Standard operating procedures for larval-based restoration of Maldivian coral reefs

version 1.0 (2023)

CSIRO

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MALDIVES MARINE RESEARCH INSTITUTE

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Acknowledgments

This work was largely funded by the Australian Government Department of Foreign Affairs and Trade (DFAT) and CSIRO. With DFAT's support of our program, our organisations have been able to provide the very first larval restoration training program in the Maldives with 16 local participants. This program has thus far been a great success and kickstarted a series of collaborations and restoration efforts in the Maldives using coral larvae.

CSIRO acknowledges the early guidance and input from Drs Mat Vanderklift, Andy Steven, and Russ Babcock; program, project and travel support from Dr Alistair Hobday and Vicki Walters; communications support from Chris Gerbing and Natalie Kikken; business and development support from Phillipa Ormandy and Robert Seymour; financial support from Sandie Cloos, Ellen Wong, and David Rehe; and contracting support from Marcus Holloway and Pixie Simmons. MMRI acknowledge the guidance and input from Shafiya Naeem and Hassan Shakeel; project, logistics and communications support from Mariyam Nazeefa; logistical and preparatory support for the hands-on workshop from Hussain Ahmed, Adam Haleem, Mohamed Shuaib and the mariculture team; and logistical support from Ahmed Yamin, Suneetha Abdulla, Aishath Raufa and Ismail Hammaz.

We had participants from throughout the Maldives participate and contribute to our program, which consisted of online workshops and a hands-on training workshop. We thank Ahmed Hadhif Hassan (MMRI – Coral Reef); Aminath Zana Shiraz, Hussain Moosa, and Mariyam Sara Hamza (MMRI - ETP); Adam Haleem and Mohamed Shuaib (MMRI – Mariculture); Fathimath Shazra Mueen (SFRDP); Abdul Raheem Mohamed (Noonu atoll); Ahmed Aslam and Ahmed Haiman Rasheed (North Male Atoll), Phillipa Roe, Ali Shareef and Miriam Staiger (Laamu Atoll); Margaux Monafred and Ramathilagam Gurusamy (Baa Atoll). Without their eagerness to learn and willingness to share valuable local knowledge, the program would not have been such a success. We also thank the students from Baa atoll Thulhaadhoo School and the Maldives National University for participating in the second online workshop.

We thank the ADh, Omadhoo Island Council for their support. The Council President and Council members greeted and welcomed us to their island, facilitating the workshop on the island and home reef, providing us with a suitable meeting space and a beautiful closing ceremony. We acknowledge it is the council members and their people who depend on the reef, so their genuine interest and support in reef restoration gives the Maldives a lot of hope. Finally, we acknowledge the services from local providers during the hands-on workshop at ADh. Omadhoo Island which enabled us to provide a seamless workshop experience for all participants. We acknowledge Drift Divers, Green Leaf and Omadhoo Inn, Kirulhiya Maldives and Turtle Garden, as well as Sikundi corporation for their excellent coverage of the project.

Executive summary

Coral reefs in the Maldives play a vital role in supporting the livelihoods of its citizens. The reefs of the 25 atolls that make up the Maldives are central to its economic prosperity and sustainability, with 58% of the population being employed via reef-based tourism and 98% of exports derived from reef-associated fisheries. In recent years, the Maldives has suffered major impacts to the health and resilience of its coral reef communities from severe mass coral bleaching events (1998, 2016, 2017, 2020) and increasing pressures from land reclamation during island developments. These disturbances have had significant negative impacts on the health of local coral reefs and the livelihoods and wellbeing of many Maldivian people.

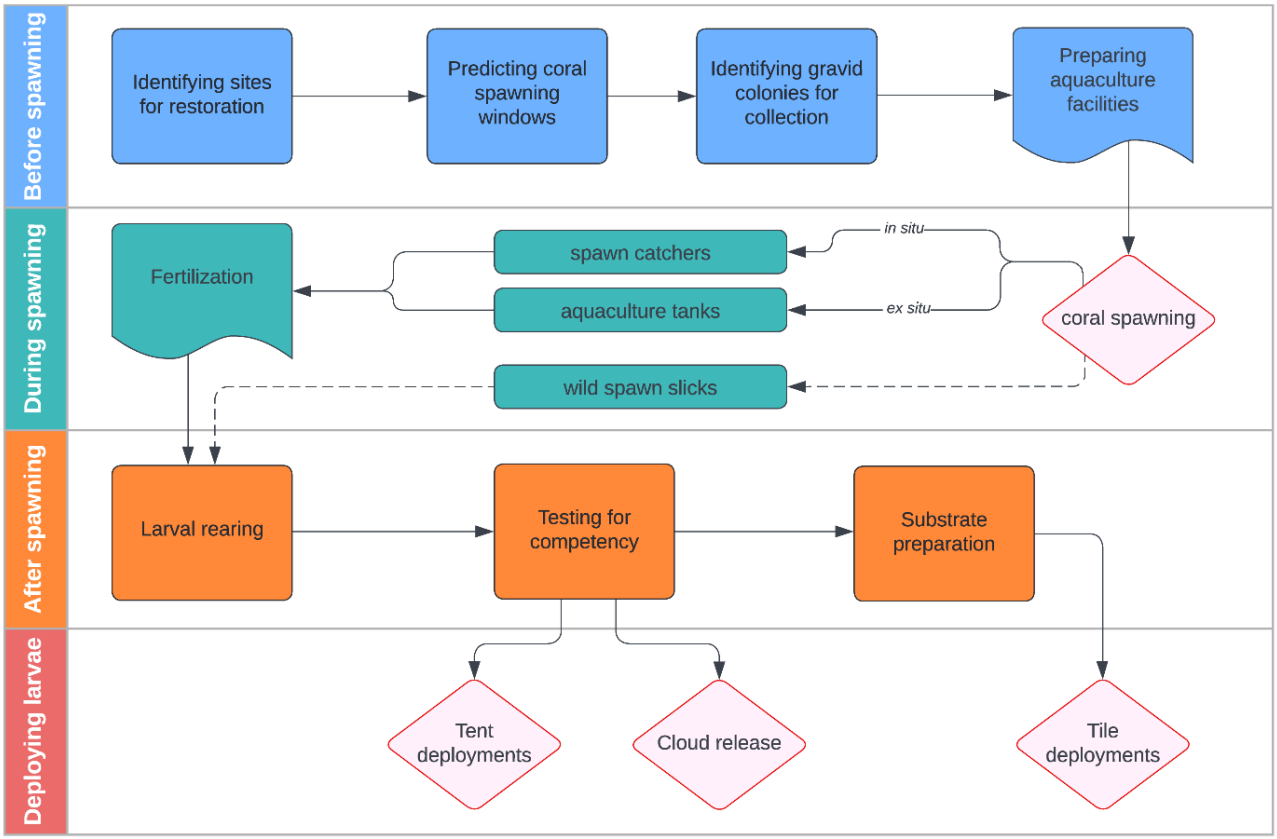
In response to recent reef declines, the Maldives Marine Research Institute (MMRI) initiated a 5-year Coral Reef Restoration and Rehabilitation program, which aims to develop a nationwide plan to safeguard, restore and rehabilitate the country's reefs. The program assesses and implements methods of reef restoration and rehabilitation based on the understanding that not all methods are equally as effective at every location. As part of the program, MMRI identified restoration of coral reefs using coral larvae as a low impact and scalable approach to reef restoration. This method involves harvesting coral larvae, culturing them, and settling them on devices or releasing them directly onto the reef. While this method has been practiced in other parts of the world, larval-based restoration has never been properly attempted on Maldivian reefs until recently.

To help with this program, MMRI sought advice from the Commonwealth Scientific and Industrial Research Organisation (CSIRO), which has extensive experience in developing the use of larvae for restoration and rehabilitation on the Great Barrier Reef in Australia. CSIRO is at the cutting-edge of applied science and scalability in using coral propagules for reef restoration and rehabilitation, with CSIRO methods of harvesting coral slicks showing potential for large-scale restoration with low costs per colony, bypassing limitations of other methods.

To apply this method to the Maldives, MMRI and CSIRO have taken a staged approach. The first stage involves quantifying the exact timings and spatial variability of coral spawning across atolls in the Maldives and quantifying when and where larval supply may be limited. Following these characterisations, the next stage involves conducting small-scale trials of collection, cultivation, and release of coral larvae onto reefs, followed by short-term monitoring to determine the best approach to be used for scaling. **This Standard Operating Procedure has been developed to provide a practical guide for the use of larval-based restoration in the Maldives and is also suitable for other Indo-Pacific coral reef nations.**

A series of online and in-person workshops were conducted to train interested parties in the process of coral larvae restoration. The first 2-day online workshop involved 17-participants from 7 different atolls and focussed on coral reproduction and recruitment ecology theory, technical aspects of collecting, culturing, and settling coral larvae, with case studies using coral larvae for scaling reef restoration. The second 2-day online workshop involved 21-participants from the same atolls and focussed on larval restoration in the Maldives i.e., what corals to collect, how to collect coral eggs and sperm, how to cultivate the larvae, how to deliver larvae to the reef, what equipment is needed. The hands-on workshop occurred over 14-days with 16-participants. The workshops have been successful in transferring knowledge to participants and provide a basis for future training and application methods for coral larvae restoration in the Maldives and elsewhere. **The information in this manual captures all the major learnings from these workshops.**

Document outline



Workflow for the Standard Operating Procedure is separated into four main sections: 1) Before spawning, 2) During Spawning, 3) After Spawning, 4) Deploying larvae and evaluation. As with the workshops, the emphasis is on “hands on” activities and procedures related to the spawning and culturing of coral larvae during the restoration process.

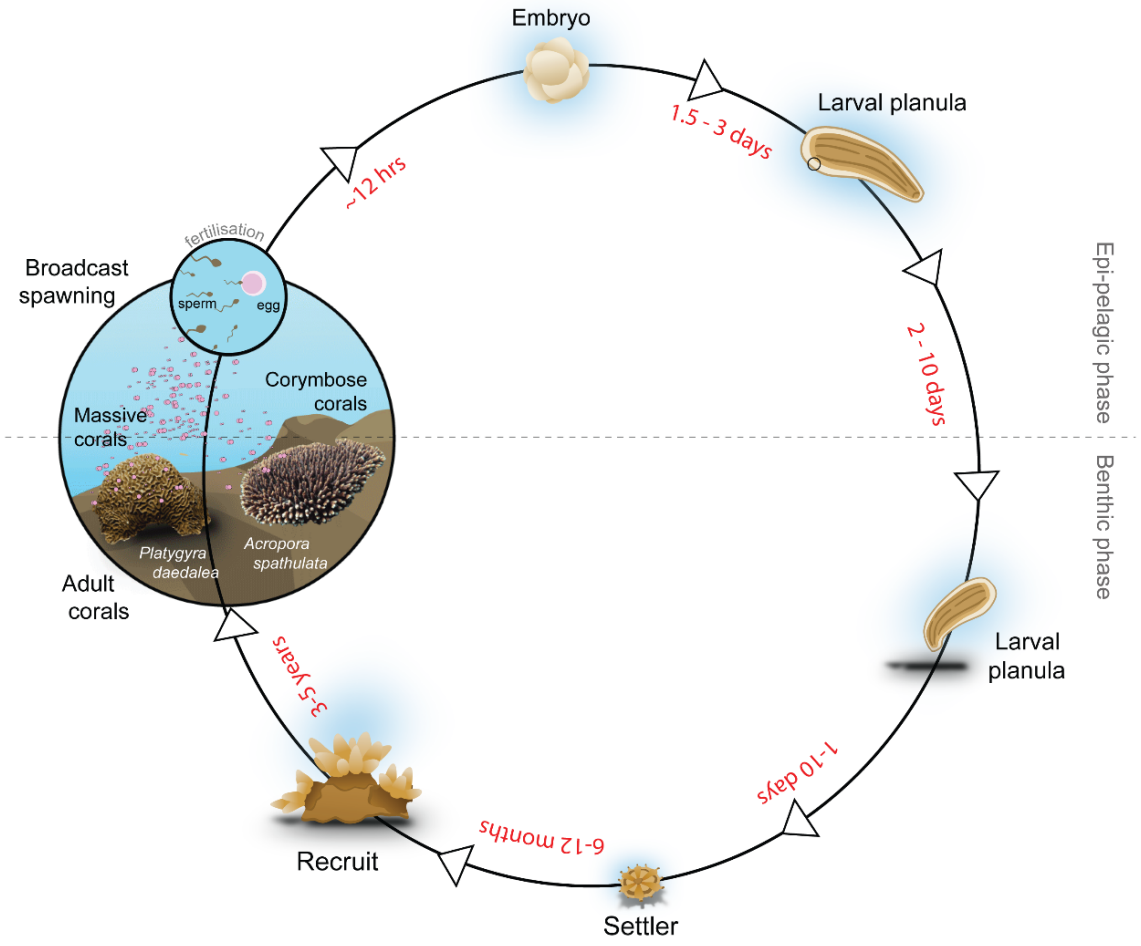
Throughout the Standard Operating Procedure, established field-methods (e.g., reef survey techniques) that are either already outlined in detail elsewhere or not specific to the Maldives region are referred to in the *Key references* for further details. Specific protocols relating to procedures for coral larval restoration in the Maldives are outlined in detail as a “hands on” guide to coral spawning and are specific to local materials and availability (see highlighted grey boxes marked “Procedures”).

CONTENTS

Aquaculture facilities at ADh. Ohmadoo Island, the location of the MMRI-CSIRO training workshop on larval-based coral restoration techniques

Coral larval biology

Most scleractinian coral species in the Maldives and the Indo-Pacific region are “broadcast spawners”, meaning that they release large numbers of eggs and sperm into the water column. These corals (such as tabular and corymbose *Acropora*) have a high reproductive output and typically recruit rapidly following disturbance, making them ideal corals for larval restoration. Broadcast spawning corals typically reproduce through “mass spawning”, where corals of the same species release their gametes in synchronised events. In the Maldives this is thought to occur twice a year: first, in the period of March-April, and secondly in last quarter of the year, but the exact times for most coral species are unknown and differ within and across different atolls. Once corals have spawned, the eggs and sperm then mix in the water column and undergo fertilisation to become developing embryos. The embryos then undergo metamorphosis and develop into free-swimming planula larvae that travel on the ocean currents to find new reefs.



After 3-10 days, the planula larvae are competent to settle so search for suitable substrate on the reef. Larvae are able to sense healthy coral reefs and avoid reefs with high sediment or abundant macroalgae. Once larvae find a permanent home, they settle onto the reef and metamorphose into a single coral polyp and start to deposit a skeleton and divide to form a coral colony. For broadcast spawning corals, few corals survive beyond the first year (~99% mortality of early settlers). Where the coral settlers survive for the first ~two-six months after settlement and become visible, they are termed “recruits” and continue to grow into adult corals unless they are consumed by predators or overgrown by algae or other competitors.

1. Before coral spawning

Using coral larvae for reef restoration activities requires planning in relation to social and governance requirements, environmental considerations, knowledge about coral biology for local coral spawning windows, and facilities available for local operations.

Social and governance aspects involve the inclusion of relevant stakeholders who may have interest in the reefs that activities are occurring and how they may be involved or impacted; as well as permitting requirements from local, state, and federal levels. In the Maldives, permitting approvals are required from the following governing bodies:

1. A project permit from the Ministry of Fisheries, Marine Resources and Agriculture (MoFMRA). The development and conduct of any marine restoration projects, including coral restoration and coral gardening project, requires a project permit from the MoFMRA (Regulation No: 2020/R-91).
2. A permit from the Environmental Protection Agency and an EIA dependent on the scale of the project (Regulation no: 2012/R-27).
3. Dependent on the framing and objectives of the project, a marine research permit may be required before proceeding with the project (Regulation no: 2020/R-100).
4. Dependent on the framing and objectives of the project, an aquaculture license may be required before proceeding with the project (Regulation no: 2020/R-94).

Environmental considerations need to include whether the area of reef aiming to be restored is appropriate for receiving coral larvae, whether it previously supported coral communities, and whether there are any future impacts that will influence whether it will be amenable to supporting coral communities following restoration.

Depending on the levels of involvement of various stakeholder groups, complexities of governance, the size of the project, and the levels of detail of ecological knowledge about the reefs and corals being considered, the planning phase is likely to require 6-months to 2-years.

1.1. Identifying sites for restoration – stakeholder involvement

Integrating stakeholders across all stages - from planning through to implementation - is key to the long-term success of restoration projects. The first step in establishing a program is to involve local stakeholders to understand needs and identifying potential sites for restoration.

Objectives	1. *Educate, involve, and gain support of community stakeholders in activities* 2. *Identify preferred areas for restoration* 3. *Organise permitting requirements and begin documenting the process*
Equipment	• None
Protocol	• Contact community Island Council for area of intended operation and arrange meeting to discuss: a. project goals

	b. traditional knowledge of local coral reefs and preferred locations for restoration c. potential concerns (e.g., conflict with aquaculture projects, areas of cultural significance, popular dive sites) d. angles for collaboration: support, employment, and education e. other potential added benefits to the stakeholders • If necessary, contact Local, State or Federal agencies to discuss the project and organise any necessary permits Contact the Maldives Marine Research Institute to communicate the type of restoration activity being undertaken, where, and when. Communicate with MoFMRA to follow the required permitting process for marine restoration activities.
Innovations	1. Early discussion and collaborations among local communities, scientists, government, and NGO agencies to maximise project success 2. Establishment of required regulations for best practice approach 3. Wholistic collaboration of nation-wide restoration projects 4. Documentation of the restoration process from the initial stages to contribute to a nation-wide restoration and rehabilitation program
Key references	• Fadli et al. 2012 *Oryx* • Suding et al. 2015 *Science* • Hein et al. 2019 *Biological Conservation* • Saunders et al. 2020 *Current Biology*

1.2. Identifying sites for restoration – environmental assessment

Once stakeholders have identified potential sites for restoration on local reefs, the next stage is to assess the environmental conditions across these sites to establish baseline conditions and to identify whether larval restoration is likely to lead to successful outcomes.

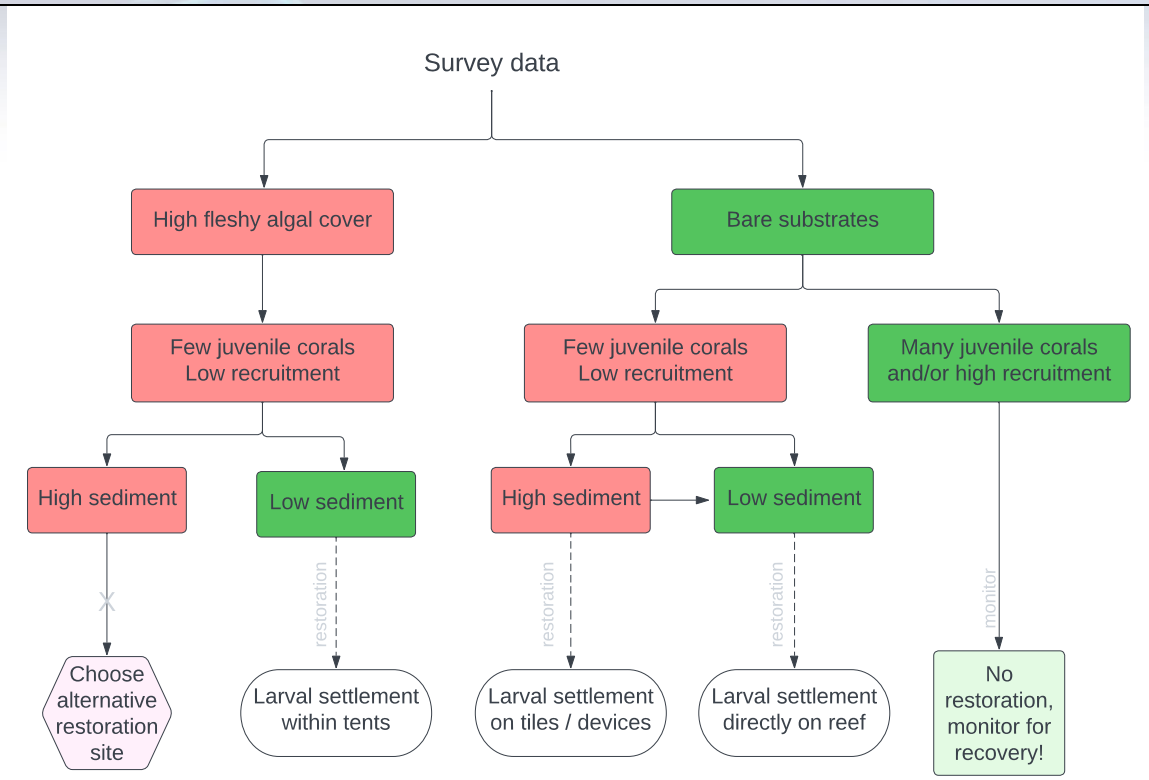
Baseline reef surveys are essential for understanding the current state of coral communities at proposed restoration sites, and additional optional surveys of coral settlement and juvenile corals surveys can give important insights into factors that can limit recovery (e.g., are larvae arriving at the site but are dying in early stages due to macroalgal overgrowth?). Consideration for the historical disturbance regime and the persistence of pressures which may limit the effectiveness of any proposed restoration activities should also be examined – e.g., sediment deposition. As sedimentation on coral reefs (both natural and from dredging/building) can strongly limit larval settlement, optional assays of sediment deposition will better inform the project as to whether larval restoration is likely to be successful. These surveys function as scoping surveys to help determine ideal locations to carry out the planned work.

Once sites for restoration work have been identified, it is critical that surveys should be carried out to flesh out any additional requirements that are needed to meet the standards set out by the required permits (e.g., EIA clearance for the project to proceed).

Objective	1. *Assess environmental conditions of restoration site(s) to see if larval restoration methods are suitable*
Equipment	**Reef surveys (corals, algae, invertebrates)** • Quadrat • Underwater camera • Underwater notebook/paper • Data sheets for invertebrate or fish surveys **Juvenile coral surveys** • Underwater notebook/paper • Plastic callipers/ruler **Coral recruitment / larval settlement** • Settlement tiles • Concrete nails • Cable ties **Sediment deposition** • Sediment traps (PVC tubes with caps)

Protocol	***Essential** – community cover surveys*
	• Assess quality of reef substrate via photo-quadrats
	• Randomly place quadrats around the reef area being considered for restoration
	• Photograph each quadrat
	• Using reefcloud.ai or other software, analyse photographs to give percent cover of major groupings:
	  ○ live coral
	  ○ dead coral
	  ○ crustose coralline algae
	  ○ bare rock
	  ○ sediment
	  ○ fleshy macroalgae
	  ○ turf algae
	  ○ other invertebrates (sponge, ascidian, bryozoan)
	
	***Optional** – juvenile coral surveys*
	• On areas of hard substrate (bare rock, crustose coralline algae), place quadrats
	• Within each quadrat, search for small corals (< 5 cm maximum diameter)
	• Count the number in each quadrat, measure their width across the longest diameter, and identify them to highest possible taxonomic resolution (e.g., *Acropora, Porites*, Fungiidae etc)
	
	***Optional** – coral recruitment (larval settlement tiles)*
	• On areas of hard substrate, attach tiles (~10 x 10 cm) to reef ~6 weeks prior to anticipated coral spawning

	• Between 6 - 12 weeks following spawning, remove tiles • Upon removal, rinse tiles in fresh water and place in mild bleach for ~24 hours to remove all organic material (e.g., macroalgae), freshwater rinse, and sun dry • Count the number of coral settlers on each tile (preferably using a microscope, otherwise under close inspection by eye / magnifying glass). ***Optional –*** *Invertebrate surveys* • In the same area and depths as the photo quadrat survey, carry out invertebrate surveys following the National Coral Reef Monitoring Framework protocols • Key invertebrate species and groups are tallied along a belt transect  *MMRI staff measuring coral juveniles in the field* ***Optional –*** *Marine fauna surveys* • *In the same area and depths as the photo quadrat survey, carry out Marine fauna surveys following the National Coral Reef Monitoring Framework protocols* • *Key coral reef and coral reef associated fish and fauna groups are tallied along a belt transect* ***Optional*** *– sediment traps* • At potential sites for restoration, attach 2-3 sediment traps (e.g., 20 cm long x 5 cm diameter PVC) to stake in a vertical orientation • After ~3 months, collect the traps, empty the contents, dry for ~48 hours, and weigh contents to determine dry weight of sediment being deposited on reef
Innovations	• *Complimentary environmental and ecological assessments to help inform the preferred restoration approach on a low coral cover reef.* • *Development of baseline data to inform a future decision framework for restoring coral reefs in the Maldives, for example:*



Key references

- Mundy 2000 *Coral Reefs*
- Abelson 2006 *Bulletin of Marine Science*
- dela Cruz & Harrison 2017 *Scientific Reports*
- AIMS 2020 *Great Barrier Reef monitoring Standard Operating Procedures*
- Gouezo et al. 2021 *Journal of Environmental Management*

1.3. Predicting coral spawning windows

While our understanding of reproductive cycles of corals is expanding throughout the Indo-Pacific, there is limited data for the Maldives for when corals spawn. As forecasting spawning cycles is essential to the success of larval restoration programs, understanding the major spawning patterns among coral species and how they vary throughout the region is of key importance.

Objective

1. *Forecast when coral spawning will occur at various locations in the Maldives for planning restoration activities*

Equipment

- Hammer
- Chisel
- Pliers (long nose better)
- Underwater tags & stainless steel nails
- Cable ties
- Underwater Notebook (optional)
- Underwater camera (if available)

	• Underwater magnifying glass (if available)

Protocol	**Field Guide: Establishing a program to determine coral fecundity**
	
	<u>Prior to the full moon</u>
	• Select approximately 10 colonies from the most common coral species at one or two local sites
	• Tag colonies by either:
	  ○ Attaching tag with cable tie to colony, OR
	  ○ Attaching tag to nail next to colony with cable tie
	<u>3 days prior to the full moon</u>
	• Sample 1-3 small fragments from centre of each tagged colony (see sampling procedure in next section)
	• Document observation of egg status (see phases in next section), options:
	  ○ visually
	  ○ using magnifying glass
	  ○ close-up image
	  ○ microscope
	<u>Repeat sampling each month</u>
	• *Note: This type of sampling will require a marine research permit before it can be carried out.*
	• Share observations to the Maldives Marine Research Institute (via the Coral Database) to communicate observations and contribute to Maldives-wide database
	
	Databases
	• Knowledge on coral spawning timings in the Maldives is limited and timings differ among locations and coral species
	• Current knowledge highlights major spawning in (Feb-) March-April (-May) and Nov-Dec in the Maldives
	• Best database to refer to is hosted by MMRI, available by request to data@mmri.gov.mv and the Coral Database (coraldatabase.gov.mv)
	• Also see the online Indo-Pacific Coral Spawning Database

Innovations	• *Contribute to an open Maldives-wide database on coral spawning times to better inform future restoration project*
Key references	• MMRI hosted coral spawning database for the Maldives (Coral Database) • Harrison et al. 1984 *Science* • Babcock et al. 1986 *Marine Biology* • Keith et al. 2016 *Proc B* • Gouezo et al. 2020 *Coral Reefs* • Baird et al. 2021 *Scientific data*

1.4. Identifying gravid coral colonies for collection

Identifying gravid adult coral colonies for collection of eggs and sperm during spawning is important for larval restoration projects focusing on in-situ collections and aquaculture-based spawning. In many broadcast spawning corals, it is relatively easy to determine reproductive status by fragmenting corals and examining the presence/absence and condition of eggs within polyps. Tagging corals and repeating surveying for reproductive status over time can provide essential information on spawning windows and reproductive cycles for Maldivian corals.

<table>
<tr><td>Objective</td><td>

1. *Identify broadcast spawning corals from brooding corals*
2. *Identify different stages of fecundity*
3. *Sample colonies in the field for fecundity*

</td></tr>
<tr><td>Equipment</td><td>

- Hammer
- Chisel
- Pliers (long nose better)
- Underwater Notebook
- Underwater camera (if available)
- Underwater magnifying glass (if available)
- Preservative (1:10 ratio of formalin to seawater) if keeping for later lab dissections.

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<tr><td>Protocol</td><td>

Identifying colonies:

- *Species:*
  - Target broadcast spawning corals (see Appendix 4 and the Indo-Pacific coral spawning database for more details on coral species)
  - *Acropora* spp. (tabular, corymbose, digitate, branching – see Appendix 4) have high reproductive output and high recruitment success
  - Massive corals (e.g. *Platygyra, Goniastrea* – see Appendix 4) also have high reproductive output
- *Size:*
  - Avoid small colonies (<15 cm maximum diameter) as they are less likely to have eggs
  - Limit to <40 cm wide colonies to avoid damage during transport
  - choose largest colonies to maximise collection of eggs and sperm
- *Health*
  - Select healthy colonies (no signs of colony damage or tissue loss)
  - Avoid colonies covered by macroalgae or turf algae

</td></tr>
</table>

Protocol

Field Guide: Sampling colonies to determine fecundity

Branching corals

- Use pliers or chisel and snap fragment at approximately 5-10 cm from the top of the branch
- Sample from the centre and bases of branches (tips are often not reproductive)
- Sample from the centre of colony (edges are often not reproductive)
- Look for eggs within the broken fragment and on the colony
- Observe egg status either by eye (if possible), magnifying glass, underwater camera, or microscope
- If the sample is to be kept for fecundity assessments in the lab, store in preservative (1:10 ratio of formalin to seawater)
- Otherwise, carefully place the broken fragment back onto colony and it can reattach

- Important: sample a total of 2-3 branches per colony and record the egg status in each branch. If pink eggs are observed in the first branch, then no further branches are needed.

Massive corals

- Use hammer and chisel to break open an ~2 x 2 cm fragment
- Sample close to the centre of the colony (edges are often not reproductive)
- Look for eggs below the tissue layer of the fragment and colony
- Carefully place the broken fragment back onto colony and it can reattach
- Sample a total of 1-2 fragments per colony

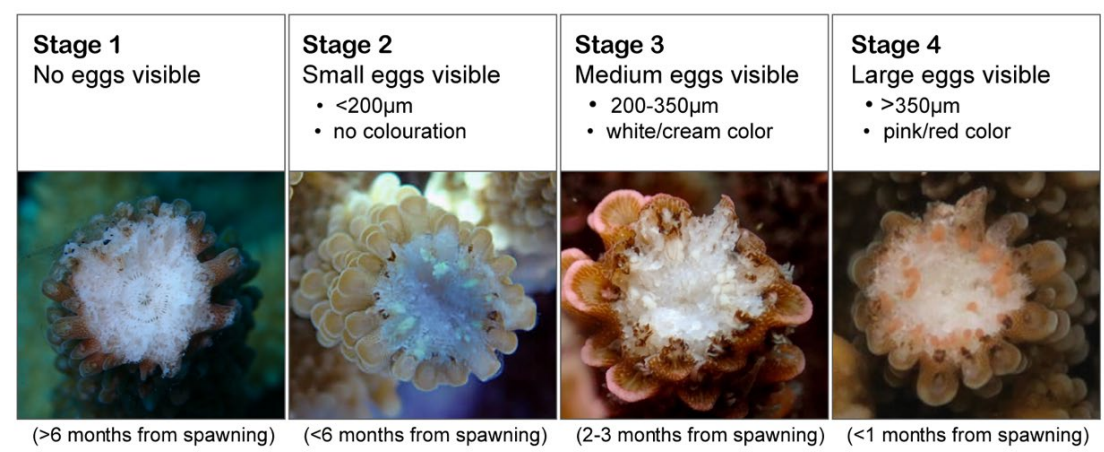
Examples of fragmenting small branching (digitate) and massive corals for egg checks

Record keeping

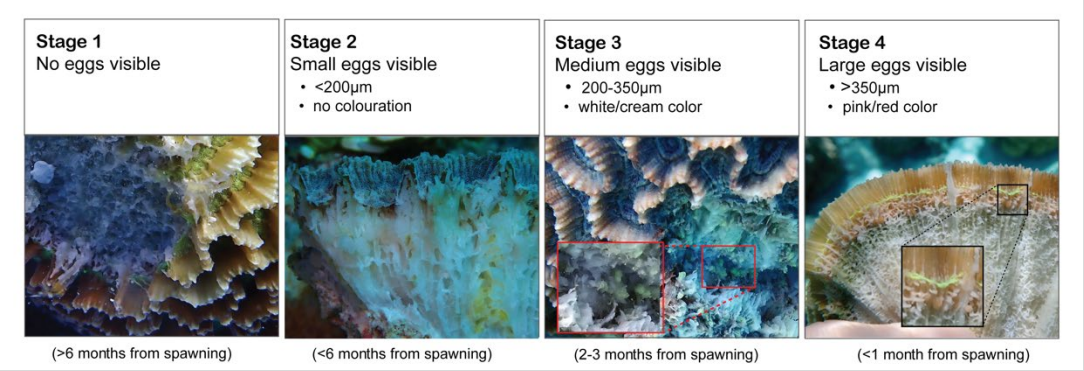
- For each colony, record:
  - Date
  - Location
  - Depth
  - Genus
  - Species (if known)
  - Size (maximum width)
  - Number of branches with eggs (include stages 1-4 as above)

Four main phases of egg development

Branching corals



Massive corals



All close-up images taken underwater using an Olympus TG6 camera

- See Appendix 5 for further examples of egg colouration in branching and massive species from the Maldives
- Send observations to the Maldives Marine Research Institute to communicate observations and contribute to Maldives-wide database

Innovations

- *Contribute to an open Maldives-wide database on coral spawning times to better inform future restoration projects*
- *Sharing of knowledge among stakeholders of reproductive states and timing of coral spawning among local reefs*

Key references

- MMRI hosted coral spawning database for the Maldives on the CoralDatabase
- Harrison et al. 1984 *Science*
- Babcock et al. 1986 *Marine Biology*
- Keith et al. 2016 *Proc B*
- Gouezo et al. 2020 *Coral Reefs*
- Baird et al. 2021 *Scientific data*

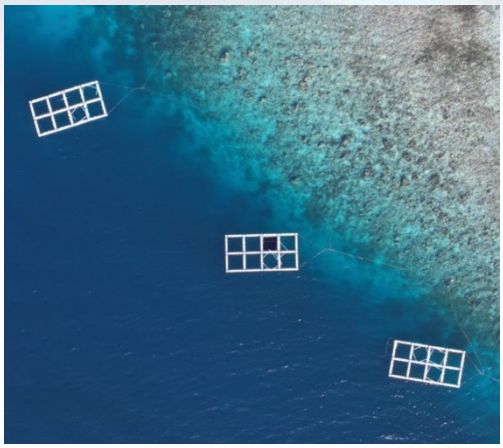
1.5. Preparing aquaculture facilities

As spawning occurs over a limited time window and fertilisation is time sensitive, preparation in advance of equipment and aquaculture facilities prior to spawning is key to project success. Ensure that preparation begins 1-2 weeks before spawning by organising and cleaning equipment, and that culture ponds / cages are deployed 1-2 nights prior to a known spawning event or on the full moon (ready for potential spawning) if uncertain.

a) Preparing aquaculture facilities - *in-situ* coral larvae culturing

Objective	1. *Preparation of in-situ culture facilities for larval rearing prior to coral spawning (1-2 weeks prior to spawning)*
Equipment	• Floating culture ponds or cages (as available, either aquaculture ponds or custom made – 120-250 micron mesh size) • Buckets with lids (10-20 litres) • Large containers (50 litres, for transfer of gametes) • Pipettes (or 50 ml syringes as available) • Small sample containers (500 ml to 1 litre)

Protocol	**Aquaculture preparations**
	• Check that nets are located in a suitable environment
	  ○ Sheltered from high wave energy (e.g. lagoonal environment)
	  ○ Moderate to high water movement to ensure throughflow
	  ○ Deep water to avoid entanglement
	  ○ Securely anchored to the substrate
	  ○ Close enough to land facilities to allow easy and repeat access by day and night
	  ○ Open air exchange at the surface to allow for gas mixing
	• Ensure that nets are cleaned and rinsed in freshwater prior to spawning
	• Cages are deployed 1-2 days before a known spawning event or on the full moon prior to spawning to reduce algal growth.
	• Once deployed, where possible keep algal growth to a minimum on the external sides of the mesh as it will limit light and water transfer. If nets show signs of algal build up and spawning is yet to occur, then change nets every 1-2 days to ensure clean facilities following spawning.
	Cleaning
	• ALL equipment must be clean and soaked in seawater prior to coral spawning
	• If rinsing in buckets ensure free flowing or large volumes of seawater
	• ALL containers / nets / pipettes must be clean and air-dried before spawning
	• Arrange all equipment for each evening's spawning during the day to ensure a smooth operation during spawning

	   *An example of spawning culture pools adapted from MMRI grouper spawning facilities*
Innovations	• *Repurposing grouper grow out aquaculture farms for coral larvae production for restoration* • *Expanded knowledge base and facility development for coral restoration facilities throughout the Maldives*
Key references	• Heyward et al. 2002 *Marine Ecology Progress Series* • Omori & Iowa 2014 Coral reef rehabilitation guide. Page 11 • Edwards et al. 2015 *Marine Ecology Progress Series* • Harrison & dela Cruz 2022 *Coral larval restoration* • Miller et al 2022 *Restoration Ecology*

b) Preparing aquaculture facilities – *ex situ* coral larvae culturing

Objective	1. *Preparation of ex-situ culture facilities for larval rearing prior to coral spawning*

Equipment	• Concrete blocks (to raise corals from substrate)
	• Buckets with lids (10-20 litres)
	• Large containers (50 litres, for soaking equipment)
	• Stand pipes
	  ○ PVC piping
	  ○ Mesh (>120 micron, <250 micron, either aquaculture mesh or muslin/organza)
	  ○ Air hose / air stones
	• Pipettes (or 50 ml syringes as available)
	• Small sample containers (500 ml to 1 litre)

Protocol	**Aquaculture preparations** • Drain all aquaculture tanks to be used for holding coral colonies *prior* to collection

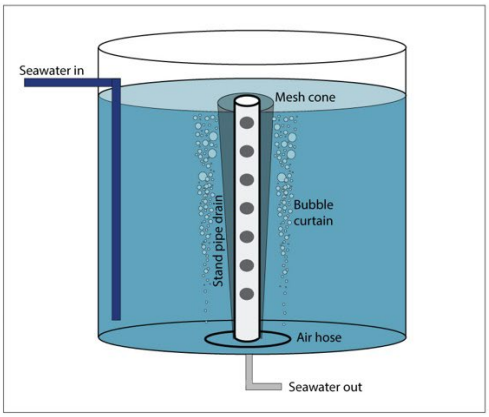
- Disinfect with ozone or chlorine and ensure tanks are subsequently neutralised
- Ensure clean free-flowing seawater at all times

- Make sure water temperature / water quality and water supply are as stable as possible, check light levels for holding tanks to ensure minimum stress on collected colonies
- Use air pumps and air stones where possible to ensure gas exchange and maintain flow within holding tanks

Cleaning

- All equipment must be clean, soaked in seawater and air dried prior to coral spawning
- If rinsing in buckets, ensure free flowing or large volumes of seawater to dilute potential contaminates
- Arrange all equipment for each evening's spawning during the day to ensure a smooth operation during spawning
- Prepare standpipes for the centre of the tank with holes for drainage

- Prepare mesh screens (minimum 120 micron) to ensure that larvae aren't accidentally drained
- If air sources are available from pumps, utilise an air curtain surrounding the standpipe to deter larvae from being lost during larval rearing phase (not required during the coral holding phase)



Preparing aquaculture facilities for coral spawning at Omadhoo, schematic of air curtains and mesh cone within aquaculture tanks to deter loss of larvae with seawater outlet

Innovations

- *Repurposing grouper fingerling grow out facilities for coral larvae production for restoration*
- *Expanded knowledge base and facility development for coral restoration facilities throughout the Maldives*

Key references

- Omori & Iowa 2014 Coral reef rehabilitation guide. Page 10
- Pollock et al. 2017 *Peer J*
- Doropoulos et al 2019 *Frontiers in Marine Science*
- Hancock et al 2021 *Marine Ecology Progress Series*
- Harrison & dela Cruz 2022 *Coral larval restoration*

2. During coral spawning

Once the facilities and equipment have been prepared prior to full moon, the next step is to successfully observe and collect spawn from corals for larval rearing.

2.1 Spawn collection

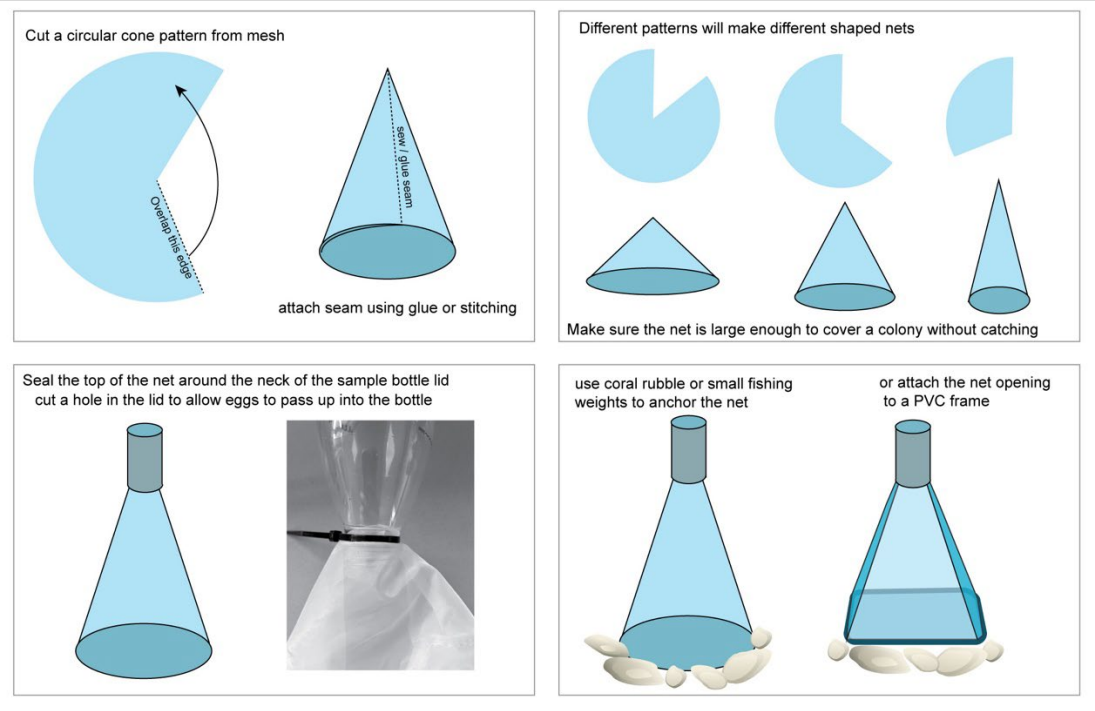
In this Standard Operating Procedure, we outline three different approaches to collecting larvae: *ex situ* using spawn catchers, *in situ* from colonies maintained in aquaculture facilities, or from wild sampled from coral spawn slicks collected from local reefs.

2.1.1 *ex situ* collection using spawn catchers

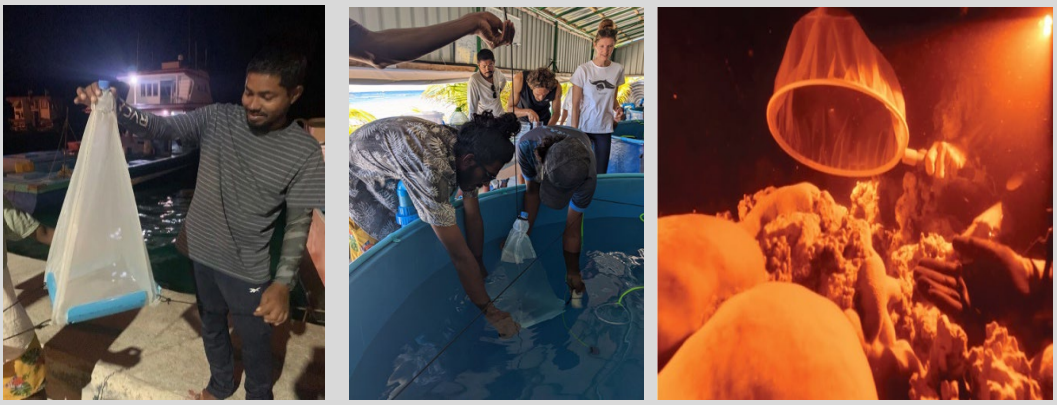
Objectives	1. *Collect egg bundles in-situ from corals using spawn catchers* 2. *Record timings of coral spawning for Maldivian coral taxa through field and aquaria observations*
Equipment	• Spawn catchers / hand nets • Small (1-2 litre) containers for rinsing nets • Large (>50 litre) storage containers • Torches, notebook to record spawning times
Protocol	• Larval spawn catchers are “tents” deployed underwater to catch positively buoyant egg bundles as they are released from colonies • Once released, the egg bundles float upwards into a container for collection • Smaller spawn catchers (<50 cm wide) can placed over individual corals, allowing estimates of total reproductive output per individual • Larger spawn catchers (1-1.5 m nets) can be placed over areas of reef and catch eggs from multiple colonies • A broad range of different approaches have been used, and below is an approach used in the previous workshop to make single colony spawn catchers using locally sourced materials.

Field Guide: Larval spawn catchers for in-situ coral collection

- From locally sourced material, obtain ~1-2 m of fine mesh net with a minimum mesh size of 120-250 micron
  - Ideally aquaculture mesh with a known hole size
  - Alternatively, muslin or organza cloth from local stores will capture most egg sizes
- Use an inverted “bottle” to catch the egg bundles when released
  - can be a plastic drink bottle (~500 ml)
  - cut a hole in the lid to allow eggs to float into the bottle
  - attach the mesh to the bottle lid with glue/ties for each removal of bottle
  - attach a float (e.g.,) polystyrene) to the top of the bottle to make sure the net is positively buoyant and doesn’t tangle with coral



- For single colonies, the diameter of the net at the base should be wider than colony to avoid the net getting tangled (30-50 cm)
- For larger colonies or sections of reefs, the base should be 1-1.5 m wide



Constructing and testing spawn catchers during spawning at Omadhoo using repurposed local materials (plastic pipe, muslin cloth)

Larval separators

- Larval separators are sieves used during the spawning process to concentrate eggs, rinse under clean saltwater, and transfer between containers
- Separators can be large (~40 cm wide) or small (5cm wide) depending on the task and number of larvae
- Separators are constructed from a plastic tube (e.g., PVC pipe) with mesh attached to the base opening using glue / zip ties

Larval separator with muslin cloth used during the workshops

General protocol

- Make sure the collection container of the spawn catchers are positively buoyant so the net does not get tangled in coral due to currents
- When deploying the spawn catchers, make sure to clearly mark the site on GPS and make note of identifiers in the water during daylight. It may be of use to deploy a marker or buoy that would be clearly visible at night to mark the site
- If deploying at multiple sites, choose sites close enough to monitor spawning times and collect eggs once spawning occurs
- If deploying at multiple sites, where possible deploy as late as possible prior to spawning (18:00) and collect as early as possible (23:00 the evening of the spawning) to avoid impacts on colonies
- When colonies spawn, record the genus (and species) if known and the timing of when the coral spawned during this event

Individual colonies

- Snorkelers or divers will need to be in the water checking to see whether the corals are spawning during the potential spawning period. Make sure observations are frequent (hourly checks)
- If deploying spawn catchers over single colonies, make sure gametes are collected either immediately after spawning if observing or at the next observation point to avoid sperm toxicity and self-fertilisation
- If necessary, replace collection containers during spawning to make sure they are not full
- Record spawning times and species for each colony
- Where possible estimate reproductive output by quantifying how full the spawn catcher containers are with eggs (by volume)

Multiple colonies

- If deploying over multiple colonies of the same species, spawn catchers can be left for longer periods of time across multiple sites. As with individual colonies, observe as frequently as possible when catchers are deployed to reduce risk of spawn toxicity
- Ensure spawn containers are large enough to collect large volumes of eggs
- Where possible record exact spawning times and species for each colony based on direct observations

	Hand nets • Monitor coral colonies underwater following sunset with torches • When spawning is observed, collect gametes using hand nets • Place contents of hand net into large container with seawater either (i) floating in the water or (ii) on the boat
Innovations	• *Successful in-situ collection of spawn from local reefs to minimise the impact of spawn collection and restoration on surviving coral colonies* • *Repurposing and recycling local products to construct spawn collecting equipment*
Key refs	• Edwards et al 2010 *Reef rehabilitation manual.* Page 84 • Omori & Iowa 2014 Coral reef rehabilitation guide. Page 6 • Suzuki et al 2020 *Restoration Ecology* • McLeod et al 2022 *PLoS One*

2.1.2 *in situ* collection using aquaculture tanks

Objective	1. *Collect egg bundles from gravid corals in aquaculture facility for larval rearing* 2. *Record timings of coral spawning in captivity for Maldivian coral taxa*
Equipment	• Small (0.5-1 litre) cups for skimming larvae from the surface • Red torches and headlamps • Notepad, pen
Protocol	**Preparation** • Any broadcast spawning corals for this purpose should be collected in accordance with regulations and guidance by MMRI, MoFMRA and the EPA. • From dusk (18:00 onwards) ensure that white lights are OFF in the facility and that indirect weak red lights are used to not disturb corals during spawning • Turn off water flow and remove air supply, reduce water level to below standpipe / drain to ensure no gametes are lost • Monitor corals every 20-30 minutes for signs of spawning (setting of egg bundles within polyps) • If no signs of spawning by 23:00 turn on waterflow and air supply

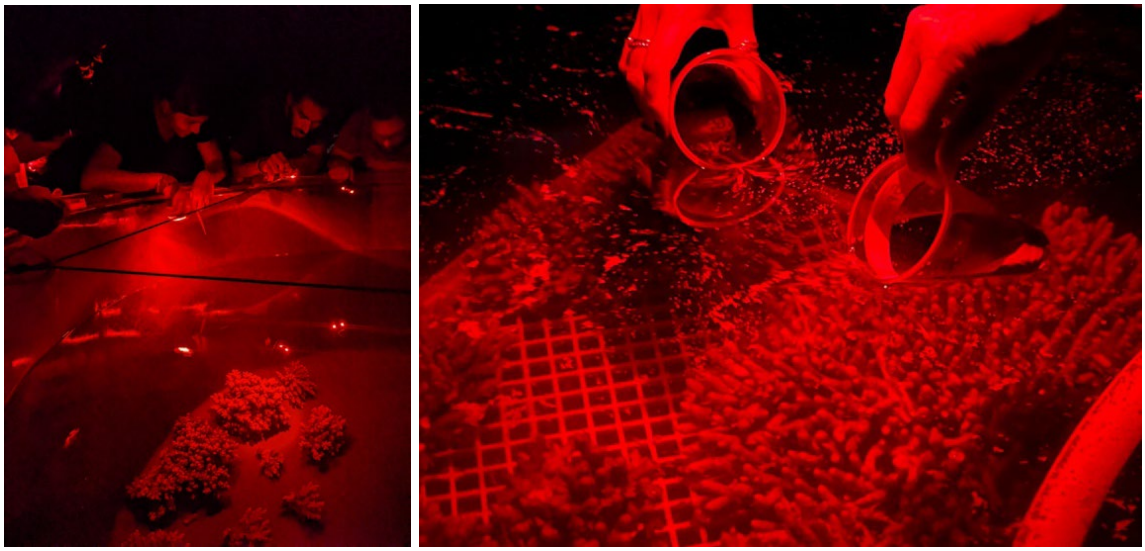
Collection and holding of gravid colonies in facilities prior to spawning (corals were returned to the reef following spawning)

Spawning

- If corals start to spawn, note the time spawning begins for each colony
- Successful fertilisation requires more than 1 colony (ideally 3 or more colonies) to avoid self-fertilisation and ensure genetic diversity
- Collect egg bundles from the surface using small cups to concentrate egg bundles

After spawning

- Once corals have spawned, return the spawned colonies back to the reef or lagoon where they were collected from on the following day. They should be ideally returned to the exact location and depth on the collection reef.



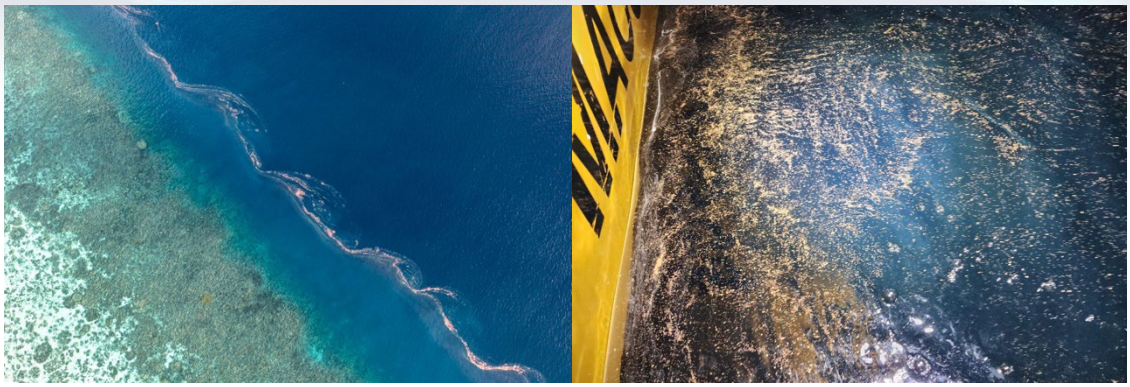
Spawning observed under red lights and collection of egg bundles following spawning

Innovations	• *Successful collection and spawning of aquaculture held corals to restore future generations of Maldivian corals*
Key refs	• Edwards et al 2010 *Reef rehabilitation manual.* Page 86 • Omori & Iowa 2014. Coral reef rehabilitation guide. Page 5

2.1.3 Wild spawn slick collection

Objectives	1. *Collect egg bundles from wild spawn slicks for larval rearing* 2. *Record timing and locations of coral spawning (where possible)*
Equipment	• Nets (minimum mesh size: 110 micron) • Small (1-2 litre) containers for rinsing nets • Large (>50 litre) storage containers • Torches (if at night) • Notebook and GPS (record position if possible)

Protocol	**Field collections**
	• Depart via boat ~19:00 to local reefs and start search for signs of surface spawn slicks
	  ○ Signs of spawn slick include specks of particles in the water, reddish formations on the surface of the water
	  ○ If possible, identify potential sites with large numbers of fecund coral colonies PRIOR to spawning and target these for collection
	• Once slicks are identified, reduce boat speed to not disturb collection
	• Use scoop nets to collect spawn from the surface of the water
	• Once collected, transfer spawn to large collection containers ½ full of seawater on the boat and rinse nets with seawater after transfer
	• Record timings of spawnings and locations
	• Continue until ~22:30 or as late as possible
	• Check colonies for gametes the next day when possible to confirm colonies have spawned.



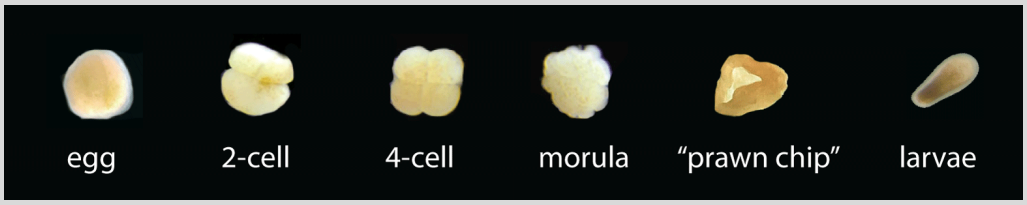
Drone photograph of a coral spawn slick during the daytime, and coral spawn visible on the surface by boat at night-time

Innovations	• *Collection of wild coral spawn slicks removes any impact of moving colonies* • *Successful wild coral spawn from local reefs to maximise the taxonomic and genetic diversity of larval restoration programs in the Maldives*
Key references	• Edwards et al 2010 *Reef rehabilitation manual.* Page 83 • Omori & Iowa 2014. Coral reef rehabilitation guide. Page 7 • Doropoulos et al. 2019 *Frontiers in Marine Science* • Tabalanza et al. 2020 *Aquaculture* • Harrison & dela Cruz 2022 *Coral larval restoration*

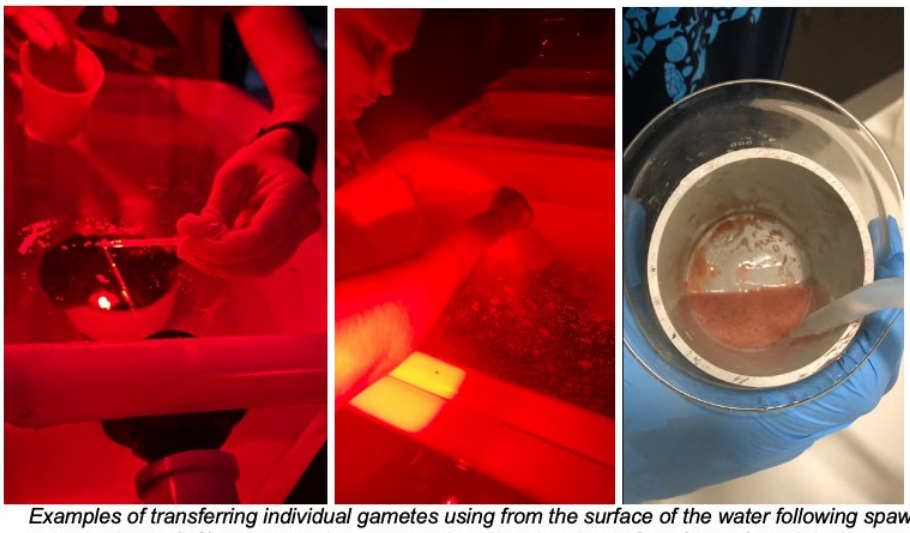
2.2 Fertilising

Objective	1. *Successful fertilisation of larvae from wild or aquaculture spawned corals*
Equipment	• Egg-sperm separators with ~120-250 micron mesh size • Large containers (50 litres) & small containers (0.5 - 1 litre) • Microscope or phone/camera with good macro function • Lights (torch or microscope) • Small dishes (petri dishes, or small 20-40 ml shallow dishes) • Pipettes
Protocol	**Field Guide: Collecting and fertilising eggs** • Collect egg-sperm bundles from the top of collection containers by skimming the surface of the water with a small container (~0.5-1 litre) • Transfer into large (~20 litre) container and mix the surface to separate egg-sperm bundles • This promotes cross fertilisation between eggs and sperm from separate coral colonies

- The concentration of eggs and sperm should be quite high during this time period of cross fertilisation (i.e., the water will look 'milky' from the sperm, the egg surface layer may be 5-10 layers thick)
- Fertilised eggs will start to divide after ~2-3 hours and become a "prawn chip" stage after ~12hrs
- Every 30 minutes or so, take a small sample of eggs using a pipette and observe under a microscope for signs of cleavage (dividing into 2-4 cells)



- Once ~30-50% eggs are observed under the microscope to show cleavage (typically ~2 hours after fertilisation), the eggs need to be separated from the sperm
- Skim eggs from surface of the large containers using a smaller container trying to minimize any sperm collection
- Once collected, **gently** rinse the eggs with filtered seawater in a mesh collector to remove excess sperm



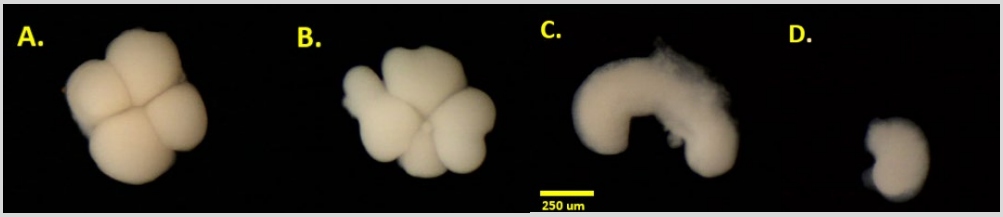
Examples of transferring individual gametes using from the surface of the water following spawning using a pipette (left), concentrating gametes by skimming the surface (centre), and rinsing eggs in the separator sieve to avoid excess sperm concentration (right)

- Count the density of larvae using 1-2 ml subsamples under the microscope
  - e.g., if a subsample (1 ml) from a 500ml container of concentrated eggs contains 120 eggs, then there are 60,000 eggs total
- Transfer clean eggs to larval rearing tubs/containers
- Aim for ~30% surface cover of eggs if microscopes are unavailable

- Keep track of samples / species / containers
- Note species, date, time spawned on rearing tub using tape labels

Field Guide: Post-fertilisation and larval development

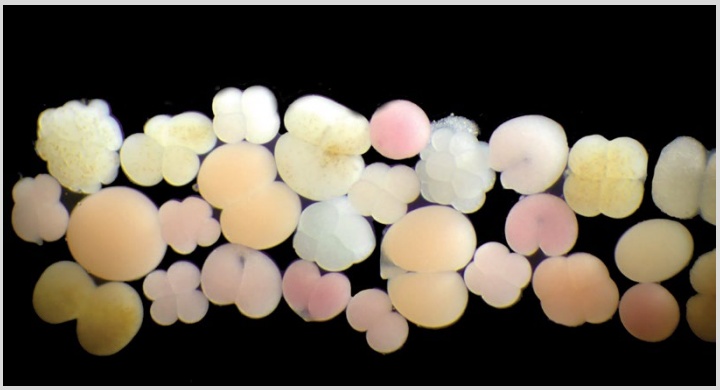
- Do NOT disturb the larvae during early development (2-12 hours after spawning)
- The following morning after spawning, collect fertilised gametes with a pipette and investigate under the microscope using a ~1 ml subsample
- Record diversity of fertilised eggs / larvae (colour, approximate size) and variability among stages. Note any deformities.



Examples of A) normally developing embryos (Acropora spathulata), b) deformed embryo during development, c) damaged embryos during experiments, d) fragmented embryos (photos: Elizabeth Buccheri)

- After 24 and 48 hours, take a sample of larvae (~20-50) and estimate how many larvae are actively moving vs inactive
  - Convert this to a percentage to quantify fertilisation success
  - For example, if 16/22 larvae are active and moving then developmental success = 73% (16/22 * 100)

- Document the timings of each step where possible to enhance local knowledge



Wild spawn at approximately 00:30 following collection from a slick (~4.5hrs prior) showing diversity of colours, sizes, and stages of cell division

Innovations

- *Key insights and continual development of fertilisation protocols from Maldivian spawned coral taxa*

Key refs

- Heyward & Babcock 1986 *Marine Biology*
- Oliver & Babcock 1992 *The Biological Bulletin*
- Willis et al. 1997 *Coral Reefs*
- Pollock et al. 2017 *Peer J*

3 After coral spawning

Once corals have spawned, gametes collected, eggs fertilised, and early development completed, the new coral larvae require between 1.5 to 10 days (depending on species and temperature) to develop and become competent before larval metamorphosis and deployment can occur. For many common *Acropora* and 'massive coral' species this can be much faster, with larvae becoming motile and competent within 2-3 days. Ensuring high survival during larval rearing is key to maximising the output of reseeding projects. Understanding competency is key to timing of reseeding larvae onto reefs.

3.1 Larval rearing and production

Larval rearing requires careful attention to the water quality and monitoring larval densities over time.

Objective	1. *Successfully rear larvae for reseeding deployments*
Equipment	• Large containers (50 litres) • Microscope • Lights (torch or microscope) • Small dishes (petri dishes, or small 20-40 ml shallow dishes) • Pipettes • Cling wrap / paper towel

Protocol	• During the larval rearing phase, take daily measurements of larval densities at the same time during the larval rearing phase to quantify larval survival
	• An initial stocking density of around 2 larvae per mL / 30% surface cover is recommended
	• Monitor conditions to try and help determine the causes of mortality events if they occur (for example temperature fluctuations, contaminants)
	
	The approach to larval rearing depends on whether it is an *ex-situ* system aquaculture system (with water and air flow) or an *in-situ* pond system:
	
	Water and air flow – *ex situ* system
	• No water or air flow for first ~12 hours
	  • conduct ~half 'manual' water change ~12 hours
	  • at this stage, most of the developing larvae are still floating on the surface and it can be done by siphoning the water from the bottom of the tank with a mesh filter on a hose / using an external standpipe
	• After ~12 hours, once **reached 'prawn chip' phase** (see section 2.4)**,** begin water flow and very light air flow; slowly increase air every ~6 hours
	  • set the rate of water flow so that each tank has 1-2 complete changes every 24 hours
	  • set the rate of air flow to reduce any sticking of the developing larvae on the meh screens of the central standpipe (see section 1.5b)

- Once larvae are swimming, air flow can be high
- Often after ~48 hours an oily 'scum' may develop on the surface of the culture
    - This can be cleaned by placing and moving plastic wrap all over the surface of the water and then removing it. The oil sticks to the plastic wrap
    - Alternatively, thick paper towel can be used in a similar way

Water and air flow – *in situ* system

- Some *in situ* systems do not use pumps or air for water exchange, but rely on natural exchange with the environment (e.g., Harrison & dela Cruz 2022)
- Some systems do include pumps for water exchange, and air for gas exchange, so this is always an additional option (e.g., Heyward et al. 2002)

Field Guide: Larval density measurements

To estimate the total number of larvae in an individual culture:

1) Make sure the larvae are well mixed prior to sampling
2) Sample (50-100 ml) of each culture in a small plastic jar and mix
3) Take a subsample of 1-5 ml (depending on larval densities) and count the number of larvae in each subsample
4) Repeat for 3-5 subsamples and standardise to larvae per ml
5) With the size of the culture tank (litres) calculate the total density of larvae

Larval density counting each day is important to estimate the number of larvae and mortality over time

Innovations

- *Maximising reproductive output for coral restoration programs in the Maldives by enhancing rearing of coral larvae and minimising larval mortality rates*

Key refs

- Heyward et al. 2002 *Marine Ecology Progress Series*
- Edwards et al 2010 *Reef rehabilitation manual.* Page 90
- Omori & Iowa 2014 Coral reef rehabilitation guide. Page 8
- Pollock et al. 2017 *Peer J*
- Harrison & dela Cruz 2022 *Coral larval restoration*

3.2 Testing for competency

After 3-10 days, the planula larvae are competent (ready to settle) and search for suitable substrate on a coral reef. Identifying when larvae are competent is key to successful deployment on reefs.

<table>
<tr><td>Objective</td><td>

1. *Test larvae for competency during larval rearing phase to identify settlement windows*

</td></tr>
<tr><td>Equipment</td><td>

- Settlement substrate:
  - CCA (Crustose Coralline Algae) fragments (1x1 cm, same species) or small fragments (1x1cm) of conditioned tiles / rubble
- Small containers (20-50 ml)
- Pipettes
- Microscope

</td></tr>
<tr><td>Protocol</td><td>

- Conduct assays for larval competency at the same time each day during the larval rearing (e.g., 10am):

Field Guide: Larval competency assays

- Take ~100ml of seawater from the larval rearing pools
- Using a microscope and pipette, count ~20 larvae and pipette into a small container of seawater (20-50 ml)
- Place small CCA/tile/rubble fragment in with the larvae
- Observe for settlement behaviour (searching and probing the substrate)
- After 6-12 hrs, count under the microscope:
  - the number of swimming larvae
  - the number of settled larvae
  - the number of inactive (dead) larvae

- Repeat this process each day at the same time to understand when larvae are ready to settle
- Larvae are ready to deploy when they are actively searching substrates and can settle rapidly (<2 hours)
- When >50% of larvae are competent, get ready to deploy larvae or add substrates

</td></tr>
<tr><td>Innovations</td><td>

- *Development of a standardised protocol to test for settlement competency for Maldivian restoration programs*

</td></tr>
</table>

Key refs	• Connolly & Baird 2010 *Ecology* • Pollock et al. 2017 *PeerJ* • Doropoulos et al. 2018 *Coral Reefs*

3.3 Substrate preparation

Coral larvae respond to settlement cues and are able to "sense" healthy reefs to settle. Preparing artificial substrates prior to spawning by conditioning them on reefs with high cover of CCA will enhance settlement rates and maximise the potential for restoration success. Similarly, collecting and preparing coral rubble for settlement that is free of macroalgae and with crevices for coral to settle will ensure strong survival during the first 1-12 months of life.

Objectives	1. *Prepare and condition artificial substrates prior to spawning* 2. *Collect and prepare coral rubble for larval settlement*
Equipment	• Coral deployment devices (plugs, tiles, etc) if required for deployment • Hammer • Chisel • Scrubbing brush • Large containers (50 litres) for transporting rubble • GPS and notebook

Protocol	**Substrate preparation**
	
	Artificial substrates
	• Make sure artificial substrates (coral frag plugs, tiles, etc) are clean and soaked in saltwater prior to deployment
	• Make sure you have required fastening equipment (drill holes, cable ties, concrete nails, etc)
	• Condition artificial substrates on the reef 4-12 weeks PRIOR to deployment in high flow and medium light environment (e.g., often ~5 m depth)
	
	Natural substrates
	• Note location and details of collection sites
	• Identify coral rubble for deployments:
	  ○ Size: 15-45 cm maximum width for handling and transfer
	  ○ Flat dimensions to lodge in reef crevices
	  ○ Shallow sites (<5 m depth, similar to restoration sites)
	  ○ High CCA cover, low turf cover, no macroalgae or invertebrates
	• Remove rubble from reef and transport back to aquaria
	• Use a scrubbing brush to lightly remove algal turfs and high sediment
	• Maintain rubble in through-flow aquaria in preparation for larval settlement

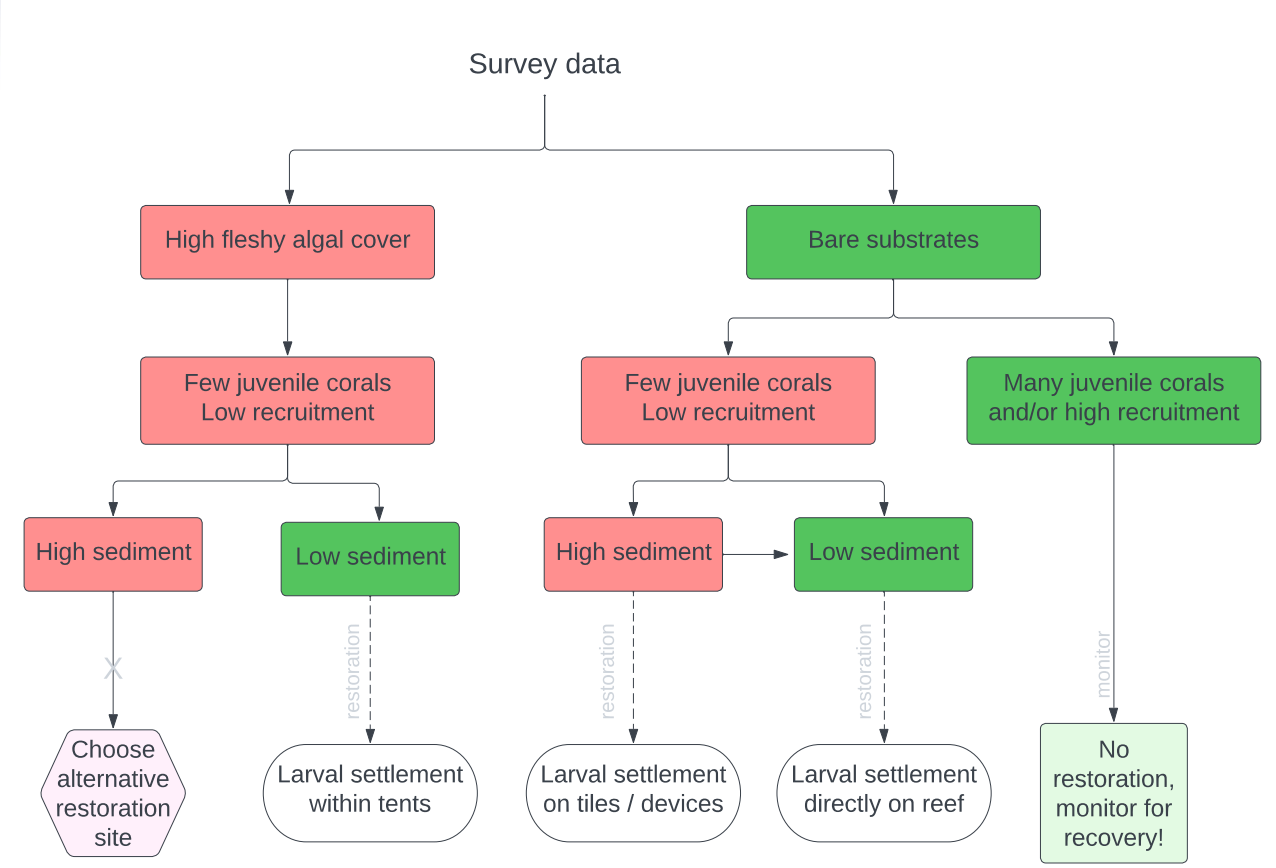
	 *Preparing coral rubble substrates prior to deployment and positioning in aquaculture tank for reseeding*
Innovations	• *Development of a standardised optimised protocol for settlement on natural substrates for Maldivian restoration programs* • *Enhanced understanding of settlement preferences for Maldivian coral taxa*
Key refs	• Mundy 2000 *Coral Reefs* • Petersen et al. 2005 *Marine Biology* • Golbuu & Richmond 2007 *Marine Biology* • Edwards et al 2010 *Reef rehabilitation manual.* Page 92 • Omori & Iowa 2014. *Coral reef rehabilitation guide.* Page 13 • Doropoulos et al 2016 *Ecological Monographs* • Doropoulos et al 2022 *Ecological Applications*

4 Deploying coral larvae

Once the larvae are competent, the final stage of larval restoration begins: deploying larvae back onto damaged reefs. Unless specified by permit, the larvae being deployed on to the damaged reef are typically larvae collected from colonies found within that atoll. There are three ways of approaching this: constrained settlement over a limited area using larval “tents”, unconstrained releases of larvae into the water column above reefs in a “cloud deployment”, and deployment of tiles with pre-settled larvae that can either be secured or attached to the reef substrate. The choice of approach may depend on the ecological state of the reef (see flow diagram below).

This phase of larval restoration is undergoing active research at a global scale, and there are many uncertainties as to the optimal way to conduct larval deployments, especially over large spatial scales. Constraining larvae within “tents” is a demonstrated approach but can be limited in the amount of area that can be restored (tens of metres). Unconstrained “cloud releases” can reseed

large areas of reef with millions of larvae, but care must be taken into considering tides and currents at restoration sites to maximise the potential for larvae to be retained at the site of interest.

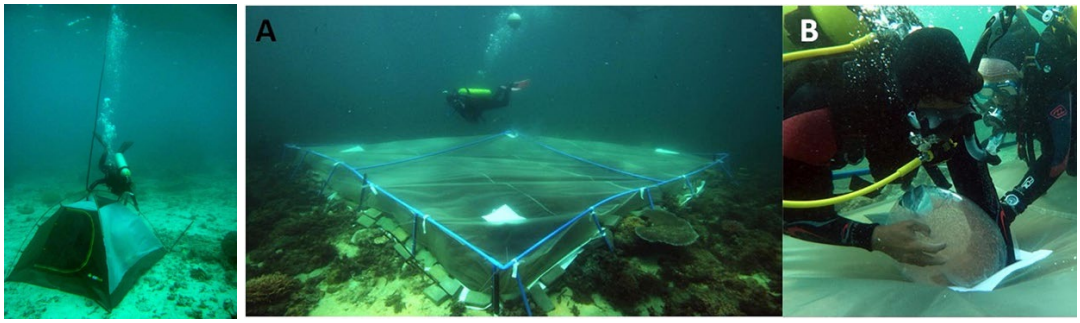


4.1 Tent deployments

Deploying coral larvae on reefs into larval tents is a demonstrated approach to enhancing settlement and adult coral densities at scales of 1 to 25 m^2. Monitoring sites and including controls will give improved understanding of the impact of restoration activities in the Maldives.

Objective	1. *Deploy cultured larvae onto reef substrates using larval 'tents'* 2. *Mark and monitor deployment sites for quantifying restoration impact*
Equipment	• Larval tents • Rocks / chain to secure tents to substrates • Larval sieves (125-250 micron) • Bottles (2 litres) or large zip-lock bags to transport larvae collected from within the atoll of the restoration site or from coral colonies sourced from the atoll of the restoration site
Protocol	**Preparation of larval tents** • Tents need to be: ○ Secured to the substrate so larvae are not lost

- Either floating or fixed with a tent-like structure so that:
  - there is space for larvae to swim and settle
  - net does not entangle on the reef
- Mesh panels (125-250 micron) large enough so that water can pass through and maintain water quality but fine enough to constrain larvae
- Large enough to cover the area of reef to be restored
- Strong enough to last for 2 days underwater



Examples of larval reseeding in Palau using a tent to trap larvae (left image, Edwards et al 2015), and purpose made low-profile larval tents (5 × 5m area, 60 cm height) for reseeding larvae in the Philippines (centre and right images, Harrison et al 2021).

Larval deployment

Prior to deploying larval tents:

- Count the number of recruits (and where possible taxonomic identity) within each plot as a pre-restoration baseline
- Where possible establish identical control plots without larval reseeding to quantify the increase in coral recruits in subsequent years

Once tents are established at restoration sites prior to deployment:

- Confirm larvae are ready for settlement (i.e., >50% competency)
- Concentrate larvae from rearing tanks using larval sieves
- Count how many larvae there are in a subsample (e.g., larvae per ml) and estimate how many larvae will be delivered at each site

- Quantify the number of larvae per area of settlement (aim for between 50,000-100,000 larvae per m^2)
- For example: 2 x 2 m larval tent = 4 m^2
  - Area * optimum density = 200,000 (4 m^2 x 50,000 larvae) to 400,000 (4 x 100,000) larvae

On the day of deployment:

- Transfer larvae using bottles / containers / zip lock bags
- Release larvae into the tent
- Keep the tent in place for 1-2 days to maximise larval settlement
  - Ensure waterflow between tent and surrounding sea water

- Ensure the larval deployment site is marked for future monitoring visits

Innovation	*Direct application of larvae to reef substrates in the Maldives to enhance local recovery*
Key refs	• Heyward et al. 2002 *Marine Ecology Progress Series* • Edwards et al. 2015 *Marine Ecology Progress Series* • dela Cruz & Harrison 2017 *Scientific Reports* • Harrison et al. 2021 *Frontiers in Marine Science*

4.2 Cloud release

While potentially the most promising method of large-scale restoration of coral reefs, the protocols for cloud releases are still being developed and many aspects of coral larval ecology are not well understood. As with larval tents, monitoring sites and including controls will give improved understanding of the impact of restoration activities in the Maldives.

Objective	1. *Deploy cultured larvae onto reef substrates using cloud releases* 2. *Mark and monitor deployment sites for quantifying restoration impact*
Equipment	• Larval sieves (125-250 micron) • Bottles (2 litres) or large zip-lock bags to transport larvae collected from within the atoll of the restoration site or from coral colonies sourced from the atoll of the restoration site • Funnel and transfer hose (2-3” diameter, ~10m length)
Protocol	<u>Prior</u> to deploying larvae: • Establish permanent quadrats on the target reef and count the number of recruits (and where possible taxonomic identity) as a pre-restoration baseline • Where possible establish identical control quadrats at adjacent sites without larval reseeding to quantify the increase in coral recruits in subsequent years • Identify sites with good local retention to ensure that larvae are maintained in one location and not diluted in the open ocean <u>Prior to deployment</u> and release of larvae: • Confirm larvae are ready for settlement (i.e., >50% competency) • Concentrate larvae from rearing tanks using larval sieves • Count how many larvae there are in a subsample (e.g., larvae per ml) and estimate how many larvae will be delivered at each site • Quantify the number of larvae per area of settlement (aim for between 50,000-100,000 larvae per m^2) • Check the tides and water flow prior to deployment: for high retention, deployment should ideally occur when there is least difference between high and low tides. Aim to release the larvae when the current is very low to maximise larval retention, usually 1 hour prior to low tide

	During deployment: • Ensure that larvae are handled as gently as possible during transport to make sure larvae are ready to settle once reaching the reef • Place the delivery tube as close to the bottom of the reef substrate as possible • Identify potential current patterns by using food colouring before releasing • As carefully as possible, pour the larvae into the funnel at a slow rate, and dilute with seawater • If deployment occurs from a boat, do not use engines as this may cause currents that will disperse larvae. • Wait 1-2 hrs or for the boat to drift before engaging engines • Ensure the larval deployment site is marked for future monitoring visits
Innovations	• *Direct application of larvae to reef substrates in the Maldives to enhance local recovery*
Key refs	• Doropoulos et al. 2018 *Restoration Ecology* • Doropoulos et al. 2019 *Frontiers in Marine Science*

4.3 Tile deployments

In section 3.3, substrates (either artificial or natural) were prepared prior to spawning. Larval settlement can occur by adding the substrates to the larval culture tank. Where possible, leave the substrates with the competent larvae for 24-48 hrs for larvae to settle and slightly calcify and make sure either i) water flow is off during this time, or ii) air curtains and/or mesh drains are used if water flow is maintained to avoid loss of swimming larvae. Newly settled larvae can be maintained in "grow out" aquaculture facilities for days to months to maximise survival and minimise exposure to predators, but care must be taken that macroalgae / turf algae do not outcompete coral settlers.

Objective	1. *Deploy pre-settled larvae onto reef substrates*
Equipment	• Substrates (either natural or artificial) • Seawater containers for transport (20-60 litres) • Attaching equipment (glue, nails, drills)
Protocol	• Where possible, count the number and size of settlers using a microscope prior to deployment to understand survival and growth over time

	• On the day of deployment, carefully transport substrates to site to avoid abrasion or mortality in newly settled corals  *Options for manufactured reef substrates (left: "lattice grids" - Randall et al 2021, centre: McLeod et al 2021, right: "tetrapods" – Chamberland et al 2017) designed to enhance settlement in microhabitats* • Carefully attach substrates to the reef or lodge within crevices to make sure substrates are not lost • Avoid deploying loose structures above coral rubble fields • Avoid deploying substrates in shallow areas of the reef that may be subject to sedimentation
Innovations	• *Direct application of pre-settled larvae to reef substrates in the Maldives to enhance local recovery* • *Development of novel settlement structures for Maldivian coral reef restoration*
Key refs	• Okamoto et al. 2010 *Fisheries Science* • Chamberland et al. 2017 *Scientific Reports* • Randall et al 2021. *Frontiers in Marine Science* • McLeod et al 2022. *PLoS ONE*

4.4 Monitoring deployments and evaluating restoration success

Ongoing monitoring of deployed larvae and quantifying growth and mortality of coral recruits through to adulthood is critical in evaluating the success of restoration projects. Section 1.2 "Identifying sites for restoration" covers the protocols for community cover surveys, juvenile coral surveys, and coral recruitment surveys. Ensure that all data and images at each monitoring time point are well organised and stored, and ensure ongoing discussions with MMRI as to analysis of the data. The monitoring process should also adhere to any stipulations and conditions required by the permitting process and organizations.

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Appendix 2

A. Coral spawning observation data table example from the **Indo-Pacific coral spawning database**[1] allowing for the recording of sites, observations, genus/species identification, spawning time, days after the full moon, data/time, and whether eggs were present / absent. (**Col** = colony, **spp_morph** = species, **Obs.time** = observation time, **Col.size** = colony size, **G.obs** = general observations, **Egg.col** = egg colour, **Egg.abd** = egg abundance, **Egg.size** = approximate egg size)

B. Coral fecundity status data table example from the **Indo-Pacific coral spawning database**[1] allowing for the recording of sites, observations, genus/species identification, data/time, and whether eggs were present / absent.

[1] https://doi.org/10.6084/m9.figshare.13100552

CORAL FECUNDITY ASSESSMENTS

National Coral Reef Monitoring Framework
Maldives

Site:					Cam #		Latitude:		Longitude:	
Obs. date:			Date of nearest FM:				Team members:			
Col#	Genus	spp_morph	TagID	Depth	Obs.time	Col.size	G. obs	Egg.col	Egg.abd	Egg.size
1										
2										
3										
4										
5										
6										
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CORAL SPAWNING OBSERVATIONS

Site:		Cam #		Latitude:			Longitude:		
Obs. date:		Date of nearest FM:				Team members:			
Genus	spp_morph	TagID	Depth	#SpCols	Sp. Start	Sp. Start time	Sp. End	Sp. End time	G. rels

Appendix 3 - Glossary

aquaculture: Culture of, propagation, keeping, raising and ranching of aquatic living resources

baseline: a point in time to base restoration against

bleaching (coral): the loss of symbiotic algae due to stress (typically warm temperatures)

brooders: corals that reproduce through internal fertilisation of eggs and sperm within coral polyps

broadcast spawners: corals that reproduce through external fertilisation of eggs and sperm in the water column

cleavage (egg): mitotic cell divisions of a fertilized egg that results in a multicellular embryo

colony (coral): an individual coral composed of multiple polyps.

condition: to place substrates on the reef to encourage growth of organisms from neighbouring substrates

community (coral): a group of species commonly found together

competency: developed larvae that are ready to settle on substrates

CSIRO: Commonwealth Scientific and Industrial Research Organisation (Australian Government agency responsible for scientific research)

culturing: large scale production of coral larvae

crustose coralline algae: rock-hard calcareous red algae that can act as a settlement cue for coral larvae

deployment: return of larvae (or settled corals) to reef substrates

ex situ: off site or away from the natural location

EIA: environmental impact assessment

embryo: the stage after fertilisation of an egg before development into larvae

fecundity: a state of being reproductive with developed eggs or sperm

fertilisation: when sperm fertilises an egg forming an embryo

fleshy macroalgae: large upright algae commonly found on coral reefs

gametes: mature eggs or sperm

genetic diversity: the range of inheritable traits in a species

gravid: containing mature eggs

in situ: on site in the natural location

larva: free-swimming life history stage following fertilisation and embryo formation

life-history: pattern of reproduction in an organism (often “early-life history stages” refers to the time before corals become adult)

metamorphosis: change in physical structure, in the case of corals the change between a free-swimming pelagic larvae and an attached benthic polyp

MoFMRA: (Maldives) Ministry of Fisheries, Marine Resources and Agriculture

MMRI: Maldives Marine Research Institute

morphotype: group of different individuals in the same population

quadrat: small areas of habitat (typically 1 x 1m^2 or 0.5 x 0.5m^2) selected at random to assess the distribution of plants/animals

permit: required paperwork to conduct restoration or research activities

pipette: (medical or scientific) small tube used to transfer small quantities of liquid by hand

planula: a free-swimming larvae (e.g planula larvae)

polyp: a single coral individual with a mouth surrounded by tentacles

rearing: low-scale production of coral larvae

recruitment: the addition of new corals to a population, typically by sexual recruitment

restoration: the addition of new corals to an environment

sedimentation (and deposition): the addition of terrestrial or marine sediments to an environment either naturally occurring (from the land) or from human activities (e.g. through dredging activities)

settlement: the process by which planula larvae attach to substrates prior to metamorphosis into a polyp

setting: the stage prior to spawning where individual coral polyps bring egg/sperm bundles to the tips of their mouths and hold them there until the whole colony is ready to release

spawn: eggs and sperm typically in the wild following mass reproduction

spawning: the process of release of egg and sperm bundles

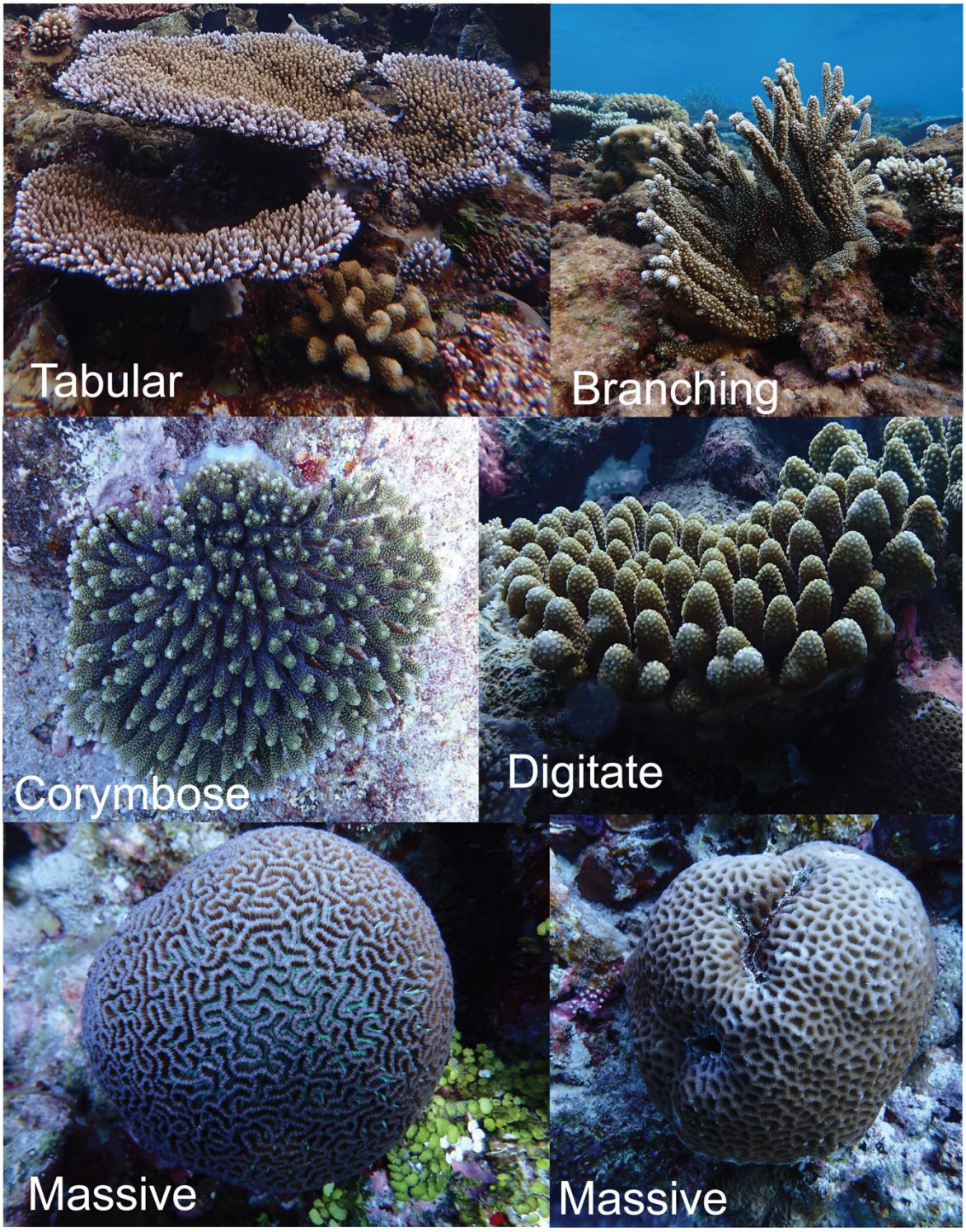
stakeholders: a person or group with an interest or concern in a process

standpipe: a vertical pipe acting as a drain in aquaculture systems

substrate: a hard surface on a coral reef

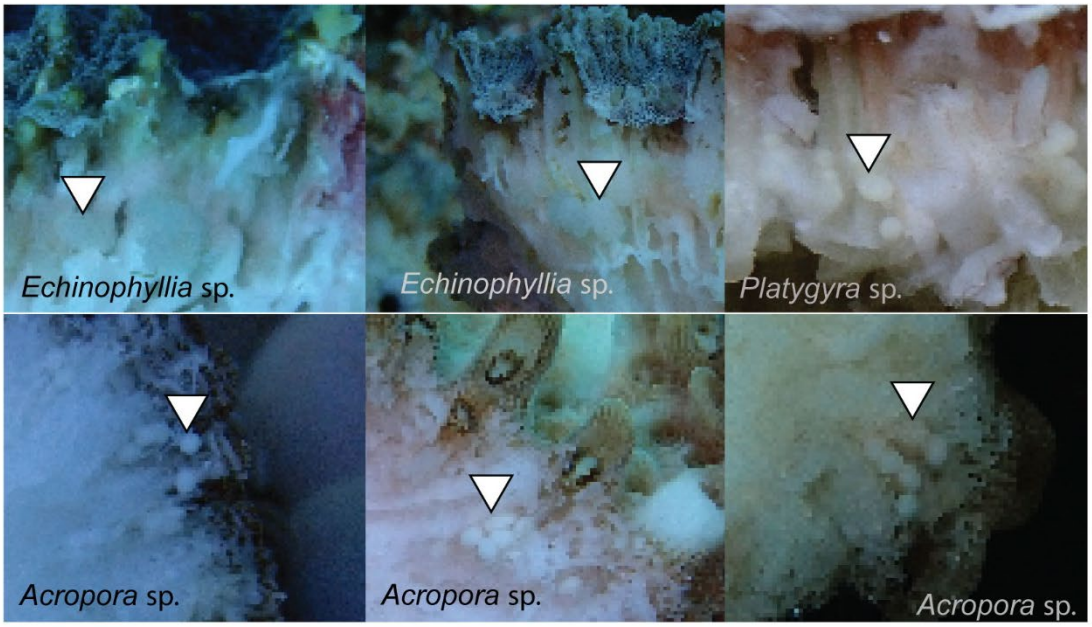
turf algae: dense, multi-species communities of filamentous algae typically less than 1 cm in height

Appendix 4 Examples of coral growth forms in the Maldives for coral fecundity sampling



Appendix 5 Examples of coral eggs from Maldives corals

White/cream eggs



Red / pink eggs