

# Best Practices for Coral Reproduction in Hawai‘i



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# Introduction

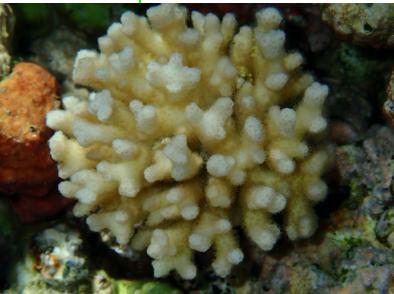
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This step by step manual aims to outline the Gates Coral Lab's best practices for the sexual reproduction of three species of corals in an ex-situ rearing facility: *Montipora capitata*, *Pocillopora acuta*, and *Porites compressa*. These methods were developed in Kāne‘ohe Bay, O‘ahu at the Hawai‘i Institute of Marine Biology, University of Hawai‘i at Mānoa.

Below you will find a brief introduction to each of these three distinct species.



***Montipora capitata*** (the rice coral) is one of the primary reef-building corals in the Hawaiian Islands. Mass spawning occurs on or near the night of the new moon in the summer months **between May and September**. *M. capitata* is a **hermaphroditic broadcast spawner** that release gametes in pinhead-sized bundles, which break apart to release eggs and sperm. This provides unique opportunities for collection and measurement of gametes **from individual colonies or community** both in the field and in the laboratory. (Photo credit: Keoki Stender)



***Pocillopora acuta*** (the lace coral) is a brooder, releasing swimming larvae directly into the water column. Although *P. acuta* in Hawai‘i are able to planulate year round, peak spawning occurs on the first quarter moon in the summer months, usually in the nighttime hours. Spawning work is therefore best completed with isolated colonies in the laboratory. (Photo credit: Ariana Huffmyer)



***Porites compressa*** (the finger coral) is a stony finger coral endemic to Hawai‘i, and is one of the primary reef-building corals in Kāne‘ohe Bay. Mass spawning occurs on or near the night of the full moon in the summer months. *P. compressa* are **gonochoric** broadcast spawners. **Individual colonies will release either neutrally buoyant eggs or sperm** into the water column, which quickly become diluted. Gamete collection is therefore best completed in **the laboratory where colonies can be isolated**. (Photo credit: Keoki Stender).

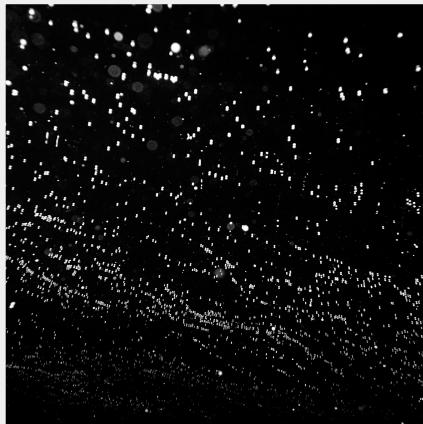
# *Montipora capitata*

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## TIMING

Mass spawning for *Montipora capitata* corals will take place on or near the night of the new moon in the summer months, especially in June and July, which are considered “peak” spawning months. In order to maximize gamete collection:

- Observe spawning on the day before, day of, and day after the new moon.
- The peak of spawning events usually occurs at approximately 9pm.



*Montipora capitata* mass spawning (left) and bundle slick on the ocean surface (right) in Kāne‘ohe Bay.

Photo credit: Mariana Rocha de Souza

## LOGISTICS

The logistical challenges of collecting gametes are best met with the following preparations and considerations:

- Access to spawning “slicks” - the masses of floating gametes released by *Montipora capitata* - is best from a motorized boat.
- Gametes will float past an anchored boat on the leeward (downwind) side of patch reefs.
- Collection will require direct access from the boat to the water’s surface.

## COLLECTION

At peak gamete release, bundles can easily be scooped from the ocean surface with handheld nets.

- Gently scoop the bundles from the ocean surface.
- Rinse bundles from nets into 5-gallon buckets using 1um filtered seawater.
- Collect as many gametes as possible in 15 minutes.
- Gametes should be handled as gently as possible, avoiding breaking bundles apart before they are aliquoted for fertilization.

## FERTILIZATION

Immediately aliquot concentrated bundles in 50L falcon tubes.

- 5mL bundles in 30mL filtered seawater is an effective ratio of bundles to seawater per falcon tube.
- Bundles must be put into fertilization contains before they break apart.
- Bundles will begin to break apart approximately 15 minutes after collection. Allow bundles to completely break apart in falcon tubes.
- Gentle agitation of the tubes, such as inverting or swirling, is encouraged to help bundles break apart.
- Allow eggs to fertilize up to 1.5 hours.



*M. capitata* bundles in a 5 gallon bucket (left) and aliquoted into falcon tubes (right).

### Sample Fertilization Schedule:

- 9pm - 9:15pm collect gametes
- 9:15pm - 9:30pm aliquot bundles
- 9:30pm - 11pm fertilization

## EMBRYOGENESIS AND LARVAL REARING

Rearing System: Newly fertilized embryos are reared within indoor, temperature controlled, flow through conical aquaria of 15L capacity. These aquaria must have:

- Filtered seawater supply (1um) and turn over control (1-5L per hour)
- Drain cover (banjo filter) with 153um mesh. (See image below)

Rinsing and Transferring Embryos: Newly fertilized embryos must be rinsed.

- Remove concentrated sperm supernatant and excess bundle material from falcon tubes using stereological pipette and discard.

Larval *Montipora capitata* inside flow through conical aquarium with banjo filter, which prevents embryos from draining out of the conical (left). Lab setup of 15L conical aquaria (right).

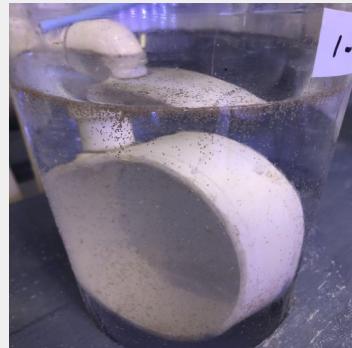


Photo Credit: \_\_\_ and Valerie Kahkejian

Embryogenesis: Embryos will grow and develop into larvae within 72 hours post fertilization (HPF).

- Turnover should be kept low ( $\leq 1\text{L}/\text{hour}$ ) during the first 24 hours.
- Turnover should be increased to  $\leq 2\text{L}/\text{hour}$  between 24 and 72 hours.
- Protein/lipid biofilms will accumulate at the water's surface as some embryos die. Gently collect the biofilm that accumulates by skimming the surface of the water in each conical with clean kimwipes and discard.

Larval Rearing: At 72 HPF, embryos will have developed into swimming larvae.

- Turnover should be increased at this time to  $\leq 4\text{L}/\text{hour}$ .
- Larvae that stick to the sides of the conical or drain filter should be regularly rinsed off with a 1um filtered seawater squeeze bottle.
- Larvae should remain in conical aquaria for 3 additional days. Larvae which settle or metamorphose inside the conical or water column cannot be used for downstream settlement.

## SETTLEMENT

Settlement Chambers and Setup: Larvae are settled inside mesh bottomed chambers, which can be easily made from 2L plastic Tupperware and 153um mesh.

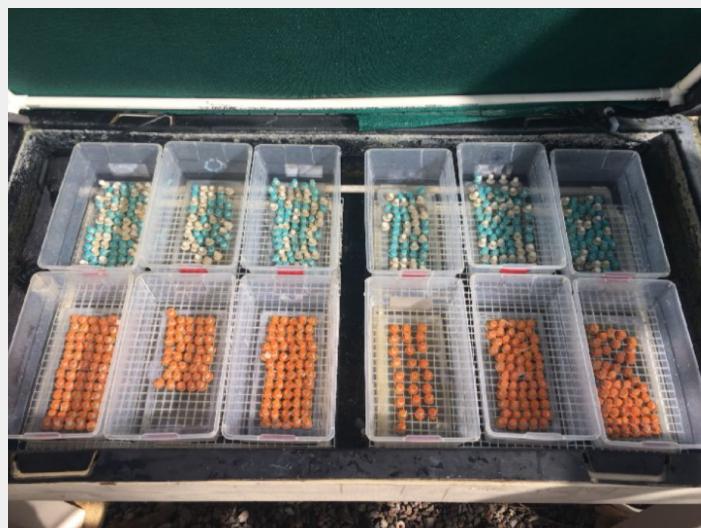
- Cut out the entire bottom section of the Tupperware, glue (2 part epoxy, cyanoacrylate or contact adhesive) 153 um mesh to the bottom, pulling mesh taught while the glue sets.

Chambers should be placed in a flow through seawater aquarium at a depth which limits volume and maximizes contact of larvae with settlement substrate.

- Mesh bottom chambers enable a continuous exchange of seawater with the aquarium, but prevent larvae from escaping the chamber.
- By limiting the depth of water to the height of the substrate material, larvae are encouraged to settle on substrate.

Chambers should be shaded (30% exclusion) from direct sunlight.

- Larval *Montipora capitata* demonstrate a preference to settle in the dark, which avoids larval stress.
- By taking advantage of natural overhead sunlight, we encourage larvae to settle on the undersides of substrate.



Settlement chambers inside flow through aquarium. Aragonite plugs are used for substrate. A large shade cover is kept over all settlement chambers.

Photo Credit: \_\_\_\_\_

Substrate and Conditioning: Aragonite/crushed coral frag plugs should be used as settlement substrate.

- Plugs should have a flat surface, which should be placed face down in direct contact with mesh bottom of the chamber. Larvae will preferentially settle on this surface.
- Plugs should be conditioned within the aquarium system in which juveniles are intended to be grown out.
  - Light and flow levels should be identical to flow out conditions.

Pooled *Montipora capitata* larvae (left). Intern Andrew Barrows transferring larvae into settlement chambers (right).

Photo Credit: \_\_\_\_



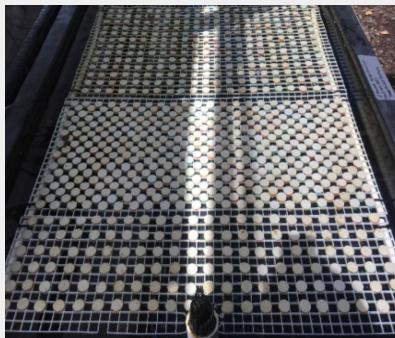
Transferring Larvae into Settlement Chambers: Larvae must be carefully transferred from conical rearing aquaria to settlement chambers.

- Siphon/drain conical, which will pull swimming larvae out. Use small diameter hose to reduce the speed of draining water and minimize physical damage to larvae
- Larvae must be drained into a sieve (153um) which is partially submerged in water, which prevents larvae from being smashed and left dry on the sieve mesh while conical drains.
  - Turn off input seawater to conical.
  - Siphon/drain conical into partially submerged 153 um mesh sieve.
  - Rinse sides of conicals with 1um filtered seawater squeeze bottle to collect any remaining larvae.
  - Quickly and gently transfer the larvae from the sieve into a beaker by rinsing the sieve with 1um filtered seawater.
- Larvae can now be quantified volumetrically and transferred int preferred densities into settlement chambers.

Monitoring Settlement: Larvae should be given 3 days to settle.

- Every 24 hours, each substrate unit (plug) should be checked for settlement.

Plugs with 5 or more settlers should be removed from settlement chambers and placed in a grow out tank.



Settled *Montipora capitata* larvae on aragonite plugs (left). Thousands of aragonite plugs in outdoor flow through seawater aquarium with newly settled larvae (right).

Photo Credit: \_\_\_\_

## GROW OUT

Conditions: Juvenile *Montipora capitata* need a specific amount of light, clean seawater, and flow to survive and grow.

- Clean Seawater
  - Juveniles can be raised in outdoor flow through seawater aquaria.
  - Filtered seawater can reduce nutrient loading and sedimentation.
  - Tank turn over (seawater input) should be high and consistent across tanks, approximately equal to 1 entire tank volume per hour.
- Light
  - Juveniles are sensitive to light and overgrowth by algae.
  - 6000 lux has been demonstrated to reduce algal overgrowth and promote growth and survivorship.
- Flow - Juvenile *Montipora capitata* can survive and grow in a variety of flow regimes:
  - High flow environment, 25 times tank turn over per hour, encourages CCA growth, which reduces macroalgal competition with the young recruits. To create high flow within a grow out tank, additional power heads should be added in addition to seawater input.
  - Low flow environments, 0 times tank turn over per hour, enable young recruits to increase polyp extension and potentially feed more.

Feeding: Coral juveniles should be fed.

- Heterotrophy is an important component of coral health.
- Juvenile corals benefit from food supplementation:
  - Dose tanks with Reef Chili (or other preferred coral food brand) according to the size of the aquarium and number of corals inside. See the instructions for more details.

Long-term Monitoring and Coral Husbandry: Juvenile corals will survive and grow into adults with proper long term care.

- Water quality, light levels, feeding, and flow should be maintained through time.
- Additional coral husbandry may be required, such as removal of benthic competitors or pests, sediment removal, etc.

Growth Rates: Sexually reproduced *Montipora capitata* can be expected to grow anywhere between 1 - 3 square centimeters in the first year following the above protocol.

- Future Coral Resilience Lab coral experiments will continually refine and improve this method.



# *Pocillopora acuta*

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## TIMING

Planulation of *Pocillopora acuta* in Hawai'i occurs in the full moon quarter with peak release in early to mid summer, from May to August.

- Larvae will be released in the nighttime hours, with occasional low frequency release occurring during daylight hours.
- Larval release varies by colony.

## LOGISTICS

Collection of larvae is best achieved in the laboratory as colonies should be isolated to best capture larvae.

- Larvae are positively buoyant, but will actively swim and can be found throughout the water column
- Larval collection with nets in the field is also possible, but logistically more challenging.

## COLLECTION

Prior to evening hours, adult colonies should be isolated in the laboratory.

- Gently place adult colonies (>10-15 cm diameter) in a pitcher or bucket with a stem or hose for outflow. Water should be continually fed at a reduced flow rate to prevent colony mortality.

- Place the stem or hose for outflow in a mesh bottom cup. Larvae will flow out of the bucket once released and will be concentrated in the mesh bottom cup for collection.
- The following morning, flush any remaining larvae out of the parent colony bucket/container and gently transport larvae in the mesh bottom cups for distribution.



Larval collection setup for *Pocillopora acuta*. A continual water source feeds the buckets containing individual colonies, with mesh bottomed containers filtering outflowing water.

Photo Credit: Arianna Huffmyer

## LARVAL REARING

Larvae can be reared and will survive in conical containers, buckets, or glass or plastic containers for days to weeks.

- Provide gentle water motion, aeration, and water changes as appropriate for the water volume.
- Be aware settlement may occur immediately (see next section).

*Pocillopora acuta* larvae, pictured to the right, are competent to settle immediately after release by the parent colony.

Photo Credit: Arianna Huffmyer



## LARVAL SETTLEMENT

Larvae are immediately competent and ready to settle once released from the parent colony.

- Settlement may occur immediately, and larvae can be placed in desired settlement systems with appropriate substrate.
- Approximately 50% of larvae will settle within 24 hours, while others may settle up to a week after release.
- Larvae will settle on all materials. As unintended settlement is common, surface area of desired substrate should be maximized.

Maximum larval settlement is achieved by orienting desired settlement substrate surface upside down in mesh bottom containers. Larvae will “crawl” under the substrate and settle on the upside down surface.

- For instructions on how to make mesh bottom settlement chambers, see protocol under SETTLEMENT for *Montipora capitata*, page \_\_\_\_.

Chambers should be placed in a flow through seawater aquarium at a depth which limits volume and maximizes contact of larvae with settlement substrate.

- Mesh bottom chambers enable a continuous exchange of seawater with the aquarium, but prevent larvae from escaping the chamber.
- The water depth inside of the chamber dictates the space larvae have to swim around.
- By limiting the depth of water to the height of the substrate material, larvae are encouraged to settle on substrate.

Chambers should be shaded (30% exclusion) from direct sunlight.

- By taking advantage of natural overhead sunlight, we encourage larvae to settle on the undersides of substrate.

## SUBSTRATE CONDITIONING

Substrate conditioning for *Pocillopora acuta* is identical to the protocol for substrate conditioning for *Montipora capitata*. For details, see the Substrate and Conditioning section of the *M. capitata* protocol on page 9.

## GROW OUT

Conditions: Juvenile *Pocillopora acuta* are fast growing juveniles and require favorable conditions to survive.

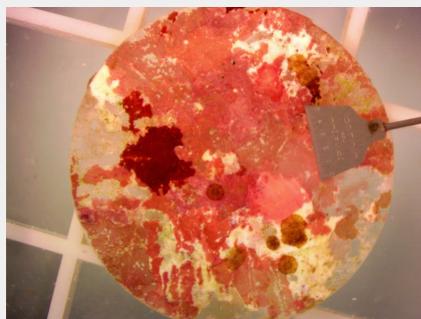
- Clean Seawater
  - Juveniles can be raised in outdoor flow through seawater aquaria which tract closely to ocean conditions
  - Filtered seawater can reduce nutrient loading and sedimentation within the grow out tanks
  - Tank turn over (seawater input) should be high and consistent across tanks, approximately equal to 1 entire tank volume per hour
- Light
  - Juveniles are sensitive to light and overgrowth by algae
  - Moderate shading is recommended with occasional removal of turf algae through manual cleaning or grazing organisms (fish, snails, etc.)
- Flow - Juvenile *Pocillopora acuta* can survive and grow in a variety of flow regimes:
  - High flow environment, 25 times tank turn over per hour, encourages CCA growth, which reduces macroalgal competition with the young recruits. To create high flow within a grow out tank, additional power heads should be added in addition to seawater input.
  - Low flow environments, 0 times tank turn over per hour, enable young recruits to increase polyp extension and potentially feed more.

Feeding: Coral juveniles should be fed.

- Heterotrophy is an important component of coral health.
- Juvenile corals benefit from food supplementation:
  - Dose tanks with natural plankton (preferred), hatched brine shrimp, Reef Chili (or other preferred coral food brand) according to the size of the aquarium and number of corals inside.

Long-term Monitoring and Coral Husbandry: Juvenile corals will survive and grow into adults with proper long term care.

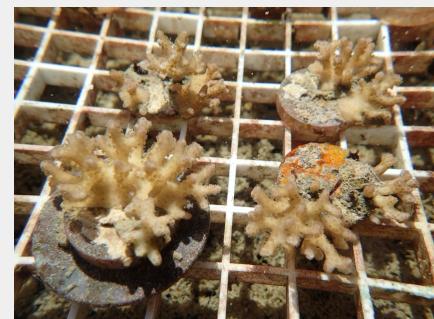
- Water quality, light levels, feeding and flow should be maintained through time.
- Additional coral husbandry may be required, such as removal of benthic competitors, pests, sediment removal, etc.



Less than 1 month



3 months



1+ years of growth

Stages in the rearing process for *Pocillopora acuta* juveniles. (PC: Arianna Huffmyer)



# *Porites compressa*

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## TIMING

Mass spawning for *Porites compressa* occurs on or near the night of the full moon in the summer months, with peak spawning events occurring in June and July.

- Observe spawning on the day before, day of, and day after the new moon in order to gauge and maximize gamete collection.
- The release of eggs and sperm will begin at roughly 11pm.

## LOGISTICS

Collection of gametes is best achieved in the laboratory since colonies release clouds of individual eggs and sperm directly into the water column.

- Eggs and sperm that are released into the water column can become diluted very quickly, thus making field collection a far more challenging and impractical task.

## COLLECTION

Prior to evening hours, adult colonies should be isolated in the laboratory.

- Isolate colonies in 1 or 5 gallon buckets by 10:30pm on the night of spawning. Colonies should be submerged in standing water throughout spawning, so as not to dilute and drain the released sperm and eggs.
- Scan the buckets regularly for sperm release. The sperm can become diluted very quickly.
- Use a sterile disposable pipet to concentrate sperm into a 50mL falcon tube.

- Eggs can remain in the bucket. Colonies should be removed once spawning has been completed. Take care not to contaminate other buckets, especially when doing selective breeding or measuring fertilization success among colonies.

## FERTILIZATION

Fill each bucket a quarter of the way with 1 micron filtered seawater to provide some clean water for the newly released eggs.

- Add sperm to each bucket.
- Check for cleavage after 30 to 45 minutes.

## LARVAL REARING

## LARVAL SETTLEMENT

Larval settlement for *Porites compressa* is identical to the protocol for *Montipora capitata*. For details, see the SETTLEMENT section of the *M. capitata* protocol on pages 8-10.

- Settlement for *P. acuta* is typically observed by the fourth day post fertilization.

## GROW OUT