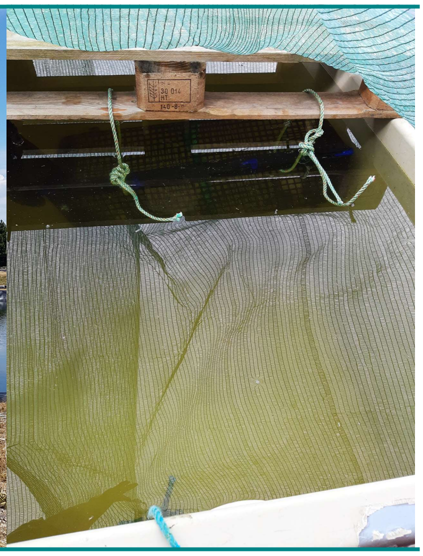
A guide to small-scale and large-scale pond production of flat oysters (*Ostrea edulis*)





CENTRUM FÖR FRAMTIDENS SJÖMAT

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Contents

Background

There is a general shortage of the European flat oyster (*Ostrea edulis*) seed both for oyster production and for the restoration sector (Zu Ermgassen et al. 2023). The three main techniques for seed production are sea-based seed collection using collectors, spatting ponds, and hatcheries (Colsoul et al. 2021). Spatting ponds have several advantages, including being less intensive (and consequently less resource demanding) compared to hatchery production, and they maintain high genetic diversity of the produced oyster seed (Strand and Sühnel 2022). In contrast to sea-based seed collection, which generally requires less resources than spatting pond production, ponds also provide means to partly control some of the most important environmental factors impacting reproduction, seed development, and larval survival (salinity, temperature, pH and alkalinity, food availability and oxygen). The performance of spatting ponds is, however, unstable with some ponds crashing every year without obvious causes. Moreover, the knowledge regarding pond production procedures is available mainly as industry know-how and is therefore often not transferable between regions.

To enable the expansion of flat oyster seed production in Europe a spatting pond production protocol was developed as part of a need identified by industry stakeholders. This work was part of case study eight in the Horizon 2020 AquaVitae project (Project No. 818173) (2019-2023), (https://aquavitaeproject.eu) which aimed to optimise flat oyster seed production (for more information see: https://aquavitaeproject.eu/case-study-on-improving-seed-availability-and-grow-out-of-native-and-non-native-oysters/) as well as the Blue Food Center (2021-2024), which supports the production of sustainable seafood in Sweden (for more information see: https://www.bluefood.se/). The protocol is based on two studies by Strand et al. (2018) and Wensveen (2022), and two sets of data. Strand et al. (2018) developed a protocol for pond production of flat oyster seed by interviewing spatting-pond operators and visiting multiple pond production sites. Following Strand et al. (2018), a pilot experiment was conducted in Sweden in 2021 to produce flat oyster seed in small on-land tanks. The pilot experiment failed to produce seed but provided valuable insights and data supporting an increased understanding of seed production in small-scale systems (Hannon et al. in prep.). Wensveen (2022) further contributed insights into how temperature affect flat oyster reproduction in both small-scale and large-scale ponds (Hannon et al. in prep.). Data for the large-scale ponds were obtained from Cartron Point Shellfish LTD (Cartron point New Quay, Ireland) through the AquaVitae project (Hannon et al. 2021). Together these studies suggest different protocols are needed for small-scale and large-scale systems. Therefore, this report aimed to compile a comprehensive set of methodologies for flat oyster seed production in both large-scale and small-scale pond systems.

The purpose of the protocols developed in this study are to serve as a guide for the initiation or optimisation pond production of oyster seed. It is worth noting that the success of production, and the details of the management strategies, will depend on the local context, such as environmental conditions. Although the protocol for large scale production is based on a commercial operation, the small-scale protocol has not yet been demonstrated at commercial scale, hence the suggested protocols should be validated on site and adapted to local contexts.

Glossary

Broodstock: Mature *Ostrea edulis* individuals used in aquaculture for the purpose of breeding.

Brooding: *Ostrea edulis* females with larvae developing in the mantle cavity.

Degree days: The accumulated temperature heat above a threshold temperature over time required for reproductive process to occur (e.g., spawning and swarming).

Green water culture: Addition of nutrients to a tank or a pond to produce microalgae that can be fed to broodstock, oyster larvae, or oyster seed.

Larvae: The developmental stages from fertilized eggs to settling planktonic stage of *Ostrea edulis* that follows swarming. Larvae feed and grow in the water column the before they settle on a suitable substrate.

Cultch: Whole shells or shell fragments used as substrate for seed settlement.

Seed: Newly settled juvenile *Ostrea edulis* oysters that have metamorphosed from the larval stage to a sedentary oyster. Seed have typically attached themselves to a hard surface where they begin to grow and develop into mature oysters.

Sickness: The morphological indicator of larval presence and developmental stage within the mantle cavity of the female oyster.

Spatting pond: a land-based structure constructed through excavation of soil to form a shallow basin with a high area to depth ratio. The basin is normally lined with some type of plastic lining to reduce leakage of water from the basin.

Spawning: The process in which male *O. edulis* release sperm into the water, leading to the internal fertilization of eggs within the female oysters. This includes the subsequent development of fertilized eggs into larvae, which are later released into the water column.

Spat-on-shell: oyster seed settled on cultch.

Swarming: The event where *O. edulis* larvae that have developed from the fertilized eggs inside the oyster females are released into the water column from broodstock females. This release marks the transition of larvae into their planktonic phase, where they disperse and feed before settling.

Weathering: The practice of leaving materials such as shell or cultch exposed to the air and environmental conditions for a period to reduce the risk of biosecurity threats by allowing natural degradation of potential harmful organic material.

Introduction

The study is divided into five chapters, guiding the reader from preparation of the ponds to harvest of the oyster seed, including information about how to manage the production. The chapters include:

(1) Preparations
(2) Pond maintenance
(3) Broodstock considerations
(4) Swarming and larvae settlement
(5) Seed harvest

Using the protocol, a producer should be able to prepare existing ponds for production, select suitable broodstock for larvae production, monitor larvae presence, abundance, development, harvest the seed after settlement, as well as manage and maintain the ponds throughout the operation.

Pre-conditions for starting flat oyster seed production

Before starting flat oyster seed production, several aspects should be considered, here referred to as pre-conditions. The pre-conditions can be divided into three categories: technological, environmental, and socioeconomic resources.

Infrastructure pre-conditions include access to land, good quality sea water, and access to other technical equipment. Ponds are normally excavated into the ground, but tanks of different sizes can also be used. The latter is preferred for small-scale production. Ponds are often lined with butyl rubber or other plastic materials and have a water inlet for filling at the beginning of the season and water renewal to compensate for evaporation. Butyl rubber has been the preferred pond liner choice in the past due to its flexibility and resistance to UV degradation. However, other plastic materials, such as HDPE, are now commonly used in pond aquaculture. HDPE offers strength, can be hot welded to form durable seams, and is a more cost-effective alternative to butyl rubber. The production site should also be in proximity to the ocean, as access to a supply of high-quality seawater is needed (Figure 1).

Figure 1. Oyster spatting pond in Rossmore, Ireland. The pond is located close to the sea, lined with butyl rubber and has an inlet for water. Picture Åsa Strand

Having access to the equipment used to operate the farm is also necessary. This includes tools to monitor the progress of seed production, for example sampling equipment, microscopes, counting chambers, and Secchi discs, as well as equipment necessary to maintain the ponds such as pumps, filters, and substrates for seed settlement. Access to electricity is needed for the use of electrical equipment like pumps. Heavy machinery, like tractors or forklifts, may be used, depending on the scale of the operation.

Natural pre-conditions include abiotic conditions that will affect the oysters' ability to reproduce and for larvae and seed to survive. Key factors to consider are temperature, salinity, oxygen, pH, food availability, and absence of polluting substances and environmental toxins. Ponds provide means to adjust some environmental parameters but does not allow as fine of control as hatcheries, hence selecting a site where the environmental conditions are suitable for flat oysters is key. Access to broodstock oysters, ideally locally sourced, is also a requirement. Translocations of oysters infers significant biosecurity issues such as dispersal of non-native and invasive species, pathogens, and diseases, and should if possible be avoided.

Socioeconomic resources include access to workforce and markets as well as existence of regulatory structures. A well-functioning operation will need access to competent personnel. Legal requirements for building and or operating spatting ponds must be followed and may differ between countries. To have a financially successful operation, the producer also needs access to capital and to a market for the produced seed, including options for easy logistics and distribution of the product to the market or to grow-out areas.

Overview of the reproductive biology of flat oysters

To understand how to best produce flat oysters seed, it is advantageous to understand the oyster's reproductive biology. Flat oysters are sequential hermaphrodites, which means that individuals alternate between being male and female and may change sex several times during a single season (Orton 1936, FAO 2023). During the reproductive season, males will release their sperm into the water, known as spawning. Females consequently filter the water, inhaling the sperm, in the same way as they filter for food. Inside the female, the eggs produced by the female will be fertilized. The fertilized eggs are stored in the pallial (mantle) cavity for a duration of about 8-10 days, called brooding, after which the larvae are released into the water, called swarming (Colsoul et al. 2021, Rendle et al. 2023; Figure 2). Depending on temperature, the time between spawning and swarming is about 9-11 days (Gustafsson et al. 2023). The planktonic larvae will then spend about 11-18 days in the water column before settling (Gustafsson et al. 2023).

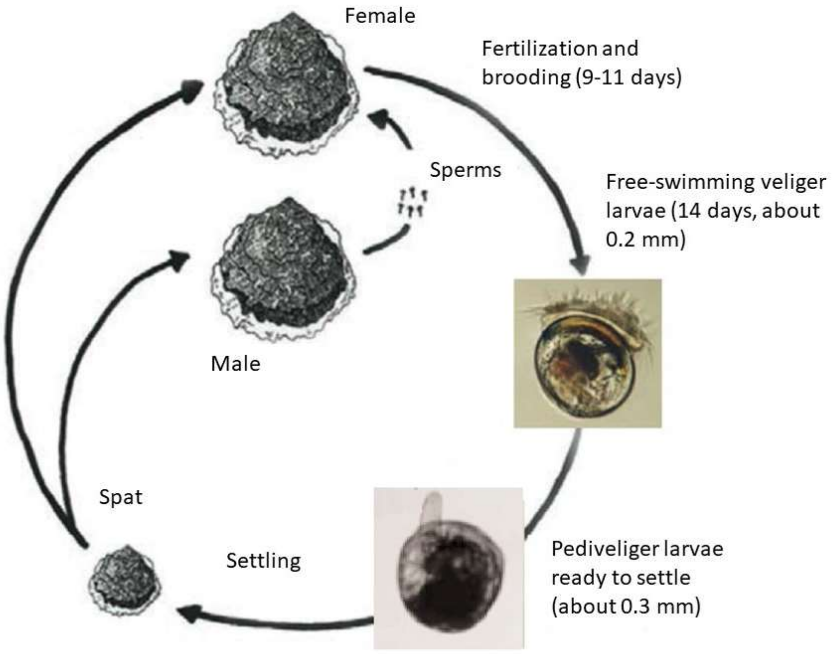

Figure 2. The life cycle of Ostrea edulis (modified from Lindegarth 2012).

Reproduction in flat oysters is triggered by temperature. The heat exposure required for flat oyster spawning and swarming can been expressed as "degree days". Degree days represent the accumulated heat above a reproductive threshold temperature over a period of time. There are two models that predict flat oyster spawning based on temperature; one predicts spawning after 150 degree-days (10 °C threshold for gamete formation and maturation, Cole 1942, Marteil 1976), while the other predicts spawning after about 400 degree-days (7 °C threshold for gonad development, Mann 1979). Further accounting for the temperature dependent brooding time, the models can be used to predict larval swarming in the wild (Maathuis et al. 2020) and in a pond setting (Wensveen 2022).

In large scale ponds in Ireland, flat oyster swarming occurred after 504-531 degree days, aligning with the Mann's model of spawning (Mann 1979) and an estimated brooding time of 100 degree days (Wensveen 2022). The first larvae peaks (>15 larvae/L) in the ponds showed the best fit to the swarming model (Wensveen 2022). In a small-scale pond setting in Sweden, however, the correspondence between predicted and observed larval occurrences varied. While an initial swarming event was delayed (about 830 degree days) relative to the predicted (559 degree days), a second swarming event aligned well with the predictions (Wensveen 2022).

In Scandinavia, flat oysters typically enter the reproductive season in June when temperatures reach about 16 – 18 °C, but the spawning onset temperature varies between regions and ecotypes (Korringa 1957, Strand et al. 2018). For instance, flat oysters in Norwegian breeding polls spawn at a temperature of 25 °C, whereas flat oysters in Spain have been reported to spawn already at a temperature of about 13 °C (Colsoul et al. 2021). Food availability is another critical aspect, with high food availability of suitable microalgae species promoting gonad formation and high-quality gametes (Helm et al. 2004). Spawning and swarming may also be triggered by high food availability. Reproduction is also limited by salinity and is unlikely to occur below 15 psu (FAO 2023).

Description of large-scale and small-scale pond production

Large-scale and **small-scale ponds** differ in their physical, chemical, and biological properties. In this protocol, large-scale ponds are defined as > 500 m^3. Most commercial ponds in Europe have a volume of 500 – 1 000 m^3 (often 1-2 m deep, Strand et al. 2018). Small-scale ponds are defined as < 100 m^3 in volume. It is important to note that the differences inferred by size of the ponds causes a successive transition from one state to the other, hence the general descriptions provided should be viewed with this in mind.

Characteristic of **large-scale ponds** are that they establish their own ecosystem, including primary production of microalgae and denitrifying bacteria, and require relatively little maintenance after the initial preparation. The ponds have a natural stability (but see below) and maintain the desired conditions even if environmental factors fluctuate (e.g., temperature). Operations can be run with water exchange rates ranging from replacing only evaporated water to a continuous flow (although this may infer reductions in cost efficiency) until larvae are detected, at which point water exchange is stopped. Inlet water can be used either untreated or filtered to minimize the presence of fouling organisms. A large surface area and shallow depth is beneficial as it allows for wind-driven water mixing and subsequent oxygenation (Strand and Sühnel 2022), as well as quick heating of the water by the sun. This is facilitated by the ponds often being lined with black butyl rubber which absorbs sunlight effectively.

Large-scale ponds can be operated using naturally available nutrients and microalgae in the seawater to sustain the production. Nutrients can, however, also be supplemented to boost the naturally occurring microalgae production and thereby supporting oyster larvae with food (Strand and Sühnel 2022). High food availability and microalgae of the right types is essential for successful production, and normally the system maintains a good balance; however, in some cases, harmful algae may proliferate in the ponds causing variability in the success of pond production. Ponds can hold many broodstock individuals, which leads to a high genetic diversity of the produced seed and reduces the likelihood of reproduction failure. Large-scale ponds also have a proven record of successfully producing seed in commercial settings. The disadvantage of large-scale ponds is that as semi-open systems they are exposed to variations in environmental conditions and other factors (for example extreme temperatures, rainfall, and contamination) for which there are few mitigation strategies except water exchange. They also require a large land area and a relatively large initial investment.

The size of **small-scale ponds** infers different challenges compared to large ponds. Small ponds are normally not dug from the ground but constitutes different sizes and shapes of land-based tanks. For simplicity we refer to these as ponds from here on. One aspect is the low volume of water, making the pond more vulnerable to environmental changes. The low volume of water also contains limited nutrients and microalgae for the oysters to feed on, hence requiring different solutions to increase food availability for broodstock, larvae, and seed. Alternatives to mitigate the lack of food is to use a flow-through system to renew food supply (with a banjo filter covering the outflow of the tank to prevent larvae from being washed out), provide microalgae from a feeding tank to the larvae tanks, or fertilizing the tank to enhance production. The low water volume also increases the risk of suboptimal microalgae composition for the larvae and unstable microalgae communities where opportunistic (and sometimes harmful) microalgae may thrive. To combat sub-optimal microalgae communities, tanks can be inoculated with the "right" microalgae species. Despite these challenges, water quality challenges in small-scale ponds can more easily be mitigated (although close monitoring is required to detect changes in time) and small-scale ponds are relatively inexpensive and have a smaller area footprint compared to large ponds. Appearance and performance of larvae is also easy to monitor. However, much remains to be explored in terms of management of small-scale tanks and the recommendations provided in this protocol should therefore be evaluated further before initiation of commercial production.

Protocol overview

This section provides a summary of each of the five chapters of the pond production protocol. Each chapter begins with a section outlining general guidelines applicable to both **large-scale** and **small-scale** pond systems. Specific recommendations for large-scale systems and small-scale systems are then provided in separate paragraphs where relevant. The chapters are illustrated in figure 3, showing at which time of the year each pond production step should take place. The timing may need to be adjusted to local conditions, but the relative order of activities remain across regions. More information is provided in each chapter.

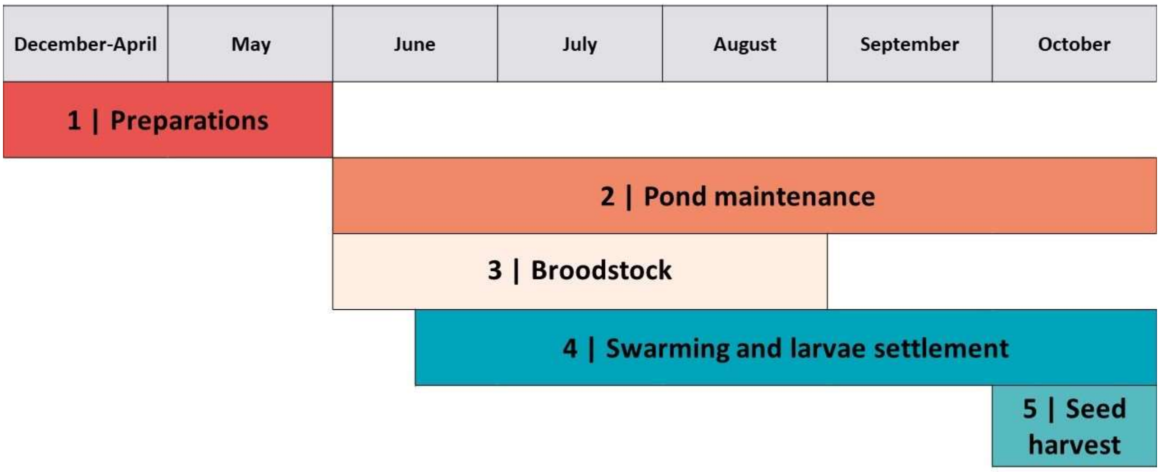

Figure 3. Annual timeline for a flat oyster seed pond operation. Time frames are given for an operation in Sweden and may change depending on local environmental conditions. Numbers correspond to the chapters in the protocol.

Preparations

December-April:

- Keep ponds empty of water over winter between production seasons. Use the time to repair ponds if needed.
- Prepare cultch using weathering if shells are to be used as substrate for seed settlement

April-May:

- Pond preparation
  - Clean ponds from sediment and other debris.
  - Fill ponds with water in spring to allow for establishment of a natural ecosystem including denitrifying bacteria and a biofilm.
  - Filter intake water through a 30 µm filter to remove larvae of fouling organisms when filling the pond. If the pond will be inoculated with microalgae, a filter of 2-5 µm should be used.
  - To achieve an efficient heating in **large-scale ponds**, flow should be maintained low. For **small-scale ponds**, adjust the water flow to ensure stable temperature conditions as temperature may increase during the day and decrease during night. Temperature may be increased using heaters. Also monitor microalgae composition closely in small ponds and empty and refill the pond if a bloom of harmful microalgae is detected.
- Settlement substrate preparation
  - Prepare the chosen settlement substrate, either cultch or collectors such as lime coupelles four weeks in advance of spawning (in Sweden, spawning typically starts in June). If cultch is used make sure that it is clean and that no biological residues remain before it is deployed in the ponds.

- Put the substrate in the water one to two weeks before spawning to allow formation of a biofilm on the collector surfaces before settlement. Collectors can be hung from ropes mounted across the ponds, supported by buoys. Cultch can be placed directly on the bottom or hanging in bags.

For more information about pond preparation see chapter 1.

Pond maintenance

June-October:

- To enable successful seed production, ensure that the environmental conditions in the ponds (salinity, temperature, pH and alkalinity, food availability and oxygen) are maintained by regular monitoring (optimally daily) from the start to the end of the production cycle. **Small-scale ponds** are more prone to suboptimal environmental conditions and food shortage than **large-scale ponds**. Continuous water flow and the use of banjo filters to prevent larvae being flushed out from the tank may be used to reduce this risk.
- Act if deviations from optimal conditions are noticed (see chapter 2 for suggested solutions to common problems)

For more information about pond maintenance, see chapter 2.

Broodstock

May-June:

- Monitor progression of spawning and brooding of oysters in wild populations, preferably adjacent to the location of the ponds, by initiating sampling of wild broodstock early in the season (in Sweden in May but may vary depending on region). To assess whether females are in the brooding stage, inspect them for "sickness" (i.e. larvae in the pallial cavity and around mantle and gill) by shucking several oysters.
- Once brooding oysters are detected, collect broodstock (7-10 cm in length) for the ponds. In Scandinavia, this normally occurs in June when sea temperatures reach about 16 – 18 °C.
- Broodstock can be placed in oyster production baskets hung in the pond or placed on the bottom of the pond (only recommended for **large-scale ponds**). Do not overlap broodstock oysters as this may cause stress or death. In **large-scale ponds**, distribute one oyster per m^3 water. In **small-scale ponds**, distribute up to 10 oysters per m^3 to ensure enough females in the pond but make sure to monitor oxygen conditions and food availability closely.
- Ensure that the ponds have high food availability of suitable microalgae species to maintain the condition of the broodstock and prepare for swarming.

June-August:

- Remove broodstock oysters after swarming. If the broodstock oysters are placed on the bottom of **large-scale ponds** it is best to leave them until the seed are harvested to not contaminate the ponds during oyster extraction.

For more information about broodstock, see chapter 3.

Swarming and larvae settlement

June:

- Sample the pond water daily to monitor the occurrence, quantity, size, and settlement of larvae. Larvae will be released in the water within days to weeks after broodstock are placed in the pond depending on sickness-stage at transfer from the sea and temperature in the pond.
- Ensure that settlement substrates are deployed in the pond, free-swimming larvae typically settle within 11 - 18 days.
- Deploy and retrieve settlement substrate (ceramic plates, shells or other substrate) on a regular basis (weekly) to monitor settlement.

For more information about larvae monitoring and settlement see chapter 4.

Seed harvest

September-November:

- Harvest the settled seed three to four months after settlement.
- Transport the seed in room temperature in moist conditions for a maximum of 48 h, or in cold storage (air temperature 5-15 °C) for no more than 4 days, to ensure high seed survival. Do not expose the seed to very low temperatures (< 0 – 5 °C), to very high temperatures (> 20 - 25 °C) or expose them to air for longer periods.

For more information about seed harvest, see chapter 5.

1 | Preparations

Pond preparations

After a production cycle is finished, the ponds should be emptied and cleaned from sediment accumulated on the bottom of the ponds and thereafter the ponds should be kept empty during winter (November to April). Repairs can be made during this period. In April to May, approximately one month prior to the start of production, the ponds should be filled with surface seawater. By filling the ponds well ahead of initiation of production, a natural ecosystem is allowed to form, which helps to stabilize the environment in the pond. This includes, for instance, the establishment of denitrifying bacteria and establishment of a biofilm.

The inlet water may be filtered, though not all commercial operations follow this step. Filtering to 30 µm will remove most organisms that can have a negative effect on the seed production, such as zooplankton and eggs or larvae of fouling species and predators. If Pacific oysters as a fouling species is not of concern, then 40 - 60 µm filter may be sufficient. If the pond will be inoculated with microalgae, a filter of 2-5 µm should be used. Drum filters are often used in spatting-pond operations, but other filters like sand filters are also used. Multiple filters may be necessary to fulfill the capacity need of the production, depending on size and number of the ponds.

For **large scale ponds**, filters should ideally have a capacity to filter 125 m^3 water per hour per pond. This allows a large-scale pond of 1 000 m^3 to be filled in half a working day, and to exchange 25% of its volume (250 m^3) in two hours. Both metrics are important for rapid response to suboptimal water quality conditions if needed during the production period. Filling up the ponds ahead of production also allows the pond water to heat up. Ponds are often lined with black butyl tarp to help attract and maintain heat. High temperatures help the oysters' reproduction and the larvae development and survival. To achieve an efficient heating from the sun during the preparation period, flow should be maintained low after the initial filling.

The capacity of filters used in **small-scale ponds** should correspond to the needed exchange rate, which in turn is impacted by food availability, and water quality characteristics. Therefore, the filter capacity should be dimensioned for a worst-case scenario where the pond volume must be exchanged once or even several times, during a day. The smaller the tank, the more likely that a higher exchange rate is needed to maintain food availability. For a 10 m^3 pond with an exchange rate of 100% per day, a filter capacity of just over 400 liters per hour will be required. This could also supply a 5 m^3 pond with 100% water exchange twice a day, or a 20 m^3 pond with an exchange rate of 50% per day. It may be difficult to achieve an increase in water temperature in small-scale ponds, depending on pond size, as the water in small ponds will increase in temperature during the days, but will lose heat during the nights if air temperature drops below the pond water temperature. This is not critical during the preparation phase, but a stable temperature should be maintained once broodstock is deployed in the ponds, hence the preparation time can be used to adjust the flow of the water to ensure stable temperature conditions. It may be possible to regulate temperature in small-scale ponds using heaters (see section 2.2). Microalgae composition in the pond should also be monitored closely, and ponds should be emptied and refilled if a bloom of harmful microalgae is noticed.

Settlement substrate preparation

Different settlement substrates can be used in oyster seed production in ponds. The choice of substrate depends on the scale and application of the production and personal preferences.

For restorative or bottom-culture production purposes, it is common to use cultch, whole or crushed shells of oysters or blue mussels, as settling substrate. Seed settled on cultch may be referred to as "spat-on-shell" and often several seed settle on one piece of substrate, potentially creating a cluster of oysters as they grow. Shells sourced for the use as cultch may contain organic materials (oyster or mussel meat residues and fouling), hence constituting a biosecurity risk that needs to be managed (zu Ermgassen et al. 2020). Shells intended for use as substrate in pond production therefore should be cleaned of organic material before the start of production. This can be achieved by weathering, where shells are placed outdoors and exposed to the elements for 6 - 12 months. The shells should be turned every two months (zu Ermgassen et al. 2020). There may be specific rules related to weathering (for example related to waste treatment or animal by-products or waste) and therefore local authorities should be approached before weathering is initiated. If cultch is used make sure that it is clean and that no biological residues remain before it is deployed in the ponds. Cultch can be placed directly on the bottom in the ponds or hanging in bags ropes mounted across the ponds, supported by buoys.

For grow-out of oysters in more intensive production systems, single seed are often preferred. Production of single seeds can be achieved using different seed collectors. Collectors referred to as coupelles are commonly used for sea-based seed-collection (Figure 4). Coupelles should be prepared at least two, preferably four weeks ahead of expected broodstock placement in the ponds by dipping the collectors in a lime (Calcium oxide), or lime and cement, mix. Cement mixtures are more stable and are often used for suspended, sea-based, deployment, where waves and currents expose the collectors to rough conditions that can make the lime come off. The lime-cement mixture may consist of 36 - 45% cement (B. Colsoul, personal communication; Strand et al. in prep.). Limed coupelles need two weeks to dry and harden (depending on the weather conditions) and should be left to harden in the shade as direct sunlight may cause the lime cover to crack due to drying too quickly, and protected from rain, or even indoor in a well-ventilated building. There are also other types of collectors that can be used, see Strand and Sühnel (2022).

Figure 4. Limed coupelles hung to dry in preparation for deployment. Picture IVL

All collector types should be placed in the ponds two weeks before introduction of broodstock to allow for formation of a biofilm. Bacterial biofilms enhance larval settlement success (Tamburri et al. 2008, Rodrigues-Perez et al. 2019), but extensive biofouling should be avoided as that can have the opposite effect (Korringa 1940). Biofouling is of lesser concern if the inlet water to the ponds have been filtered. Collectors can be hung from ropes mounted across the ponds or supported by buoys. The entire pond area may be filled with collectors as long as it does not inhibit access to the pond or broodstock, nor prevent circulation of water in the pond. Coupelles can also be left standing on the bottom of the pond in racks. Before putting coupelles (or any lime material) in **small-scale** ponds, the collectors should be soaked in freshwater or in filtered seawater to reduce the risk of changes in water pH and alkalinity which may be more pronounced the smaller the pond is. In **large-scale ponds**, coupelles can be placed in the pond as soon as they are dried and hardened. Due to the large volume of water, pH or alkalinity will not be affected significantly.

2 | Pond maintenance

There is a range of abiotic (e.g. temperature, salinity, pH, alkalinity, oxygen) and biotic (food availability and quality) factors that need to be managed during the production of oyster seed. The factors, how they can be monitored, their optimal ranges, and suggested management, are presented in tables 1 and 2.

Table 1. Key environmental factors that should be managed during pond production of oyster seed, how to measure them and monitoring frequency.

Factor	How to measure	Frequency of measurement
Salinity	Salinity meter, multimeter, other	Weekly
Temperature	Thermometer, HOBO logger	Daily-Weekly
pH and alkalinity	pH meter, multimeter	Daily-Weekly
Oxygen	Dissolved oxygen meter, both at bottom of pond and top	Daily
Food	Secchi disc, chlorometer, light logger, microalgae counting chamber	Daily

Table 2. Key environmental factors that should be managed during pond production of oyster seed, their optimal ranges, and possible mitigation actions if the factors deviate from the desired range.

Factor	Optimal range	How to manage
Salinity	25 - 35 psu	Increase salinity by evaporation, by limiting freshwater input (rain, estuarine source) or by adding salt. Reduce salinity by exchanging water in pond. Do not add freshwater. Larvae are more sensitive to salinity changes than adults.
Temperature	18 - 24 °C (depending on the ecotype)	Increase temperature: fill ponds early in the season, use dark colored materials, and/or use coil heaters (for **small-scale ponds**). Reduce temperature: Provide shading and exchange water. Avoid rapid and large fluctuations in temperature.
pH and alkalinity	pH > 8, ideally 8.2. Alkalinity 50 - 150 mg/L (or 150 ppm) of $CaCO_3$ equivalents. Alkalinity should never go below 20 mg/L.	Alkalinity is more important than pH. Na_2CO_3 (sodium carbonate) can be added to increase pH and alkalinity. 18 g of Na_2CO_3 in 1 m^3 of water will increase alkalinity by 10 ppm.
Oxygen	> 70 % dissolved oxygen in all parts of the pond	Increase oxygen availability: increase water flow, exchange water or provide oxygenation through bubbling air.

Food	< 1 m visibility using Secchi disc. 0.1 – 1 mg/l chl a	Increase food availability: increased flowthrough of surface water. Supplement of microalgae. Fertilization (can cause low pH or harmful algae species).
Water flow	Continuous water movement	Increase circulation of water: increase inflow, use pumps, add paddle wheel.

It can be more challenging to maintain good water quality in small-scale compared to large scale ponds due to the low water volume. Automatic monitoring using digital solutions, including alarm functions for suboptimal levels, facilitates water quality management but are expensive.

2.1 | Salinity

Salinity is often referred to as practical salinity units, psu. Salinity in the ponds should be kept > 25 psu and < 35 psu (Strand et al. 2018). Oysters can survive salinities below 20 psu and above 35 psu, but their growth may be severely affected and at prolonged exposure of sub-optimal salinities oysters may die (Hutchinson and Hawkins 1992). Larvae are more sensitive than adults (McFarland et al. 2022). Pond salinity may decrease due to inflow of freshwater through rainfall or if the supply of water to the pond is near a river or in an estuary. Salinity may increase due to evaporation of water when there is little or no exchange of water in the pond. Salinity levels should be monitored weekly using a salinity meter in different parts of the ponds. Although rare, the ponds may become stratified by salinity. By measuring in different parts of the pond and not just the surface, stratification can be detected and mitigated. Stratification may result in suboptimal oxygen conditions.

The easiest way to manage too low or too high salinities in a pond is to exchange water unless the intake water is brackish (see above). For low salinities, salt can also be added. Salt is recommended to be sodium chloride and not contain additives like iodine. To achieve a salinity of 35 psu, 35 g of salt should be added to one liter of water. If increasing the salinity of a pond using salt, it is recommended to dissolve the salt across the pond. Alternatively, it can be dissolved into a small volume of water beforehand, and thereafter poured into the pond. This is done to provide an even salinity throughout the pond. Monitoring of weather forecasts for high temperatures, causing evaporation, or rainfall, causing freshwater input, can enable prediction of salinity changes and early action. This may be beneficial for large ponds as it may be challenging to exchange large amounts of water rapidly.

In **small-scale ponds**, aspects influencing salinity may cause large fluctuations due to the low water volumes in the ponds. Often, however, small ponds are managed with a continuous water flow, reducing the risk of salinity variations. The ponds may be protected from rain by covering although long term cover may impact plankton production (as microalgae production is dependent on access to light) and plastic cover may also act to create a greenhouse effect in the pond, which could increase temperature. To prevent a greenhouse effect, covers can be raised above the ponds, allowing for air circulation.

2.2 | Temperature

Temperature is a critical aspect in pond production to ensure successful reproduction and larvae development. The desired temperatures can differ for different populations of oysters of the same species. In Sweden, the optimal pond temperature is approximately 20 °C and the pond should be at least 18 °C in June and July and not exceeding 25 °C (Strand et al. 2018). Temperatures outside this range can impact spawning, swarming, larvae development and survival and settlement success (Prado et al. 2016, Eymann et al. 2020). Larvae are more sensitive than adults to rapid temperature fluctuations (Korringa 1940). The temperature should be monitored daily using a thermometer or a temperature logger, preferably in different parts of the ponds. Like salinity, ponds may become stratified by temperature and affect oxygen conditions, so temperature should be measured in different parts of the pond and not just the surface to detect stratification.

Monitoring the weather forecast may aid in predicting changes in temperature. To reduce the temperature of a pond, water can be exchanged. Alternatively, the pond can be shaded from direct sunlight by using shrubs or trees (**large-scale ponds**) or cover (**small-scale ponds**). Shading is relatively straightforward and simply aims to prevent increased temperatures from solar radiation. For **small-scale ponds**, tarps or shading cloths can be installed above the ponds. Care should be taken so that the cover material does not collect rainwater that is then spilled into the pond.

Temperature in ponds can be increased by having a large surface to depth area and dark materials for lining the ponds. For **small-scale ponds** which normally have a higher water exchange rate than large-scale ponds, electrical spiral coil heaters or other water heat exchanges may be used to increase temperature. If heaters are used, water flow must be maintained to prevent to enable an even temperature throughout the entire pond.

2.3 | pH and alkalinity

The optimum pH for oyster seed production is 8.0-8.2 (Strand et al. 2018). If the pH is above 8, no adjustments are needed. Alkalinity and water hardness is more crucial. Alkalinity is the buffering capacity of the water to neutralize acids, i.e. preventing the water from becoming acidic. The alkalinity should be between 50 - 150 mg/L (or 50 - 150 ppm, mg/L and ppm can be used interchangeably) of $CaCO_3$ equivalents and should not go below 20 mg/L. Alkalinity can also be expressed as dKH (degrees of carbonate hardness). One dKH is equal to 17.9 mg/L $CaCO_3$ equivalents. The water hardness represents the availability of calcium- (Ca^{2+}) and magnesium- (Mg^{2+}) ions, which are both important for oyster shell formation. The water hardness should be between 75 - 200 mg/L (Strand et al. 2018). pH and alkalinity can be measured with a pH meter or a multimeter and should also be measured in different parts of the pond.

Alkalinity and hardness are difficult to manage in ponds as adding $CaCO_3$ may increase or decrease alkalinity depending on the water chemistry. If it is necessary to adjust the pH or alkalinity, sodium carbonate (Na_2CO_3) may be used to increase pH. Adding 18 g of Na_2CO_3 to 1 m^3 of water will raise alkalinity by 10 ppm. To reduce pH or alkalinity, water exchange is recommended, as seawater normally has the appropriate pH and alkalinity levels. As for the other factors, **small-scale ponds** are more sensitive to actions that may impact their pH or alkalinity such as fertilizing than large-scale ponds.

2.4 | Oxygen

Oxygen should be kept above 70% saturation, or 3.5 mg/L, at all times (Strand et al. 2018). Oxygen saturation can be measured using an oxygen sensor. As for the other abiotic conditions, oxygen levels should be measured in all parts of the pond, but particularly in bottom waters. If the oxygen saturation is approaching 70%, then it should immediately be managed. Regular monitoring will enable early action against low oxygen conditions. For instance, if oxygen levels are decreasing continuously, preventive measures such as increased water exchange for a period of time may be warranted to prevent oxygen levels falling below the 70% saturation threshold. Since dissolved oxygen levels are inversely related to temperature, warmer water holds less oxygen, making regular monitoring particularly important during periods of high temperatures when oxygen depletion is more likely.

Normally, wind mixing of the water is enough to ensure good oxygen conditions in **large-scale ponds**; however, in conditions with low wind, or during periods of high biological activity such as algal blooms, methods to prevent oxygen deficiencies may be needed. Flow-through of water and active mixing of the water column using pumps or paddle wheels are effective ways to provide oxygenation of the water (Strand et al. 2018). Paddle wheels are also very energy efficient. For example, a paddle wheel designed by Guernsey Sea Farms can move 30 000 liters of seawater per hour using a 1Kw motor (Syvret pers. communication). Paddle wheels are large turning wheels that continuously disturb the water surface, thus oxygenating the water. Paddle wheels are a popular method to prevent deoxygenation in many forms of land-based aquaculture.

For **small-scale ponds**, water exchange and pumps generating bubbles of air or oxygen in the pond are common ways of ensuring good oxygen conditions. Air bubbling is not commonly used in large-scale ponds. In the case of bubbling, it is important to ensure gentle bubbling as excessive turbulence can disturb free-swimming larvae and potentially kill them. Bubbles can be placed under banjo filters in small-scale ponds to reduce the risk of clogging.

2.5 | Food availability

Access to food is a limiting factor for the survival and success of both adult oysters, larvae and oyster seed. Food availability can either be measured as chlorophyll-α concentration, or by observing water transparency using a Secchi disc. Chlorophyll concentration should be between 0.1 – 1 mg/L. Chlorophyll can be measured using a chlorometer. A Secchi disc or a light sensor can be used to determine the transparency of the water, which is an indication of presence of phytoplankton in the water. If the Secchi disc is not visible beyond 1 m depth, then there is a high concentration of phytoplankton in the pond.

There are several ways to ensure the oysters and larvae are sufficiently fed. One way is to maintain a continuous flow of water to provide the oysters with a continuously renewed inflow of microalgae and nutrients. However, in high oyster densities, the flow required to provide sufficient food may be very high, and alternative ways may be needed. Fertilization of ponds will provide nutrients, thereby promoting production of microalgae. Nutrient supplementation can be achieved by addition of artificial or natural fertilizers to the ponds, such as crushed crab shell, chicken feces or cow manure. In the case of natural fertilizers, they should be added in quantities of 5 kg per 100 m^3 of water in netted bags, allowing them to be easily removed. Natural fertilizers should be removed as soon as chlorophyll-α levels are back to a desirable level (Strand et al, 2018). Artificial fertilizers should be supplied according to the Redfield molecular ratio, which is 106:16:1 C:N:P (Redfield, 1958). While this ratio is not perfect for all primary producers, it provides a good basis for nutrient supplementation to the ponds. Certain species of microalgae will prefer a slightly different ratio or have more specific requirements. Diatoms for example also need addition of silica. In this case, the ratio used should be 106:15:16:1 (C:N:P:Si; Brzeski and Newkirk 1997).

To reduce the risk of contamination of the ponds with fertilizers, it is possible to also cultivate microalgae in specific algae ponds or tanks (depending on the quantities needed) that can be fed to the oysters in the spatting-ponds. If the production is based on the natural occurring microalgae, this is often referred to as "green water culture". It is also possible to inoculate ponds or tanks with specific species. Both methods rely on having separate ponds or tanks available for algae production. Green water culture is an extensive method, in which the pond is fertilized, as described above, with the aim of creating a natural bloom of microalgae. To maintain light penetration during the microalgae production, the pond should have a water depth of about 1 m, with thorough mixing of the water. The inlet water to the pond should be filtered as described in chapter 1. As the water is sourced from the ocean, and the ponds are open to the air, green water culture is prone to contamination and will always contain many different species of algae. Typically, the water in the green water culture is fertilized 3-7 days before planned use of the biomass.

When inoculating with one or several specific species, the inlet water to the ponds should be filtered to 2 μm. In doing so, only the selected species with a known nutritional profile will be produced and later fed to the oysters. Production of specific species requires a more complex pond production set-up, more investment, and more time to manage the microalgae production. Green water culture may be preferred for producers that do not have expert knowledge on the topic. All forms of food supply enhancement to the oysters also infer increased risks. The use of fertilizers may create large blooms of microalgae, which later results in a system crash. The crash reduces the food supply and may result in anoxic conditions and a decrease in pH as dead algal cells are degraded or consumed by other microorganisms.

In addition to food supply, the quality of food and the diversity of microalgae species should also be considered as they are important for successful larvae and seed production. Oyster larvae have been shown to only utilize naked flagellates as food, while unicellular green algae are unsuitable due to their cellulose or hemicellulose cell walls, which cannot be digested efficiently. For settled seed, certain green algae, such as *Coccomyxa littoralis*, can support growth, although pure cultures of this alga may not be optimal. Common microalgae normally fed to flat oysters in hatchery production include flagellates and diatoms, which consequently are types of microalgae that should be in high abundance in the ponds. Specifically flagellates such *as Isochrysis galbana, Pavlova lutheri,* and *Tetraselmis sp*., and diatoms such as *Chaetoceros calcitrans, Chaetoceros muelleri, Chaetoceros gracilis, Thalassiosira* *pseudonana*, and *Nannochloropsis sp.*, the dinoflagellates in genus *Dinophysis sp.*, and the cryptophytes *Rhodomonas spp*., are beneficial (Strand et al. 2018, Sühnel and Strand 2022). Observations indicate that food quality may decrease with time in terms of abundance and composition (personal observation, M. Steinke). Food quality, i.e. microalgae species composition, can be assessed using water samples and a microscope.

Harmful microalgae can also proliferate in the ponds, causing mortalities of larvae and oyster seed. Examples include *Prorocentrum cordatum*, which can disrupt oyster larval digestion (Wikfors and Smolowitz 1995) and lead to high mortality during the early feeding period (Luckenbach et al. 1993). *P. cordatum* e.g. dominated the microalgal community in the small-scale production pond experiment in Sweden, causing extensive larvae mortalities (Wensween, 2022).

3 | Broodstock

Ideally, the oysters should spawn in the ocean, and brooding females can then be collected and transferred to the ponds before swarming. This reduces the problems with low genetic variability and the need to produce food for the broodstock. In Scandinavia, spawning typically occurs in June when temperatures reach above 16 °C but may be earlier or later depending on geographical region (see introduction). It is therefore recommended that the progression of maturation, spawning and brooding of oysters in wild population, preferably located adjacent to the location of the ponds, is monitored by initiating sampling of wild broodstock early in the season (in Sweden in May). Broodstock oysters should be between 7 - 10 cm in length (Walne 1979, Goulletquer 2004, Strand et al. 2018). To assess whether females are in the brooding stage, they can be inspected for "sickness" by shucking several oysters. Sickness (white, grey, black) is a term to describe the occurrence of larvae within the oysters (Figure 5). Consequently, if larvae are visible on the gills (which are colored black or grey), then the oysters are approaching swarming and should be collected.

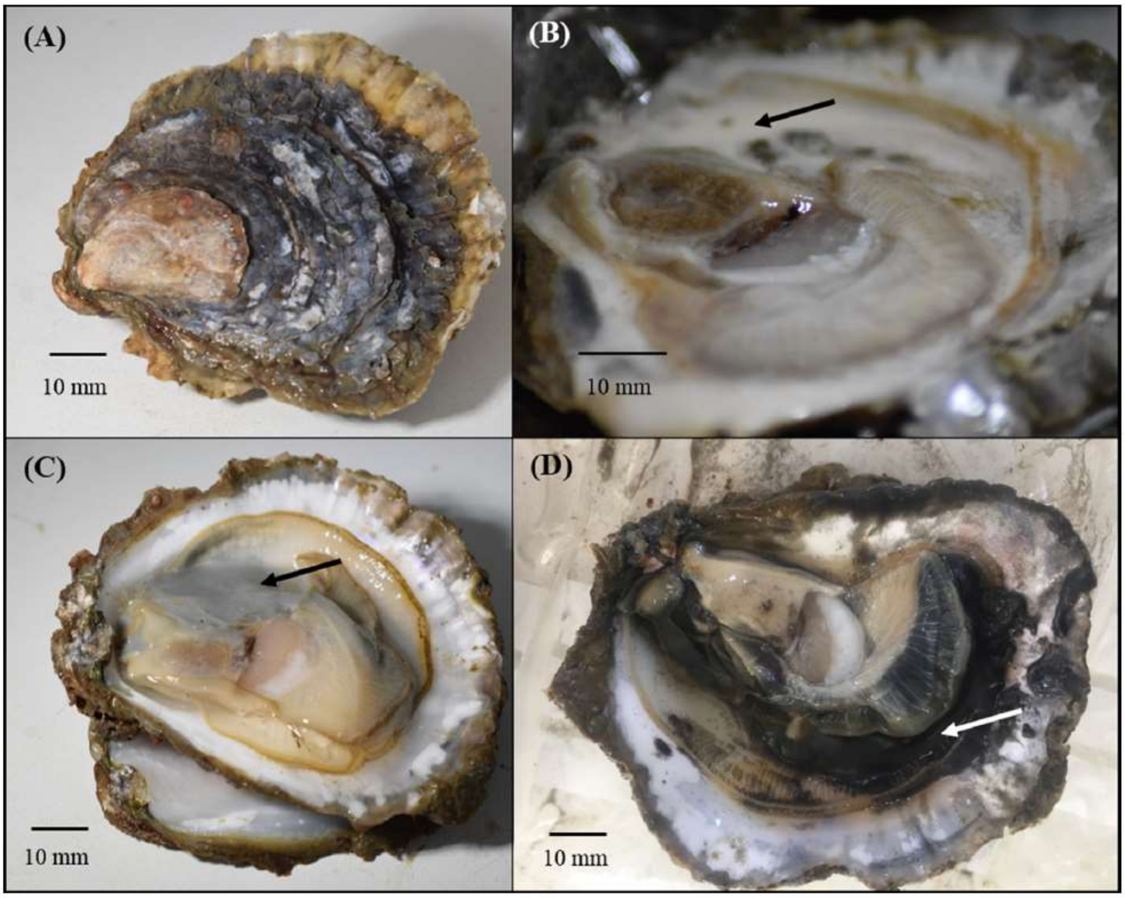

Figure 5. From Helmer et al (2020). "Sickness"-stages displayed in mature Ostrea edulis individuals. (A) Brooding oyster prior to shucking; brooding oysters containing larvae referred to as (B) white "sick", (C) grey "sick" and (D) black "sick" stages of development. Larvae within the pallial cavity in and around the gill and mantle structures, indicated by arrows.

Once collected, broodstock can be placed in standard oyster production baskets hung in the pond or placed on the bottom of the pond (only recommended for **large-scale ponds**). The broodstock individuals should lie separately in the cage or on the bottom and not be stacked or overlap to reduce stress. Placing broodstock directly on the bottom of the pond is cheaper but makes them harder to remove once swarming is complete. By not removing broodstock after swarming, they will compete with the larvae for food, which is not desirable. In **large-scale ponds**, one oyster per m^3 water should be used. In **small-scale ponds**, up to 10 oysters per m^3 can be used to ensure enough females in the pond (Wensveen, 2022), but care must be taken not to overstock the pond, which could lead to rapid depletion of food and oxygen.

4 | Swarming and larvae settlement

Swarming (i.e. release of larvae) should occur after about 10 days after placing the broodstock in the pond if temperature is about 18-20 °C (Figure 7, Strand et al. 2018). Time to swarming will however depend on the maturation status of the females which may differ between individuals, and pond temperature, with faster release of larvae at higher temperatures. Once larvae are released, the pelagic larval stage spans about 11 - 18 days (Gustafsson et al. 2023) but is normally about 14 - 16 days at a temperature of 20 °C (Strand et al. 2018).

Larvae should be monitored in terms of occurrence, quantity, sizes, and settlement through sampling of pond water. To monitor the larvae, water should be sampled daily (preferably) until few, or no free-swimming larvae are observed. Sampling is done by extracting a known volume of water, between 300 – 1 000 ml for large ponds and about 250 ml for small ponds, in different areas and at different depths in the ponds. This will give a representation of what is present in the pond. The sampled water should be filtered using a 100 µm filter to collect the oyster larvae, which are then gently rinsed into a container and counted using a counting chamber (Figure 6) and a stereo microscope at 10x magnification. The water in the counting chamber should be shallow, which prevents larvae overlapping each other, making counting easier.

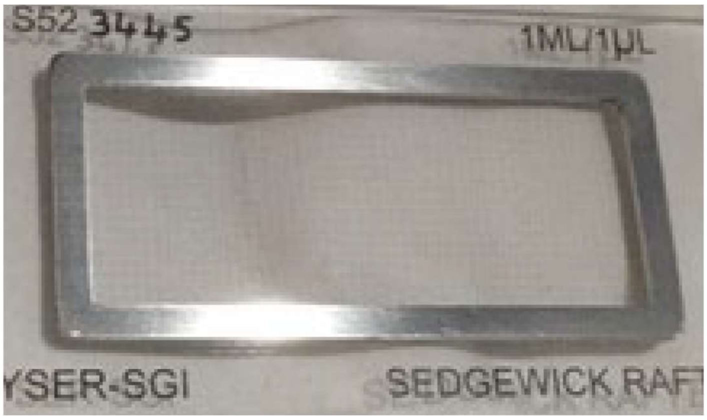

Figure 6. Counting chamber used for quantifying the number of larvae after sampling the water of the pond (modified from Strand et al. 2018).

By monitoring the number of larvae, swarming peaks can be identified. Good numbers of larvae during a swarming peak are 15-30 larvae per liter (Wensveen, 2022, Figure 7). There can be a single larvae peak, multiple peaks, or continuous production throughout the season depending on environmental conditions in the ponds and the obtained broodstock. Typically, populations from northern Europe produce few, large larvae peaks, whereas populations further south have smaller peaks and continuous production (B. Colsoul, personal communication).

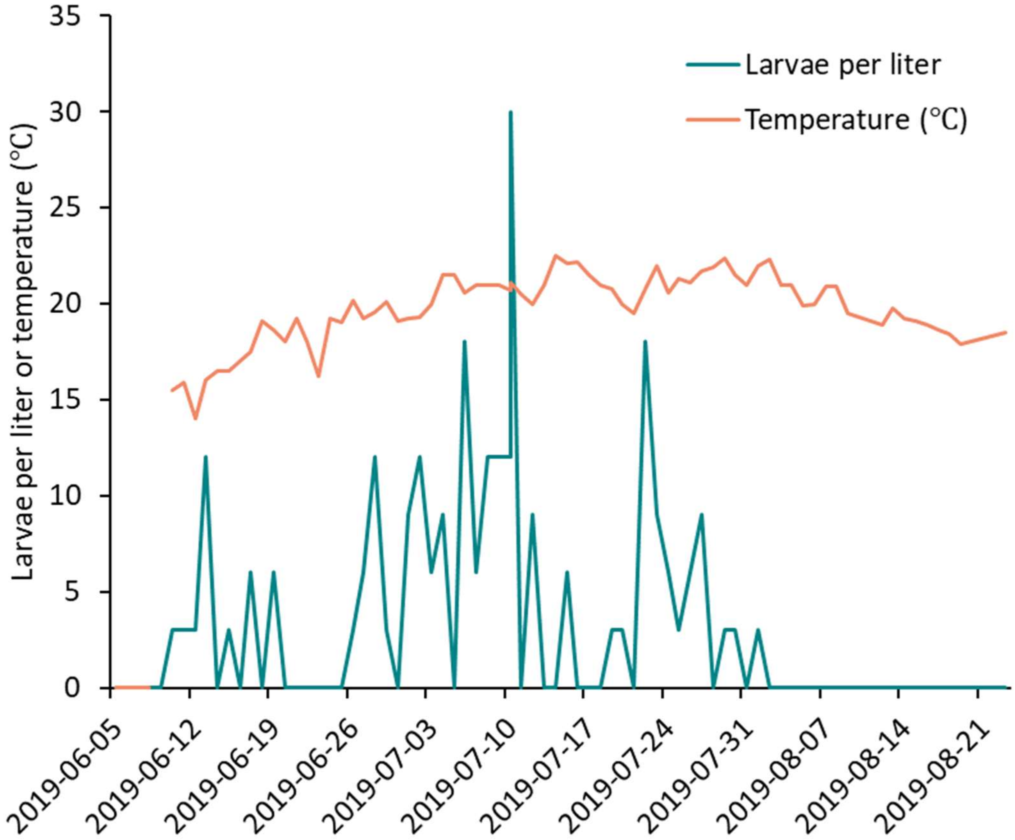

Figure 7. The number of O. edulis larvae in relation to temperature in a large-scale pond in Ireland (Cartron Point Shellfish LTD, Cartron point New Quay, on the South shore of Galway Bay). Data collected in the AquaVitae project 2019 (raw data, Hannon et al. 2021, Wensveen, 2022).

When the pediveliger larvae are about 0.3 mm they are ready to settle (Strand et al. 2018, Gustafsson et al. 2023). To monitor the larvae settlement, shells of either blue mussels or oysters, plastic or ceramic plates, or collectors, should be placed in the pond (Figure 8). The substrate should be placed in different parts of the pond and at different depths. Every 2-3 days the substrate should be removed and inspected for settled larvae by placing the shell under a stereo microscope with 10x magnification. Every time a substrate is removed, new substrate should be put in the pond to replace it.

Figure 8. Newly settled flat oyster seed (small dots) settled on a blue mussel shell used as substrate in a spatting pond.

5 | Seed harvest

3-4 months after the larvae have settled on the used substrate, they are ready to be harvested. Collectors can be removed from the pond, stripped of seed and placed in cages for continued growth. If cultch was used as settlement substrate, shells with living seeds can be transferred to a grow-out or restoration area (Strand et al., 2018). During transport to grow-out areas, seed should be stored in room temperature in moist conditions for a maximum of 48 h, or in cold storage (air temperature 5-15 °C) for no more than 4 days, to ensure high survival (Hedensjö and Strand 2025). Do not expose the seed to very low temperatures (< 0 – 5 °C), to very high temperatures (> 20 - 25 °C) or expose them to air for longer periods. The ponds should be emptied after harvest.

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References

Broquard, C., Martinez, A. S., Maurouard, E., Lamy, J. B., Dégremont, L. (2020). Sex determination in the oyster *Crassostrea gigas*-A large longitudinal study of population sex ratios and individual sex changes. Aquaculture, 515: 734555.

Brzeski, V., Newkirk, G. (1997). Integrated coastal food production systems—a review of current literature. Ocean & Coastal Management, 34: 55-71.

Cole, H. (1942). Primary sex-phases in *Ostrea edulis*. Journal of Cell Science, 2(331), 317-356.

Colsoul, B., Boudry, P., Pérez-Parallé, M. L., Bratoš Cetinić, A., Hugh-Jones, T., Arzul, I., Mérou, N., Wegner, K. M., Peter, C., Merk, V. M., & Pogoda, B. (2021). Sustainable large-scale production of European flat oyster (*Ostrea edulis*) seed for ecological restoration and aquaculture: a review. Reviews in Aquaculture, 13(3), 1423-1468.

Eymann, C., Götze, S., Bock, C., Guderley, H., Knoll, A. H., Lannig, G., Sokolova, I. M., Aberhan, M., & Pörtner, H. O. (2020). Thermal performance of the European flat oyster, *Ostrea edulis* (Linnaeus, 1758)—explaining ecological findings under climate change. Marine biology, 167, 1-15.

FAO 2023. *Ostrea edulis*. Cultured Aquatic Species Information Programme. Text by Goulletquer, P. Fisheries and Aquaculture Division [online]. Rome. [Cited Tuesday, August 8th 2023]. https://www.fao.org/fishery/en/culturedspecies/ostrea_edulis?lang=en

Goulletquer, P. (2004). Cultured aquatic species information programme: *Ostrea edulis*. In FAO Fisheries and Aquaculture Department. Rome, Italy: Food and Agriculture Organization of the United Nations. Updated 1 January 2004.

Gustafsson, M., De Wit, P., Robert, C., Wrange, A.-L., Laugen, A.T., Strand, Å. (2023). Kunskapsunderlag för en enhetlig förvaltning av OSPAR-listade *Mytilus*- och *Ostrea*-bankar. Del 3 – Underlag för bedömning av bevarandevärde av *Mytilus*- och *Ostrea*-bankar. Rapport C731, IVL Svenska Miljöinstitutet, 49s.

Hannon, C., Jones, C., Suckow, B. (in prep). Report on final development phase in Case Studies WP1. Deliverable D1.6. AquaVitae, Horizon 2020 BG-08: Part C, GA 818173.

Hannon, C., Jones, C., Suckow, B. (2021). Report on first development phase in Case Studies WP1. Deliverable D1.2. AquaVitae, Horizon 2020 BG-08: Part C, GA 818173.

Hedensjö, A., & Strand, Å. (2025). How to transport *Ostrea edulis* seed (Report No. C886). IVL Svenska Miljöinstitutet. ISBN 978-91-7883-654-3.

Helm, M. M., Bourne, N., & Lovatelli, A. (2004). The hatchery culture of bivalves: a practical manual. FAO.

Helmer, L., Hauton, C., Bean, T., Bass, D., Hendy, I., Harris-Scott, E., Preston, J. (2020). Ephemeral detection of *Bonamia exitiosa* (*Haplosporida*) in adult and larval European flat oysters *Ostrea edulis* in the Solent, United Kingdom. Journal of Invertebrate Pathology, 174: 107421.

Hutchinson, S., & Hawkins, L. E. (1992). Quantification of the physiological responses of the European flat oyster *Ostrea edulis* L. to temperature and salinity. Journal of Molluscan Studies, 58(2), 215-226.

Korringa, P. (1940). Experiments and observations on swarming, pelagic life and setting in the European flat oyster, *Ostrea edulis* L (PhD thesis). Archives Néerlandaises de Zoologie. Amsterdam, pp. 249.

Korringa, P. (1957). Water temperature and breeding throughout the geographical range of *Ostrea edulis*. Colloque international de biologie marine station biologique de Roscoff. L'Année Biologique, 33(5–6), 1–17.

Lindegarth S. (Ed.) (2012). Handbok för ostronodlare – ett resultat av Projekt Nord-Ostron 2009 – 2012. Göteborgs universitet. Billes tryckeri. Pp. 36.

Luckenbach, M., Sellner, K. G., Shumway, S.E., Greene, K. (1993). Effects of 2 bloom-forming dinoflagellates, *Prorocentrum-minimum* and *Gyrodinium-uncatenum*, on the growth and survival of the Eastern oyster, *Crassostrea-virginica* (Gmelin 1791). Journal of Shellfish Research, 12: 411.

Maathuis, M.A., Coolen, J.W., Van Der Have, T., Kamermans, P. (2020). Factors determining the timing of swarming of European flat oyster (*Ostrea edulis* L.) larvae in the Dutch Delta area: implications for flat oyster restoration. Journal of Sea Research, 156: 101828.

McFarland, K., Vignier, J., Standen, E., & Volety, A. K. (2022). Synergistic effects of salinity and temperature on the eastern oyster *Crassostrea virginica* throughout the lifespan. Marine Ecology Progress Series, 700, 111-124.

Mann, R. (1979). Some biochemical and physiological aspects of growth and gametogenesis in *Crassostrea gigas* and *Ostrea edulis* grown at sustained elevated temperatures. Journal of the Marine Biological Association of the United Kingdom, 59: 95-110.

Marteil, L. (1976). La conchyliculture francaise II, biologie de phuttre et de la moule. Institut Scientifique et Technique Ptches Maritimes, 319 p.

Orton, J.H. (1936). Observations and experiments on sex-change in the European oyster, *Ostrea edulis* L. A simultaneous study of spawning in 1927 in two distinct geographical localities. Memoirs du Musee Royale d'Histoire Naturelle de Belgique, Series 2, 3: 997-1056.

Prado, P., Roque, A., Pérez, J., Ibáñez, C., Alcaraz, C., Casals, F., & Caiola, N. (2016). Warming and acidification-mediated resilience to bacterial infection determine mortality of early *Ostrea edulis* life stages. Marine Ecology Progress Series, 545, 189-202.

Quayle, D. B. (1988). Pacific oyster culture in British Columbia. Canada: Department of Fisheries and Oceans.

Strand, Å., Sühnel, S. (2022). Module molluscs topic oysters concept 2 - Sea based seed production and spatting ponds. Zenodo. https://doi.org/10.5281/zenodo.6593769

Strand, Å., Wrange, A.-L., Hogström, P., Nielsen, J. W., Persson, P., & Persson, K. (2018). Produktion av ostronyngel (*Ostrea edulis*) i havsbaserade tankar: Biologisk och teknisk förstudie (Report No. C 380), Pp. 69.

Tamburri, M.N., Luckenbach, M.W., Breitburg, D.L., Bonniwell, S.M. (2008). Settlement of *Crassostrea ariakensis* larvae: effects of substrate, biofilms, sediment and adult chemical cues. Journal of Shellfish Research, 27: 601-608.

Thompson R.J., Newell R.I.E., Kennedy V.S., Mann R. (1996). Reproductive processes and early development. In: Kennedy V.S., Newell R. I. E., Eble A. E. (eds.). The Eastern Oyster *Crassostrea virginica*. Maryland: Maryland Sea Grant Publications. Pp. 335-370.

Redfield, A.C. (1958). The biological control of chemical factors in the environment. American scientist, 46: 230A-221.

Rendle, E. J., Hunt, E. L., & Bicknell, A. W. J. (2023). A three-step approach for co-locating nature-based solutions within offshore wind farms. Frontiers in Ecology and Evolution, 11, 690382.

Rodriguez-Perez, A., James, M., Donnan, D.W., Henry, T.B., Møller, L.F., Sanderson, W.G. (2019). Conservation and restoration of a keystone species: Understanding the settlement preferences of the European oyster (*Ostrea edulis*). Marine Pollution Bulletin, 138: 312-321.

Walne, P.R. (1964). Observations on the fertility of the oyster (*Ostrea edulis*). Journal of the Marine Biological Association of the United Kingdom, 44: 293-310.

Wensveen, S. (2022). The influence of temperature on flat oyster husbandry: A case study in Ireland & Sweden (Master's thesis). University of Gothenburg.

Wikfors, G.H., Smolowitz, R.M. (1995). Experimental and histological studies of four life-history stages of the eastern oyster, *Crassostrea virginica*, exposed to a cultured strain of the dinoflagellate *Prorocentrum minimum*. The Biological Bulletin, 188: 313-328.

zu Ermgassen, P., van den Brink, A., Bromley, C., Brown, J., Colsoul, B., Fabra, M., Frankicc, A., Glover, A., Hansen, J. H., Khan, J., Alves Monteiro, H. J., Mortensen, S., Pogoda, B., Renton, D., Sanderson, W., & Strand, Å. (2020). Understanding Biosecurity in Native Oyster Restoration. In Z. E. P. S. E., G. C., D. A., C. B., F. M., S. W. G., S. Å., & P. J. (Eds.), EUropean Guidelines on Biosecurity in Native Oyster Restoration (pp. 1-18). Zoological Society of London

zu Ermgassen, P., Strand, Å., Bakker, N., Blanco, A., Bonačić, K., Boudry, P., Brundu, G., Cameron, T. C., Connellan, I., da Costa, F., Debney, A., Fabra, M., Frankic, A., Gamble, C., Gray, M. W., Helmer, L., Holbrook, Z., Hugh-Jones, T., Kamermans, P., Magnesen, T., Nielsen, P., Preston, J., Ranger, C. J., Saurel, C., Smyth, D., Stechele, B., Theodorou, J. A., & Colsoul, B. (2023). Overcoming *Ostrea edulis* seed production limitations to meet ecosystem restoration demands in the UN decade on restoration. Aquatic Living Resources, 36, 16.

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This report has been reviewed and approved in accordance with IVL's audit and approval management system.