ReCoral

Standard Operating Procedures

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1 Overview

We aim to develop a non-invasive methodology to grow healthy coral colonies on offshore wind turbine foundations, using surplus coral larvae from broadcast spawning corals. During the event of the annual synchronized coral spawning event (triggered by the darkness before moon rise that occurs at and after full moon in conjunction with increased sea surface temperatures at beginning of summer /1/), it is possible to collect surplus coral larvae that are carried onshore by current and wave activities /1/,/3/. Our aim is to develop a method that enables us to grow coral on offshore structures located in environmental conditions that are not significantly influenced by global warming, creating safe havens, where coral thrives and from where coral spawn can be facilitate the restoration of naturally occurring coral reef systems.

As part of Ørsted's 2030 strategy, we have pledged to build renewable energy in balance with nature. Specifically, we aim to have a net-positive biodiversity impact in all renewable energy projects we commission from 2030.

We have invested a significant amount of time and resources into the development of the ReCoral by Ørsted™ undertaking and our findings will be made accessible to the coral conservation community to support other large-scale coral restoration efforts.

Broadcast spawning corals release their offspring in a synchronized event that occurs around full moon at the beginning of summer /5/. The coral spawn released contains sperm and eggs /8/ in the form of egg bundles. These egg bundles are positively buoyant and can therefore be collected on the sea surface /4/, /8/. Coral larvae are forming over 96 hours past the release from the mother coral colony, undergoing fertilization and a 12 stepmetamorphosis (see Figure 1.1). These coral larvae are mobile and they actively seek adequate growth substrate to permanently settle upon and to further develop into a sessile adult coral colony.

This document presents a standard operating procedure covering the key steps that were developed and followed during the proof-of-concept phase of ReCoral by Ørsted™. ReCoral by Ørsted™ relies on a coral seeding approach that uses surplus coral spawn and that aims for the greatest potential for industrial-scale coral reef restoration /4/,/1/.

The three key ReCoral by Ørsted™ procedures are described below and are made freely available to facilitate the wide distribution and application of this approach. In order to maximise the chances of successfully replicating the ReCoral approach outlined herein, we recommend utilising a team of coral researchers who have expertise in reef restoration and who are trained in coral larvae husbandry.

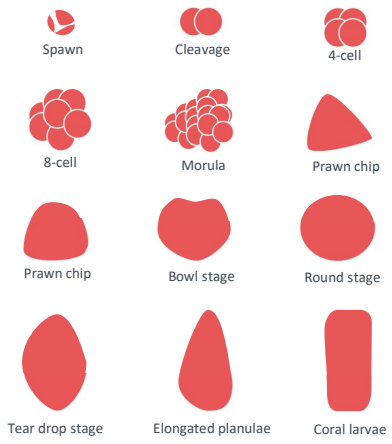

Figure 1.1: The 10 metamorphosis stages of coral larvae development.

2 Collection of surplus coral spawn and rearing setup

2.1 Preparation of coral larvae culturing equipment

To prepare for the culturing of coral larvae, equipment should be cleaned of industrial traces (if new containers/ equipment are used) or biological traces (if equipment is being reused).This will support the successful culturing of coral larvae from collected surplus coral spawn. Hereafter, the term 'coral propagules' will be used to refer to the various stages that are being cultured – the collected coral spawn (egg bundles), and developmental stages towards formation of coral larvae (see Figure 1.1) /12/.

1. Equipment should be rinsed with dilute household bleach (1%) to rinse off any agents that could deteriorate, or otherwise negatively impact, the coral larvae culture.
2. Rinse equipment thoroughly with freshwater to remove remaining bleach and let it dry (preferably in the sun to utilize UV for extra sterilization).
3. To support effective daily coral larvae maintenance, additional cleaned rearing containers should be contained in a dust-free environment, ready for usage.

2.2 Coral larvae culturing setup

Seawater used to rear coral propagules, should be filtered through a 0.1 µm filter to remove organic particles. The critical initial fertilisation stages occur in the first 24 hours after coral spawn has been released coinciding with coral spawn collection. During the following developmental phase up to 96 hours the coral larvae are developing (see Figure 1.1) /12/,/6/.
For the culturing of coral larvae beyond 96 hours, the seawater used for rearing should be prepared by filtering it through a 0.2 µm filter.
To maximise the chances of successful coral larvae rearing, the rearing container should provide sufficient surface area and meet the minimum tank diameter as outlined in Table 1.1.

Figure 1.2: Bubble stone attached to airpump.

Adequate tank surface area, aeration via an air pump, as well as water recirculation within the rearing tank, will help to ensure sufficient oxygenation. During the culturing process, the seawater should be aerated by means of an aquarium air pump attached to a flat disk bubble stone (minimum diameter 10cm) (see Figure 1.2). Furthermore, the seawater used for rearing should be recirculated with a pump within the culturing container.

Lighting conditions when culturing should (a) be based on natural lighting at an outdoor location (if flow-through systems are available to ensure adequate temperature regulation), or (b) follow a 12-12 light-dark rhythm with ambient light intensity ranging between 100 – 300 µE and using a light source that can provide photosynthetically active radiation (400 – 700nm).

In general, the condition of the seawater used for rearing, in terms of temperature, salinity, and pH, should resemble the conditions of the coral spawn collection site.

Table 1.1: Recommended tank size, surface area, and diameter, for rearing propagules (eggs, embryos or larvae) before seeding (adopted from Edwards, A.J. (2010)).

Tank volume (L)	100	200	500	1,000	1,500	2,000
Min. tank surface area (m^2)	0.08	0.16	0.38	0.75	1.13	1.50
Min. tank diameter (cm)	31	46	69	98	120	138
Total number of propagules	30,000	60,000	150,000	300,000	450,000	600,000

2.3 Coral spawn collection

Coral spawn is collected from the shoreline at sites that are windward facing. Weather conditions will have to be checked prior to coral spawn collection to confirm wind direction and to select collection sites based on the information retrieved.

Equipment for coral spawn collection

- Plastic collection cup (see Fig. 1.3)
- Collection container (20L volume)

Coral spawn collection

1. Collect coral spawn close to the shoreline at water depths of up to 0.5m (to avoid the collection of damaged coral spawn).
2. Immediately place coral spawn into a transportable container.
3. Bring coral spawn to the culturing location.
4. Temparature control coral spawn (to within +/- 0.1 degrees of the temperature at the collection site) during transport and avoid any erratic movements of the collection container that might damage the coral spawn.

Figure 1.3: Plastic collection cup used for coral spawn collection.

2.4 Coral larvae culture setup

In brief, coral spawn should be reared in large volume containers, aerated and stirred using water recirculation (see section 2.2). The following culture quality checks should be carried out on a daily basis or throughout the day where indicated.

Coral propagules should be reared at a density that's between 300 – 1,300 propagules L^{-1}.

Rearing activities

1. Prepare adequate volumes of excess filtered seawater. Set this aside to allow for seawater exchanges (if prepared in advance, place the seawater container into a cooler to avoid bacterial growth – allow the seawater to warm up to culturing temperatures prior to water exchange).

2. Exchange the seawater that's used for rearing once a day.

3. Check the quality of the seawater that's used for rearing every few hours to avoid any contaminant-related deterioration of the coral larvae culture. Typical indicators of culture contamination are seawater cloudiness, and/or foam formation on the surface of the culture. If such characteristics are observed, water exchange should be carried out immediately (see below).

Seawater exchange procedure

For seawater exchange, the 'propagules' need to be treated with great care as they are relatively fragile. To execute a seawater exchange, pour the propagules onto a 100µm plankton net, taking care to ensure that the propagules remain submerged at all times. The seawater should be poured away slowly to avoid shear stress. Once the rearing container has been fully emptied, the coral larvae can be gently transferred into a freshly prepared rearing container.

3 Coral larvae 'competency test'

After the collected coral spawn has developed into coral larvae (about 96hrs), the coral larvae will gain 'competency', meaning that they will be mature enough to sense adequate growth surface substrate that will induce settlement. The development phase of the coral larvae will have to be closely monitored in order to facilitate the planning of offshore deployment operations.

Equipment for competency test

- Glass beaker filled with filtered (0.45 µm filter) seawater
- Stones or coral rubble encrusted with crustose coralline algae (can be sourced from the mother coral colony)
- Scoop (cleaned prior to usage following the procedure described in 2.1)

Competency test

Stones, or coral rubble encrusted with coralline algae, can be used as settlement substrate. Place the settlement substrate into a glass beaker filled with filtered seawater immediately before the competency test. Place the beaker in a sunlit, or an artificially lit environment.
Source a subsample of the cultured coral larvae from the rearing container using a pre-cleaned scoop. Place actively swimming coral larvae from the rearing container into the beaker and monitor for settling activity.

If coral larvae actively swim towards the settlement substrate within 20 mins of being placed into the beaker, then the coral larvae can be deemed 'competent'.
If coral larvae continue to randomly move around and do not target the settlement substrate, then the coral larvae will require additional culturing time. The competency test should be repeated after 24hrs.

4 Coral larvae transport and deployment

For coral larvae transportation, ambient rearing parameters (temperature, oxygen, salinity) should be controlled. These parameters should approximate those observed at the initial coral spawn collection site. This will support larvae fitness and settlement success at the offshore marine infrastructure. Once arrived at the deployment site the bags holding the coral larvae should be placed in buckets containing freshly collected seawater from the deployment site. This will allow to adjust to the local temperature regime before deployment.

Equipment required

- Salinity measuring device (refractometer or conductivity meter)
- Plankton net (100µm)
- Pre-cleaned beaker (see 2.1)
- 30 L medical-grade plastic bags
- Medical-grade oxygen cylinder
- Gaffer tape
- Styrofoam boxes (need to fit filled 30L bag)
- Freshly prepared sterile filtered seawater (0.45 µm filter)

4.1 Preparation of coral larvae for transportation

Preparation should commence no earlier than 2hrs before the planned transportation time. This will reduce the amount of time that the coral larvae are contained within the transportation enclosure.

For each foundation two 30 L medical-grade plastic bags with final concentration of maximum 0.4 ± 0.1 larvae ml^{-1} should be prepared as follows:

1. Place a 100µm plankton net in front of the outlet of the rearing container (attach a hose if not streamlined)
2. Release the seawater used for rearing from its container catching the coral larvae on the plankton net
3. Fill the 30 L medical-grade plastic bags with filtered seawater to about the half-way mark
4. Use the coral larvae stock to create a concentrated culture (0.4 larvae ml^{-1}) by flushing the coral larvae from the plankton net into a beaker
5. Release the quantity of concentrated coral larvae into the prepared plastic bag required to achieve the recommended density
6. Purge the overhead space with medical-grade oxygen, holding the opening tight around the gas inlet
7. Once the plastic bag bulges in volume close the bag with a tight clip and gaffer tape the remaining fold so that the bag is sealed completely
8. Initiate transport to the offshore deployment site immediately after closing
9. To avoid a reduction or loss of activity, and to help to ensure that the larvae remain viable, when they reach the deployment site, transportation time should not exceed 12 hours

The coral larvae can now be transported to the offshore site for release into an enclosure attached to the foundation (see below).

4.2 Coral larvae enclosure

Current regimes at offshore locations can be quite strong. A temporary enclosure can help to ensure safe low-current conditions that can support the settlement of larvae onto the offshore structure. For the ReCoral deployment, temporary net-cage enclosures were designed. These fitted into brackets that had been welded into place on the foundation legs.
For the release activity, it's recommended that a team of at least 4 people, including a rope-access team, are deployed in order to ensure a safe operation.

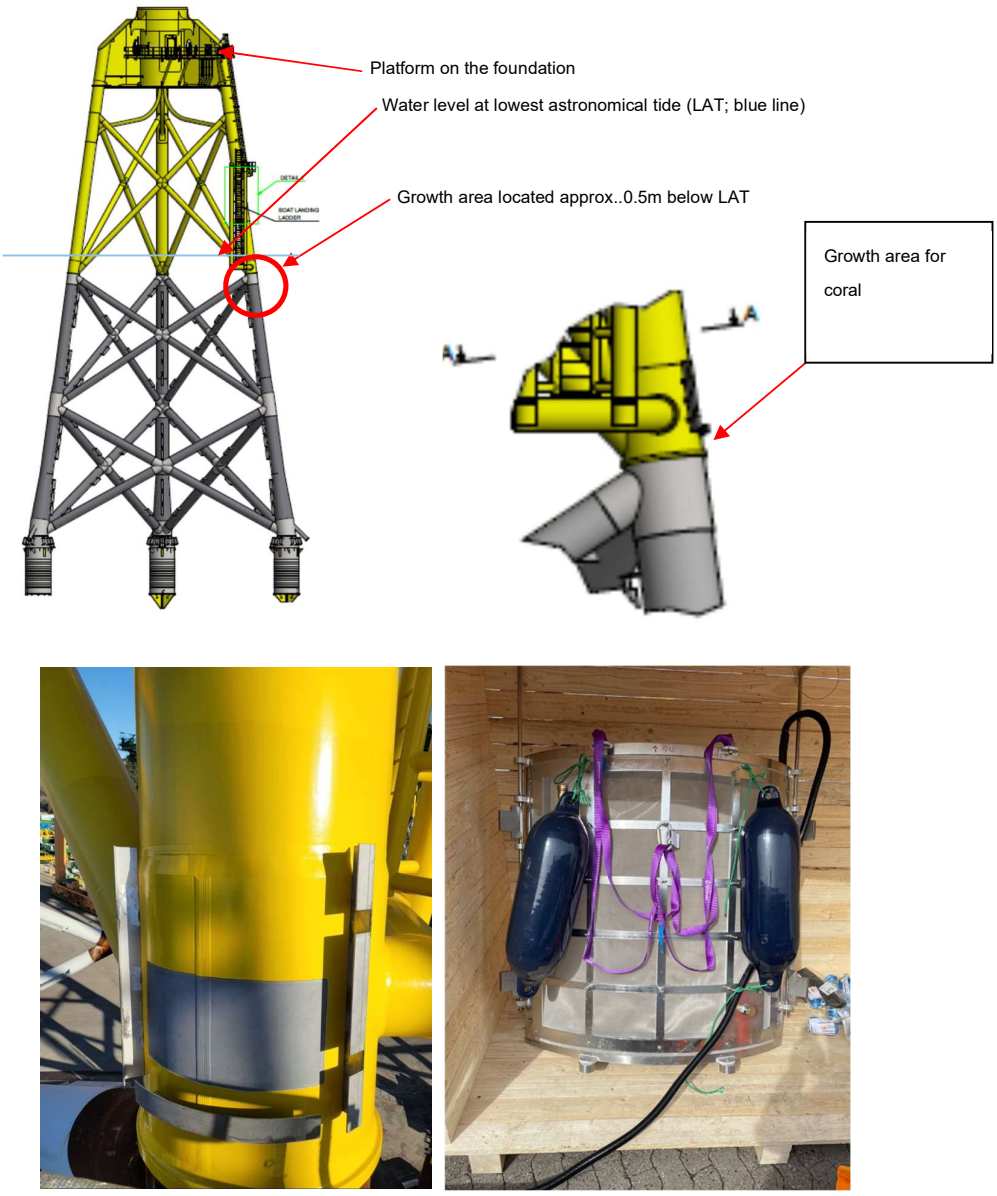

Figure 1.4: Drawing of a foundation indicating the platform at the foundation, the lowest astronomical tide level (blue line), and the location of the enclosure (above left) as well as a close-up of the coral growth area (above right). Images of the enclosure brackets and the surface area, here half covered with a concrete surface paste - see following text (below left) and the custom-made enclosure mesh (below right).

The enclosure, designed to facilitate the coral larvae settlement on an offshore marine foundation, has the form of a square and open “back-end” aluminum box, - or “net-cage” (Fig. 1.4 below right and Fig. 1.5). The front, facing the sea is made of stainless-steel wire mesh with a mesh size of 150 µm. Coral larvae will be contained behind the mesh, close to the settlement substrate of the

foundation. At the same time, the mesh will ensure that an adequate amount of oxidized fresh seawater and sunlight reach the larvae. This is especially important given thatlarvae activity is directly linked to light availability /9/.
The enclosure covers 1 m^2 of the foundation surface making this area available for the coral larvae as settlement ground. The surface area of choice for ReCoral on the foundation should ideally be 'aged', meaning that it has crustose coralline algae growing on the surface and/or a solid layer of calcium carbonate formed due to the presence of an anti-corrosion anode system. Such surface substrates are favourable to coral larvae as settlement ground. Alternatively, an artificial surface layer can be applied such as concrete plaster (e.g. Sika Monotop 610), which is an acrylic modified concrete plaster specifically developed to be applied in thin layers. Concrete surface layers can provide three-dimensional surface structure that's preferred by coral larvae, and concrete has been shown to be adequate settlement ground in coral restoration efforts /6/, /5/.

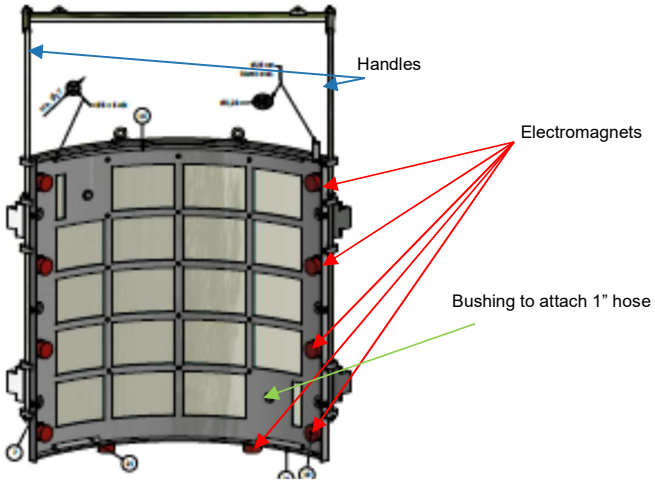

Figure 1.5: Lay-out of net-cages.

The net-cage of the enclosure is supplied with 2 handles to temporarily lock the net-cage into the brackets welded onto the foundation legs, and further fitted with 10 electromagnets that will hold the net-cage tight to the surface of the foundation for the duration of the larvae settlement period.
The electromagnets are powered by a battery power pack installed on the upper access platform. The batteries are powered by temporary solar panels and also supported by a temporarily installed small wind turbine.
All remaining equipment and installations are of a temporary nature, that are brought to the site just before the trial, installed, - and dismantled again when the trial is completed.

4.3 Coral larvae release

Equipment required

- Funnel (30cm diameter and an insert with decreasing diameter from 3cm)
- Clear hose (20m range with 2.5 cm diameter)
- Coral larvae contained in transport container

- Scissors
- Radio connection to the rope-access team for coordination
- 4 x Bucket (5L) with seawater (collected at the offshore site)

The rope-access team and the equipment are transferred on to the foundation. After the rope-access team has deployed the net-cage, ensured a good fit, and activated the electromagnets, the release activity can be executed as follows.

The coral larvae contained in the transport containers (see section 4.1) are placed on the platform at the foundation (see Fig. 1.4).The activity of the coral larvae is observed and recorded prior to the release operation in order to support scientific reporting.

1. The net-cages are fitted with a 12 mm bushing to which a clear hose is connected
2. Attach the hose to the enclosure and bring the end of the hose to the container holding the coral larvae (approx. 12 meters above the enclosure area)
3. The angle of the hose towards the enclosure should be kept below 30° at all times
4. Attach the funnel to the plastic hose (ensure a tight fit)
5. Place the plastic bag containing the coral larvae above the funnel
6. Clip the edge of the plastic bag containing the coral larvae culture
7. Carefully release the coral larvae culture into the enclosure (ensure that the angle of the hose will slow the flow rate of the larvae culture into the enclosure)
8. Open the emptied plastic bag
9. Fill the empty plastic bag with seawater collected at the offshore site (whilst holding above the funnel) to rinse any remaining coral larvae from the plastic bag.
10. Release the seawater into the enclosure via the hose
11. Pour the remaining buckets of seawter into the enclosure via the hose

The net-cage component of the enclosures will remain attached to the foundation for 4 days (the settlement phase) to allow coral larvae to settle. During the ‘settlement phase’ each net-cage is inspected daily to ensure the functionality of the equipment.

After completion of the settlement period all portable equipment including the net-cages are carefully dismantled and brought to shore. The equipment has been custom-made and tested during concept trials and is available for re-use during follow-on ReCoral initiatives.

5 Perspective on reef restoration

Coral reef restoration efforts that are based on 'coral seeding' spread coral larvae and encourage its settlement on degraded reef sites, or on other adequate growth ground, with the aim of restoring and encouraging growth of coral /3/. As well as enabling new coral growth locally, these reef restoration initiatives can also add to the diversification of the gene pool locally and at inter-connected reef areas (e.g. coral triangle, Kuroshio triangle).

The following figures highlight the potential positive impact that ReCoral could have on the resilience and growth of natural reef systems. One mature adult coral colony (15 - 20 cm diameter) can yield up to 17,300 coral larvae per annual spawning event /1/. Natural seeding density for reef restoration with the highest settlement success is 1000 coral larvae per square meter /10/.

If one assumes that a successful ReCoral deployment yields 20 adult coral colonies per 1 square meter of wind turbine foundation, then the combined coral larvae output would support natural reef restoration at a ratio of 1:340 (i.e. 1m^2 of ReCoral grown mature corals can revitalize 340m^2 of coral growth areas). This interpolation of the potential positive impact of ReCoral will increase as the corals grow and the larval output increases.

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