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# Feeding coral spat produced in aquaculture – Standard Operating Procedure

2 June 2025

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Cover Images: Coral Reef, Credit: Gary Cranitch, Queensland Museum

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The RRAP partners acknowledge Aboriginal and Torres Strait Islander Peoples as the first marine scientists and carers of Country. We acknowledge the Traditional Owners of the places where RRAP works, both on land and in sea Country. We pay our respects to elders; past, present, and future; and their continuing culture, knowledge, beliefs, and spiritual connections to land and sea Country.

We specifically acknowledge and thank the following Traditional Owners of sea Country that this report relates to:

Location	Traditional Owner Group
Australian Institute of Marine Science, SeaSim	Wulgurukaba and Bindal
Palm Islands, John Brewer Reef	Manbarra

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# 1 Executive Summary/Abstract

This Standard Operating Procedure (SOP) presents a protocol for feeding coral juveniles produced in aquaculture facilities for deployment on the reef as part of reef restoration initiatives. The described protocol involves batch feeding of corals every afternoon with a mixture of newly hatched *Artemia* and enriched rotifers, which need to be produced daily and in continuous culture, respectively. The described feeding protocol aims to support and improve the survival, growth and condition of the produced corals before deployment as a step towards enhancing the survival rates of coral one year after deployment. The protocol is system agnostic and can be adjusted for use with a series of tank sizes and coral spat densities. The presented protocol is intended to be a working document, with updated versions expected as evidence-based improvements in dosing, supplements, and species-specific adjustments to feeding protocols become available.

## 2 Background

Live feeds can provide supplemental nutrition to increase tissue and skeletal growth (Ferrier-Pagès et al., 2003), and it has been demonstrated that feeding of coral juveniles can increase survivorship before and after deployment (Schutter et al., 2023; Toh et al., 2014). Live feeds that are commonly used to feed coral spat in aquaculture facilities include *Artemia*, rotifers and microalgae (Banaszak et al., 2018; Brown & Blackburn, 2013; Conlan et al., 2017) .

Here, we provide a protocol for feeding coral spat used for reef restoration in an aquaculture facility, involving batch feeding every afternoon with a mixture of newly hatched *Artemia* and enriched rotifers starting as soon as possible after settlement and photosymbiont acquisition. The presented protocol is similar to the standard protocol used to feed coral juveniles held at The National Sea Simulator (Ramsby et al., 2024), however microalgae have not been included in the recommended standard live feed mixture.

Newly hatched *Artemia* are recommended as an important component of the live feed mixture as *Artemia nauplii* can be ingested by coral juveniles of many coral species (Geertsma et al., 2022), and increased survival and growth have been reported relative to other feed-treatments and no-feed controls (Lewis, 1974; Petersen et al., 2008), with benefits extending to after deployment (Schutter et al., 2023; Toh et al., 2014). Moreover, *Artemia* is commercially available as dehydrated cysts, which presents logistical advantages as newly hatched *Artemia* (nauplii) can be produced overnight from an off-the-shelf product. *Artemia nauplii* are generally in the size-range 400-500  $\mu\text{m}$  and are slower swimmers than on-grown *Artemia*, making them easier to capture and ingest for early life stages of many marine species (Hill et al., 2020).

Enriched rotifers (*Branchionus*) are also recommended to be included as part of the live feed mixture. While rotifers have regularly been used to feed coral juveniles (Craggs et al., 2019; Ionata, 2021; Mendoza Quiroz et al., 2023; Ramsby et al., 2024), only a few controlled feeding studies have been reported. While effects on growth relative to no-feed controls are generally marginal or not significant (Conlan et al., 2017). This has also been observed for coral fragments (Lavorano, 2013; Osinga et al., 2012), however other studies have found that feeding coral fragments with rotifers may be beneficial through enhanced stress resilience (Connolly et al., 2012; Towle et al., 2015). The small size of rotifers (90-340  $\mu\text{m}$ ), their ability to stay in the water column, high reproductive rate, and capability to be cultured in large volumes mean that they are

commonly produced and used as live feeds in aquaculture facilities (Hill et al., 2020; Kandathil Radhakrishnan et al., 2020; Pan et al., 2022). Their nutritional value reflects what they consume, and they are routinely enriched with microalgae or microalgae-based products (Hill et al., 2020; Kandathil Radhakrishnan et al., 2020).

At this time, we do not recommend including live microalgae in the feed mixture. While some microalgae species were reported to support growth of coral juveniles, feeding with cultured microalgae generally does not support faster growth than feeding with *Artemia* (e.g Petersen et al. 2008), and we have not seen reports suggesting that feeding with microalgae can enhance coral stress resilience. Since the production of microalgae is labour and resource intensive, and live microalgae may contribute to algal fouling of grow-out tanks, we do not see that the additional cost is warranted until further evidence of their benefit is available. If microalgae cultures are available at the facility, they can optionally be added at a concentration of 2000 cells/mL (Ramsby et al., 2024).

In a recent experiment performed under RRAP-ECT3-2.2, we experimentally validated that daily feeding of juvenile *Acropora millepora* and *Platygyra daedalea* with a mixture of newly hatched *Artemia* and enriched rotifers improved growth after 11 weeks in the facility relative to non-fed controls (Supplementary Figures 1 and 2). In this experiment, a liquid supplement (Continuum Microblast; minerals, vitamins, pigments and marine lipids) was added on its own or in combination with the zooplankton mixture, leading to stronger pigmentation in *P. daedalea* spat as compared to the zooplankton only treatment (Supplementary Figure 1). Coral spat from this experiment have been deployed on John Brewer Reef, and their survival and growth will be monitored over the next few months. Depending on these field results, the addition of supplements may be added to an updated version of this SOP.

The presented SOP is a working document, with updated versions expected as evidence-based improvements in dosing, supplements, and species-specific and age-specific adjustments to the feeding protocol become available.

### 3 Objectives and Scope

This Standard Operating Procedure provides a description of feeding protocols that are appropriate to use with a range of coral species from early life stages to adult colonies, with the intention to guide RRAP industry partners engaged to produce and grow coral juveniles in nurseries until deployment on the reef. The scope of the SOP is to describe feeding protocols only, and other steps in the coral production cycle can be found in other SOPs and training materials. Specifically, SOPs developed by the National Sea Simulator for production of Artemia and enriched rotifers are included as Appendices to this SOP. This SOP intends to be general, and the information presented can be adapted and applied to several types and sizes of coral holding tanks.

## 4 Pre-requisites



The production of the live feed organisms themselves is covered in existing SOPs prepared by the National Sea Simulator, provided as Appendices to this SOP. These Sea Simulator SOPs include overviews of health, safety and environmental risks, overviews and photos of the tools, equipment and resources required, and step-by-step protocols. Note that we recommend feeding coral juveniles with newly hatched rather than enriched Artemia (see section: Steps for implementation, Procedure), hence the enrichment part of the Artemia SOP listed below is superfluous here.

- National Sea Simulator Artemia Hatching, Harvesting & Enrichment Protocol (Appendix 2)
- National Sea Simulator Rotifer Culture Protocol (Appendix 3)

The personnel preparing live feeds and delivering them to the coral tanks are required to have read this and the above listed SOPs and relevant risk assessments before commencing these tasks. Competency should be confirmed by the relevant supervisor before these tasks are undertaken unsupervised. Personnel are also required to have undergone any relevant inductions.

## 5 Identified Risks and Hazards

Table 1. Significant health and safety risks

Hazard	Risk	Control
Wet Floor	Slips	<ul style="list-style-type: none"> <li>Continual assessment of workplace to identify hazards.</li> <li>Dry floor with mop when finished and sign with wet floor signage.</li> <li>Place slip hazard sign when appropriate.</li> </ul>
Trips and falls	Strain injury	<ul style="list-style-type: none"> <li>Maintain good housekeeping practices.</li> <li>All equipment after each use will be stored correctly.</li> <li>All walkways to be kept free of obstructions.</li> </ul>
Working with hands in aquaria e.g. manipulating organisms, husbandry, taking water samples, cleaning tanks	Biological hazards	Cuts, bruises, musculoskeletal injury, skin irritation
Chemical exposure to Biosan 	Skin, eye, and potential respiratory irritation.	<ul style="list-style-type: none"> <li>Read and understand the CRA and SDS.</li> <li>Store stock solution locked up in well-ventilated area.</li> <li>Open stock solution container in fume hood only.</li> </ul>
Chemical exposure to stock solution: O-CHLOR: 10-30% sodium hypochlorite; or working solution (10% dilution of stock) 	Skin, eye, and potential respiratory irritation.	<ul style="list-style-type: none"> <li>Make sure to have easy access to safety shower and eyewash station.</li> <li>Personal protective equipment (PPE) must be worn (enclosed shoes, dishwashing gloves and safety glasses).</li> <li>Avoid contact with eyes.</li> <li>Prepare minimal volumes required to reduce risk of exposure.</li> <li>Only use a diluted working solution for cleaning equipment (1-3%).</li> <li>Ensure all containers have an appropriate lid cover and are clearly labelled with hazard pictograms and phrases, username and date prepared.</li> <li>Keep away from incompatible chemicals e.g. acid</li> </ul>

		<ul style="list-style-type: none"> <li>• Complete works in accordance with CRA and SDS - including disposal.</li> </ul>
Lifting heavy containers	Manual task-related injury (Strains/sprains/back injury)	<ul style="list-style-type: none"> <li>• Use mechanical aids (e.g. trolleys) where possible.</li> <li>• Two person lift heavier items if required.</li> <li>• Follow Manual Handling and Ergonomics Procedures.</li> </ul>
Broken glass	Cuts, laceration, infection	<ul style="list-style-type: none"> <li>• Handle glass equipment with care. Use of glass log to record movements of glass equipment in and out the room. Careful clean up and proper disposal if breakage of glass equipment.</li> </ul>
Microscopy	Musculoskeletal injury	<ul style="list-style-type: none"> <li>• Ensure posture and seated position are comfortable.</li> </ul>
Chemical exposure to Iodine	Very toxic to aquatic organisms. Risk of serious damage to eyes. Possible skin sensitizer.	<ul style="list-style-type: none"> <li>• Read and understand the CRA and SDS.</li> <li>• PPE must be worn (enclosed shoes, dishwashing gloves and safety glasses).</li> <li>• Avoid contact with eyes.</li> </ul>

# 6 Equipment and Materials

## 6.1 Procedure equipment

- Sea water
- Freshwater and hose
- Fridge with aeration
- 20 L Bucket
- 1 L Beaker
- Freshly hatched live Artemia
- Live rotifers
- Iodine
- Microscope
- Pipette
- Sedgwick rafter chamber
- Bogorov Plankton chamber
- Pipettes
- Biosan or other suitable disinfectant

## 6.2 Personal protective equipment (PPE) and other safety equipment

- Enclosed shoes
- Safety glasses
- Nitrile gloves
- Dishwashing gloves

## 7 Steps for Implementation

Coral spawning, larval settlement, inoculation with photosymbionts, transfer to holding tanks, and general culture conditions and husbandry should be performed as described in other SOPs and training materials provided.

- **Procedure preparation**

- This standard operation procedure to feed coral spat, can be used in Flow-through (Semi-open) systems and Recirculating systems.
- Aquarium systems must be set up for holding coral spat settled on tiles or settled on any other substrate for coral restoration.
- The coral spat settled on tiles, should be held in tanks with water flow and circulation.

- **Important considerations**

- It is important to start feeding the coral spat as early as possible after settlement and symbiont inoculation.
- Coral spat should be fed at least once a day, 7 days of the week.
- As corals get used to the feeding, this should be carried out at around the same time every day.
- Circulation and water flow should remain on during and after feeding, this will promote the Artemia and rotifers to be suspended in the water column and will facilitate being trapped and eaten by the coral spat.
- Depending on the tanks used, the water level may be dropped before feeding to reduce the amounts of live feeds needed. This information should be included in the specific training materials provided.
- All equipment and material used to feed the coral spat must be rinsed with fresh water, dipped in a solution of Biosan followed by a freshwater rinse as described below.

- **Procedure**

1. Refer to the National Sea Simulator Artemia Hatching, Harvesting & Enrichment Protocol (Appendix 2) to hatch and harvest Artemia according to the sections Prepping the Hatch Tank (p. 6), Hatching Artemia (p. 9), Harvesting Procedure (p. 4), and Cleaning after harvesting (p.

- 6). *Artemia nauplii* should be stored in a refrigerator with aeration to slow their metabolism and growth if not fed immediately after harvest.
2. Refer to the National Sea Simulator Rotifer Culture Protocols (Appendix 3) to culture, enrich and harvest enriched rotifers.
  3. Once *Artemia nauplii* or enriched rotifers have been harvested, determine the density of the harvest and calculate the total number of harvested individuals, then calculate the volume of treated seawater needed to resuspend the concentrated, harvested individuals to reach stock concentrations of ca. 4000 *Artemia nauplii*/mL or 5000 rotifers/mL (steps 4 and 5, respectively). Lastly, determine the volume of feeding stocks to add to each coral tank to reach the final feed concentration of 0.5 individuals/mL (step 6).
  4. To determine the density of the harvested *Artemia* stock, take 10 mL of the sample for counts, and from that take an aliquot of 1 mL and pour it into a Bogorov counting chamber, perform counts under a stereoscope.



*Figure 1. Bogorov counting chamber under a stereoscope to perform Artemia counts (Photo credit: Sara Godinez-Espinosa).*

- a) To calculate the density of the harvested *Artemia* stock, use the following formula:

$$\text{Artemia concentration (individuals/mL)} = \text{Artemia count (individuals)} / \text{Volume harvested (mL)}$$

**Example:**  $\text{Artemia concentration} = 100 \text{ Artemia} / 1 \text{ mL} = 100 \text{ Artemia/mL}$

- b) To calculate the total number of harvested *Artemia*, use the following formula:

Total number of harvested Artemia (individuals) = Artemia concentration (individuals/mL) \* Harvested volume (mL)

**Example:** Total number of harvested Artemia = 600 Artemia/mL \* 50,000 mL = 30,000,000 Artemia

c) To calculate the volume of treated seawater needed to resuspend the harvested Artemia (concentrated in a net) to reach the **desired feeding stock concentration of ca. 4000 Artemia nauplii /mL**, use the following formula:

Volume of treated seawater (mL) = Total number of harvested Artemia (individuals) / Feeding stock concentration (individuals/mL)

**Example:** Volume of treated seawater = 30,000,000 Artemia/ 4000 Artemia/mL = 7,500 mL

5. To determine the density of the harvested rotifer stock, take 1 mL of the sample for counts and pour it into a Sedgewick Rafter counter then add a drop of Iodine. Place the glass slide on to the counter and using a microscope in 4x magnification to count all rotifers in the Sedgewick Rafter counter (Figure 1).

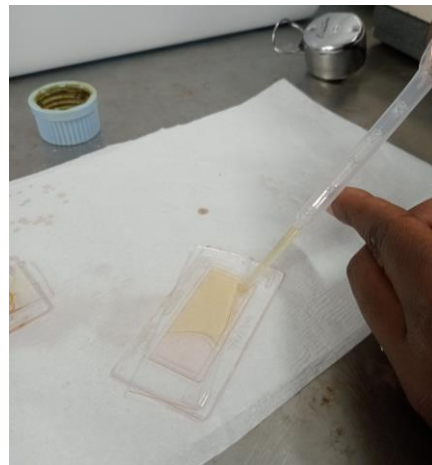


Figure 2. Loading 1 mL of rotifer harvest to the Sedgewick Rafter Counter (Photo credit: Sara Godinez-Espinosa).

1. To calculate the density of the harvested rotifer stock, use the following formula:

Rotifer concentration (individuals/mL) = Rotifer count (individuals) / Volume counted (mL)

**Example:** Rotifer concentration = 600 rotifers / 1 mL = 600 rotifers/mL

2. To calculate the total number of harvested rotifers, use the following formula:

$$\text{Total number of harvested rotifers (individuals)} = \text{Rotifer concentration (individuals/mL)} * \text{Harvested volume (mL)}$$

**Example:** Total number of harvested rotifers = 600 rotifers/mL \* 2,500 mL = 1,500,000 rotifers

3. To calculate the volume of treated seawater needed to resuspend the harvested individuals (concentrated in a net or on a screen) to reach the **desired feeding stock concentration of ca. 5000 rotifers/mL**, use the following formula:

$$\text{Volume of treated seawater (mL)} = \text{Total number of harvested rotifers (rotifers)} / \text{Feeding stock concentration (rotifers/mL)}$$

**Example:** Volume of treated seawater = 1,500,000 rotifers / 5000 rotifers/mL = 300 mL

6. **The desired live feed densities to be presented to coral spat is 0.5 individuals mL<sup>-1</sup> of each zooplankton species.** The amount of feeding stock needed per tank depends on the tank's water volume. To calculate the volume of feeding stock to add to a coral tank, refer to Table 2 or use the following formula:

$$\text{Volume of feeding stock (mL)} = \text{Water volume in coral tank (mL)} * \text{Desired feed concentration (individuals/mL)} / \text{Concentration of feeding stock (individuals/mL)}$$

**Example Artemia:** for a tank with 1000 L water: Volume of feeding stock (mL) = 1,000,000 mL \* 0.5 Artemia/mL / 4000 Artemia/mL = 125 mL

**Example rotifers:** for a tank with 1000 L water: Volume of feeding stock (mL) = 1,000,000 mL \* 0.5 rotifers/mL / 5000 rotifers/mL = 100 mL

Table 2. Feeding stock volumes needed to reach end concentration of 0.5 individuals/mL

Water volume in coral tank	Amount of Artemia feeding stock (ca. 4000 nauplii/mL)	Amount of rotifer feeding stock (ca. 5000 rotifers/mL)
100 L	12.5 mL	10 mL
500 L	62.5 mL	50 mL
1000 L	125 mL	100 mL
5000 L	625 mL	500 mL

7. Aliquot the correct volume of each live feed organism into a separate container for each tank and pour gently into the tank near the circulation pump.

- ***Notes on special handling, transport, and storage***

All equipment and materials used to feed the coral spat must be rinsed with fresh water and dipped in a solution of Biosan.

#### Biosan Bath

1. Wear appropriate PPE (safety glasses and nitrile gloves)
2. Drain all the old Biosan solution from the bath container.
3. Rinse with fresh water and refill the bath container with fresh water.
4. Add Biosan powder to the bath container to have a final concentration of 1%.
5. Rinse all equipment and materials with fresh water before placing into the Biosan bath.
6. All equipment and materials must be left in the Biosan bath for a minimum of 1 hour.
7. After removal from the Biosan bath, rinse all equipment and materials with fresh water before use.

- ***Monitoring, maintenance, and assessment procedures***

- During the holding/grow out period of the corals, health should be monitored daily.
- All tile surfaces need to be cleaned on a weekly basis or as needed according to other provided SOPs or training materials.
- The husbandry of corals is essential for survival and growth, to keep the algae down, brush gently the surfaces to remove superficial diatoms, green algae, brown algae or crustose coralline algae (CCA). These organisms can compete with coral spat and over grow the corals which can lead to restricted growth or mortality.

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## 9 Acronyms

CCA Crustose coralline algae

CSIRO Commonwealth Scientific and Industrial Research Organisation

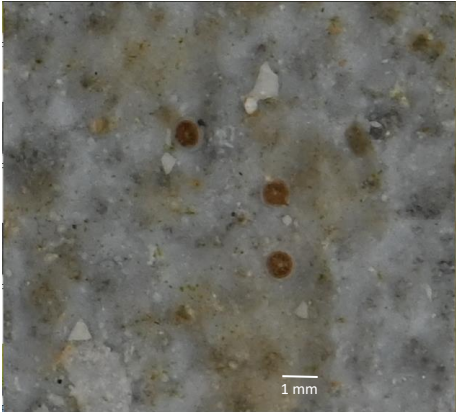

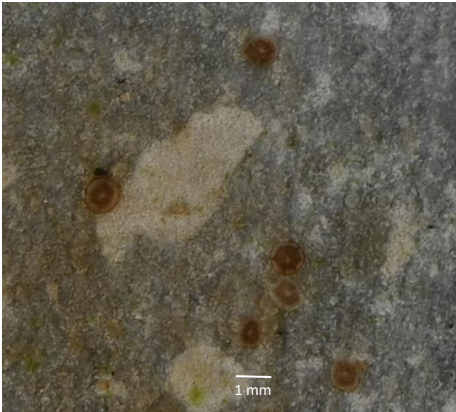
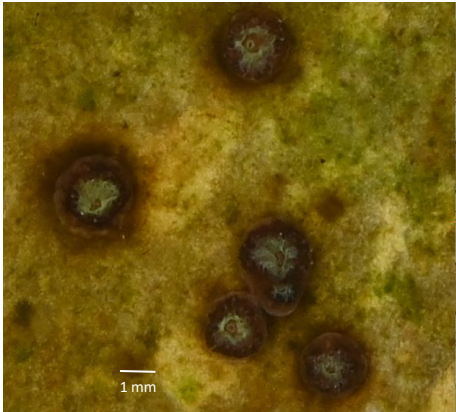
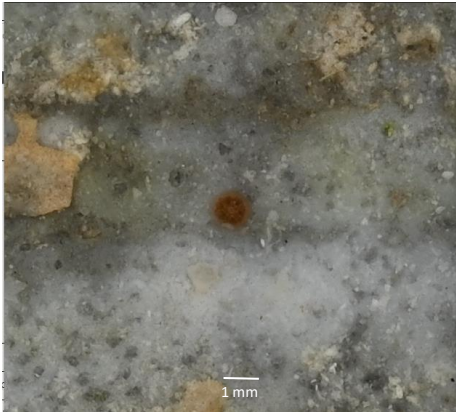

PPE Personal Protective Equipment

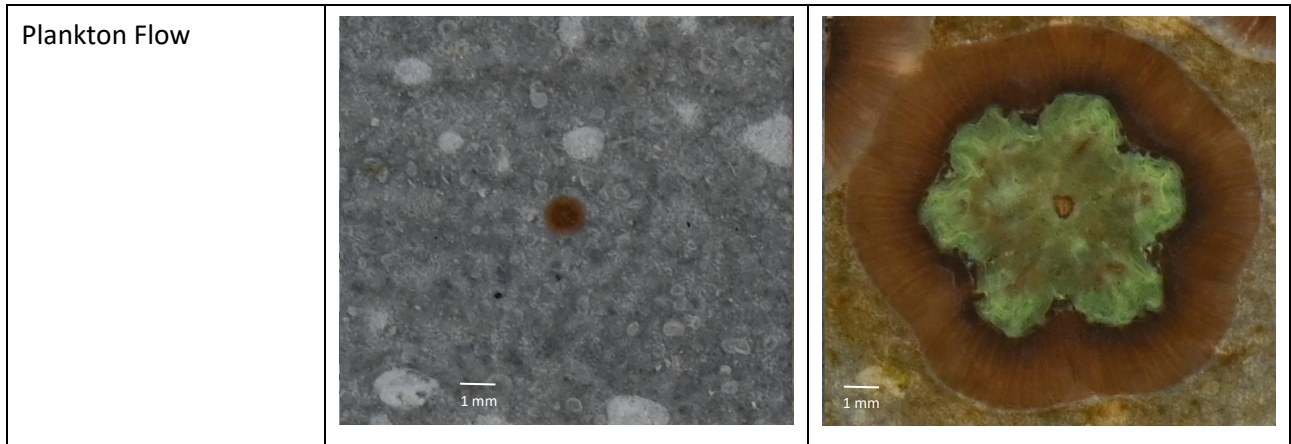
RRAP Reef Restoration and Adaptation Program

SOP Standard Operating Procedure

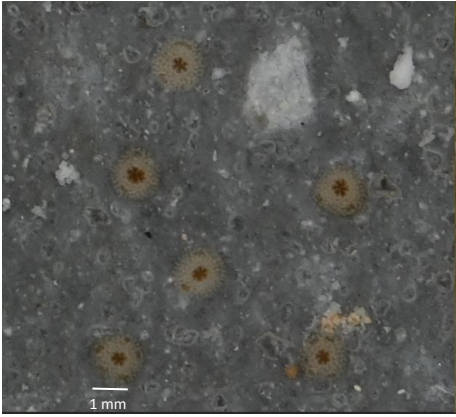

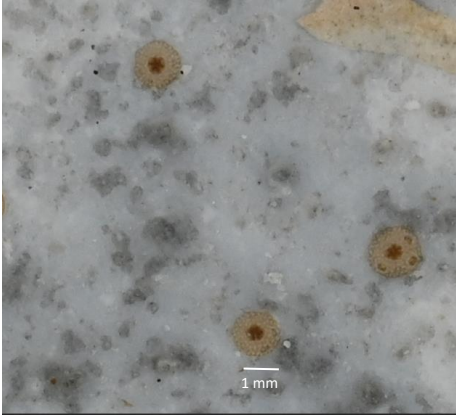
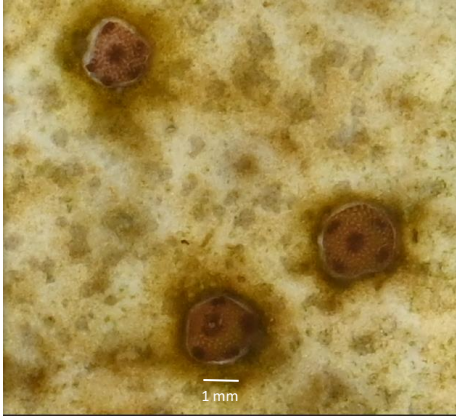
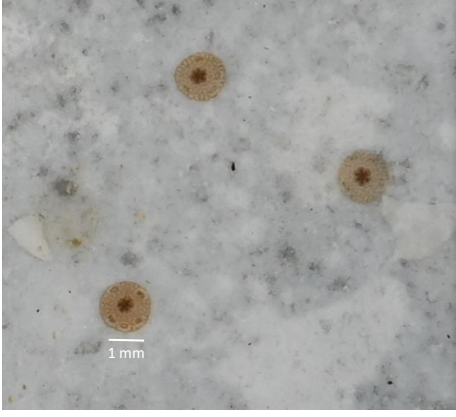

# Appendices

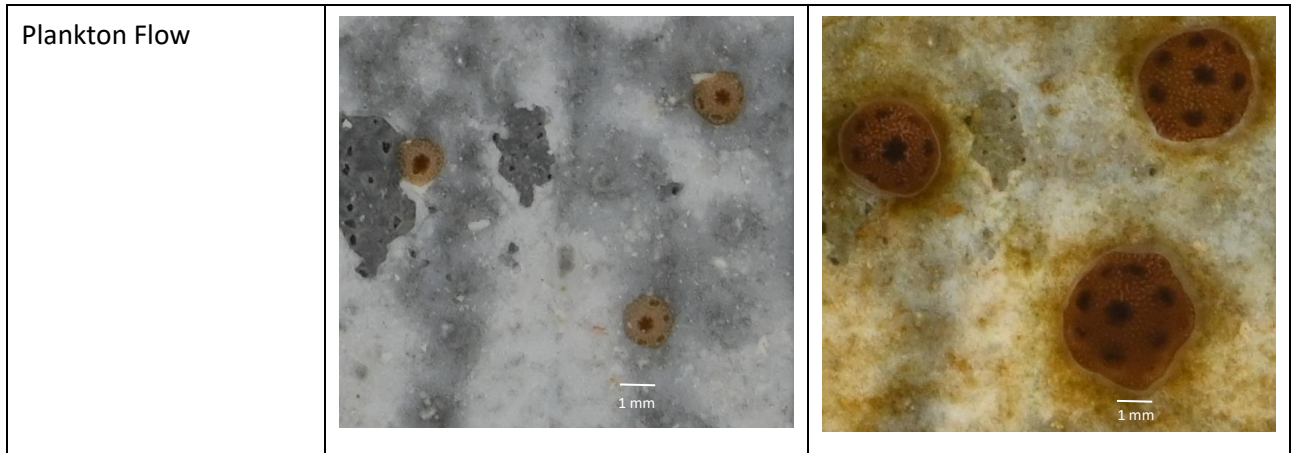
## Appendix 1: Supplementary figures

<i>Platygyra daedalea</i>		
Treatment	T0 (16 days after settlement)	T2 (80 days after settlement)
Control Flow (no feed)		
Dissolved Nutrients Flow		
Mix Flow		



*Supplementary figure 1. Representative photos of Platygyra daedalea spat fed with one of four diet treatments. Photos were captured at two time points: T0 (16 days after settlement) and T2 (80 days after settlement).*

<i>Acropora millepora</i>		
Treatment	T0 (12 days after settlement)	T2 (76 days after settlement)
Control Flow (no feed)		
Dissolved Nutrients Flow		
Mix Flow		



*Supplementary figure 2. Representative photos of Acropora millepora fed with one of four diet treatments. Photos were captured at two time points: T0 (12 days after settlement) and T2 (76 days after settlement).*



# Appendix 2: SEASIMULATOR ARTEMIA HATCHING, HARVESTING & ENRICHMENT PROTOCOL

## AIMS National Sea Simulator Facility

AIMS-SOP-(NSS)-##


### Document Details

<b>Authored by:</b>	Shanae Read	<b>Authorised by:</b>	
<b>Version No:</b>	4	<b>Responsible Officer:</b>	
<b>Date of Issue:</b>		<b>Review date:</b>	

### Tasks Included in this SOP

1. Instructions for harvesting Artemia in the National Sea Simulator Facility
2. Instructions for enrichment of Artemia in the National Sea Simulator Facility
3. Instructions for hatching Artemia in the National Sea Simulator Facility

### Significant Health, Safety & Environmental Risks

Hazards	Risk	Control
Lifting of heavy items (harvesting magnets & feed-out buckets)	Possible back, arm and/or shoulder strain/injury.	Ensure you are comfortable lifting the Artemia harvest magnets, if not please inform your supervisor so we can assign other team members to this task.  Use the correct lifting posture and techniques when lifting/carrying magnets & feed-out buckets.  If buckets are above a comfortable weight, please use a trolley for transport around the National Sea Simulator Facility.
Wet Floor area	Possible slippage	Ensure proper footwear is worn throughout the National Sea Simulator Facility, including the Artemia preparation area.  Do not run through the facility.
Chemical 	Possible chemical burns from bleach.	Always wear eye protection when using bleach (O-Chlor)  Ensure you have read and understood the chemical SDS relating to O-Chlor.  Pour required amount of bleach into a small beaker at bench height to reduce risk of pouring bleach into hopper at extension.



Trips/Falls	Strain/Injury	<p>Ensure hoses and Artemia harvesting cannon are placed out of the way when not in use. Be aware of your surroundings when working in the harvesting area.</p> <p>Use a step stool when cleaning the Artemia hoppers. Do not stand on the FRP.</p>
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**Personnel Prerequisites & Competencies**

Personnel are required to have read this SOP and the relevant TRA before commencing this task. Competency of undertaking this task must be undertaken by Supervisor before commencing work unsupervised.

Personnel must be inducted into the SeaSim Precinct.

**Tools & Equipment**

- Artemia harvesting hopper, with temperature control & aeration.
- Artemia harvesting separator set-up. Complete with harvesting cannon, harvesting magnets, harvesting spout, hose connection from hopper-cannon, catchment tub, net stand and harvesting net.
- Enrichment hopper with aeration
- Fridge with aeration.
- General Purpose Ambient Seawater hose
- Freshwater hose
- 20L bucket with defined volume lines
- 3L jug
- 5L jug
- 1L beaker
- 100mL measuring cylinder
- Custom spoons for feeding out Artemia (30mL & 140mL)

**Abbreviation & Definition**

GPA (General Purpose Ambient Seawater)  
 HUFA (Highly Unsaturated Fatty Acids)  
 RRAP (Reef Restoration and Adaptation Program)



Pictures of Equipment

Artemia harvesting hopper, with temperature control and aeration	Complete artemia harvesting set up	Enrichment hopper with aeration
		
Fridge with aeration	Water Supply	
		
20L bucket with volume lines	3L jug	5L jug
		
1L beaker	100ml measuring cylinder	Custom spoons for artemia out feeding (30ml & 140ml)



## Procedure

### Title

#### General Overview

Artemia needs to be hatched and harvested daily, with the cysts placed in the hopper the day prior to harvest. We alternate between Hopper 1 and Hopper 2 (Figure 1) as a hatching vessel. The other hopper is used as a chlorine bath overnight to sterilise equipment used in this area.

The Enrichment hopper is bleached each night and then cleaned each morning to enrich artemia during the day. We harvest artemia in the morning and place the regular batch in the fridge over the course of the day. The fridges where we keep the harvest bucket of artemia during the day are located on the other side of the wall in which the hoppers back on to (beside CMMG aquarium rooms).

#### Harvesting Procedure (8am).

1. Turn off aeration and temperature control for the Artemia harvesting tank. The left switch controls the temperature for Hopper 1, the right switch for Hopper 2. These are labelled (Figure X).
2. Prepare the SeptArt harvesting cannon as pictured in Figure (X). Carefully place the magnets within the harvesting cannon, with the handle facing towards the opening. Take care to not damage the magnets, as it can cause internal swelling and rusting if they are damaged. Push the SeptArt cap onto the harvesting cannon, with the cut out facing upwards to reduce leaks. Flush the harvesting cannon with General Purpose Ambient (GPA) seawater, ensuring that there is no dirt within the set-up.
3. Below the opening of the SeptArt cap, place the catch container, with the outlet orientated towards the floor drain. Place the PVC frame over the catch container (Figure X). Carefully check the harvesting net for any signs of damage, and if there are none present place this on the PVC frame, with the lining facing outwards to prevent Artemia catching in it (Figure X).
4. Attach the small harvesting hose to the hatch tank, and place the open end inside the SeptArt cannon. Open the valve slowly, allowing the flow to reach 4L/min. This flow rate will ensure only the live artemia flow into the net while the magnetised cysts are captured by the magnets. If the flow rate is set too high, cysts will run through the magnets and into the hatching net. If you are unsure about your flow rate, it is better



to slightly reduce flow. Measure your flow with a beaker and a timer, ensuring you are operating it correctly.

5. Allow the hatch tank to completely drain through the magnets, checking the harvest net throughout the process to ensure no cysts are coming through, and that no Artemia are leaking through small holes in the net. You should not adjust the hatch tank valve through the harvesting process as it can put a burst of water through the cannon disrupting the cysts on the magnets.
6. Once the harvest hopper has drained, place the GPA hose into the tall end of the separator tube, turning on the seawater to the same flow rate (4L/min) established when harvesting. Flush for approximately 20 mins, ensuring most of the artemia have been flushed to the harvesting net. Set this flow outside of the harvesting cannon, ensuring that excess water flow does not disrupt the cysts on the internal magnets. While flushing the SeptArt cannon with seawater, place the freshwater hose on a low flow (~4L/min) into the catch container. This reduces this risk of a bacterial spike from the hatch tank making its way into the Feedout bucket. Artemia can survive in low salinity conditions such as these. Leave the freshwater hose in for an additional 5 minutes after the GPA seawater hose has been removed from the cannon.
7. After flushing Artemia with seawater, rinse all Artemia to the bottom of the net using the freshwater hose. Do not fully lift the net out of the catch container when it is full of water. Slowly swirl the water out as you lift to reduce the chance of breaking the net. Swirl the Artemia in the netting, creating a condensed, thick paste.
8. Gather the net above the Artemia paste holding the opening to the net shut and invert the net into the bucket. The opening of the net should be facing the bottom of the bucket with all the Artemia paste gathered in a pocket on top of the opening.
9. Let go of the net, releasing the Artemia paste into feeding bucket. Make sure to rinse all Artemia from the net to the feeding bucket with GPA.
10. Top up feeding bucket with GPA to indicated volume on Artemia sheet to create your SeaSim stock solution. Check the Artemia sheet for the nominated volumes for enrichment and remove this to add to the enrichment hopper, covered below. Check the Artemia sheet and remove the volume specified for researchers (RRAP) and place this into the outdoor fridge, alongside the bucket of remaining solution with an airstone within each set to a gentle bubble.

#### **Enrichment hopper (8am-10am)**

1. While the harvest tank is draining, empty the enrichment hopper, filled with diluted bleach. Use a long hose to drain this directly to the floor drain. Rinse with freshwater to remove any residual bleach.
2. Rinse the ball-valve on the enrichment hopper, remove it from the tank by unscrewing the back segment. Then place the freshwater hose against this, on the "shower" setting while opening and closing the valve. This will dislodge any Artemia caught within the mechanism. Replace the ball-valve on the hopper and close it.
3. Fill the enrichment hopper with GPA to the designated amount (~20L). Turn on aeration.
4. Checking the Artemia sheet, identify the quantity of microalgae and HUFA (Highly Unsaturated Fatty Acids), designated for enrichment purposes. Collect microalgae



from the “Algae Lab” and the HUFA from the “Diet Room” fridge. Add these both to the enrichment hopper.

5. Upon the completion of the harvesting of Artemia, take from the full Artemia bucket the specified volume of Artemia required for Enrichment listed on the Artemia sheet and add it to the enrichment hopper. Turn on aeration.
6. Leave this to enrich until it is harvest time (315pm).

#### **Prepping the Hatch Tank (8am-10am)**

1. Remove items inside the hopper and rinse well with freshwater. Set aside on racks to dry.
2. Drain the hopper filled with diluted bleach. Use a long hose to drain this directly to the floor drain. Rinse with freshwater to remove any residual bleach.
3. Re-fill the cleaned hopper with GPA to the level indicated on the outside of the tank (140L).
4. Once filled, turn on aeration and temperature control for the hatch hopper. The left switch controls the temperature for Hopper 1, the right switch for Hopper 2. These are labelled (Figure X). Check temperature coils in tanks for leaks in the John Guest components, and that all valves are open. Check that temperature probe is inside the hatching tank so that accurate temperature measurements are taken.
5. Go to the Diet Room and measure the set volume of ACE from the fridge, as indicated on the Artemia sheet. Add to the hopper filled with GPA, rinsing the measuring cylinder or beaker well.
6. This tank is now ready for the hatch to be put on at 12pm.

#### **Cleaning after harvesting (8am-10am)**

1. Once the harvest has completed, rinse the hatch tank with freshwater to remove residual cysts. Use the long hose to drain wastewater directly into drainage grate.
2. Scrub interior of the hatching tank, airline, and titanium loop with a sponge and detergent. Rinse thoroughly with freshwater.
3. Remove the ball-valve from the bottom of the hatch tank, using the freshwater hose, blast water through the valve while opening and closing the valve handle to dislodge any cysts that may have been caught in the mechanism.
4. While the ball-valve is removed, using the “Jet” function, push water up the pipework of the hatch tank, flushing cysts and other debris from the bottom pipework and aeration inlet. Rinse the inside of the tank after all other areas are cleaned.
5. Replace ball-valve to bottom of tank and close the valve. Refill hatching hopper with freshwater and add ~100 mL of bleach. Wear safety glasses and pour the bleach in slowly to avoid splashes. Add enough freshwater to completely fill the tank, this is to account of bacteria on the sides from cysts splashing up while bubbling. Add a chemical caution sign to the outside of the tank.
6. Place any small equipment used during harvesting into the bleached hopper overnight.
7. Rinse all hoses used in the harvesting process with freshwater. Hang in the designated area to dry.

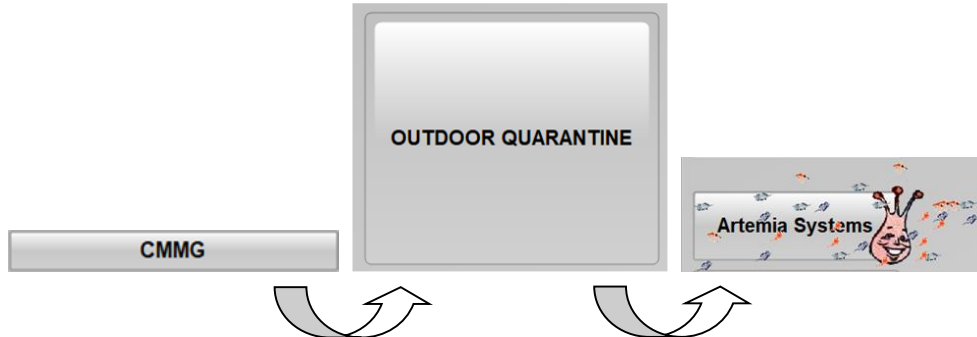


8. Rinse all components of the SeptArt harvester with freshwater, including the cap, cannon, PVC frame & catch container. Place to designated area to dry.
9. Rinse magnets with freshwater, carefully to not catch them on the concrete or FRP drain grate. After initial rinse, wipe over with a white sponge and detergent, removing the last traces of cysts. Rinse again with freshwater and leave aside to dry.
10. Carefully rinse the hatch net with freshwater, taking care that it does not touch the concrete or FRP grate which can cause holes. Once rinsed, place in the chlorine filled hopper and remove after 30 mins. Make sure to rinse with fresh water before hanging back up.
11. Hose down the floor of the area, pushing all cysts and dirt to the drain. Flush the drain after all the floors are hosed down to prevent stagnant piles of cysts forming.

### SCADA checks (morning)

The person in charge of harvesting Artemia should perform a SCADA temperature check on that days hatching vessel. These checks can be done in the morning while we wait for artemia to drain from the hatching tank, or while we flush the SepArt separator. As we only have the ability to either heat OR cool, it is important to monitor this closely during seasonal times of change throughout the year.

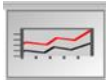
1. To view temperature profiles for artemia hoppers click on:





- The hopper with temperature control has a green valve symbol above the Sea Monkey container while the hopper with no temperature control is labelled manually isolated. This green symbol is only evident if the temperature switch is on, use the System 1 label and System 2 label which matches the hopper numbers.

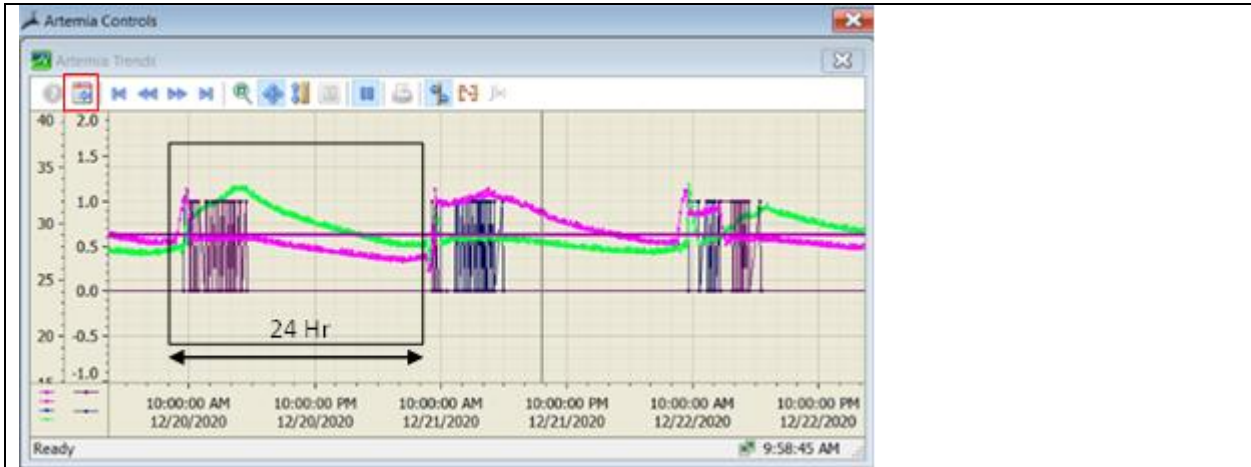


- Click on this symbol  to view temperature trends over time. Make sure the temperature does not rise significantly above or below 29°C for most of the day and night for the hatching hopper.


Below are several examples of temperature trends.



You can see the hopper with temperature control turned on (Pink line) is holding steady throughout the 24 hours it is manipulating temperature. The other hopper (Green line) decreases in temperature during the night hitting below 25°C as the hopper receives no temperature manipulation.



You can see the binary on/off dosing from the solenoid. 1.0 = on while 0.0 = off. The ratio between the frequency of dosing and temperature, as well as the time-of-day dosing occurs, can tell you a lot about how well the system is functioning and if it is functioning correctly.

Click on this symbol  to open the Trend Properties screen and change which variables are seen on the graph.

- By ticking off the boxes beside each trend you can toggle them on or off in the graph
- By selecting each trend, you can double check the assigned trend colour

**Troubleshooting Temperature**

Temperature can be incorrect for a variety of reason:

- The temperature probe was removed from the tank and is measuring air temperature instead of the water temperature. This causes the system to temperature manipulate for the wrong temperature creating a positive feedback loop.
- The solenoid is stuck in one position. This causes too much or too little water to travel through the coils causing ineffective temperature manipulation.
- Temperature manipulation was not turned on for the new hatching tank during harvest in the morning. This causes no temperature manipulation to occur. During times of the year when air temperature is steady around 29°C it is not a big problem. However, in summer months, temperatures rise too high during the day and during winter months, temperatures drop too low during the night.

If temperature seems irregular:

- Make sure the temperature probe is in the hopper
  - Make sure that temperature control is turned on for the correct hopper
- \* If all seems normal but temperature irregularities continue, find a SeaSim staff member to aid with continued troubleshooting.

**Hatching Artemia (12pm)**

1. Check the Artemia sheet for the required weight of cysts identified for hatching. In a 3L jug, weigh the required amount of cysts located in the fridge in the Diet Room.
2. Check for open tins of cysts before opening a new one, replace back in the fridge when finished if tin is not empty with the can lid secured on top. If cysts are running



low, additional boxes can be collected from the Stores facility where it is a stocked item.

3. Add approximately half a litre of freshwater to the jug. Stir the cysts and water together ensuring there is no clumps of dehydrated cysts. Check for dry spots on the sides and bottom of the jug. Once well mixed add additional freshwater to the jug.
4. Add hydrated cysts to the prepared hatch tank (see above). Rinse the cysts from the jug into the tank using the shower setting on the freshwater hose. Rinse the sides of the tank where cysts are sticking, and the surface of the water if they cysts are floating on top. Repeat until a majority of the cysts are immersed in the tank water.
5. Rinse jug and spoon and add to chlorine filled hopper for sterilisation.
6. Ensure the hatching tank has aeration and temperature control on.

#### **Bypass Systems Prior to Feeding (315pm)**

1. Set all systems throughout SeaSim to bypass mode for the evening. Refer to Backwashing SOP for details on how to do so, or check with your supervisor if you are unsure.

#### **Enriched Artemia Harvesting (330pm)**

1. Place the catch container and PVC frame directly under the enrichment hopper outlet. Place a small amount of GPA into the bottom of the catch container. Add the harvesting net to the top of the PVC frame, as was done for the initial harvest.
2. Turn off aeration to the enrichment hopper. Open the valve of the enrichment hopper fully, harvesting the Artemia directly into the net. Rinse the enrichment hopper with GPA once empty, so all Artemia are rinsed through the outlet.
3. Flush the catch container for 5 minutes with freshwater for 5 minutes. This step removes all free-floating HUFA from the water that has not been ingested by Artemia and is highly important. Multiple studies have shown that free-floating HUFA in the water column can be toxic to Acropora coral species. If water appears cloudy then please rinse for longer until it is clear.
4. Once rinsing is finished, swirl the net to remove access water while rinsing with GPA, creating an artemia paste. Follow the same procedure as outlined above in **Harvesting Procedure**.
5. Add artemia paste to 10 L bucket and rinse any remaining Artemia on netting with GPA into the bucket. Top up with GPA to specified volume identified on Artemia sheet (same volume of regular artemia added in the morning to the enrichment hopper).
6. Rinse enriched hopper with freshwater and fill to top with freshwater. Flush valve in the same procedure as used for the hatching tanks. Add ~30mL of bleach to the enrichment hopper carefully while wearing appropriate safety equipment (safety glasses).
7. Rinse the catch container and PVC frame with freshwater, set aside to dry.
8. Rinse the Artemia harvesting net with freshwater, add to chlorine bath for duration of time for feeding out. Ensure that it is removed and rinsed before going home.
9. Feed out regular Artemia and enriched Artemia to allocated systems according to the feed sheet, located on the teams channel SeaSim General and SeaSim Husbandry.
10. After completing feed outs, rinse all equipment used (feeding buckets, beakers, ladles) with freshwater before placing them in the bleach filled hatching tank



overnight. Remove harvesting net from the chlorine bath, rinse with freshwater and hang to dry.

11. Before leaving for the day ensure all equipment used throughout the day is cleaned and returned to its spot to dry overnight. Ensure all valves for freshwater and GPA are turned off and that temperature control is turned on for the hatching tank. Leave the space in a clean state in preparation for tomorrow.



### Responsibilities

It is the responsibility of the supervisor to induct the user in the work area and familiarize the user with the SOP. The supervisor should induct the user on the safe use of chemicals and equipment used in this SOP. The supervisor must deem the user competent in the task prior to the user operating on their own.

It is the responsibility of the User to read and understand the procedures outlined below and adhere to the instructions identified at induction and in this SOP. The user should feel safe when performing tasks outlined in the SOP and should seek advice from your supervisor or another SeaSim staff if in doubt.

### Environmental Consideration

O-Chlor: Very toxic to aquatic organisms. Safe to environment at low concentrations



**Chemical Disposal**

O-Chlor: The diluted working solution (<3% sodium hypochlorite) may be disposed of down the sink or drain by flushing with lots of running water. PPE must be worn (enclosed shoes, dishwashing gloves and safety glasses).

**References**

NA

**Appendices**

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## Appendix 3

### AIMS National Sea Simulator Facility

### AIMS-SOP-


#### Document Details

<b>Implementation date:</b>		<b>Authorised by:</b>	
<b>Version No:</b>		<b>Responsible Officer:</b>	Grace Othieno
<b>Date of Issue:</b>		<b>Review date:</b>	




#### Tasks Included in this SOP

Rotifer Culture Protocols

#### Significant Health, Safety & Environmental Risks

Hazards 	Risk	Control
Heavy items	Possible back, arm, shoulder, strain/injury	Lift the filled artemia buckets and magnets with your legs, not your back. If buckets are too heavy to carry, use a trolley.
Wet area	Possible slippage	Ensure proper footwear is worn and move cautiously. Walk, do not run in the Quarantine area and Artemia preparation area.
Chemical	Possible chemical burn from bleach	Make sure to wear eye protection when pouring bleach. Make sure to pour bleach slowly and carefully into the hopper to minimize splashback.
Trips/Falls	Strain/Injury	Tripping over hoses or Artemia separator. Use a step stool when rinsing/washing down hopper.

#### Personnel Prerequisites & Competencies

<p><b>PERSONAL PROTECTIVE EQUIPMENT (PPE)</b></p> <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;">  <p>Eye protection must be worn in this area</p> </div> <div style="text-align: center;">  <p>Foot protection must be worn</p> </div> <div style="text-align: center;">  <p>Safety gloves must be worn</p> </div> </div>	<p><b>DO NOT attempt to perform this task without formal instruction/training!</b></p> <p><i>This SOP is to be used in conjunction with any competency training or specific TRA relevant to this task.</i></p>
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General Seasim induction



### Required Conditions

	<ol style="list-style-type: none"> <li>1. Room temperature - 26°C</li> <li>2. Culture Water- 35ppt</li> <li>3. 1micron filtered GPA water.</li> <li>4. Aeration</li> <li>5. S-Parkle</li> <li>6. Enrichment Tanks</li> <li>7. Sedge wick rafter counter</li> <li>8. Microscope</li> <li>9. Iodine</li> <li>10. 5L Jug</li> <li>11. 500mL jug</li> <li>12. Biosan.</li> <li>13. Fresh water</li> <li>14. Harvesting screens (57 and 255µm)</li> <li>15. Nally tubs</li> </ol>
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### GPA Sea Water System

	<p>When working on the rotifer systems, use 1 micron filtered GPA. Set up as per image.</p> <ol style="list-style-type: none"> <li>1. Insert cartridge filter in the blue casing making sure it sits right in the centre.</li> <li>2. Tightly screw the casing on to the filter cap.</li> <li>3. Flush the line for 1-2 minutes before use.</li> </ol>
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## Weekend Harvesting and Enriching Rotifers (Usually Between 9:00 -10:00 am)

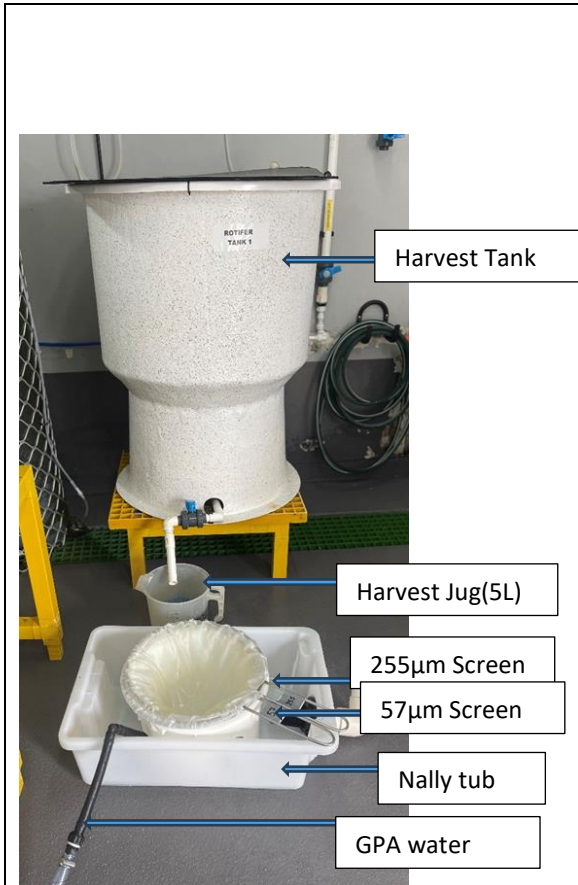


Image of harvesting set up.

1. Fresh water rinse the enrichment tank and partially fill with 2L GPA
2. Wear appropriate PPE and thoroughly rinse all gear from the Biosan tub with fresh water.
3. Use ½ nally tub with cut down bucket ring to support screens.
4. Fill the nally tub with GPA.
5. Open the bottom valve of the harvest tank for a few seconds to waste until an all-clear flow to purge the initial bottom culture.
6. Harvest 6L of the culture from the harvest tank in a 5L jug.
7. Have the smallest sized screen on the bottom. (57 micron first, the 255 micron on top).
8. Gently pour the harvested rotifers through the screens.
9. Remove the upper screen (255 micron) and put a side.
10. Rinse the rotifers thoroughly with GPA water and concentrate the rotifers.
11. Cup the underneath of the screen in your hand and invert screen into a 5L Jug filled with 1L GPA.
12. Rinse out the rotifers in the filter in the Jug to the 3L mark.
13. Transfer the rotifers to the enrichment tank.
14. Take 1ml of the sample for counts on to a sedge wick counter and add a drop of Iodine.
15. Place the glass slide on to the counter and count all rotifers in the sedge wick counter.
16. 6L harvest @250 rotifers/ml ≈ 1.5million rotifers
17. Get the S.parkle enrichment solution bottle from the fridge and shake well.
18. Measure 10mL of S.parkle enrichment solution and add to the enrichment tank.
19. Allow for a minimum of 4 hours for the Rotifers to be enriched.
20. Fold the GPA horse and put a side.

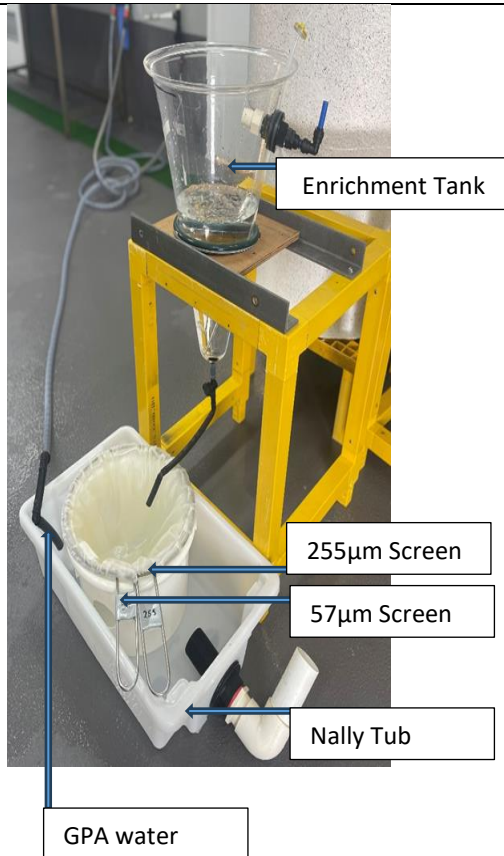
**Enrichment:**



Picture of S-parkle

1. Weigh 25g of S.parkle (~ 45mL in falcon tube).
2. Measure 700mL of RO water in a clean jug.
3. Add the weighed S.parkle in the jug and blend well using a hand blender.
4. When fully dissolved, pour the enrichment solution in a cleaned labelled bottle and store in the fridge.

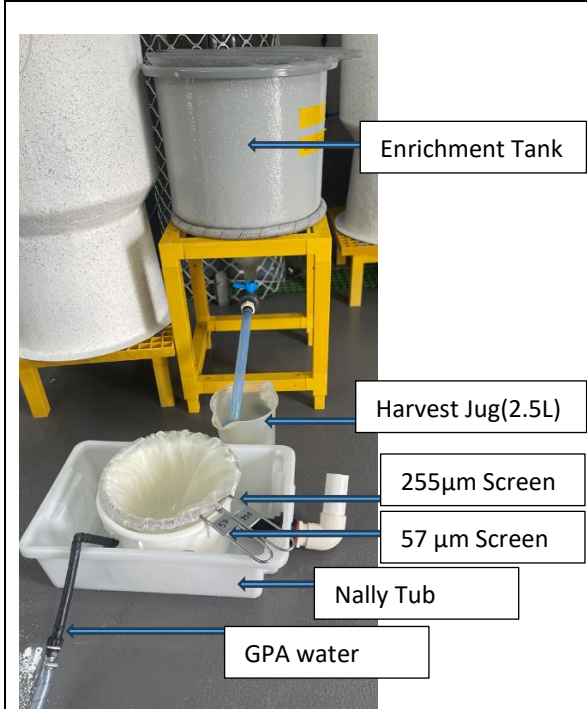
**Weekend Harvesting Of Enriched Rotifers (Before 2:00pm)**



1. See picture for set up.
2. Wear appropriate PPE and thoroughly rinse all gear from the Biosan tub with fresh water.
3. Use ½ nally tub with cut down bucket ring to support screens.
4. Fill the nally tub with GPA.
5. Have the smallest sized screen on the bottom. (57 micron first, the 255 microns on top).
6. Direct the poly pipe at the bottom over the set-up screens.
7. Open the bottom valve on the enrichment tank and harvest all the enriched rotifers.
8. Rinse the tank with 500mL of GPA water.
9. Remove the top screen and rinse the rotifers with GPA.
10. Cup the underneath of the screen in your hand and invert screen into a 500mL Jug.
11. Rinse out the rotifers in the screen with GPA and Concentrate to 300mL
12. Clean and scrub the enrichment tank with antibacterial soap. Rinse thoroughly with fresh water, close the bottom valve and add 500mL of the prepared biosan solution.
13. Take the harvested rotifers and distribute to experimental tanks accordingly.
14. Disconnect the GPA Hose and flash with fresh water.
15. Unscrew the GPA filter casing and rinse the case with fresh water.
16. Rinse the cartridge filter with fresh water and hang up to dry.



**Weekday Harvesting Enriched Rotifers (Before 2:00pm)**



**How to work out the Concentration Volume after Harvesting 2,500mL from The Grey Enrichment Tank**

1. Mix the 2,500mL of rotifers harvested and take a 1mL Sample.
2. Transfer the sample to a sedge wick rafter counter, add a drop of iodine and place the glass slide on.
3. Using a X4 magnification, count all rotifers in the sedge wick rafter counter (e.g. 600 rotifers)
4. Total number of rotifers in 2,500 mL =  $600 \times 2500 = 1,500,000$
5. To obtain a concentration of 5,000 rotifers per mL =  $1,500,000/2,500 = 300\text{mL}$

1. See picture for set up.
2. Wear appropriate PPE and thoroughly rinse all gear from the Biosan tub with fresh water.
3. Use ½ nally tub with cut down bucket ring to support screens.
4. Fill the nally tub with GPA.
5. Have the smallest sized screen on the bottom. (57 micron first, the 255 micron on top).
6. Attach the harvest hose to the bottom of the enrichment tank and direct it over the set-up screens.
7. Open the bottom valve on the enrichment tank and harvest 2.5 L of the enriched rotifer.
8. Pour the harvested enriched rotifers through the screen.
9. Remove the top screen and rinse with GPA.
10. Cup the underneath of the screen in your hand and invert screen into a 500mL Jug.
11. Rinse out the rotifers in the screen with GPA and Concentrate to 240mL
12. Take the harvested rotifers and distribute to experimental tanks accordingly.

**How to work out the concentration volume Based on rotifer counts Recorded in the Diary**

1. To Determine Concentration Volume when harvesting from the Grey Enrichment tank,
2. Rotifer count recorded in the diary (e.g. 6376R/ml), collected from Volume of 5,000mL
3. Total Number of Rotifers in 5,000mL =  $6376 \times 5,000 = 31,880,000$  rotifers
4. Total number of rotifers transferred to 40,000mL enrichment tank = 31,880,000
5. Volume to harvest from grey enrichment tank = 2,500mL
6. Number of rotifers in 2,500mL =  $31,880,000 \times 2,500 / 40,000 = 1,992,500$  rotifers
7. To obtain a concentration of 5,000 rotifers per mL =  $1,992,500 / 5,000 = 398.5$  mL of GPA



Biosan Bath

	<ol style="list-style-type: none"> <li>1. Wear appropriate PPE (goggles and gloves)</li> <li>2. Drain all the old biosan solution from the nally tub.</li> <li>3. Rinse with fresh water and refill with fresh water.</li> <li>4. Add 10 g of biosan powder.</li> <li>5. Rinse all gear used with fresh water and place in Biosan bath.</li> <li>6. Empty the GPA nally tub and rinse with fresh water and place a side.</li> </ol>
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Note:

Concentration Volume of GPA to have a density of 5000 Rotifers per mL	<p>Total counts in the sedge wick rafter Counter (i.e. 300 rotifers) X Volume in mL where the sample was taken from (i.e. 5000mL)/5000.</p> <p style="text-align: center;">=300x5000 5000 =300mL</p>
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Responsibilities

Stop task immediately and report to supervisor if experiencing any pain, tenderness, discomfort, or strains

Environmental Consideration

N/A

Chemical Disposal

N/A

References

NIL Noted

Appendices

All appendixes must be referenced within the body of the SOP

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Records

N/A

