. 55.
- Kamermans P, Blanco A, van Dalen P, Engelsma M, Bakker N, Jacobs P, Dubbeldam M, Sambade IM, Vera M, Martinez P. 2023. Bonamia-free flat oyster (*Ostrea edulis* L.) seed for restoration projects: non-destructive screening of broodstock, hatchery production and test for Bonamia-tolerance. *Aquat Living Resour* 36: 11.
- Karim M, Zhao W, Rowley D, Nelson D, Gomez-Chiarri M. 2013. Probiotic strains for shellfish aquaculture: protection of eastern oyster, *Crassostrea virginica*, larvae and juveniles against bacterial challenge. *J Shellfish Res* 32: 401–408, 408.
- Kim HJ, Giri SS, Kim SG, Kim SW, Kwon J, Lee SB, Park SC. 2020. Isolation and characterization of two bacteriophages and their preventive effects against pathogenic *Vibrio coralliilyticus* causing mortality of Pacific oyster (*Crassostrea gigas*) larvae. *Microorganisms* 8: 926.
- Lallias D, Beaumont AR, Haley CS, Boudry P, Heurtebise S, Lapégué S. 2007. A first-generation genetic linkage map of the European flat oyster *Ostrea edulis* (L.) based on AFLP and microsatellite markers. *Anim Genet* 38: 560–568.
- Launey S, Barre M, Gerard A, Naciri-Graven Y. 2001. Population bottleneck and effective size in *Bonamia ostreae*-resistant populations of *Ostrea edulis* as inferred by microsatellite markers. *Genet Res* 78: 259–270.
- Launey S, Leduc C, Boudry P, Bonhomme F, Naciri-Graven Y. 2002. Geographic structure in the European flat oyster (*Ostrea edulis* L.) as revealed by Microsatellite polymorphism. *J Hered* 93: 331–351.
- Le TS, Southgate PC, O'Connor W, Abramov T, Shelley DV, Vu S, Kurtböke Dİ. 2020. Use of bacteriophages to control *Vibrio* contamination of microalgae used as a food source for oyster larvae during hatchery culture. *Curr Microbiol* 77: 1811–1820.
- Maneiro V, Pérez-Parallé ML, Pazos AJ, Silva A, Sánchez JL. 2016. Combined effects of temperature and photoperiod on the conditioning of the flat oyster (*Ostrea edulis* [Linnaeus, 1758]) in Winter. *J Shellfish Res* 35: 137–141, 5.
- Maneiro V, Pérez-Parallé ML, Silva A, Sánchez JL, Pazos AJ. 2017a. Conditioning of the European flat oyster (*Ostrea edulis*, Linnaeus 1758): effect of food ration. *Aquac Res* 48: 4363–4370.
- Maneiro V, Santos Y, Pazos AJ, Silva A, Torres-Corral Y, Sánchez JL, Pérez-Parallé ML. 2020. Effects of food ration, water flow rate and bacteriological levels of broodstock on the reproductive conditioning of the European flat oyster (*Ostrea edulis*, Linnaeus 1758). *Aquaculture Reports* 18: 100412.
- Maneiro V, Silva A, Pazos AJ, Sánchez JL, Pérez-Parallé ML. 2017b. Effects of temperature and photoperiod on the conditioning of the flat oyster (*Ostrea edulis* L.) in autumn. *Aquac Res* 48: 4554–4562.
- Mann R. 1984. Bivalve Mollusc Hatcheries: A critical appraisal of their development and a review of their potential value in enhancing the fisheries of developing nations, in: J.E. Winter, E. Clasing, A. Gutierrez (Eds.), *Memorias De La Asociacion Latinoamerica De Acuicultura Vol. 5*. Universidad Austral de Chile, Valdivia, Chile.
- Martínez-García MF, Ruesink JL, Grijalva-Chon JM, Lodeiros C, Arreola-Lizárraga JA, de la Re-Vega FE, Varela-Romero A, Chávez-Villalba J. 2022. Socioecological factors related to aquaculture introductions and production of Pacific oysters (*Crassostrea gigas*) worldwide. *Rev Aquacult* 14: 613–629.
- McCausland MA, Brown MR, Barrett SM, Diemar JA, Heasman MP. 1999. Evaluation of live microalgae and microalgal pastes as supplementary food for juvenile Pacific oysters (*Crassostrea gigas*). *Aquaculture* 174: 323–342.
- Mineur F, Le Roux A, Maggs CA, Verlaque M. 2014. Positive feedback loop between introductions of non-native marine species and cultivation of oysters in Europe. *Conserv Biol* 28: 1667–1676.
- Möbius KA. 1877. Die Auster und die Austernwirthschaft, *Hempel & Parey, Berlin, Wiegandy*
- Ockendon N, Thomas DHL, Cortina J, Adams WM, Aykroyd T, Barov B, Boitani L, Bonn A, Branquinho C, Brombacher M, Burrell C, Carver S, Crick HQP, Duguy B, Everett S, Fokkens B, Fuller RJ, Gibbons DW, Gokhelashvili R, Griffin C, Halley DJ, Hotham P, Hughes FMR, Karamanlidis AA, McOwen CJ, Miles L, Mitchell R, Rands MRW, Roberts J, Sandom CJ, Spencer JW, ten Broeke E, Tew ER, Thomas CD, Timoshyna A, Unsworth RKF, Warrington S, Sutherland WJ. 2018. One hundred priority questions for landscape restoration in Europe. *Biol Conserv* 221: 198–208.
- Olsen OT. The piscatorial atlas of the North Sea, English and St. George's Channels, Grimsby, London.
- Orton JH. 1924. Sex change and breeding in the native oyster, *Ostrea edulis*. *Nature* 114: 191–92.
- OSPAR Commission. Background document for *Ostrea edulis* and *Ostrea edulis* beds, *OSPAR*
- Perić L, Stinga Perusco V, Nerlović V. 2020. Differential response of biomarkers in the native European flat oyster *Ostrea edulis* and the non-indigenous Pacific oyster *Crassostrea gigas* co-exposed to cadmium and copper. *J Experiment Mar Biol Ecolo* 523: 151271
- Pogoda B. 2019. Current status of European oyster decline and restoration in Germany. *Humanities* 8: 1–12.
- Pogoda B, Brown J, Hancock B, Preston J, Pouvreau S, Kamermans P, Sanderson W, von Nordheim H. 2019. The Native Oyster Restoration Alliance (NORA) and the Berlin Oyster Recommendation: bringing back a key ecosystem engineer by developing and supporting best practice in Europe. *Aquat Living Resour* 32: <https://doi.org/10.1051/alr/2019012>
- Pogoda B, Boudry P, Bromley C, Cameron TC, Colsoul B, Donnan D, Hancock B, Hugh-Jones T, Preston J, Sanderson WG, Sas H, Brown J, Bonacic K, von Nordheim H, zu Ermgassen PSE. 2020. NORA moving forward: developing an oyster restoration network in Europe to support the Berlin Oyster Recommendation. *Aquat Conserv* 30: 2031–2037.
- J. Preston, C. Gamble, A. Debney, L. Helmer, B. Hancock, P.S.E. zu Ermgassen (Eds.), *European Native Oyster Habitat Restoration Handbook*, The Zoological Society of London, London, UK 2020.
- Renzoni A. The influence of some detergents on the larval life of marine bivalve larvae. in Genovese S. (Ed.). *Proceedings of the 5th International Colloquium of Medical Oceanography*, University of Messina, Messina 1973, pp. 101–104.
- Richards GP Watson MA Needleman DS Church KM Häse CC 2015, Mortalities of eastern and Pacific oyster larvae caused by the pathogens *Vibrio coralliilyticus* and *Vibrio tubiashii*. *Appl Environ Microbiol* 81: 292–297.

- Robert R, Vignier J, Petton B. 2017. Influence of feeding regime and temperature on development and settlement of oyster *Ostrea edulis* (Linnaeus, 1758) larvae. *Aquacult Res* 48: 4756–4773.
- Sambade IM, Casanova A, Blanco A, Gundappa MK, Bean TP, Macqueen DJ, Houston RD, Villalba A, Vera M, Kamermans P, Martínez P. 2022. A single genomic region involving a putative chromosome rearrangement in flat oyster (*Ostrea edulis*) is associated with differential host resilience to the parasite *Bonamia ostreae*. *Evol Appl* 15: 1408–1422.
- Sas H, Deden B, Kamermans P, zu Ermgassen PSE, Pogoda B, Preston J, Helmer L, Holbrook Z, Arzul I, van der Have T, Villalba A, Colsoul B, Lown A, Merk V, Zwierschke N, Reuchlin E. 2020. *Bonamia* infection in native oysters (*Ostrea edulis*) in relation to European restoration projects. *Aquat Conserv* 30: 2150–2162.
- Šegvić-Bubić T, Žužul I, Talijančić I, Ugrin N, Lepen Pleić I, Žuvić L, Stagličić N, Grubišić L. 2020. Translocation and aquaculture impact on genetic diversity and composition of wild self-sustainable *Ostrea edulis* populations in the Adriatic Sea. *Front Mar Sci* 7: 84, 13.
- Sohn S, Lundgren KM, Tammi K, Karim M, Smolowitz R, Nelson DR, Rowley DC, Gómez-Chiarri M. 2016. Probiotic strains for disease management in hatchery larviculture of the eastern oyster *Crassostrea virginica*. *J Shellfish Res* 35: 307–317.
- Strand Å, Bakker N, Bird A, Blanco A, Bonačić K, Brundu G, Colsoul B, Connellan I, da Costa F, Fabra M, Hannan M, Hugh-Jones T, Humm G, Nielsen P, Stechle B, zu Ermgassen P. What restoration practitioners need to know about the oyster production industry. *NORA, Berlin* <https://nora-europe.eu/wp-content/uploads/2021/11/NORA-What-restoration-practitioners-need-to-know.pdf>
- Sutherland WJ, Adams WM, Aronson RB, Aveling R, Blackburn TM, Broad S, Ceballos G, Côté IM, Cowling RM, Da Fonseca GAB, Dinerstein E, Ferraro PJ, Fleishman E, Gascon C, Hunter, Jr., M, Hutton J, Kareiva P, Kuria A, Macdonald DW, Mackinnon K, Madgwick FJ, Mascia MB, Mcneely J, Milner-Gulland EJ, Moon S, Morley CG, Nelson S, Osborn D, Pai M, Parsons ECM, Peck LS, Possingham H, Prior SV, Pullin AS, Rands MRW, Ranganathan J, Redford KH, Rodriguez JP, Seymour F, Sobel S, Sodhi NS, Stott A, Vance-Borland K, Watkinson AR. 2009. One hundred questions of importance to the conservation of global biological diversity. *Conserv Biol* 23: 557–567.
- Thurstan RH, Hawkins JP, Raby L, Roberts CM. 2013. Oyster (*Ostrea edulis*) extirpation and ecosystem transformation in the Firth of Forth, Scotland. *J Nat Conserv* 21: 253–261.
- Utting SD, Spencer BE. 1991. The hatchery culture of bivalve mollusc larvae and juveniles. *MAFF Laboratory Leaflet Number* 68.
- Vera M, Carlsson J, Carlsson JE, Cross T, Lynch S, Kamermans P, Villalba A, Culloty S, Martinez P. 2016. Current genetic status, temporal stability and structure of the remnant wild European flat oyster populations: conservation and restoring implications. *Mar Biol* 163: 239.
- Vera M, Pardo BG, Cao A, Vilas R, Fernández C, Blanco A, Gutierrez AP, Bean TP, Houston RD, Villalba A, Martínez P. 2019. Signatures of selection for bonamiosis resistance in European flat oyster (*Ostrea edulis*): New genomic tools for breeding programs and management of natural resources. *Evol Appl* 12: 1781–1796.
- Walne PR. 1963. Observations on the food value of seven species of algae to the larvae of *Ostrea edulis* L. Feeding experiments. *J Mar Biol Assoc UK* 43: 767–784.
- Walne PR. Experiments in the large-scale culture of the larvae of *Ostrea edulis* L., Her Majesty's Stationery Office, London, 1966.
- Walne PR. 1970. Present problems in the culture of the larvae of *Ostrea edulis*. *Helgolander Wissenschaftliche Meeresuntersuchungen* 20: 514–525.
- Waugh GD. 1964. Observations on the effects of chlorine on the larvae of oysters (*Ostrea edulis* (L.)) and barnacles (*Elminius modestus* (Darwin)). *Annals of Applied Biology* 54: 423–440.
- Webster D. 2011. Education, *Shellfish Aquaculture and the Environment*, pp. 447–459.
- Wilson JH. 1979. Observations on the grazing rates and growth of *Ostrea edulis* L. larvae when fed algal cultures of different ages. *J Exp Mar Biol Ecol* 38: 187–199.
- Wilson JH. 1980. Particle retention and selection by larvae and spat of *Ostrea edulis* in algal suspensions. *Mar Biol* 57: 135–145.
- Zapata-Restrepo LM, Hauton C, Williams ID, Jensen AC, Hudson MD. 2019. Effects of the interaction between temperature and steroid hormones on gametogenesis and sex ratio in the European flat oyster (*Ostrea edulis*). *Comp Biochem Physiol, Part A Mol Integr Physiol* 236: 110523.
- Zrnčić S, Oraić D, Mihaljević Ž, Zanella D. 2007. Impact of varying cultivation depths on growth rate and survival of the European flat oyster *Ostrea edulis*, *L Aquacult Res* 38: 1305–1310.
- zu Ermgassen PSE, Bonačić K, Boudry P, Bromley CA, Cameron TC, Colsoul B, Coolen JWP, Frankić A, Hancock B, van der Have TM, Holbrook Z, Kamermans P, Laugen AT, Nevejan N, Pogoda B, Pouvreau S, Preston J, Ranger CJ, Sanderson WG, Sas H, Strand Å, Sutherland WJ. 2020. Forty questions of importance to the policy and practice of native oyster reef restoration in Europe. *Aquat Conserv* 30, 2038–2049.
- P.S.E. zu Ermgassen, C. Gamble, A. Debney, B. Colsoul, M. Fabra, W.G. S and erson, Å. Strand, J. Preston (Eds.), European Guidelines on Biosecurity in Native Oyster Restoration, The Zoological Society of London, London UK
- zu Ermgassen PSE, Strand Å, Bakker N, Blanco A, Bonačić K, Boudry P, Brundu G, Cameron TC, Connellan I, da Costa F, Debney A, Fabra M, Frankić A, Gamble C, Gray MW, Helmer L, Holbrook Z, Hugh-Jones T, Kamermans P, Magnesen T, Nielsen P, Preston J, Ranger CJ, Saurel C, Smyth D, Stechele B, Theodorou JA, Colsoul B. 2023. Overcoming *Ostrea edulis* seed production limitations to meet ecosystem restoration demands in the UN decade on restoration. *Aquat Living Resour* 36: 16.

**Cite this article as:** zu Ermgassen PSE, Albentosa M, Bakker N, Blanco A, Bonačić K, Carboni S, Brundu G, Colsoul B, Piñeiro NA, da Costa F, Dubbeldam M, Fabra M, Galley T, Gowland D, Jones N, Hernández Á, Hernández S, Laugen AT, Magnesen T, Malham S, Pogoda B, Preston J, Sas H, Saurel C, Barja JL, Kamermans P. 2023. Ten priority questions for increasing the consistency and success in hatchery production of the European flat oyster for habitat restoration. *Aquat. Living Resour.* 36: 29