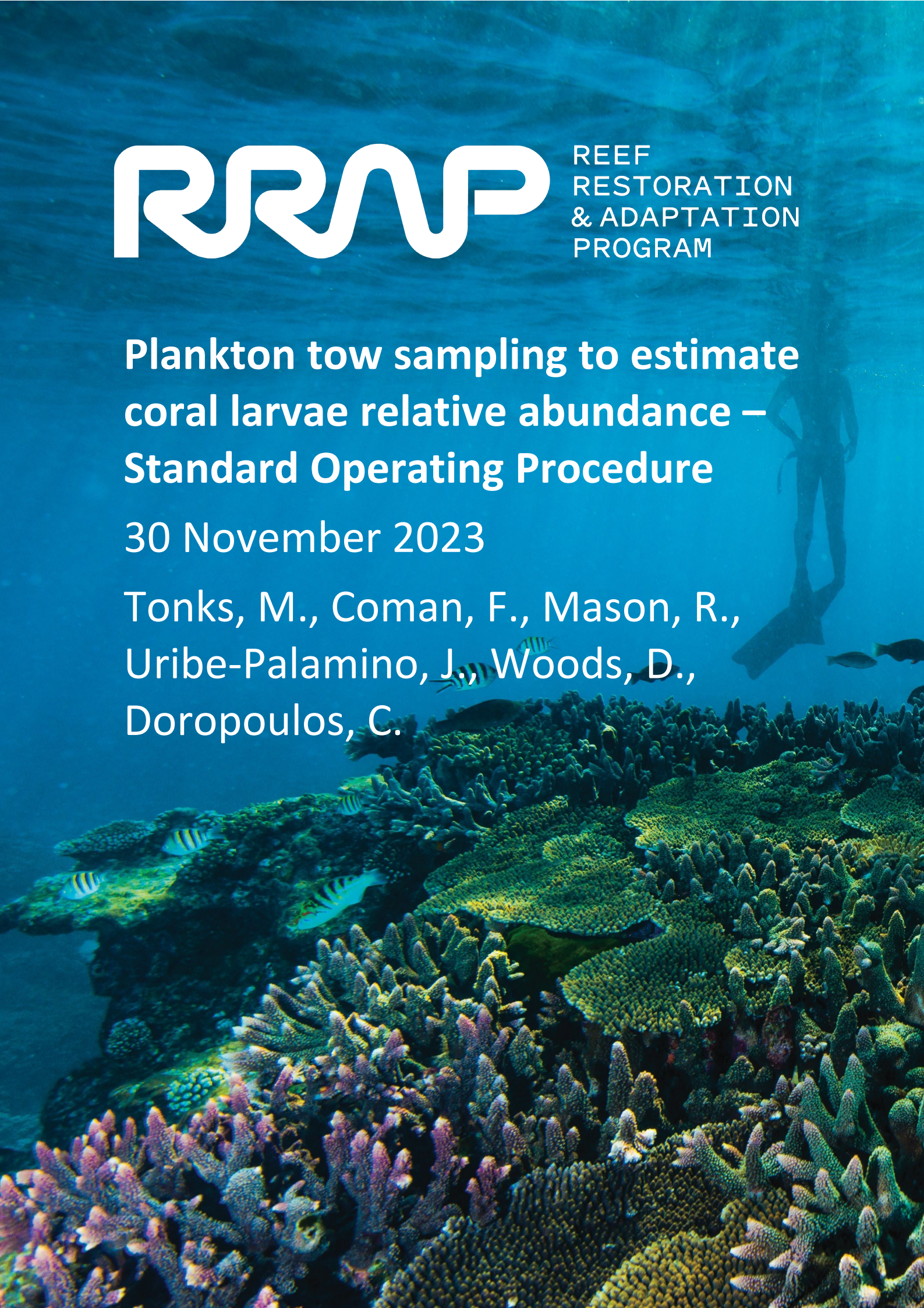
REEF RESTORATION & ADAPTATION PROGRAM

Plankton tow sampling to estimate coral larvae relative abundance – Standard Operating Procedure

30 November 2023

Tonks, M., Coman, F., Mason, R., Uribe-Palamino, J., Woods, D., Doropoulos, C.



Report Title

Enquiries should be addressed to:

Mark Tonks

CSIRO Environment
Qld Biosciences Precinct
Building 80, Services Road

St Lucia Qld 4067

(07) 38335973

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

REVISION HISTORY			
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1.0	30/11/2023	Drafting and editing of SOP	Tonks, M., Coman, F., Mason, R., Uribe-Palamino, J., Woods, D., Doropoulos, C.
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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**
Heron Island, Wistari Reef, Sykes Reef	Gooreng Gooreng, Gurang, Bailai, Taribelang Bunda
Palm Islands	Manbarra

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1 Executive Summary

Marine spatial planning and coral restoration considers larval connectivity to understand meta-population dynamics as part of the decision-making framework. Larval connectivity calculates the relative strength of larval supply from source reefs to sink reefs using biophysical models. These biophysical models use oceanographic hydrodynamics, with particles that incorporate coral spawning timing and larval behaviours (competency, mortality), to produce the connectivity networks.

Such connectivity networks are utilised across the decision modelling frameworks in the RRAP Modelling & Decision Support Subprogram (M&DS). For example, connectivity modelling outputs are used in all the ecological models in M&DS – C~scape, ReefMod, and CoCoNet, as well as ADRIA. However, coral larvae connectivity models are yet to be validated using empirical measurements on the Great Barrier Reef. Efforts to validate connectivity models are occurring in the RRAP EcoRRAP Subprogram. To do so, standardised, empirical measurements of larval supply to reefs needed to be developed and conducted.

Here, we provide a standardised approach for conducting plankton tows and analysing plankton samples to determine the relative abundance of coral larvae by volume of water filtered. A guide to differentiating between non-scleractinian larvae, potential scleractinian brooders, and scleractinian spawned larvae is also provided. The approach can be utilised to (a) empirically measure the supply of coral larvae onto reefs following major spawning events, and (b) to test the efficacy of larval connectivity model predictions.

2 Background

Coral reef restoration initiatives, such as the Reef Restoration and Adaptation Program, are investing in decision support frameworks for choosing when and where to restore reefs. Broadcast spawning of corals and their ability to disperse widely on currents means that reefs are often connected at various dispersal ranges, from 1 kilometre to 100's of kilometres. Restoration planners can make optimal use of resources by allowing larval dispersal to replenish highly connected but degraded reefs, whilst targeting restoration efforts at poorly connected, degraded reefs. The challenge is to establish high-fidelity predictions of hydrodynamic and propagule connectivity on reefs to ensure that these decisions can be made accurately.

Whilst hydrodynamic models are mature, extending these tools to wholistic models of coral larval dispersal remains a challenge. To refine and validate connectivity models on the Great Barrier Reef, we measured and modelled coral dispersal using high intensity spatial and temporal sampling of coral larvae and newly settled coral larvae in the inshore Palm Island reefs (2022) and offshore Capricorn Group reefs (2021-2023).

To estimate the supply of coral larvae, standardised plankton tows were conducted at pre-determined sites in these regions. This document outlines plankton tow and laboratory plankton sorting procedures so that future sampling techniques remain consistent.

3 Objectives and Scope

The purpose of this document is to outline the procedures utilised to sample coral larvae from the plankton using a standardised approach. It applies well developed principles from standardised plankton sampling procedures (Richardson et al. 2019) and adjusts them specifically for scleractinian larvae sampling.

The SOP guides the user through the entire process, from site selection, temporal sampling, plankton net assembly, plankton sampling, and lab sorting requirements. Specialised equipment and training are required; in particular, knowledge of coral spawning timing and for identifying coral larvae. Data are available for predicting when coral spawning occurs. Images of coral larvae from spawning taxa are provided, as well as brooded coral larvae and other larvae that may be confused as spawning coral larvae.

4 Pre-requisites

None required for this operating procedure.

5 Identified Risks and Hazards

Hazards

- Vessel not compliant with AMSA regulations (including safety equipment).
- Sampling equipment snagging on subsurface obstacles.
- Poor communication with Research Station or home base (e.g. mothership) therefore risking appropriate/timely response in an emergency.
- Exposure (sun, hot weather, windy conditions).
- Manual handling of equipment/lines.
- Interaction with wildlife attached to equipment/lines.
- Staff non-compliance with sampling procedure.

Risks:

Vessel not compliant with AMSA regulations (including safety equipment)

- Death - drowning, fire, vessel communication devices not functioning properly, poor or no response to an emergency from base contacts.

Sampling equipment snagging on subsurface obstacles.

- Uncontrolled release of pulley towards stern of vessel*.

Poor communication with Research Station or home base (e.g. mothership).

- Delay in response time in emergency,

Exposure (sun, hot weather, windy conditions).

- Sunburn, dehydration, hyperthermia.

Manual handling of equipment/lines.

- Rope entanglement, blunt force trauma to crew, rope burns.

Interaction with wildlife attached to equipment/lines.

- Stings/injury from jellyfish.

Staff non-compliance with sampling procedure.

- Lack of QA/QC of samples

**The tow pulley may present a hazard to personnel operating the net on the boat. The pulley is usually attached to a high point on the boat and may be affixed using cable ties. During a tow, if the net catches on the benthos, the resulting force will cause the pulley to snap off its fixed position and fly towards the direction of the tow net. This will occur suddenly and without enough time to allow a response. Therefore, whilst a tow is underway, personnel need to stand clear of the area on the deck of the vessel that is between the pulley and the direction of the tow net.*

6 Equipment and Materials

6.1 Procedure equipment

Tow rope length, diameter – 50 m, 8 mm braided nylon rope

Bridles – 8 mm braided nylon rope (x 3, 75 cm each length). Attaches net frame to tow rope.

Plankton net dimensions/material – (1) Length (Mouth of net to base of cod end): 167 cm

(2) Length (Mouth of net to top of cod end adaptor): 146.5 cm

(3) Length of cod end plus cod end adaptor: 20.5 cm

(4) Net diameter at mouth: 50 cm

(5) Net diameter where it meets cod end adaptor: 11.8 cm

(6) Net mesh size – 200 um

Net float and rope – 8-inch diameter foam float with 5 m tail rope (8 mm braided nylon). Tail rope attaches to a bridging rope (8 mm braided nylon) that goes between top eyelets on net frame

Net weights – size 6. ball sinkers enclosed in 20mm diameter shrink wrap sections (2 x 40 cm sections) attached to lower half of net frame within Velcro net attachment collar. 500 g dive weights can also be attached to lower net frame if additional weight required.

Pulley – stainless steel 32 mm diameter, larger diameter pulleys also an option

Esky (with ice)

Labelled cod ends as per sampling requirements (cod ends can vary but we use 100 mm diameter)

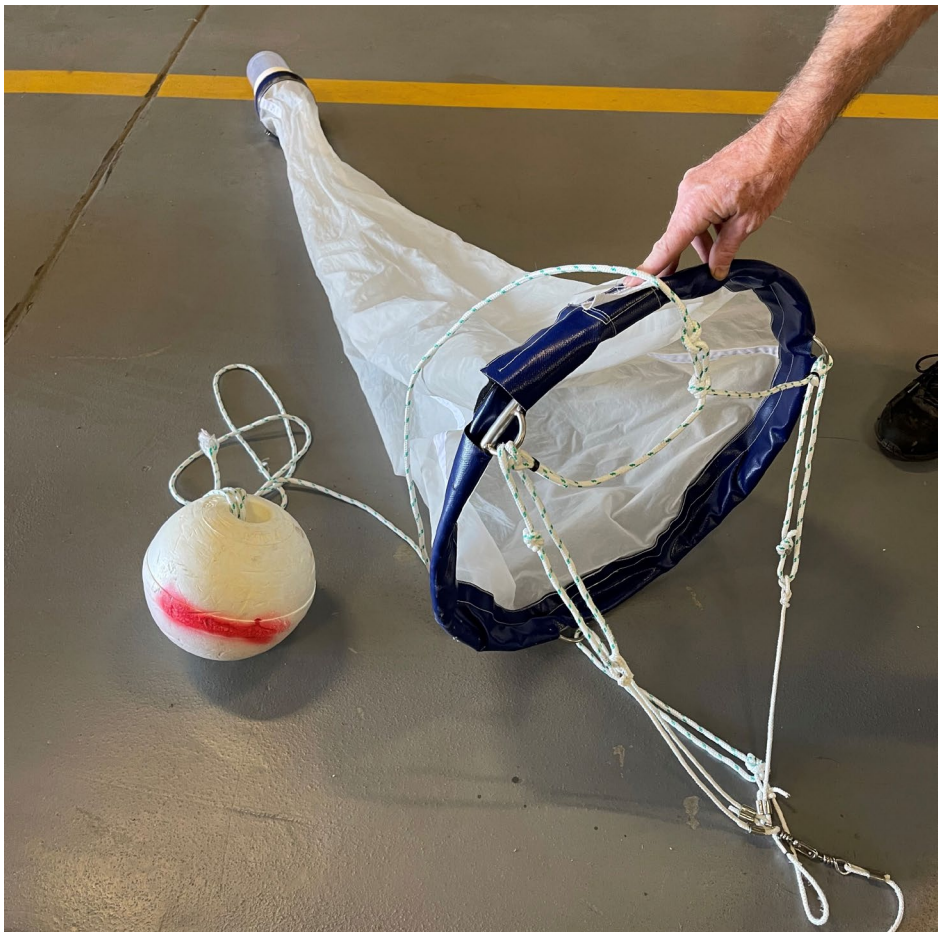
Datasheet (*see examples in Annexure*)

Handheld GPS – (e.g.,) GARMIN Etrex

Depth logger/ docking station – (e.g.,) Cera diver (load software onto 2 field computers)

Flowmeter – (e.g.,) GMBH or General Oceanics

Table 1 Details and images of numerous pieces of equipment used.

Net float and rope attached to net	
Cod end	

Cod end adapter	
32mm Pulley	

6.2 Personal protective equipment (PPE) and other safety equipment

- Gloves
- Hat
- Protective clothing
- Sunscreen
- Water
- Vinegar

7 Steps for Implementation

7.1 Site sampling methodology

Spatial site selection

Sites (sampling stations) can be selected to cover a range of potential coral larval supply. This can be predicted from connectivity modelling across high, medium, and low supply levels. If such models are not available, sites should be selected to try and cover a range of environments or project-specific needs.

Site locations should also consider the feasibility of sampling multiple sites daily from Research Stations or other platforms and addressing potential variances in larval supply predicted from models.

Temporal sampling

Scleractinian corals typically spawn from 3–7 days after the full moon from October to December on the Great Barrier Reef, with the exact timing dependent on the reef location. Spawning usually occurs over 3 consecutive nights at these times. See Baird et al. (2021) for data compiled from known coral spawning observations globally.

Plankton tow sampling requires a 'pre-spawn' sample to provide a control, baseline level of coral larvae abundance prior to spawning. This is typically conducted around the day of the relevant full moon. Daily sampling then begins the morning after the first major spawning event and continues daily. As most larvae transition from the plankton to settle on reef substrate approximately 3–14 days following spawning, plankton tows are usually conducted for a period of ~10–14 days to capture the peak period of larvae in the water column.

Personnel

A 3-person crew is most suitable to complete this work on a small vessel. The skipper will manage vessel operations and be responsible for co-ordinating approach to sites, crew deployment/retrieval of plankton net and tow length. A second crew member will be responsible for resetting of flowmeter, physically managing net deployment/retrieval and cod end sample collection. The third crew member will manage the handheld GPS and scribing of sampling metadata on datasheet, and act as an assistant to the second crew member with physical tasks.

7.2 General plankton net tow procedure

- Flowmeter Calibration is best done pre-trip. The flowmeter is towed through still water over a known distance, preferably at least 20 –25 m and the number of rotations recorded. This should be repeated at least 3 times. It is preferable to recalibrate the flowmeter regularly.
- Prior to baseline abundance tows, locate pulley in a suitable high location on boat and attach with strong capable ties or other strong anchor point.
    - Perform trial tow(s) to test and configure the net weighting/float system and tow rope length. Several trials may be required to find the adjustments that give the desired tow depth, and to ensure that the net is opening fully and is squarely facing the direction of the tow.
- Set up rope through pulley, net, etc.
- When approaching sampling site, skipper use handheld GPS to determine distance from site
- Orient boat into current, parallel to reef contour and aim to keep depth sounder reading consistently between 6–8 m along the 100 m tow
- Maintain tow speed at approximately 1.5–2 knots.
- Crew to ensure that flowmeter is zeroed (or record pre-tow reading if using a General Oceanics model)
- Skipper to alert crew at 150 m from site co-ordinates to prepare net deployment
- Manage float rope, ensure no rope entanglement
- At approximately 60 m from site co-ordinates, skipper to direct crew to start deploying net
- Cod end deployed first, release net and float rope thrown clear

Following the deployment until completion, procedures include:

- Complete datasheet for deployment (*see example in the Annexure*)
- Check for entanglement after deployment and notify skipper to proceed if all clear
- Release rope to predetermined length (based on pre-sampling trials)
- Tie rope off to cleat.
- Skipper to monitor rope angle with crew input (weather dependant)
- Skipper monitors approximate tow distance from site coordinates (aim for 100 m tow). At approx. 50 m past site co-ordinates alert crew that tow complete and commence net retrieval, reduce boat speed, and turn vessel to allow easy net access for crew.
- Complete datasheet after net retrieval (*see example in the Annexure*). Skipper to provide estimate of average depth of site.
- Net rinsed to capture any adhering larvae, and cod end removed., Appropriate labelled lid fitted to cod end and placed in esky with ice.
- Attach new cod end and proceed to next site location.
- Re-zero flowmeter logger

The approximate time once arrived at a site to complete a full cycle as detailed above is 7–8 minutes.

Figure 1 Deployed plankton net during tow along Heron Island reef slope. Note the flow meter in the centre of the mouth of the net, the weights at the bottom of the mouth of the net, and the rope coming from the top of the net has a buoy on the surface. The relationship between the buoy, the weights, and the tow speed influence the depth of the plankton net in the water column during a tow.

7.3 Laboratory analysis – plankton sorting using microscopes/imaging

Laboratory equipment

- Microscopes, with optional inbuilt camera e.g. Olympus EP50
- Sieve mesh (125–200 µm) separators for concentrating samples
- Petri dishes (with grid lines drawn)
- Bogorov trays
- Probes/forceps/Pasteur pipettes and bulbs
- Scale tags (stage micrometer)
- Optional phone and/or tablet (e.g., Samsung Galaxy TabActive4 Pro 5G 128GB) with software that connects to inbuilt camera
- Lab Datasheet (*see example in the Annexure*)

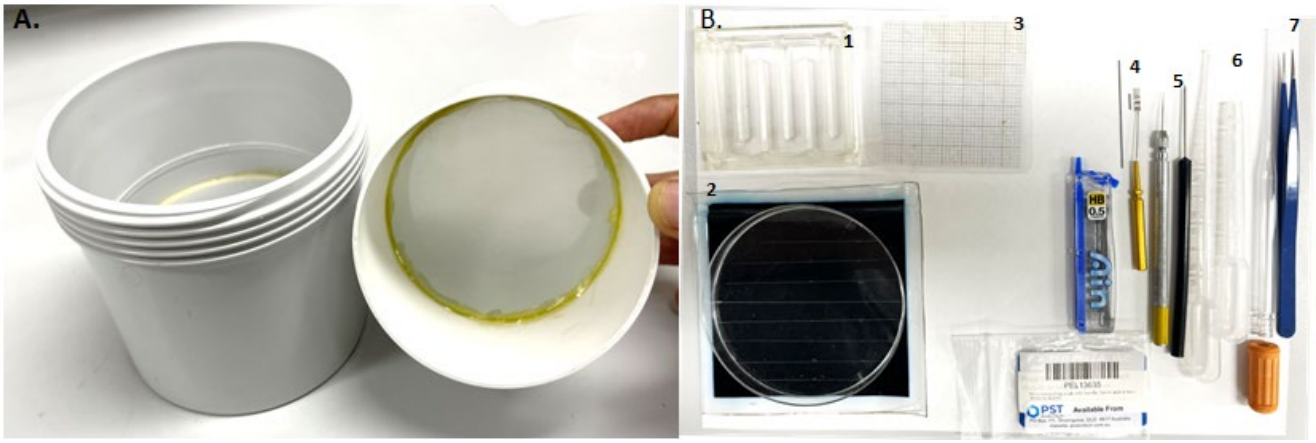


Figure 2 Sorting equipment for zooplankton samples. A. Sieves with mesh (200 µm mesh pore). B. 1. Bogorov tray, 2. alternative petri dish with drawn grid. Elements for measurement: 3. Millimetric paper grid, 4. pencil led (0.5 mm or 0.7 mm) or micro-ruler 5. sorting needles 6. plastic or glass pipettes 7. forceps.

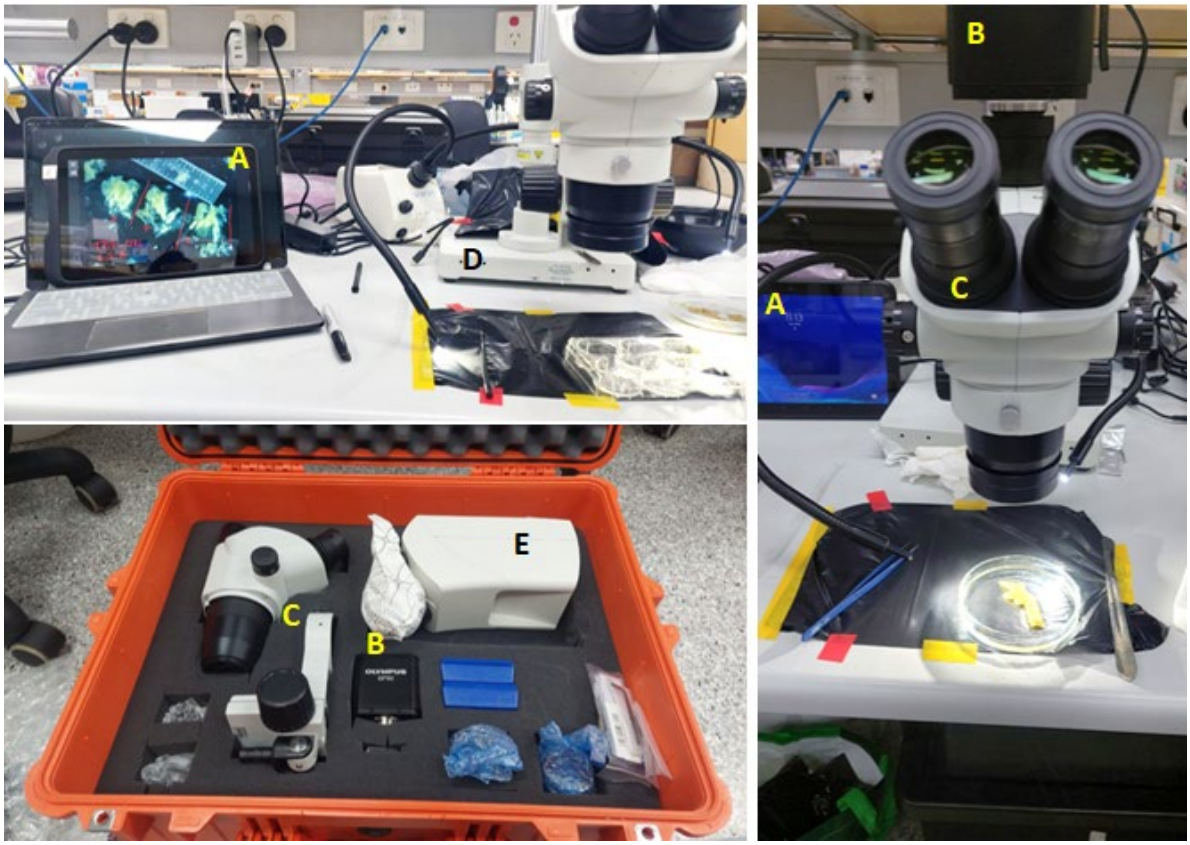


Figure 3 Field microscope set up. A. wi-fi tablet that connects to the camera (B). C. Stereo-microscope body. D. Heavy base holder for the microscope body. E. light source

Laboratory sorting/imaging procedure

- Concentrate sample in sieve.
    - Depending on larger gelatinous material, screen sample with large mesh sieve
- Transfer contents into sorting petri dish or bogorov tray to analyse.
    - Depending on concentration of plankton, add in multiple subsamples until entire sample is analysed.
- Scan for coral larvae and remove into separate dish using pipette.
- Images taken on phone or tablet and images labelled as required (date/time, sample site).
- Complete abundance and composition information
- Brooders vs spawners
    - brooders are typically identified as >800 µm in size and containing zooxanthellae. A series of lines on the surface of the larva, running from one end to the other, and often containing a concentration of zooxanthellae, are present.
    - spawners are typically identified as without zooxanthellae and between 250–700 µm in size, or <400 µm in size and containing zooxanthellae, distributed at random in the larva and with no presence of lines.

- OPTIONAL: samples preserved for molecular analysis (labelled and frozen)

The approximate time to analyse an entire sample following the steps detailed above is 15–45 minutes, which varies depending on concentration of sample and abundance of Scleractinia coral larvae.

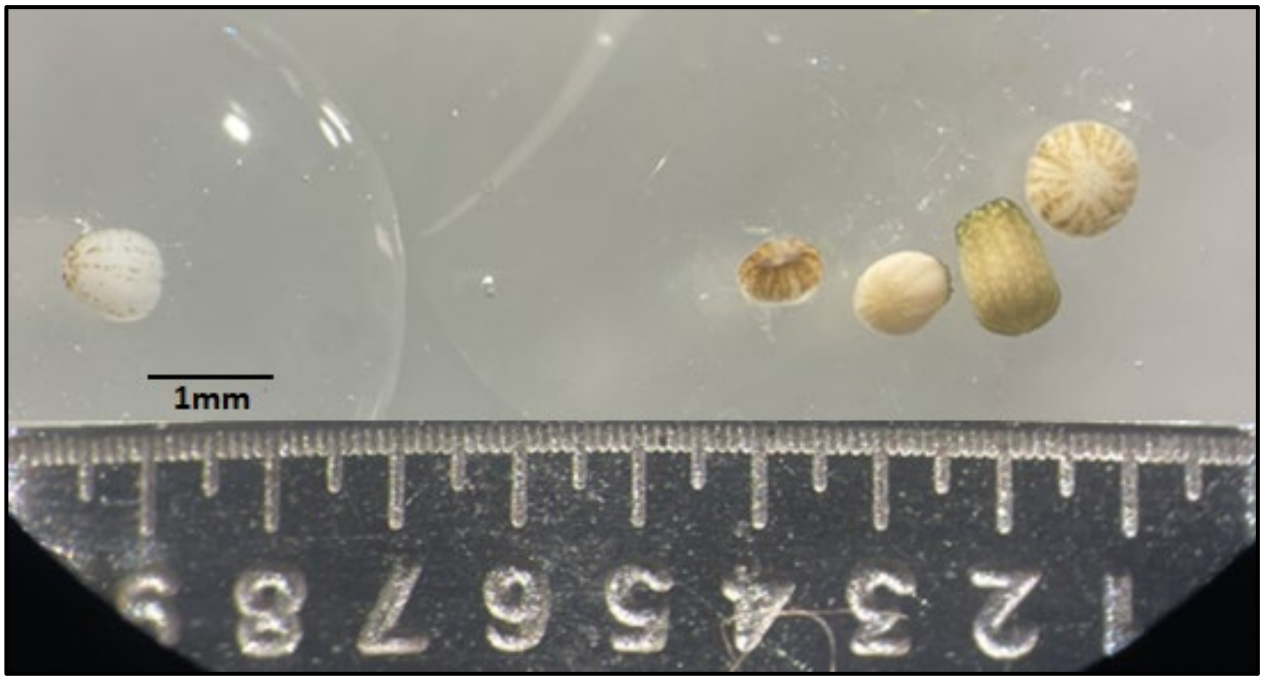


Figure 4 Example of potential scleractinian brooder (far left) and other non-scleractinian larvae (right).

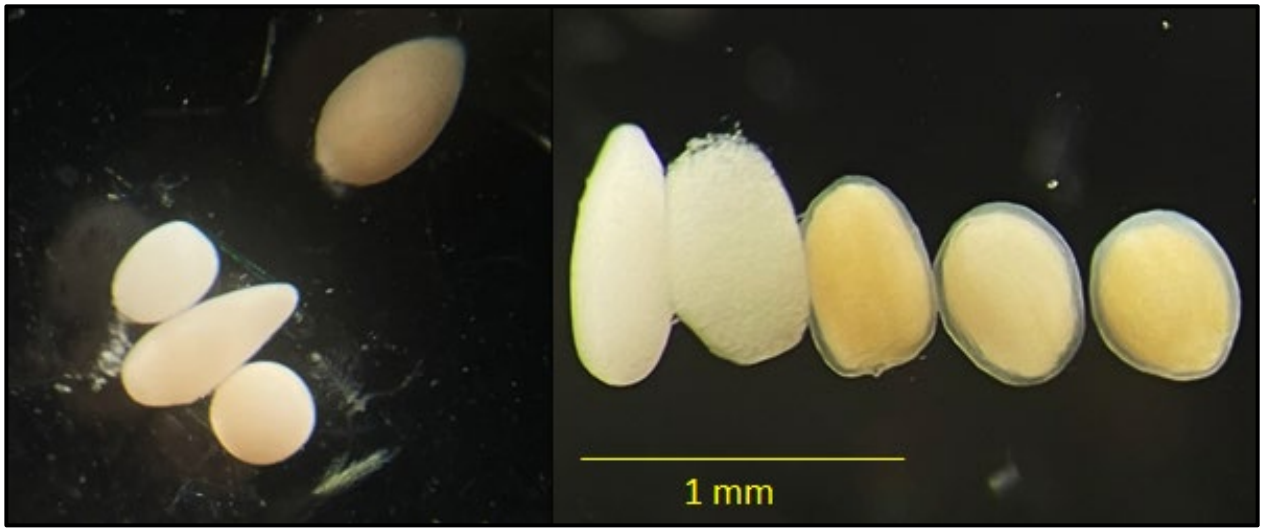


Figure 5 *Larvae of typical scleractinian broadcast spawners, containing no zooxanthellae (azooxanthellate).*

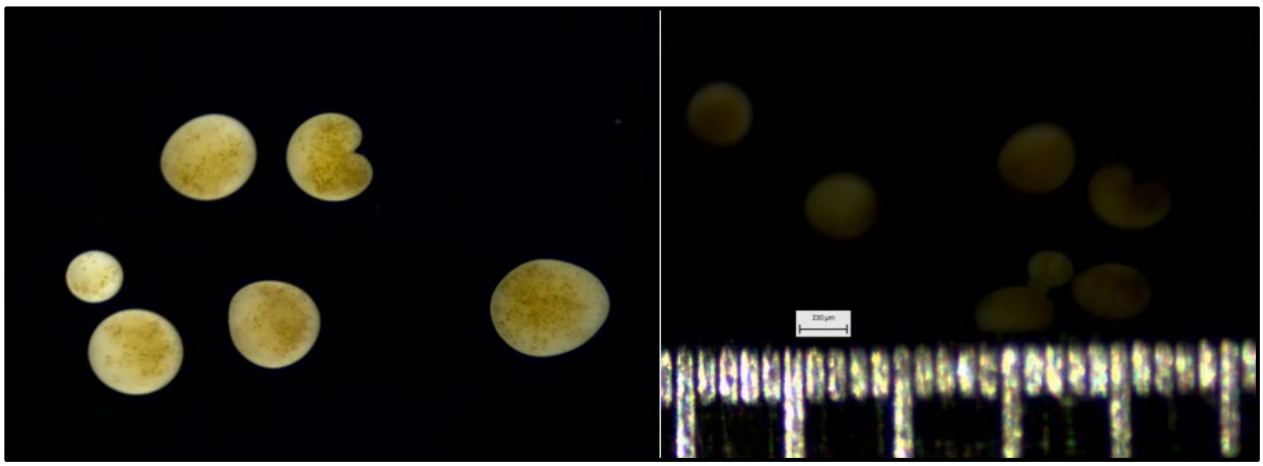


Figure 6 Larvae of scleractinian broadcast spawners containing zooxanthellae (zooxanthellate). Larvae in the left image are shown against a scalebar in the right image.

7.4 Calculating larval density

To calculate the coral larval density of a plankton tow replicate – e.g. density of spawning coral larvae per m^3 – the following procedure is followed:

Calculate the volume of seawater that has been sampled by the plankton net by using general formula for the volume of a cylinder:

$$V = \pi * r^2 * D$$

$Where$:
$V = volume\ of\ seawater\ sample\ (m^3)$
$r = radius\ of\ net\ opening\ (m)$
$D = calibrated\ distance\ of\ flowmeter\ (m)$*

*Calibrated flowmeter distance will differ when compared surface distance travelled and depends on the strength and direction of the subsurface currents encountered.

The determination of this distance from the flowmeter will vary depending on the model of flowmeter being used. Calibration tows need to be conducted in still water over a known distance to calculate how many rotations are recorded per metre travelled. For the electronic flowmeters (Hydro-Bios—no longer produced—see Figure 7), 1 rotation equates to 1 m of water passing flowmeter.

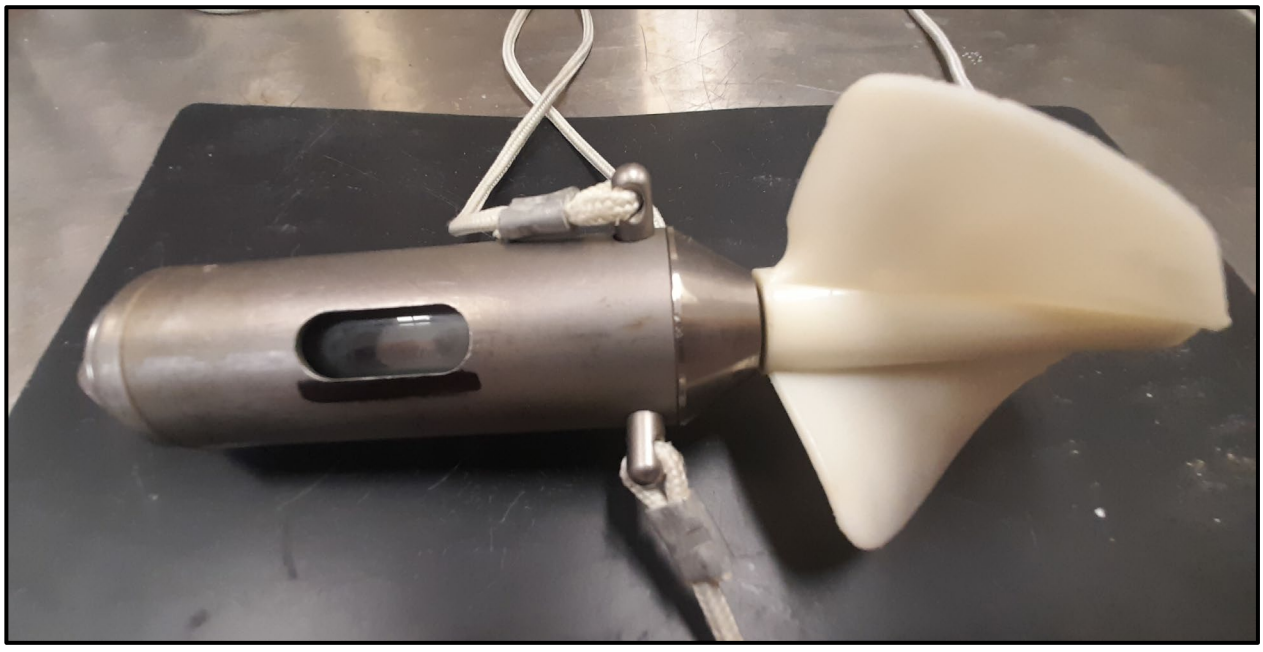


Figure 7 Hydro-Bios electronic flowmeter

To calculate coral larvae density per cubic meter, divide the number of larvae counted from a sample by the cubic meters filtered by the net for that sample.

$$Larval\ Density = Larval\ count/volume$$

Example:

Using our 0.50 m diameter plankton net we towed 98 m at site *X* according to our flowmeter reading.

$$V = \pi * r^2 * D$$

$$V = \pi * 0.25^2 * 98$$

$$V = 19.24m^3$$

Our coral larvae count for site *X* was 42.

$$Larvae\ Density = Larval\ count/volume$$

$$Larvae\ Density = 42/19.24\ = 2.2$$

Therefore, the coral larvae density for that tow was 2.2/m^3.

8 References

Baird, A. H., J. R. Guest, A. J. Edwards, et. al. 2021. An Indo-Pacific coral spawning database. Scientific data 8:1-9.

Richardson et-al. 2019. Coastal and marine zooplankton: identification, biology and ecology (Chapter 8) In: Plankton: A Guide to Their Ecology and Monitoring for Water Quality (2nd ed.), Iain Suthers, David Rissik, Anthony Richardson (eds.) (2019). Hardback, 248 pages, CSIRO Publishing.

9 Acronyms

None required for this operating procedure.

Annexure

Table 2 Example Field Datasheet for Plankton Tows

Heron December 2022 plankton tows

Date	Cod end number	Site	Average depth	GPS start mark	GPS end Mark	Start time	End time	Flow meter

Table 3 Example Lab Datasheet for Coral Larvae Abundance

Date	Cod end ID	Sampling station	Azooxanthellate coral larvae	Zooxanthellate coral larvae		Name of scorer	Image name	Which device is image on	Notes
			Number of spawners	Number of spawners	Number of brooders				

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