# Caribbean Coral Spawning for Research and Restoration

## How to raise larvae and outplant settlers without shedding (a lot of) blood and tears

NOAA / TNC Coral Restoration Webinar
Presented on 8 February 2017

Hosted by
Kristen Marhaver: CARMABI Foundation, Curacao
Valerie Chamberland: SECORE Int'l & CARMABI Foundation, Curacao
Nicole Fogarty: NOVA Southeastern Univ., Ft. Lauderdale, FL, USA

CARMABI
Caribbean Research & Management of Biodiversity

secore international

NSU NOVA SOUTHEASTERN UNIVERSITY

# Greetings, PDF downloaders

Hello and thanks for downloading this PDF from our 2017 webinar on Coral Spawning Research. We had an excellent turnout of over 150 people! Whether you attended or not, we hope that you will find these slides useful in future spawning seasons. We're all in this together! We do ask that you keep the following in mind:

**FOOTNOTES + APPENDICES:** The slides below are nearly identical to what we presented during the webinar, with two exceptions: We've added two APPENDICES on preparing materials and constructing tents, and we've marked certain things in the PDF with this orange asterisk: * This means we've added some extra information based on comments from colleagues and suggestions from webinar participants. This extra information is included in the FOOTNOTES section; see page 105.

**PHOTOS:** We've included some great coral spawning photography here, with credit to the photographers. Published figures are subject to the journal's rules. If you would like to use any other photographs for educational purposes, please ask the photographer for permission (and a hi-res photo) **before** you use his or her work. Key email addresses are listed on page 108. Thanks very much for your cooperation on this point.

**FURTHER QUESTIONS:** If we were not able to address your question(s) during the webinar, please join us online to continue the discussion at www.reefresilience.org/network. First, join the forum, then click on "Groups" and look for "Coral Restoration Working Group"... We'll see you there!

Thank you so much for your interest and feedback
(Original slides start now…)

# Your hosts

**Kristen Marhaver** Conducted spawning research in Panama & Curacao for Scripps, UC Merced, and CARMABI; helped develop ‘spawning paranoia’ methods for rearing fragile species like *Orbicella*; led CARMABI project to tackle *Dendrogyra* spawning; now isolating microbial settlement cues for coral larvae.

**Valerie Chamberland** Conducted spawning and restoration research for SECORE, CARMABI, and the University of Amsterdam; an expert in large-scale culturing, settlement, outplanting, and conducting experiments with settlers, especially for *Acropora* spp. and brain coral species; has outplanted *Acropora* spat that now spawn!

**Nicole Fogarty** Conducted spawning research in Belize, Panama, and Florida for UNCW, FSU, Smithsonian, and NOVA Southeastern; an expert in *Acropora* spawning, hybridization, and genetics, *Orbicella* species boundaries, and gamete choice and gamete incompatibility; now exploring the possible use of the hybrid species *A. prolifera* in restoration.

We don’t get all the credit… Shoutouts to ALL the pioneers and gurus of spawning research

# Who are you? Results from our survey

- 193 survey respondents (and 161 RSVPs for the webinar!)
  - 23% currently in a spawning initiative or lab:
    - About 50% coral researchers / 50% restoration practitioners
    - 20% are working beyond the Caribbean (Pacific, Red Sea, Indian Ocean)
  - 77% are not currently in a spawning group/lab, but half would like to join one!
    - ~40% Restoration practitioners
    - ~40% Researchers
    - ~20% Stakeholders (educators, divers, gov’ts, NGOs, industry, hobbyists, funders)
  - Main areas of interest
    - Logistics
      - Timing of spawning
      - Keeping larvae alive
      - Getting larvae to settle + survive
    - Application
      - Understanding the potential of spawning in restoration
      - Applying Caribbean knowledge to other systems
      - Fighting the good fight (conservation, local activism, working with gov’ts, etc…)

Photo credit: K. Marhaver

# Webinar goals

- Inspire you with what's now 'doable' in Caribbean coral spawning
- Enable you to advance your spawning efforts or join in for the first time
- Provide you with resources for future reference

We'll cover the full coral life cycle, from spawning to outplants that spawn (!)

# Webinar caveats

- We assume you know your coral species and some basic knowledge about coral reproduction
- This is *not* a comprehensive summary of coral people, places, and pubs
- We're sharing from our experience… opinions and methods *do* vary
- We'll focus on the best **LOW TECH** hacks for success in spawning work
- We'll share our slides with helpful appendices and any edits you think we should include

Photo credit: K. Marhaver

# Webinar schedule (All times Eastern Standard Time)

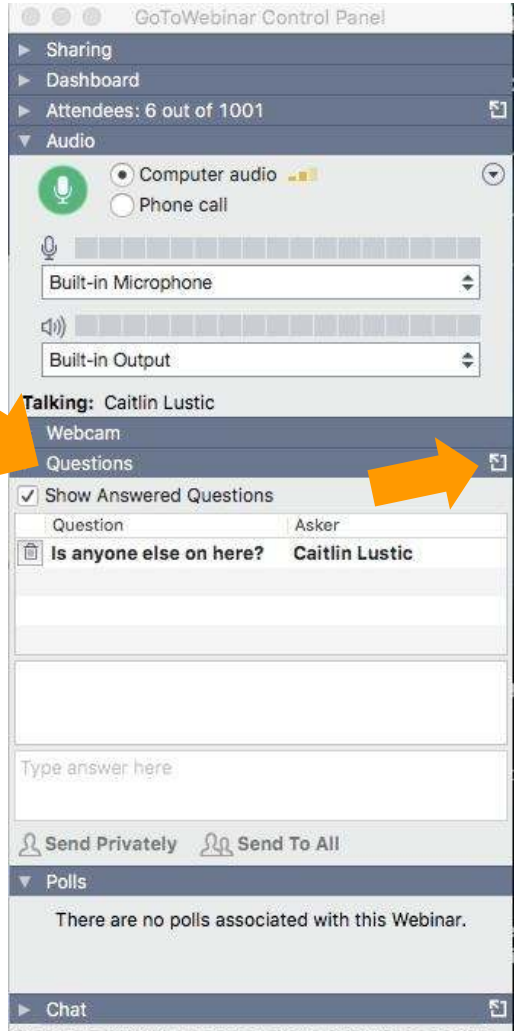
- 10:00 - 11:15 CORAL SPAWNING RESEARCH INFO + INTEL
  - Benefits
  - Choosing a species
  - Secrets to success
    - Timing, collecting, fertilizing, handling, settling, outplanting
  - State of the science
  - Resources
- 11:15 - 12:00 QUESTIONS + ANSWERS
  - Practical + technical advice
- 12:00 - 12:10 BREAK
- 12:10 - 12:40 GROUP DISCUSSION
  - Priorities for our field(s) as a whole
  - Each group's plans for upcoming seasons
- 12:40 - 1:00 UPDATE FROM NOAA

We know you are all multi-tasking humans
Feel free to join/leave as you wish

# During the webinar…

- We won't pause for questions during the talk, however:
- To add questions to the queue, type them in the GoToWebinar Control Panel
- Click this icon  to make the question panel larger
- Please delete your question if we answer it along the way

Here we go…

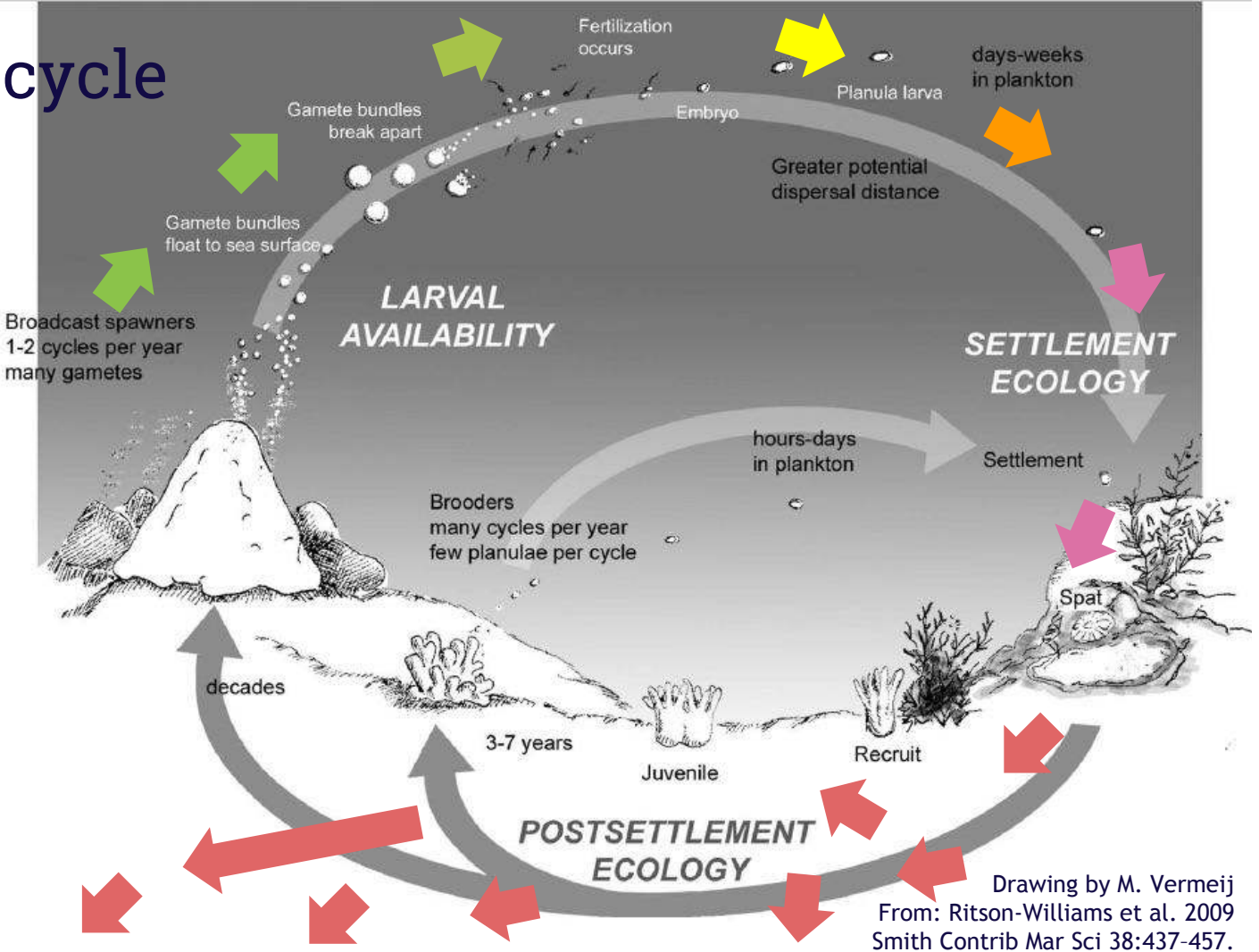


# The coral life cycle

*Fairly easy to figure out for most species*

*Fairly easy to navigate IF you can get gametes*

*Still difficult to do for most Caribbean spawners*




Drawing by M. Vermeij
From: Ritson-Williams et al. 2009
Smith Contrib Mar Sci 38:437-457.

# Spawning research used to suck...

# Spawning research used to suck

Photo credit: M. Vermeij

Diving panic, rushing to shore (or to the boat), mixing gametes in dirty parking lots (or dirty boats), driving panic

# Spawning research used to suck

Photo credit: M. Vermeij

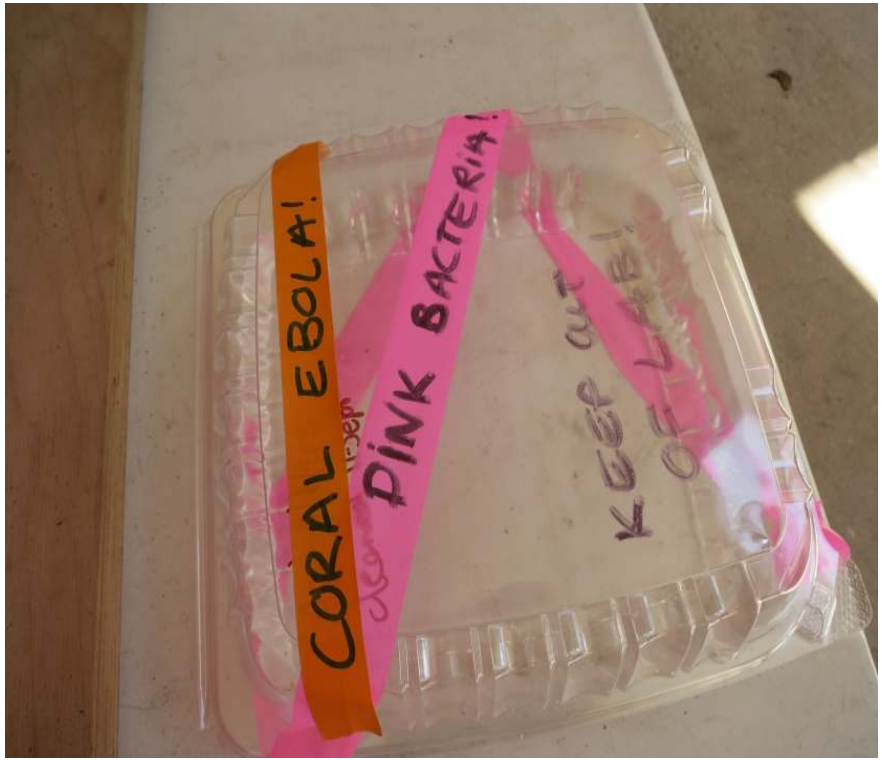
Large white Igloo coolers full of super-dense batches of larvae requiring constant watching and water changes

# Spawning research used to suck


Photo credits: V. Chamberland

Endless water filtration for water changes

# Spawning research used to suck



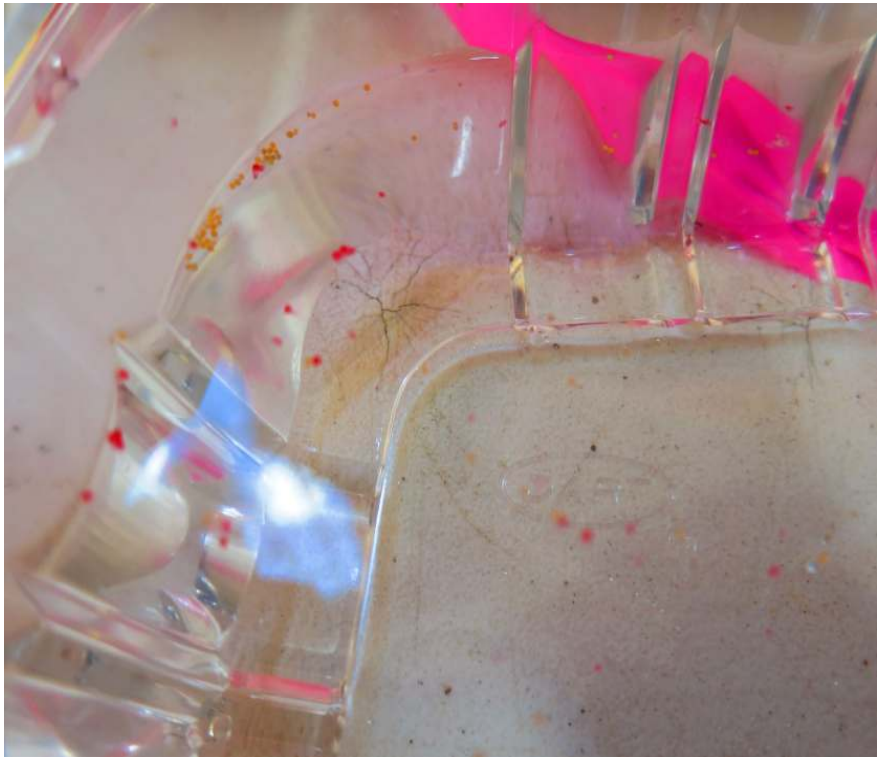



Photo credits: M. Vermeij

Contagious pink bacteria aka “coral plague”

# Spawning research used to suck

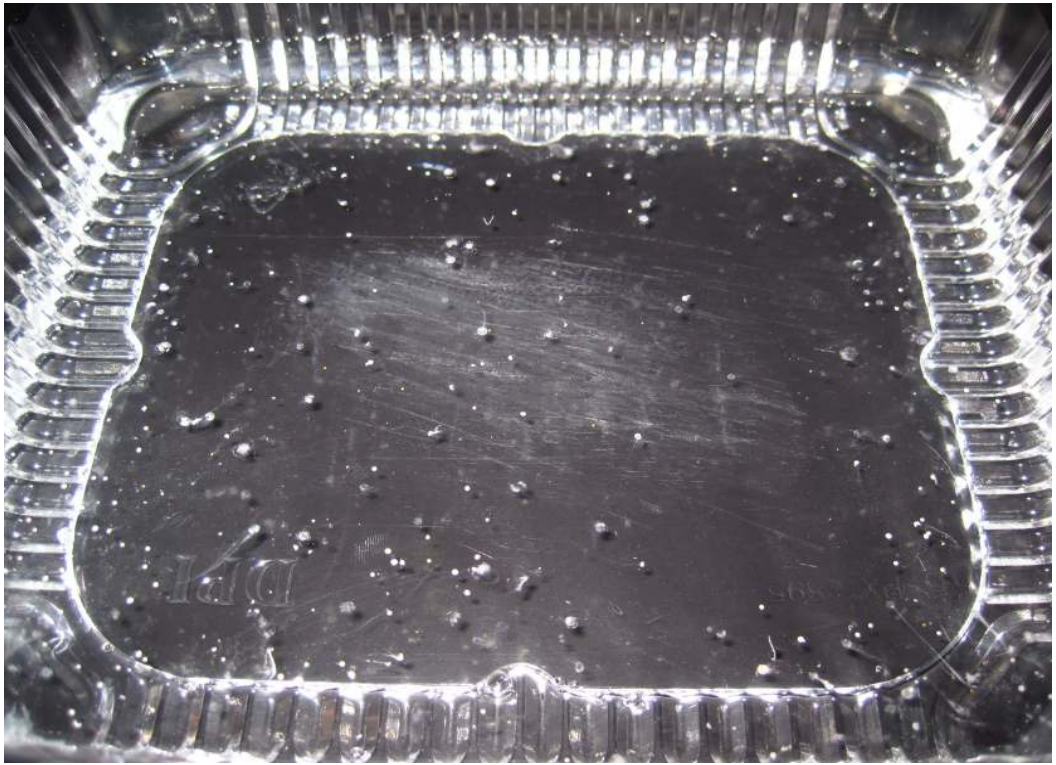
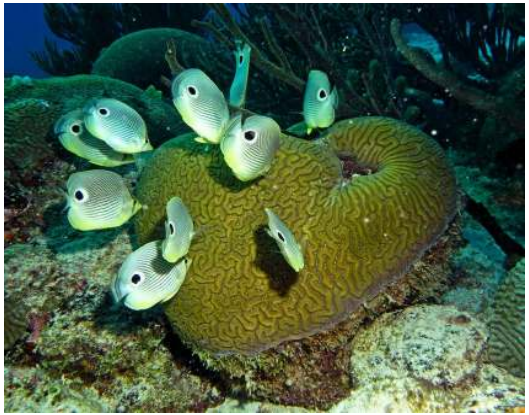
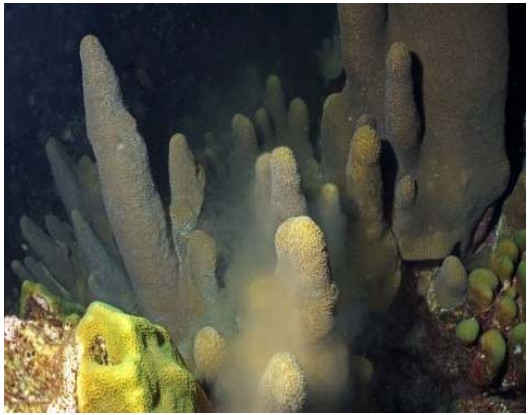


Photo credit: M. Vermeij

Larvae explosions, sudden death of entire cultures

# Spawning research doesn’t suck anymore*!

*always, as much, for as many decades…


# Spawning research doesn't suck anymore*!



Photo credits: B. Mueller

Spawning times and life histories unraveled for multiple species

# Spawning research doesn’t suck anymore*!

Photo credit: P. Selvaggio

Methods worked out for healthy larval cultures (*C. natans* shown here)

# Spawning research doesn't suck anymore*!

Photo credit: P. Selvaggio

4-year-old *A. palmata*, outplanted at the age of 1 year, now sexually mature!

Photo credit: N. Hurtado

2.5-year-old *C. natans* juvenile

Full life cycle worked out for some species!

# Benefits of spawning research

It’s worth the hassle

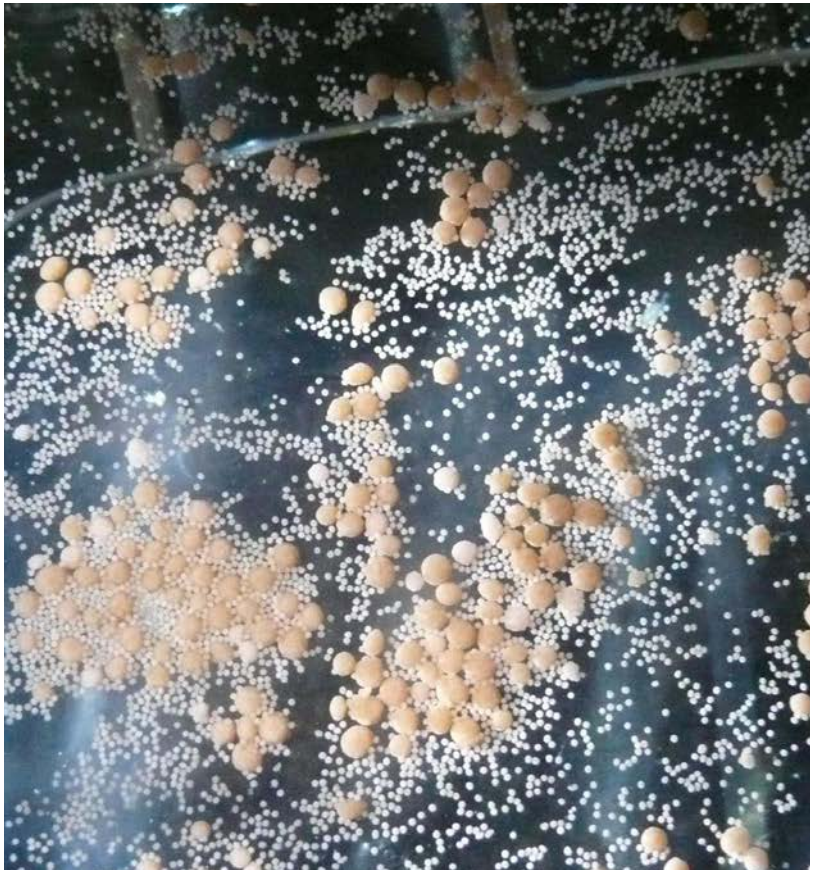


Photo credit: K. Marhaver

*O. faveolata* gamete bundles and eggs

# Benefits of spawning work: Basic research

- For many species, spawning yields a nearly unlimited supply of individuals
- Critical and fragile life stages provide key ecological and evolutionary insights
- Larvae provide fast and clear information on behavior, survival, settlement choice
- Requires relatively modest equipment (but travel can be pricey)
- Spawning is a good platform to study development, genetics, symbiosis, microbiology, molecular biology, fertilization biology, endocrinology, toxicology, ecology…

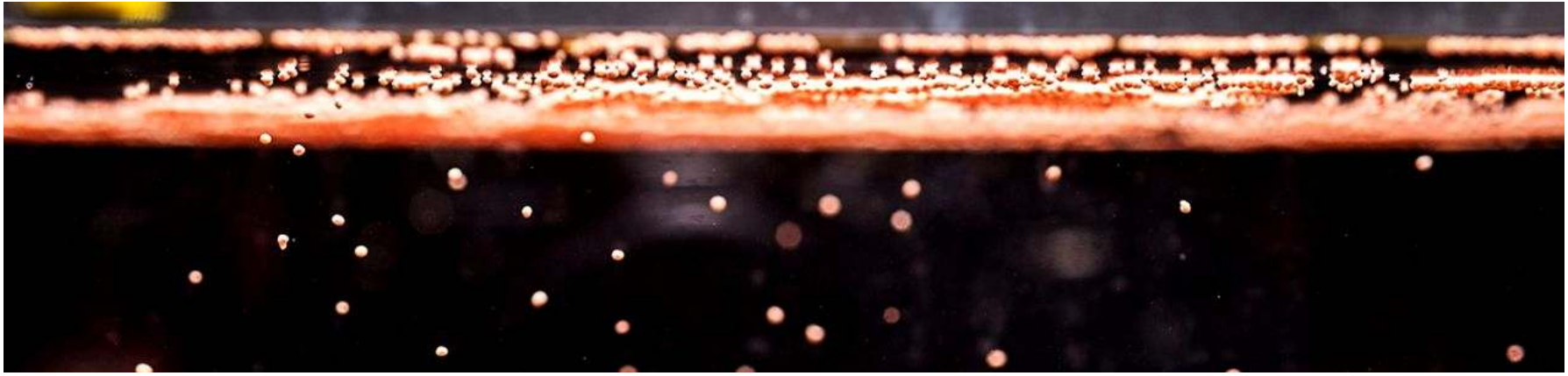


Photo credit: P. Selvaggio

# Benefits of spawning work: Restoration

- Yields thousands of individuals in a short time
- Nature does a lot of the ‘work’ for you
- Most important source of **new genetic diversity** for threatened coral species
- Juveniles may be more flexible than adults and may survive better on degraded reefs
- Individuals can be bred selectively or deliberately ‘stressed’ to conduct directed evolution
- Methods are applicable to several species, including those with massive growth forms

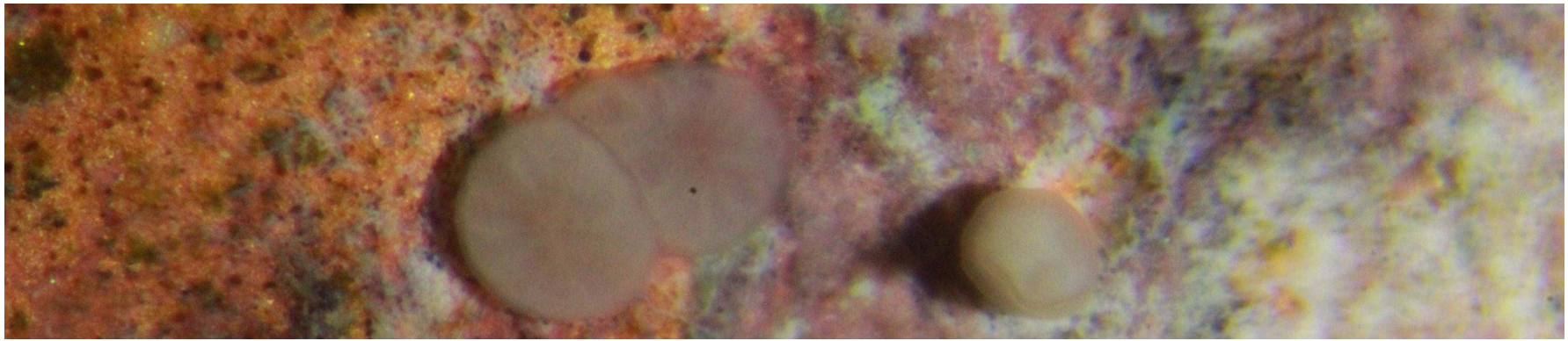


Photo credit: V. Chamberland

For long term restoration success, we need more progress *and* more people in coral spawning!

# Choosing a species to work with

It’s not just *Acropora* and *Orbicella* anymore

Photo credit: S. Snowden

*D. labyrinthiformis* releasing sperm-egg bundles

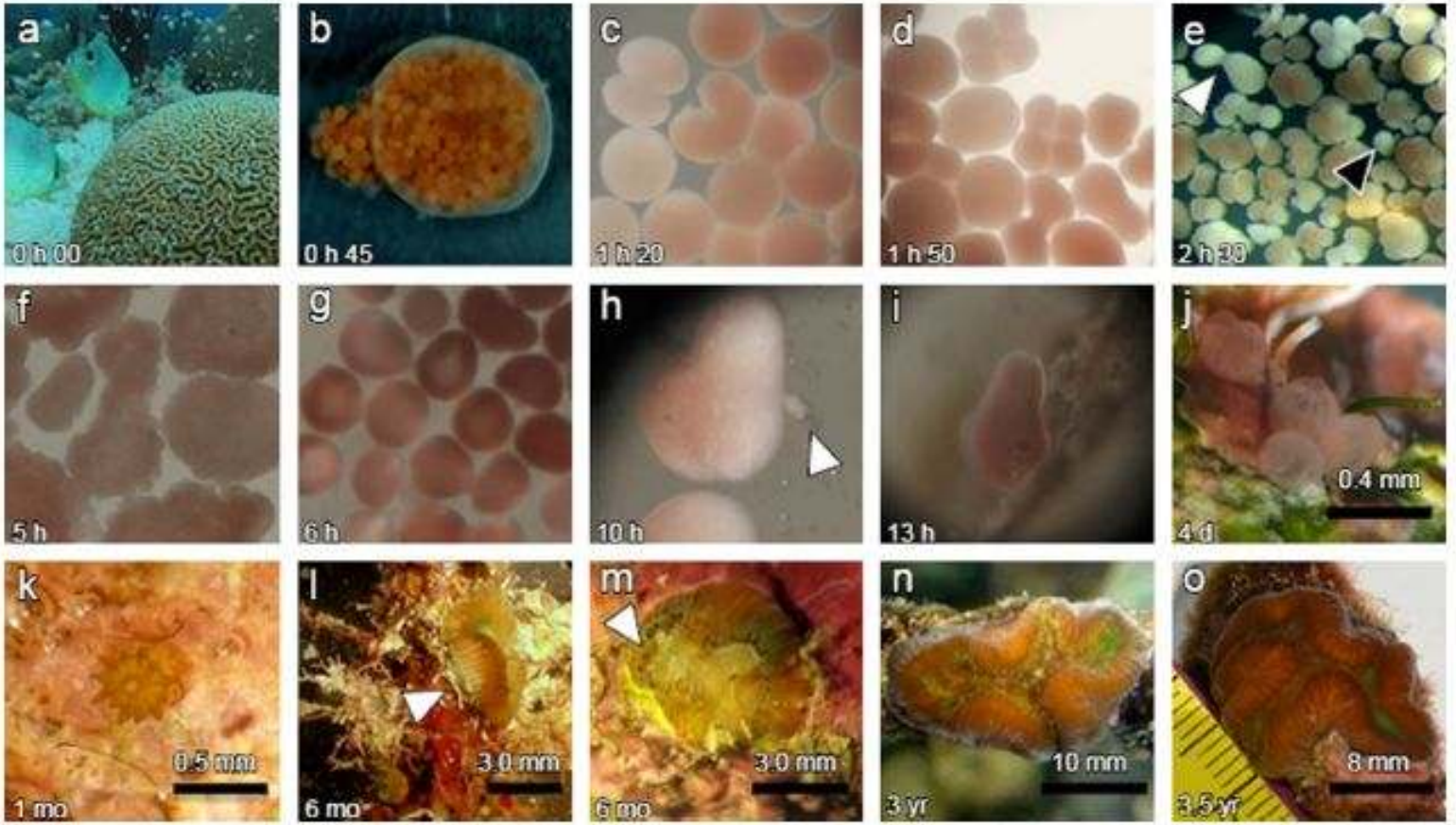



Photo credits: Chamberland et al. 2016
Coral Reefs 36:83-94. DOI:10.1007/s00338-016-1504-2

# *D. labyrinthiformis* development

# Spawners ranked from easiest to most annoying:

| Species | Predicting spawning | Gametes | Embryos | Time to motility | Time to settlement competency, larval "choosiness" | Settler survival |
|---|---|---|---|---|---|---|
| *D. labyrinthiformis* **EASIEST TO SEE SPAWN* | VERY EASY *6-7 events/yr* | BIG BUNDLES, SMALL EGGS | ROBUST | VERY SHORT | FAST, SEMI-PICKY | MEDIUM |
| *C. natans* **EASIEST TO REAR TO AGE 2* | EASY *2-3 events/yr* | BIG BUNDLES, SMALL EGGS | ROBUST | SHORT | FAST, NOT PICKY | VERY HIGH |
| *P. strigosa* **THIS MAY ALSO APPLY TO P. CLIVOSA?* | EASY *1-2 known events/yr* | BIG BUNDLES, SMALL EGGS | ROBUST | SHORT | FAST, NOT PICKY | HIGH |
| *Acropora* spp. **BIG LARVAE, GROW FAST* | NOT ALWAYS EASY *1 event/yr* | SMALL BUNDLES, LARGE EGGS | FRAGILE | VERY LONG | MEDIUM FAST, SELECTIVE | MEDIUM |
| *Orbicella* spp. **GOOD FOR LARVAL EXPERIMENTS* | VERY EASY *2 events/yr* | LOTS OF SMALL BUNDLES, TINY EGGS | VERY FRAGILE | SHORT to MEDIUM | SLOW, VERY SELECTIVE | TERRIBLE |

Our field started with fragile and difficult species; it can be much easier

# Gonochoric spawners: We're working on it...

| Species | Predicting spawning | Gametes | Embryos | Time to motility | Time to settlement competency, larval "choosiness" | Settler survival |
|---|---|---|---|---|---|---|
| *M. cavernosa* **POSSIBLY THE EASIEST GONOCHORE?* | PRETTY EASY | PLENTIFUL ♂, SPARSE ♀, ROBUST | SEMI-FRAGILE | MEDIUM | *QUICK, VERY SHORT WINDOW, PICKY | LOW |
| *S. siderea* | MEDIUM-HARD | PLENTIFUL, TINY | ROBUST | MEDIUM | SLOW, PICKY | TBD |
| *D. cylindrus* **KIND OF EASY... AFTER THE REALLY HARD PART* | EASY IF YOU CAN FIND MALES *AND* FEMALES | RARE, SPARSE, TINY | ROBUST | FAST | FAST, NOT VERY PICKY | TBD |
| *S. intersepta* | DIFFICULT? | RARE, TINY | TBD | TBD | TBD | TBD |
| *D. stokesii* | TBD | TBD | TBD | TBD | TBD | TBD |
| *M. meandrites* | TBD | TBD | TBD | TBD | TBD | TBD |

Current challenges: spawning times, finding good colonies, gamete collection

# Secrets to spawning success

We suffered a lot of the hassle so you don’t have to

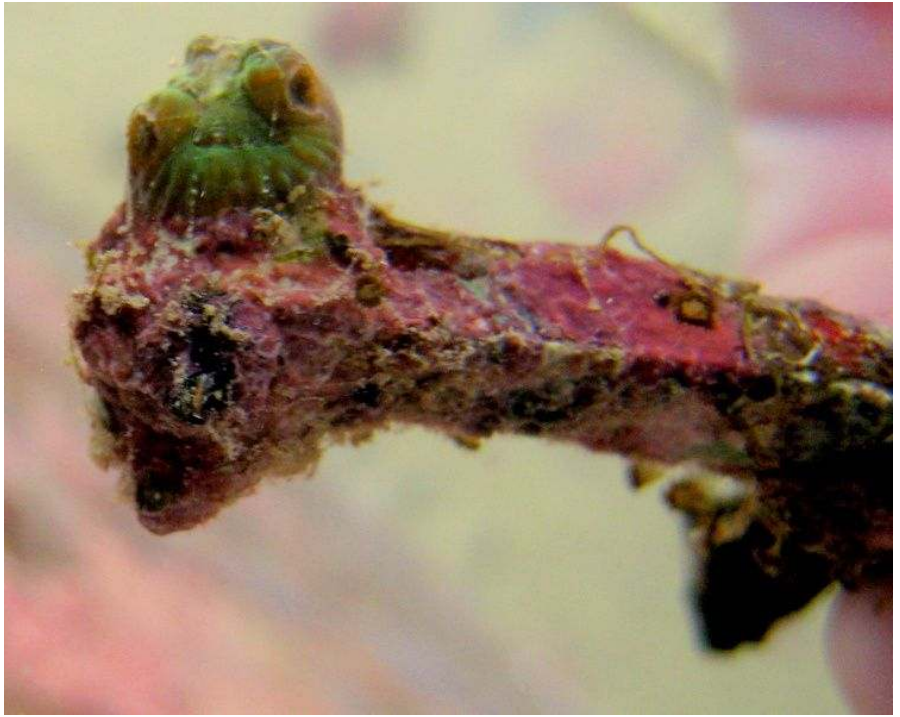


Photo credit: V. Chamberland

6-month-old *D. labyrinthiformis* juvenile, reared from spawn

# Timing of spawning

Difficult to figure out… until suddenly it’s easy

Photo credit: K. Marhaver

*O. annularis* releasing gamete bundles

# Timing of spawning: Finding gravid corals

Photo credit: R. Ritson-Williams

**Acroporids**

Photo credit: H. Noren

***Orbicella* spp.**

- Gravid corals *can* be identified prior to spawning
- Months prior to spawning: eggs will look like tiny clear sausages under a dissecting scope
- Days prior to spawning: eggs will be opaque pink/orange and visible with the naked eye
- Very helpful if bringing corals to the lab, but not worth stressing corals “just to know”

# Predicting the timing of spawning

- DAY & TIME
  - Refer to past observations *in your region* whenever possible
  - Note month, date of full moon, and timing of sunset
  - Use minutes past sunset, not time of day (daylight savings, full moon falls)
  - Translate past observations to current year's moon cycle and your location's sunset times
  - Use sun/moon data from your precise location, not global sun/moon times
  - A good resource is www.timeanddate.com
  - Watch out for time zones and daylight savings time!
    - E.g., Venezuela moved its time zone back by 30 minutes in 2007 and then 30 minutes forward again in 2016!
- MONTH
  - NOTE: Spawning occurs about 1 month earlier in the northern Caribbean vs. the southern Caribbean
    - E.g., Florida *Orbicella* spawn in Aug and Sept, Curacao *Orbicella* spawn in Sept and Oct
  - Try to dive one month early and one month late, even if just to watch

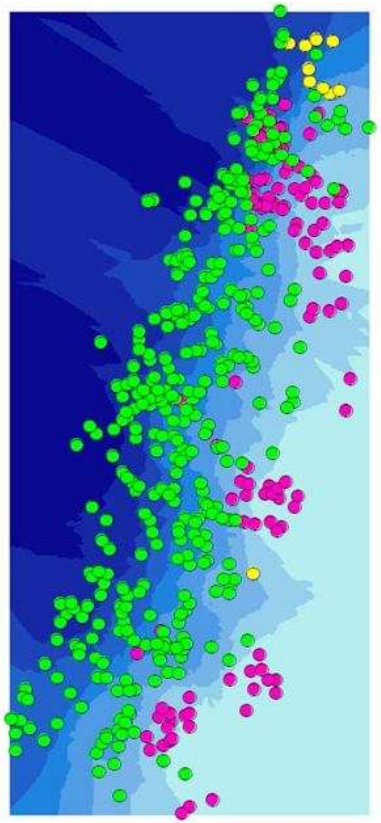
Dive as much as you can with the resources you have

# Timing of spawning: Insider tricks

- SPLIT SPAWNING
  - When the full moon occurs very early or late in the typical spawning month, expect a split spawning across two separate months
    - E.g., in Curacao, in 2015, *C. natans* spawned in early October *and* early November
- FULL MOON TIMING
  - A full moon at 0200h on 2 Sept will *feel* like a full moon to the corals on the night of 1 Sept
  - If the full moon occurs before ~0400h, we begin diving one day earlier
- PREDICTABILITY IS GOING DOWN
  - Warmer water can shift spawning earlier or cause split spawns; it's important to have as much of a "baseline" as possible for each species
  - Bleaching causes poor spawning and less synchronized spawning the next year, and in some cases for several years afterward
  - Smaller coral populations are worse at synchronizing spawning

AVOID HUBRIS: *A. palmata* can spawn on Day 1…or Day 11. Dive early & often!

# Spawning precision: an example from *Orbicella*



Levitan, Fogarty, et al. 2011
Evolution 65:2154-1270.

In Bocas del Toro, Panama, tagged *Orbicella* colonies have been monitored during spawning since 2002. Individual colonies spawn with striking consistency from one year to the next:

***Orbicella faveolata:***

Standard deviation for individual colony: 14 min

***Orbicella franksi*:**

Standard deviation for individual colony: 7 min

***Orbicella annularis:***

Standard deviation for individual colony: 10 min

The timing of spawning at the colony level seems to be largely determined by each coral's internal circadian clock. However, spawning *also* depends on whether nearby corals are also spawning. In other words, coral colonies likely communicate with chemical cues to fine-tune the timing of spawning at the last minute.

# The spawning giveaway: Setting aka staging

Photo credit: R. Ritson-Williams

Photo credit: A. Wood

Setting aka staging of gamete bundles by *O. annularis* (Left) and *A. palmata* (Right)

# Hermaphroditic broadcast spawners

Photo credit: E. Hickerson

***Orbicella franksi***
SD: Aug 1–Oct 18; peak Aug 19–Sept 12
DAFM: 5–10; peak 6–8
MAS: 100–250; peak 110–200

Photo credit: K. Marhaver

***Orbicella annularis***
SD: Aug 1–Oct 30; Aug 26–Sept 21
DAFM: 5–8; peak 6–7
MAS: 150-275; peak 190-250

Photo credit: FGBNMS

***Orbicella faveolata***
SD: Aug 3–Oct 6; Aug 18–Sept 10
DAFM: 5–9; peak 6–8
MAS: 100–275; peak 175–250

**Abbreviations: SD** = spawning dates; **DAFM** = days after full moon; **MAS** = minutes after sunset

# Hermaphroditic broadcast spawners

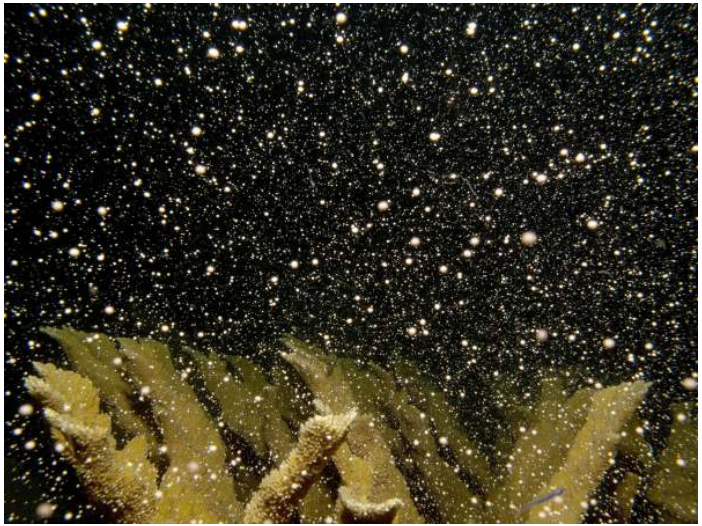


Photo credit: R. Ritson-Williams

***Acropora palmata***
SD: July 18–Sept 12; peak Aug 9–24
DAFM: 0–15; peak 3–6
MAS: 90–210; peak 140–190

Photo credit: R. Ritson-Williams

***Acropora cervicornis***
SD:July 21–Sept 3; peak Aug 6–Aug 20
DAFM: 1–15; peak: 2–6
MAS: 125–200; peak 150–190

**Abbreviations: SD** = spawning dates; **DAFM** = days after full moon; **MAS** = minutes after sunset

# Hermaphroditic broadcast spawners

Photo credit: Hickerson/FGBNMS

***Pseudodiploria strigosa***
SD: Aug 2–Oct 4; peak Aug 15–Sept 4
DAFM:5–8 ; peak 6–8
MAS: **-100**–320; peak 100–200

Photo credit: Muller & Vermeij 2011
Coral Reefs 30:1147.

***Diploria labyrinthiformis***
SD: May–Nov; peak Aug 10–Sept 9
DAFM: 7–13 ; peak 10–12
**MBS**: 0–65; peak 15–45

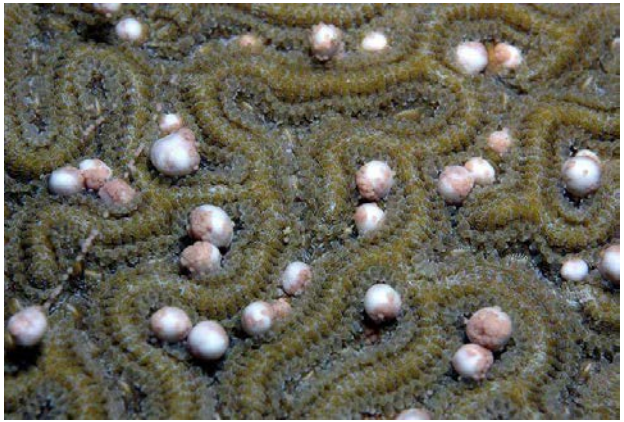


Photo credit: FGBNMS

***Colpophyllia natans*** *
SD: Aug 8–Sept 6; peak Aug 16–31
DAFM: 7–10; peak 8–9
MAS: 38–170; peak 30–123

**Abbreviations: SD** = spawning dates; **DAFM** = days after full moon; **MAS** = minutes after sunset
**Note:** *D. labyrinthiformis* spawns *before* sunset (**MBS**)

Please share your data as we work to figure out these species!

# Gonochoric broadcast spawners

Photo credit: Neely et al. 2013 Coral Reefs 32:813.

***Dendrogyra cylindrus* - males**
SD: Aug 2–Sept 30; peak Aug 12–Sept 4
DAFM: 2–5; peak 2–4
MAS: 58–134; peak 93–119

Photo credit: Neely et al. 2013 Coral Reefs 32:813.

***Dendrogyra cylindrus* - females**
SD: Aug 2–Oct 1; peak Aug 12–Sept 4
DAFM: 1–4; peak 2–3
MAS: 58–142; peak 102–134

**Abbreviations: SD** = spawning dates; **DAFM** = days after full moon; **MAS** = minutes after sunset

# Gonochoric broadcast spawners


Photo credit: B. Holland

***Montastraea cavernosa* - males**
SD: Jul 19–Oct 4; peak Aug 13–Sept 3
DAFM: 4–9; peak 6–7
MAS: **-19**–259; peak 62–154

Photo credit: FGBNMS/Schmahl

***Montastraea cavernosa* - females**
SD: Jul 19–Oct 4; peak Aug 13–Sept 3
DAFM: 4–9; peak 6–7
MAS: **-9**–245; peak 62–147

**Abbreviations: SD** = spawning dates; **DAFM** = days after full moon; **MAS** = minutes after sunset
**Note:** Spawning range includes times before sunset, depicted here as a negative number

# Gonochoric broadcast spawners

Photo credit: B. Brown

***Siderastrea siderea* - males**
SD: Aug 1–Oct 5; peak Sept 10–Oct 2
DAFM: 5–7; peak 5–6
MAS: 217–226; peak 217–226

Photo credit: B. Mueller

***Siderastrea siderea* - females**
SD: Aug 1–Oct 5; peak Sept 10–Oct 2
DAFM: 5–7; peak 5–6
MAS: 210–231; peak 215–229

**Abbreviations: SD** = spawning dates; **DAFM** = days after full moon; **MAS** = minutes after sunset

**More data are needed for these species!**

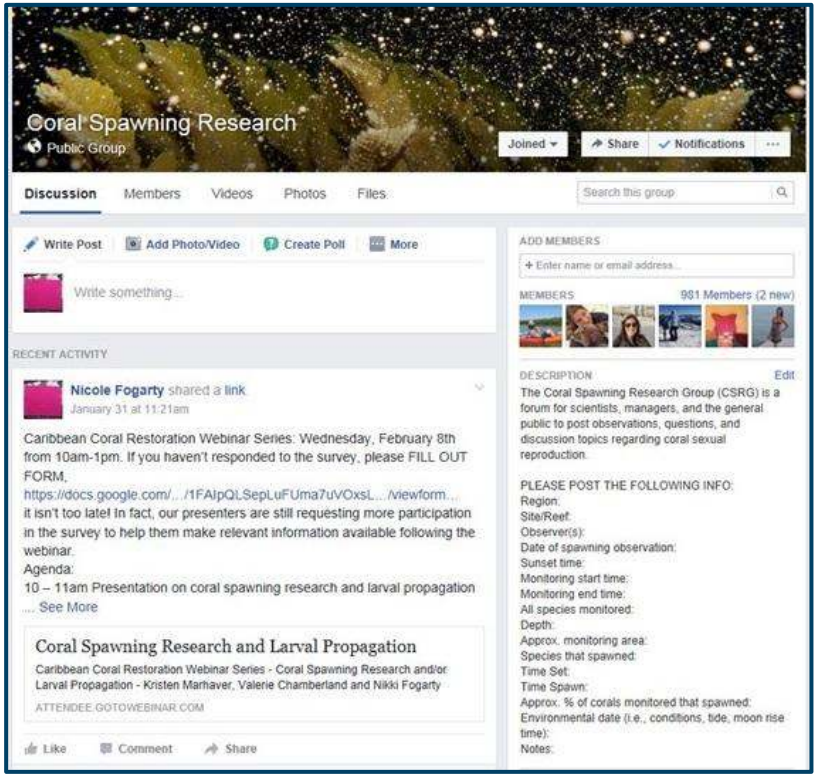
# Timing of spawning: Please report!

More than 1000 members!

The Coral Spawning Research Group (CSRG) is a Facebook Group for scientists, managers, and the general public to post observations, questions, and discussion topics on coral sexual reproduction.

**PLEASE RECORD + SHARE THE FOLLOWING DETAILS OF YOUR SPAWNING OBSERVATIONS**

Region:
Site/Reef:
Observer(s):
Date of spawning observation:
Sunset time:
Monitoring start time:
Monitoring end time:
All species monitored:
Depth:
Approx. monitoring area:
Species that spawned:
Time Set:
Time Spawn:
Approx. % of corals monitored that spawned:
Environmental data (i.e., conditions, tide, moon rise time, wind speed):
Notes:



# Collecting gametes

Don’t panic
We repeat: Don’t panic
And don’t use crappy supplies


Photo credit: K. Marhaver

# Alternate scheme: Bring the corals to you

- Gravid corals *can* be collected prior to spawning:
  - Corals will spawn in buckets in the lab
  - They don't detect chemical cues from each other, so they will often spawn later than in the wild
  - Collecting *O. annularis* heads, branches of *A. cervicornis*, or smaller *M. cavernosa* colonies works well
  - Make sure you get the necessary permits!
  - After spawning, epoxy all corals back to the reef in their original place
  - Note: Large boulder corals and *A. palmata* do NOT do well in the lab

Photo credit: A. Wood

Photo credit: H. Noren

Photo credit: R. Ritson Williams; N. Fogarty

Buckets for spawning experiment examining chemical cues, *Orbicella* setting and spawning in buckets

# Night diving: Safety and logistics*

DIVE LIGHTS

- Bigger isn't necessarily better! Look for compact LED lights with multiple brightness settings
- Secure lights with clips
- ALWAYS have a spare light
- Use glow sticks on your tank
- Don't blind your buddy! Signal by making circles with your light
- Headlights - wrist mounts are useful
- Dome lights are useful on tents

BOAT LIGHTS

- Running Lights/Anchor Lights
- Tag line with light
- Light on dive flag

NAVIGATIONAL AIDS

- Transects
- U/W buoys
- Glow sticks

*


GEAR

- Full wetsuit or skin to protect against stinging creatures
- Hood for warmth & to keep worms out of ears: Yuck!
- Streamlined gear
- Lights
- Compass
- Underwater camera


DATA RECORDING

- Glow-in-the-dark slate
- Extra pencils
- Data sheets
- Set your camera and dive computer to the exact local time!

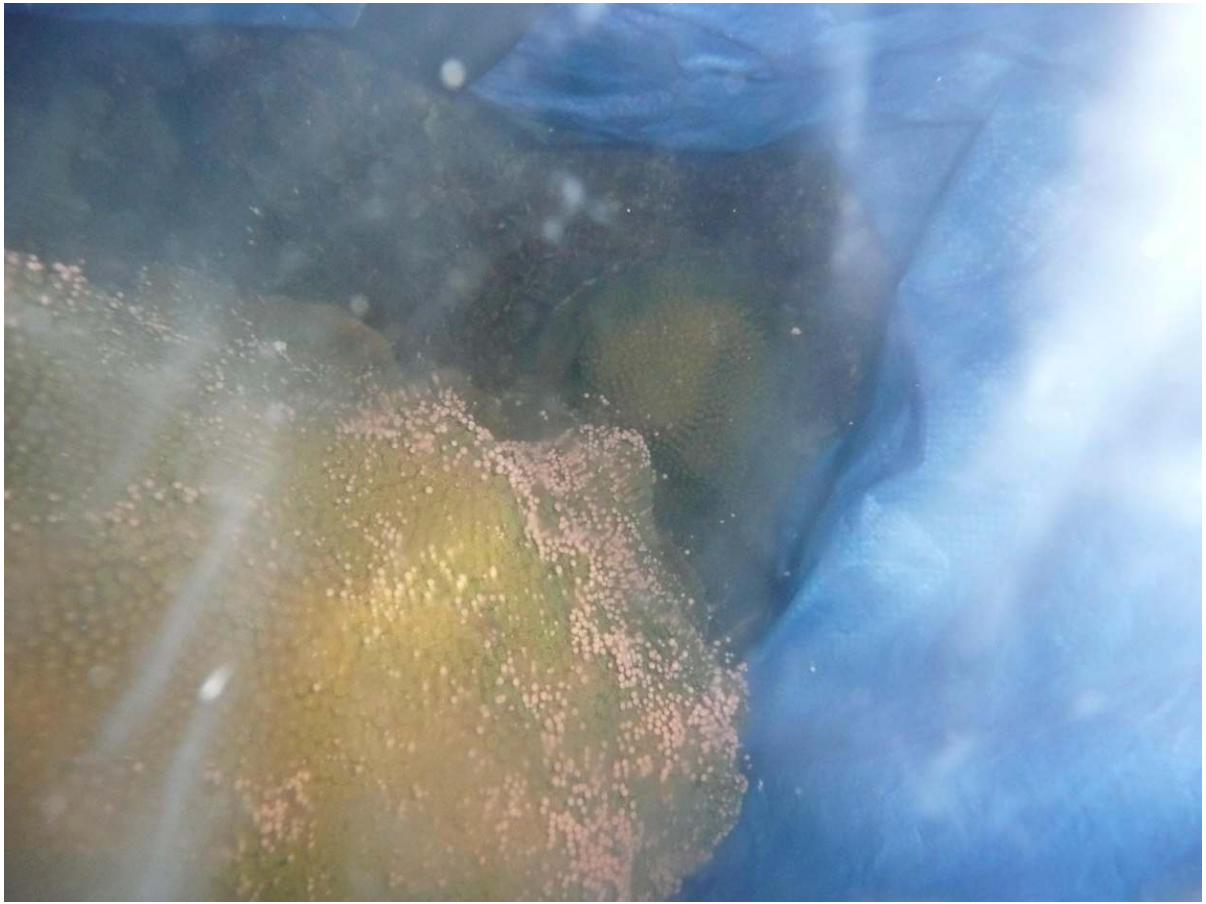


Photo credit: K. Marhaver

*O. faveolata* releasing bundles inside a spawning tent

Photo credit: K. Marhaver

Teeny-tiny *D. cylindrus* eggs in the water column

# Collecting gametes: Spawning tents

Photo credits: R.-J. van der Houwen

FOR ACROPORIDS

- Mesh tents of rip-stop nylon
- Lighter material for delicate coral branches
- Weighted cords secure tents to individual coral branches
- Bundle each w/ rubber band, carry in large mesh bag

FOR MASSIVE CORALS (+ BIG, STURDY ACROPORIDS)

- Fabric tents of nylon mesh and/or plastic tarp tents
- Heavier material stays up off colonies
- Weights on bottom keep tents wide open
- Carry in bundles with bungee cords

Well-built tents make spawning 1000X more fun+easy…
See Appendix B below for details on building all 3 types

# Collecting gametes: Key materials


TUBES

- Buy LOTS of sterile, high quality, easily-replaced tubes and caps from ONE brand
- Polypropylene or polystyrene tubes are okay (we like 50 ml BD Falcon tubes)
- DON'T make tubes and caps a precious or rare item!
- Take plenty of extras underwater
- To prevent loss, you can glue a string between cap and tubes
- Caps + tubes should be sterilized with 10% bleach after *every* dive night, then rinsed well in dilute sodium thiosulfate pentahydrate (chlorine neutralizer) and rinsed well with fresh water
    - Note: some labs NEVER use bleach
- To be safe, throw away *any* tubes that have contained spawn

BAGS

- BEST choice: small nylon mesh dive bags with D-rings
- Second choice: Zip-top FREEZER bags (Ziploc or Hefty), reinforced with Gorilla tape on the top corner
- Many projects are ruined when someone buys crappy plastic bags to save 50 cents!

# Collecting gametes: Gonochoric species

CHALLENGES

- Males often spawn before females, may live deeper or in separate locations
- Sex ratio may not be 1:1, colonies may be part male/part female

COLLECTING

- Identify and track “good” male and female colonies ahead of time
- Collect sperm w/ polypropylene syringes (60, 130, or 500 ml), a bilge pump attached to a cubitainer, or plastic freezer bags
- Put tubing on syringes to reach tight spaces, ALWAYS tie off tubing to keep contents inside
- Collect eggs with syringes or with non-mesh spawning tents
- Eggs are tiny and not very buoyant, easily get stuck in wrinkled nets

FERTILIZING

- Consider fertilizing *in situ*: e.g., tent females and transfer syringe of sperm to tented females, OR combine gametes in plastic bags underwater, OR or fill syringes with half sperm/half eggs

Tell us what works! We have only just started to figure out these species.

# Collecting gametes: Insider tricks

- Keep dive lights away from tents and tubes, it attracts annoying plankton that will cause trouble in fertilization
- Work in teams of two divers
  - One diver handles nets, tents the colonies, handles tubes
  - One diver carries nets, spots setting colonies, holds flashlights, holds tube caps
  - This is much more efficient and calm, especially in rough seas… HIGHLY recommended for shallow *A. palmata*
- Don't get greedy! Don't fill tubes more than ⅔ full; ⅓ full is even better… Don't wait on the reef for the very last bundle.
- A small air bubble in the tube is useful to prevent a slimy overflow when opening tubes back on shore
- * CLEAN CATCHES will make your life much easier for the coming days! Avoid mucusy, stressed, bleached, or sick-looking corals; throw out anything that looks gunky, clumpy, slimy, goey, congealed, jello-y
- A few ml of **clean** happy bundles from a small healthy colony is worth **far** more than a huge tube full of slimy gunk

Swarming worms
Photo credit: M. Briscoe


Photo credits: K. Correia, M. Vermeij

# Fertilizing gametes

Continue to not panic
This is the part where you don't really do *that* much

Photo credit: K. Marhaver

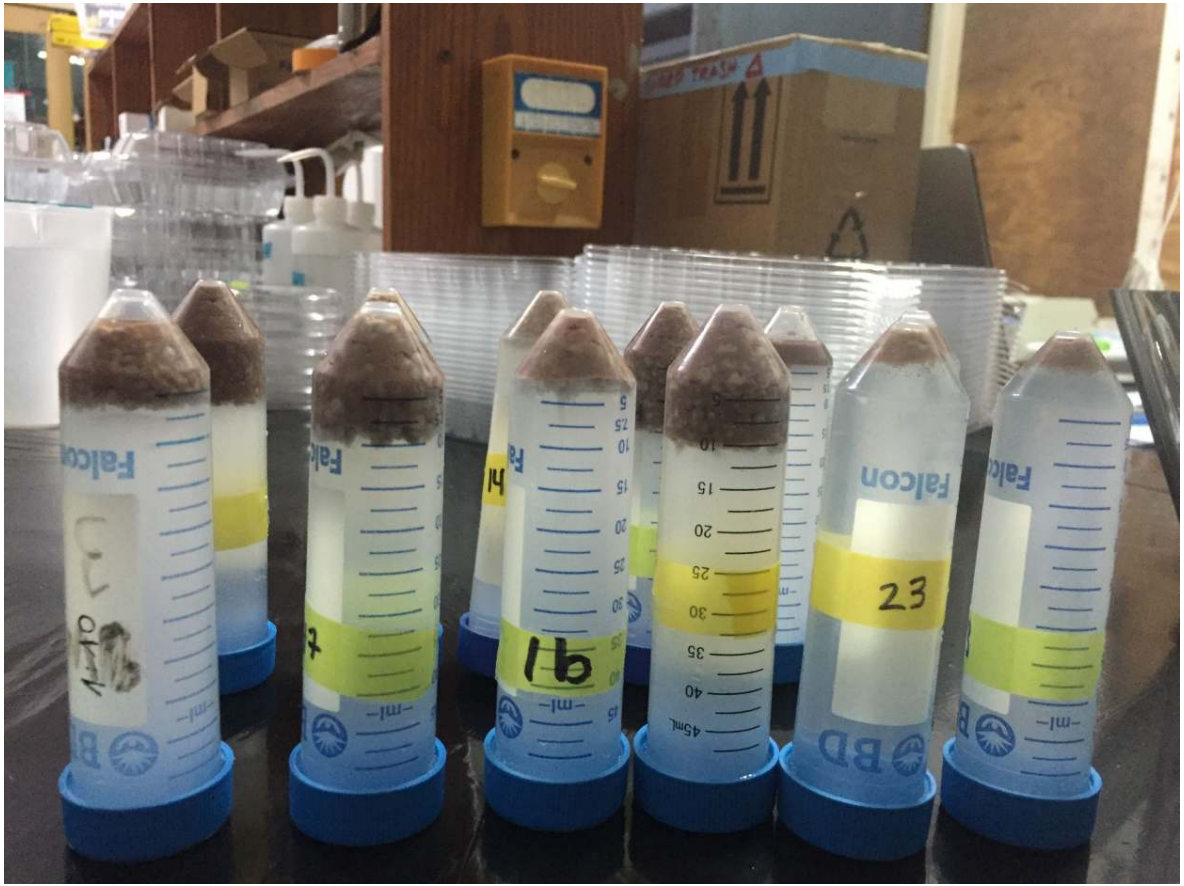


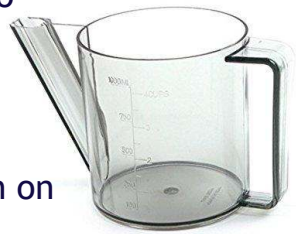

Photo credit: N. Hurtado

# *C. natans* gamete bundles upon arrival at the lab

# Fertilizing gametes: Key materials

- WELL-FILTERED SEAWATER
  - Prepare at least 100 L of filtered seawater (FSW) before spawning; we use peristaltic pumps with silicone tubing; vacuum or hand pumps also work
  - CARMABI uses 0.45 µm filters (Millipore HPWP 47 mm disc) for fragile species (*Orbicella*) and Whatman GF/F (~0.7 µm) for 'the rest'
  - Filter within 48 hours of spawning, keep within 1 degree of ambient SW temperature, store in bleached & rinsed plastic jugs (e.g. Blitz brand)
  - See Appendix A for water filtration tools and tips

- FERTILIZATION CONTAINERS
  - Fertilize gametes directly in their rinse containers: large volume fat separators; we like Norpro 4 cup size; they're large and easy to clean
  - Alternate option: fertilize in polystyrene deli containers (described in the next section on Handling Embryos)



- MATERIALS FOR MINOR ADJUSTMENTS
  - Polypropylene plastic transfer pipettes (we like sterile bags of 20)
  - Small cheap LED flashlight to inspect for any copepods, exploding eggs, or clumpy eggs
  - Polypropylene plastic squeeze bottles full of recently-filtered FSW **at ambient seawater temperature**

# Fertilizing gametes: Mixing and incubation

**LITERALLY don't put all your eggs in the same basket!**

- Prepare MULTIPLE fertilization batches with each a mix of bundles from all (or from a subset of) the parent colonies
- Gently pour sperm and eggs from each Falcon tube into each fat separator
- Ensure eggs are no more than 2-3 layers thick once the bundles break apart
- Adjust sperm density by adding FSW through the spout of the fat separator, until the water column is as cloudy as shown in picture 4
- Incubate until first cell divisions (~90 min)
- GENTLY stir with a pipette every 20-30 minutes to help bundles break apart
- Watch for gametes clumping in the meniscus at the container edge!*

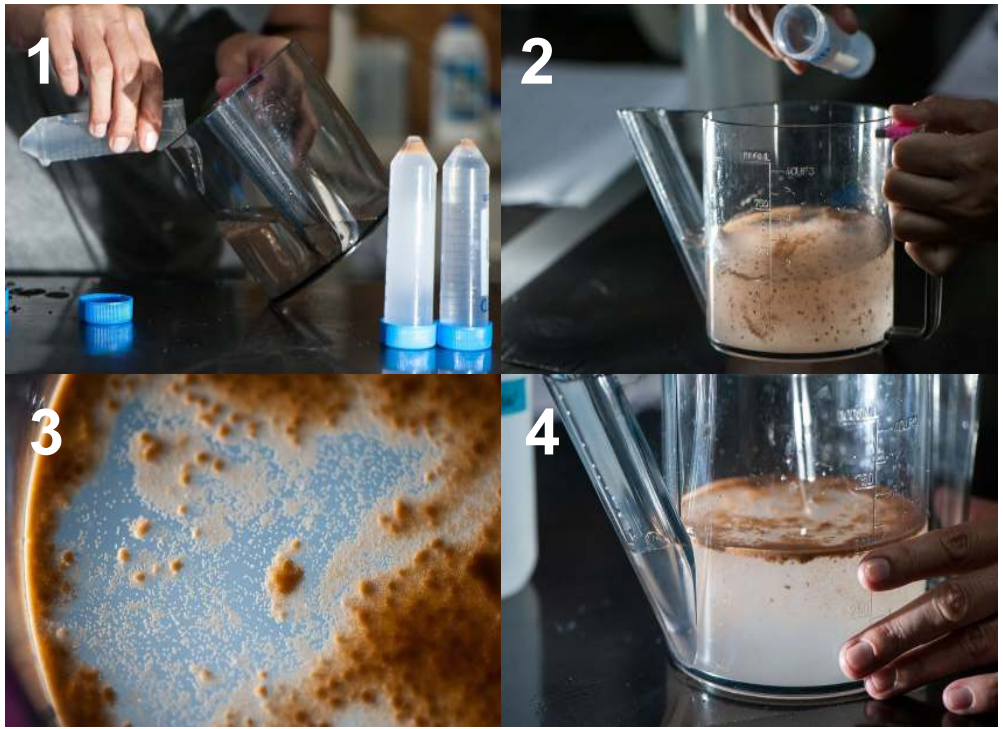



Photo credit: P. Selvaggio

Fertilizing *Orbicella* gametes


Photo credit: P. Selvaggio

If needed, rinse eggs from the sides of the fat separator by running water **down the walls** with a squeeze bottle of clean FSW. Don't spray water directly at the eggs!

# Fertilizing gametes: Insider tricks

- Most gametes stay viable for over an hour: DON'T RUSH while diving or handling tubes *
- Gamete density is *not* as important as we once thought, it's more important to have a variety of parents to achieve high fertilization (but, too high can result in polyspermy and failed development)
- Use a large flat container to maximize gas exchange between the water and the air - there is a LOT of metabolism going on and things can quickly get funky in a bad way
- Aim for a single layer of eggs at the water surface, with some water showing - although you can get away with them being 3-eggs-deep
- Water should be visibly cloudy with sperm - anywhere from a little bit cloudy to very milky will probably work OK
- If you want to quantify fertilization rates or gamete viability between different parents, you can transfer 0.5 ml of eggs + 2 ml of sperm directly from the Falcon tube into a petri dish with 30 ml of seawater; repeat x 3 for each parent-parent combination. Avoid cross-contamination of the pipettes!
- If you have very little material, you can use a small bowl or smaller polystyrene deli container (5" size) in place of a larger container

# Separating egg and sperm*

- Separating sperm and eggs into different stock solutions is time consuming and only necessary if you are conducting fertilization experiments to examine compatibility between unique genotypes
  - Once gamete bundles have broken apart, pour this mixture through a mesh filter (e.g., Nitex nylon mesh glued to bottom of a PVC tube) into a labeled urine cup; this will be your sperm stock. Urine cups are sometimes sold as “specimen cups” or “sterile vials.
    - Mesh size matters! Use 80 µm for small eggs like *Orbicella* and brain corals; 120 µm for *Acropora*
  - The eggs should be retained on the mesh and the filter should immediately be placed into a beaker of filtered seawater. Don’t let the water overflow and wash the eggs out!
    - Rinse eggs at least 4 times by gently swirling, then picking them up and moving to a new beaker
  - After rinses, the eggs should be poured into a cup with filtered seawater; this is your egg stock
    - Here you can use a squirt bottle with the tip cut off so the water stream is slow/gentle
  - Between corals, rinse the filter in fresh water to kill any sperm, then submerge in clean seawater to rinse off fresh water droplets
  - Watch out for cross-contamination: All rinse beakers must be thoroughly rinsed and refilled with seawater before moving to the next parent

Photo credit: N. Fogarty

# Fertilizing gametes en masse: nested tripours

- Small, inner containers have a Nitex nylon mesh bottom to catch eggs but allow water through
- Solid outer containers are used for quick, serial rinsing of the eggs after fertilization is complete
- These batches are **too** dense, but they did fertilize

Photo credit: A. Wood

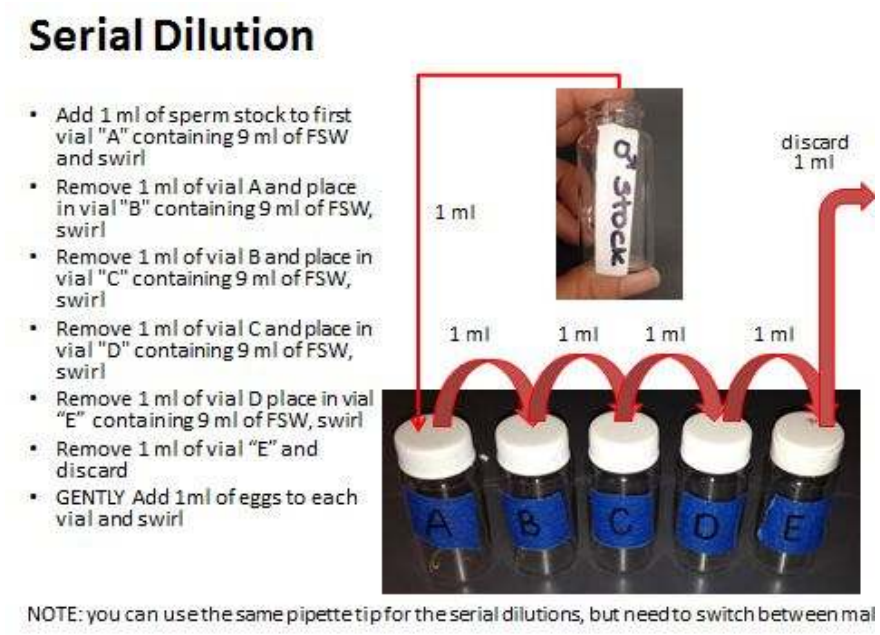
Photo credit: A. Vollmer

# Fertilizing experiments: Insider tricks

- FERTILIZATION EXPERIMENTS
  - Materials: 1 ml pipettor that has never been (and will never be!) used with chemicals
  - Glass is preferable to conduct experiments in because sperm sticks to plastic; however, plastic urine cups can be used
  - Proper sperm concentration is key, or use serial dilutions with concentrated sperm



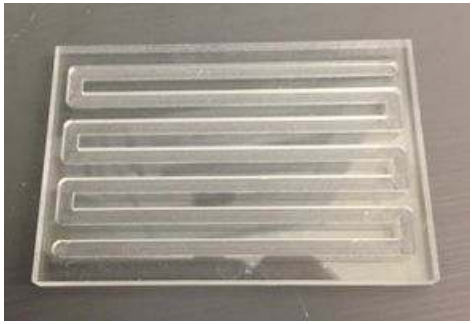
Photo credit: N. Fogarty



# Fertilizing experiments: Insider tricks

- SCORING FERTILIZATION
  - Use “maze” for ease of scoring fertilization. You can make or hire someone to make these out of plexiglass
  - You can also use slides- flat or deep well
  - Quantifying sperm concentration is key because fertilization is dictated by sperm concentration. If sperm is too low or too high it can lower fertilization and you will mistake the low fert for incompatibility between genotypes

- QUANTIFYING FERTILIZATION
  - Add 1 ml of sperm stock to vial, add 3 drops of concentrated formaldehyde, mix
  - Use hemacytometer, 8 replicate counts
  - Use manufacturer's instructions for calculating sperm/ml= most species ideal is $10^6$ sperm/ml



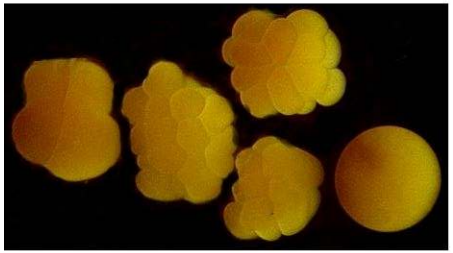
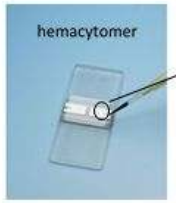
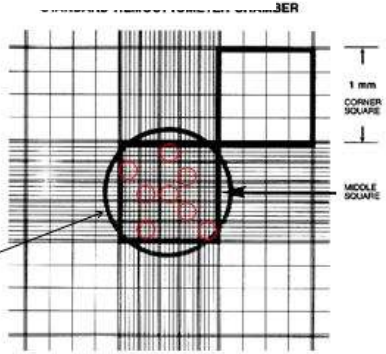


Photo credits: N. Fogarty



# Rinsing + handling embryos

BE CAREFUL!

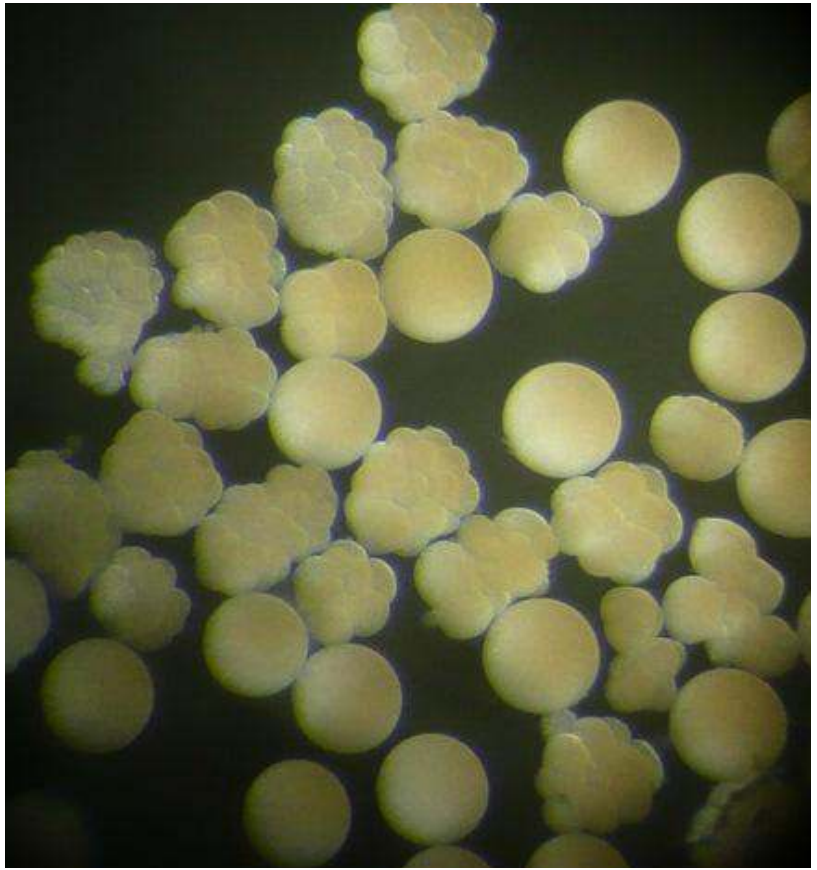
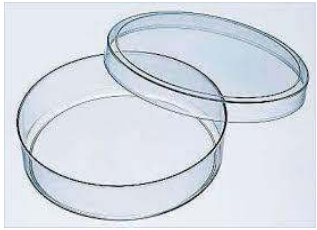


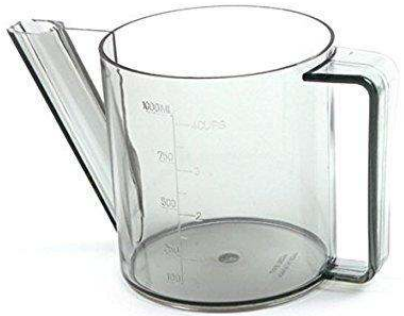
Photo credit: K. Marhaver

# Handling and rinsing embryos: Key materials

- SCOOPING EMBRYOS
  - Best: Sterile Petri dishes and lids
    - cheap, disposable, hydrophobic
  - Alternate: plastic spoons, small plastic tripour beakers
  - Less great: turkey basters, syringes, pipettes
    - More dishes to do, higher contamination risk
    - Causes shear forces on delicate embryos



- RINSING EMBRYOS
  - 30+ plastic tri-pour beakers (1000 ml volume, polypropylene)
    - Cheap and super useful
    - Label a few each for FSW, Larvae, and Scuzz
  - Large volume fat separators
    - We like Norpro 4 cup size; large and easy to clean
    - Embryos float on water surface
    - Rinses are easily done through the spout



# Handling embryos: Our FAVORITE THING

THE HOLY GRAIL OF SPAWNING MATERIALS:
Plastic larval “swimming pools”

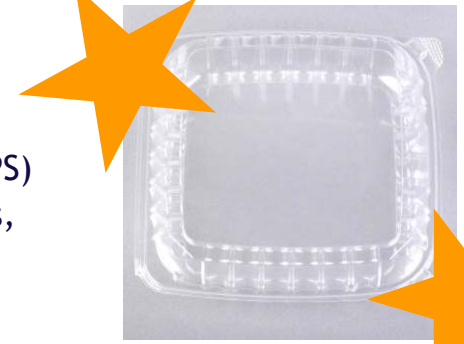
- **Clear, polystrene** clamshell food containers, 8-9” size, single internal compartment, snapping lids
- Buy ~200 for a spawning season
- Available at food and restaurant supply stores
- 9” size holds 800-1000 ml of water, this is the best size for rearing larvae
- 5” size holds 200-500 ml of water; best size for very small volumes of spawn or for replicated experiments
- Do be careful when carrying them full of water; the plastic can “taco” and dump out the water!

These deli bins work WAY better without the salads in them

Why are we so obsessed with *these* food containers?
They **really** work…

# Why polystyrene?

- In 2007, we discovered that larvae do GREAT in **polystyrene** (PS)
- PS is available as deli containers, petri dishes, multiwell plates, even parfait cups with lids
- PS binds lipids, which helps with larval ‘de-scuzzing’
- PS is cheap, clear, and disposable (sorry, we’re not sorry)
- PS items often come with lids, many are sold in sterile packages
- **Best of all,** PS is very hydrophobic; it repels water and forces the meniscus at the water surface to be very flat:




A FLAT MENISCUS means that no embryos or larvae will get stuck on the edge of the container

A CURVED MENISCUS is where coral larvae go to get stuck, dehydrate, clump together, die, get eaten by bacteria, and thereby kill all their friends

WARNING: Clear PET (#1) plastic is **not the same**, but it can look almost identical: AVOID THIS AT ALL COSTS!


If you just skipped this slide, go back and read it again

# Rinsing embryos: Timing

- The goal is high fertilization without double fertilization
  - Polyspermy messes up development, kills embryos
- While waiting: Setup deli bins with filtered seawater
- Team NOVA waits 30-60 minutes before rinsing *A. palmata*, *M.franksi* (60 mins max)
- Team CARMABI waits until the first hint of cell division before rinsing (45-90 mins after fertilization)
  - Focuses effort on bins with high % fertilization
  - Avoids wasting effort on failed batches
- Embryos become fragile quickly!
  - Aim to complete rinses before 8-cell stage
  - Later embryos likely to break apart
- Ideally, you don't handle the embryos from the 8-cell stage through late gastrulation. Instead:
  - Score fertilization % and take rad microscope photos
  - Peek at embryos for disasters, pipette out bits of scuzz
  - Beer, shower, have a nap, tell stories

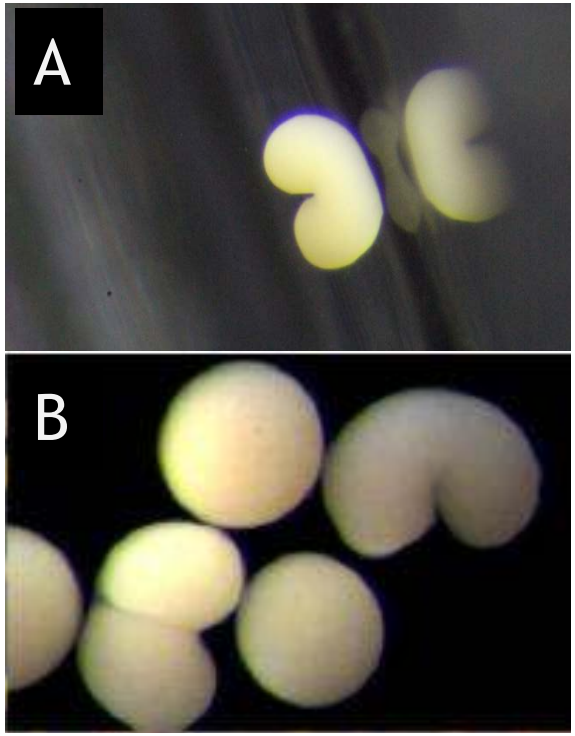



Photo credit: A) V. Chamberland; B) J. Gijsbers

Onset of 1st cell division in *S. siderea* (A) and *O. faveolata* (B)

# Rinsing embryos without stress

- ALWAYS BE EXTREMELY GENTLE
  - Avoid shear forces from turkey basters, pipettes, syringes
  - Avoid smushing embryos against container walls
  - Avoid desiccation, dust, greasy hands, perfume, bug spray
- RINSE SLOWLY AND CAREFULLY
  - Pour FSW down the **spout** of the fat separator
  - Allow embryos to float back to surface
  - Pour 80-90% of the water out through the spout
  - Repeat rinses until **very very clear** (5-6 times)
  - Use tape on each container to tally the number of rinses… you *will* lose count... It's 1 am!
- SCOOP CAREFULLY
  - Use sterile Petri lids to scoop <1000 embryos to each deli container, pre-filled with ~800 ml filtered seawater
- EARN KARMA POINTS
  - You will have way too many embryos to keep. Return your extras to the ocean for karma. **Don't get greedy.**
- SEE PREVIOUS BULLET POINT

Photo credit: K. Marhaver

# Each coral species is different…

Photo credit: A. Wood

*Acropora palmata* embryos at the ‘cornflake’ stage; such a strange shape that people have mistaken them for dead larvae and dumped out the batch

# Caring for larvae

If you did the other steps well,
this part is easyish, sort of

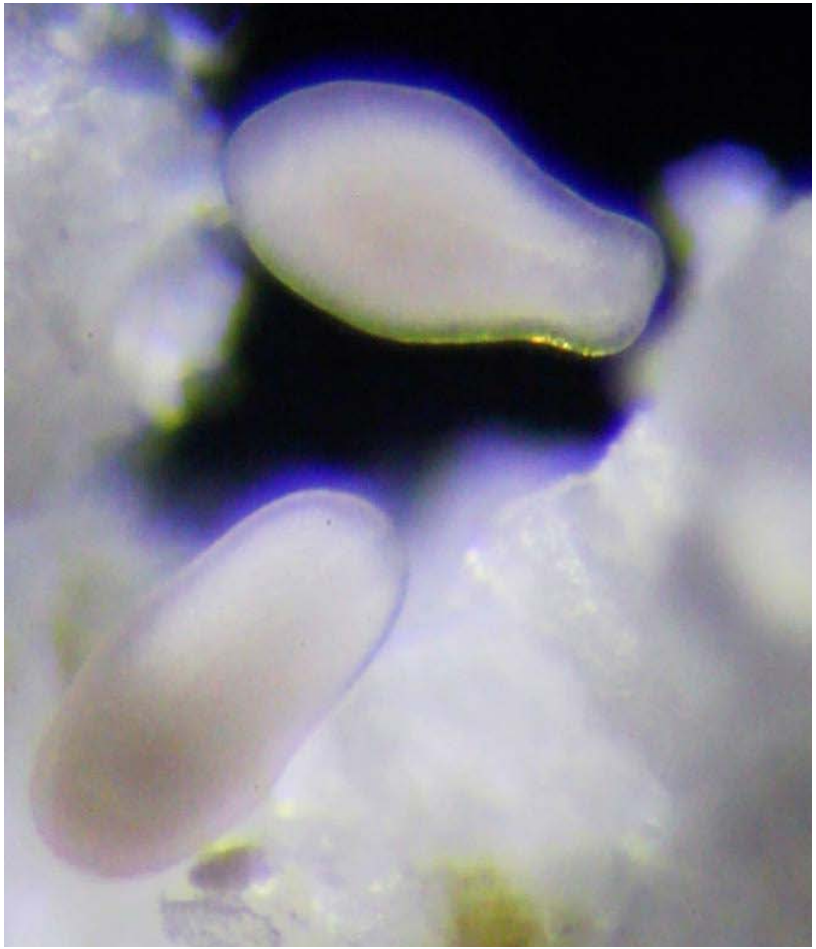


Photo credit: K. Marhaver


Photo credit: P. Selvaggio

Teamwork at the CARMABI lab to de-scuzz all those larvae

# Caring for larvae: Key materials

- BLOTTING MATERIALS
  - Dead eggs and embryos become a lipid-rich surface SCUZZ in larval cultures
  - Blot this off at least once per day, and every time you do a water change
  - We like laboratory tissues and plastic wrap
    - Small and XL Kimwipes
    - SARAN wrap
  - Different materials work for different corals and different degrees of scuzz


- SLURPING MATERIALS
  - To move and count larvae:
    - Fine-tip plastic transfer pipettes
    - Glass Pasteur pipettes
    - Turkey basters (Norpro glass)
  - To move and remove water:
    - Plastic airline tubing/silicone tubing plus a clothespin = mini siphons
    - Nitex mesh filters for separating larvae from water

# Caring for larvae: The ‘de-scuzzing’ process *

- Unfertilized eggs explode into a dangerous SCUZZ
- BUT, embryos are much less fragile once they’re motile
- The Goal: Fully separate healthy survivors from ***all*** lipids, bacteria, unfertilized eggs, and dead embryos
- Ideally, this is done after all unfertilized eggs have exploded; this requires setting up VERY low-density cultures in super clean water on spawning night
    - If your cultures are gnarly, you will have to do a full de-scuzzing before all unfertilized eggs have exploded (and possibly even before motility)
    - If your cultures are doing well, happy healthy larvae will be swimming among a thin film of scuzz ~18 h after motility and all unfertilized eggs will be exploded; one major de-scuzzing will give you beautiful, clean cultures and time to sleep
    - If your your cultures are so clean that your unfertilized eggs haven’t exploded by ~18 hours after motility, you should fully de-scuzz anyway



Photo credits: P. Selvaggio

# Caring for larvae: The ‘de-scuzzing’ process


- FULL DE-SCUZZING:
  - PART 1: BLOT all lipids and dead stuff from surface with plastic wrap or Kimwipes. You can remove very minor scuzz with a clean glass or plastic pipette
  - PART 2: Do a full WATER CHANGE by moving larvae to a fat separator, by removing water from bins using airline tubing as a tiny siphon, or by moving all healthy larvae to a new container using a pipette; this is very slow but also very clean and effective
  - PART 3: While changing water, change over to a NEW PLASTIC CONTAINER; this is key to reducing bacteria
- Repeat this process on Day 2 after embryos reach motility
- At Day 3, full de-scuzzing may only be needed every 48-72 hours, with blotting as needed in between
- Once larvae are swimming, they can be rinsed over Nitex mesh filters (80-150 µm; buy and test different mesh sizes!)

NOTE: This is a lot like fishing or gardening - it’s **much** easier to learn in person, from someone who knows


Photo credit: P. Selvaggio


Photo credit: P. Selvaggio

*C. natans* larvae at a good density with no scuzz

# Alternative rearing systems: kreisels

Photo credit: P. Selvaggio

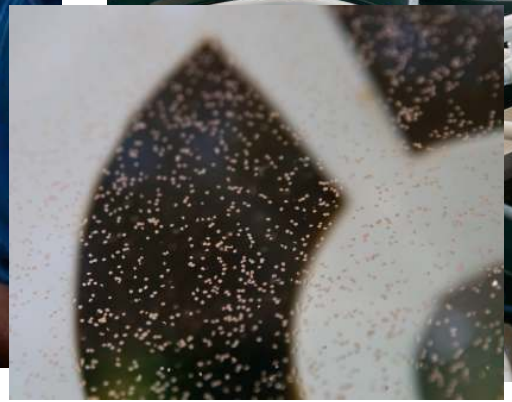




Photo credits: A. Wood

- SECORE has used kreisels to maintain 1000's of larvae with lower effort
- Caveats: kreisels require a lot of work to set up, water flow is very challenging to get right
- Caveats: fewer replicates, plus less control of temperature, dust, crowding, and water quality
- Not recommended for first attempts - very hard to troubleshoot when things go wrong

# Alternative rearing systems: in situ pools…


Photo credit: P. Selvaggio


Photo credit: N. Fogarty

- SECORE (left) and UNCW/NOAA (right) have tried rearing and settling larvae in *in situ* pools
- Larvae (or embryos!) can be added directly, with settlement substrates already in place
- SECORE pools worked for *O. fav* and *C. nat* embryos *and* larvae (Curacao; left), but the pool used in Florida (right) did not work well, possibly due to UV exposure and rain
- This method shows good promise for scaling up, but a HUGE pain to build and set up
- Virtually no monitoring possible during settlement - also not a good option for first attempts

# Fostering settlement

## Sit down, picky beasts

Photo credit: K. Marhaver

Settling *O. faveolata* spat and one very picky larva

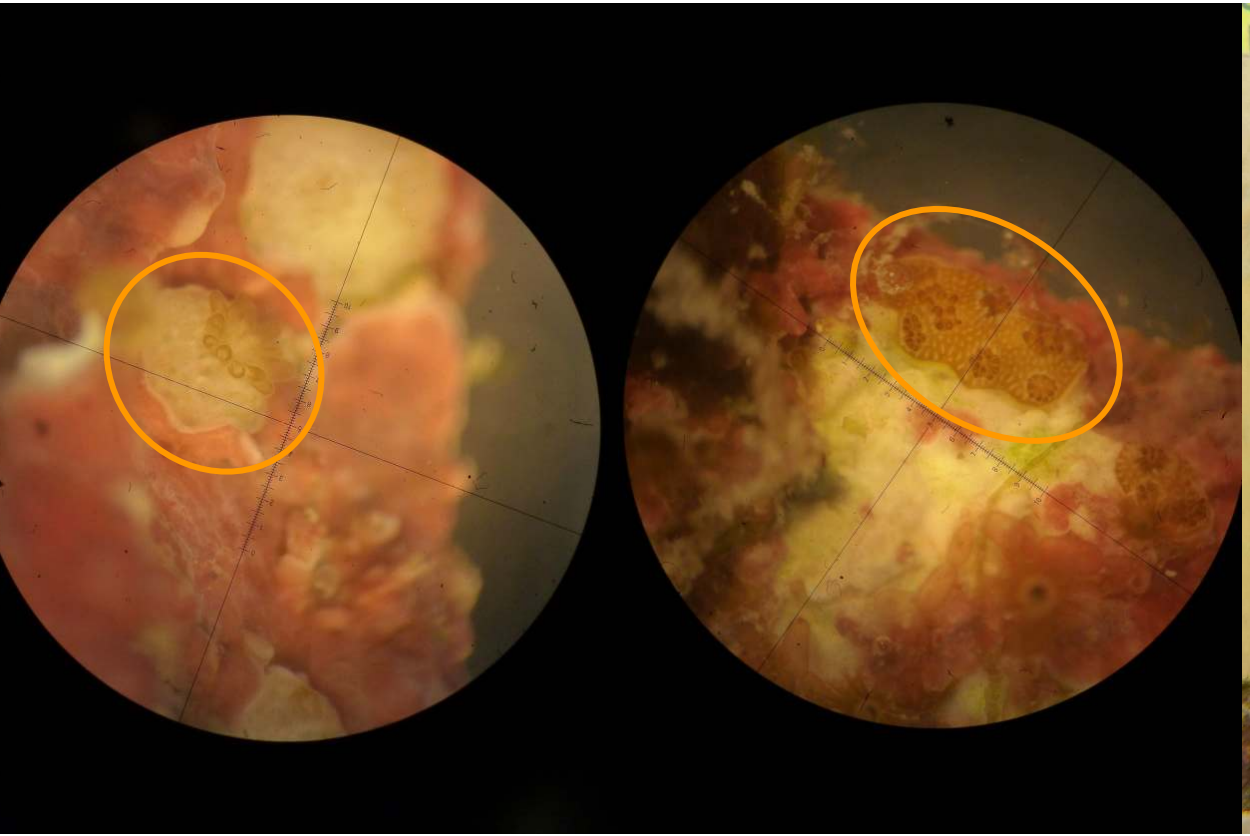



Photo credits: V. Chamberland

*Acropora palmata* settlers at 2 weeks, 6 weeks, and 1 year

# Fostering settlement: Key materials


- CERAMIC TRIPODS i.e. KILN STILTS (and CORAL FRAG DISKS)
  - Ceramic tripods are used when firing clay/pottery in kilns; available from pottery/art suppliers
  - Tripods have very few points on contact with other surfaces - minimizes “squish points”
  - Both are good options for many species, easy to handle and score

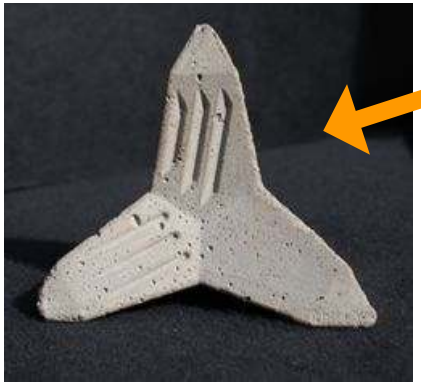
- TERRA COTTA CHIPS
  - Buy unsealed terra cotta pots or floor tiles and SMASH EM UP
  - Works great with *Orbicella* and possibly *M. cav* (but not *Acropora* or brain corals)

Googling “Terra Cotta Chips” also yields this unhelpful photo!

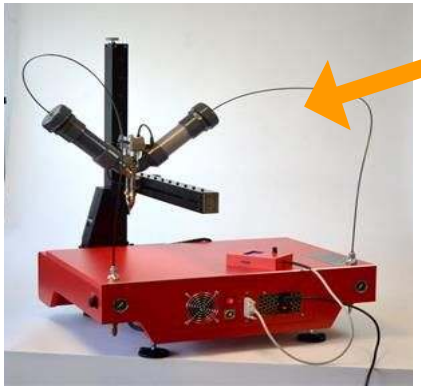

- LIMESTONE CORAL SKELETON (and ARAGONITE CORAL FRAG DISCS)
  - Corals love settling on limestone, especially freshly-cut coral skeleton
  - Chisel the outsides off a large *O. fav* skeleton and cut/chop/snip into pieces
  - Skeleton frags can be pressed into plumbing epoxy or supergued to plastic backings to reduce turf accumulation in the vicinity
  - Settlers will be HARD to see and impossible to count on coral skeleton, but survivorship may be high enough to justify the hassle

NOTE: All materials should be cured in seawater prior to use

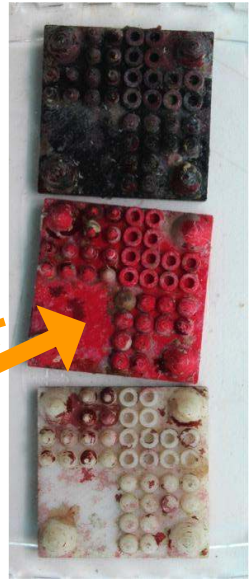
# Fostering settlement: Materials in development




- CONCRETE TETRAPODS aka SECORE Seeding Units
  - Cast using custom molds
  - No top or bottom and few points of contact
  - Grooves help attract/protect settlers
  - Tetrapod legs can be secured into reef w/o binding materials
  - **BUT** concrete is rapidly overgrown by algal turfs
  - **AND** could be improved by incorporating additional microhabitats and semi-cryptic surfaces



- 3D PRINTED CERAMIC SUBSTRATES (new designs in the making, by SECORE/CARMABI)
  - Glazed clay to reduce the growth of algal turfs and to promote CCA and settler growth
  - To be tested in the field this year
- 3D PRINTED PLASTIC SUBSTRATES (designs in revision, by K. Marhaver)*
  - ABS plastic, red/black/white, donuts/hills/spikes
  - Coral species vary in color and surface preference but almost all seek protected grooves/crevices
  - Plastic stays well-grazed, but not the best eco-material



# Fostering settlement: Conditioning the substrates

Photo credits: N. Hurtado

- **IN SITU**, look for an area with:
  - High CCA cover
  - Low turf and macroalgae cover
  - High grazing pressure
  - Good water movement
  - Shade
- Regularly move the substrates in crates to allow CCA to colonize all surfaces of the substrates and to prevent the formation of anoxic conditions
- Don't stack too many substrates on top of each other to allow water flow between them and to prevent the formation of anoxic conditions
- Don't allow thick CCA crusts to completely overgrow the substrates; those will eventually kill your settlers! *
- 3 months should suffice

Artificial settlement substrates conditioned *in situ*

# Fostering settlement: Conditioning the substrates

- **IN AQUARIA,** ensure you have:
  - Grazers (e.g., parrotfish, urchins, hermit crabs)
  - Good water circulation
  - Shade
- To promote CCA growth, collect rubble covered with CCA from the reef and intersperse it between the settlement substrates
- To avoid the formation of anoxic conditions:
  - Stack a maximum of 2-3 layers of substrates
  - Regularly siphon out sediments
  - Regularly move substrates around
  - Place substrates on egg crate grids elevated from the bottom
- Shade your tanks to suppress turf and macroalgae growth

Photo credits: N. Hurtado, N. Fogarty, D. Kuhn, Jonas, A. Estapé, K. Marks

# Fostering settlement: Experimental set-ups

- Settlement experiments in laboratory
  - Petri dishes, tripour beakers, non-toxic plastics, or floating chambers can be used for settlement assays
  - Soak all plastics for a minimum of 24 hrs prior to use to leach out chemicals
- *In situ* experiments can also be conducted using acrylic chambers with mesh ends

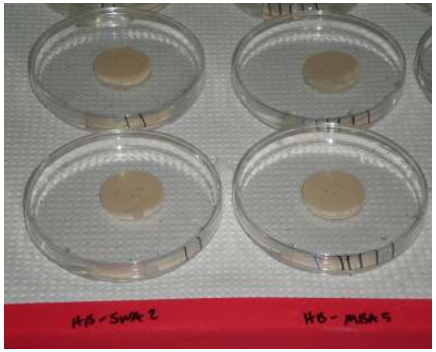


Photo credit: J. Sneed

Photo credit: H. Noren

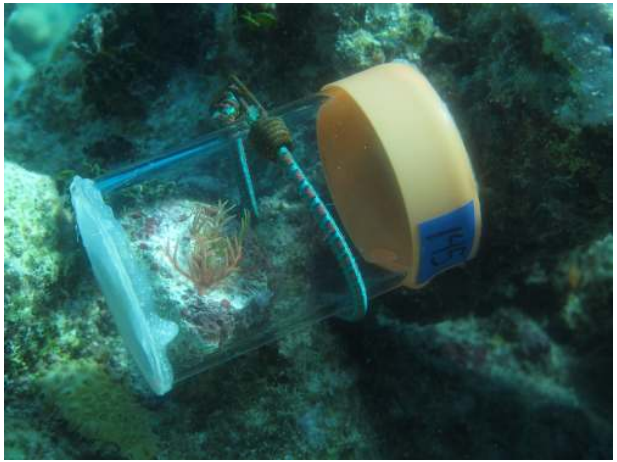


Photo credit: N. Fogarty

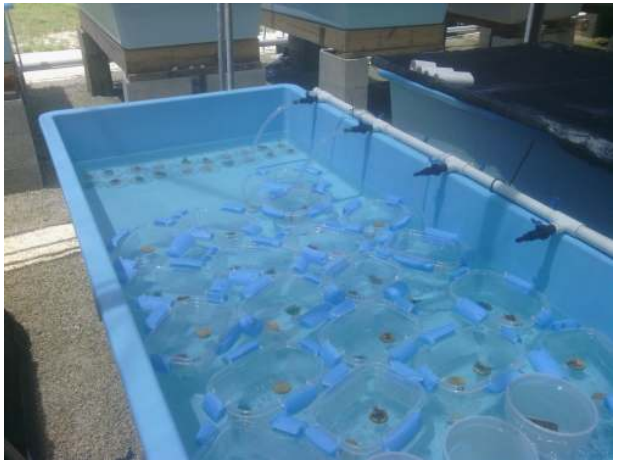


Photo credit: Justin Voss

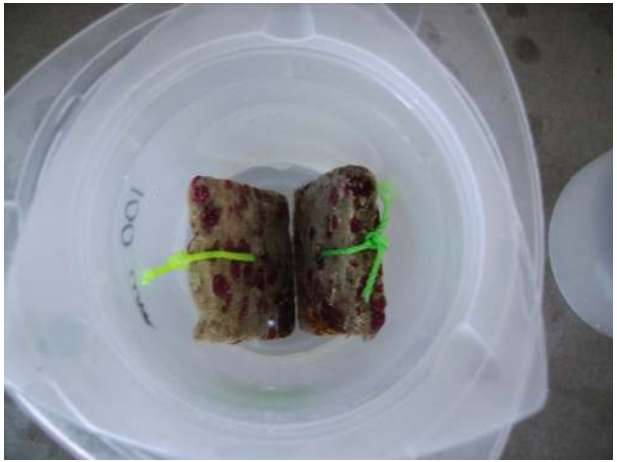
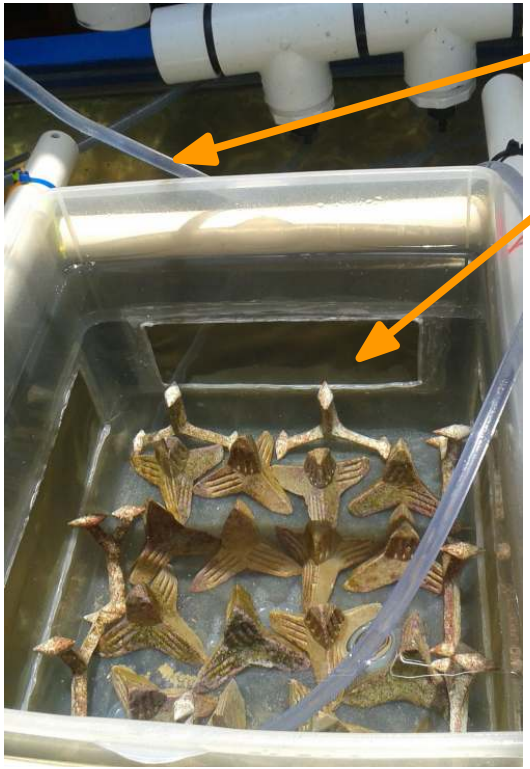


Photo credit: N. Fogarty

# Fostering settlement: Flow-through set-up

#1



- Water flow is generated through silicone tubing by a submersible pump
- Nylon mesh allows water exchange and prevents larvae from escaping the settlement container (150 µm mesh is glued to the container with a hot glue gun)
- Before first use, soak the plastic containers in freshwater for at least 24 h to allow any chemicals to leach from plastic and glue

Photo credits: N. Hurtado

Flow-through settlement set-up partially submerged in flow-through aquarium system

# Fostering settlement: Closed system

#2

Photo credits: L. Röpke

Closed set-up for larval settlement

- Water movement is generated with an air pump, airline tubing, and air stones

- To prevent the buildup of microbial communities that will kill your settling larvae:
  - Use FSW and do daily ~50% water changes
  - Prepare **many** settlement containers with **few** substrates
  - Avoid using substrates that are excessively overgrown by thick benthic communities
  - Cover your containers to prevent dust and insects from landing in them

# Fostering settlement: Insider tricks


Photo credit: V. Chamberland

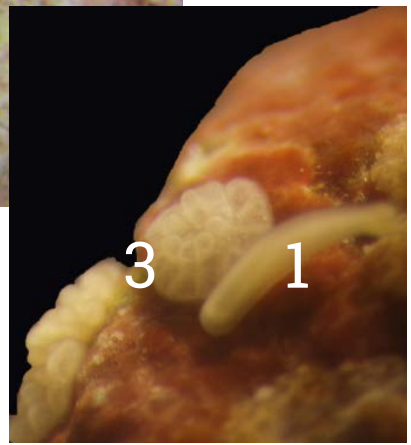



Photo credit: N. Fogarty

*D. strigosa* (left) and *A. palmata* (right) exploring (1), attaching (2), and metamorphosed (3)

- Avoid moving the substrates while larvae have not yet metamorphosed; they might detach from the substrate
- Regularly inspect 4-5 substrates under a dissecting microscope to track settlement and metamorphosis. This will help you decide when the substrates can be safely removed from the settlement containers
- The main challenge of this step is to avoid that a gazillion larvae settle on the same few substrates. To optimize settlement:
  - Do not place large numbers of larvae in a few settlement containers all at once
  - Regularly exchange substrates harboring ***enough**** settlers for new ones
  - When only a few larvae are left that have not yet settled, add a new batch of larvae

**** The optimal number of settlers per substrate will depend on the size of the substrate you are using, on the species you are rearing, and on the outplant site you are targeting (see next slide)***

# What's the optimal number of settlers per substrate?

***There is no magic number!*

**Quality of outplanting site**

Healthy

Disturbed

Algae- dominated

**Substrate size**

Small

Medium

Large

**Species' early life survival**

High

Medium

Low

NOTE: In addition, there will be settler density-dependence effects, which we have not yet tested

\- Optimal number of settlers per substrate +

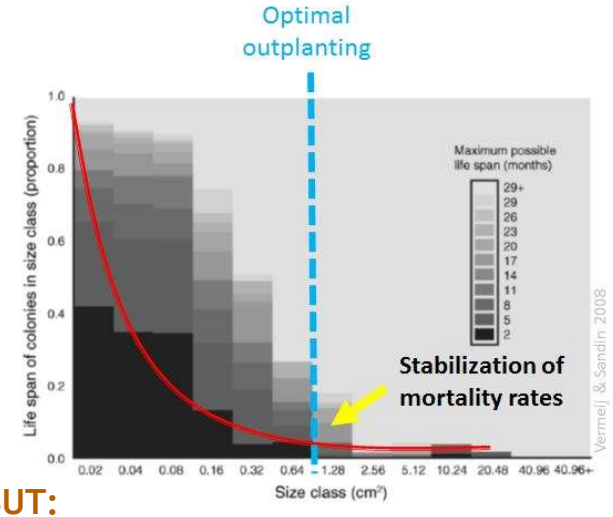
# Outplanting + monitoring settlers

When, where, and how? (We don’t have all the answers yet...)

Photo credits: P. Selvaggio

# Intermediate nursery phase: Optimal duration

Natural mortality is a negative function of size...




Keeping coral recruits under controlled nursery conditions until they reach larger sizes can increase post-settlement survival

LAND-BASED NURSERY
Photo credit: N. Hurtado

OCEAN-BASED NURSERY
Photo credit: P. Selvaggio

**BUT:**

1. Species with different growth strategies and growth rates may have different optimal nursery durations
2. Recruits outplanted to degraded outplanting sites may require longer nursery durations than for healthy sites

**Currently tested on Curaçao with 5 species, updates very soon!**

# Outplanting settlers: Key materials

**NOTE: This will very much depend on the type of substrates you are using, and in which type of environment you will be outplanting your settlers**

- ZIPLOC BAGS to transport substrates to the outplanting site (carry one substrate per bag in a large volume of water)
- LARGE MESH BAG to transport ziploc bags (do not carry too many at a time/use several mesh bags to avoid that settlers are crushed by the weight of other bags/substrates)
- BRUSHES to scrub off unwanted algae and remove sediments from the substratum before proceeding to the outplanting
- MARINE EPOXY PUTTY works well in wave exposed habitats where nooks and crevices are not abundant, e.g., reef flats and breakwalls (red putty works better than green putty; Magic Sculpt for bigger jobs)
- CABLE-TIES work well in habitats where nooks and crevices are present
- If you are outplanting at a reef site characterized by medium to high structural complexity and exposed to low wave energy, you can use the SECORE seeding units; simply wedge them into crevices without using binding materials


or


or

# Outplanting settlers: Insider tricks

- Outplant in habitats characterized by (a)biotic conditions similar to those in which your target species naturally occurs. Take into account the following parameters:
  - Depth
  - Light intensity
  - Wave exposure
  - Benthic community
- Avoid outplanting super sensitive species (e.g., *Orbicella*) in very degraded* habitats; opt for sturdier species that were shown to survive well in such conditions (e.g., *C. natans*, *D. labyrinthiformis*)
- Most species have clear settlement preferences between cryptic (undersides) and exposed (topsides) surfaces; keep the same substrate orientation throughout all phases, from settlement to outplanting
- When outplanting, handle the substrates very gently to avoid crushing settlers. When using tripods, you can mark the “pod” with the least settlers by attaching a cable tie at its end. During outplanting, handle the substrate by that pod and avoid touching other pods
- Select and mark outplanting locations before proceeding to the outplanting phase

Photo credit: M. Vermeij

Two 1.5-year-old *C. natans* on the underside of a SECORE substrate outplanted at a degraded reef

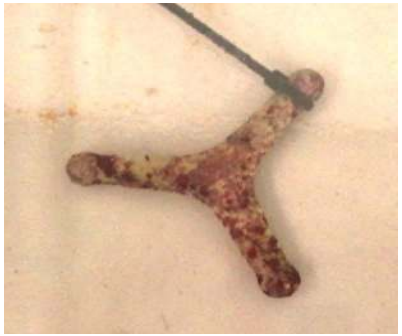
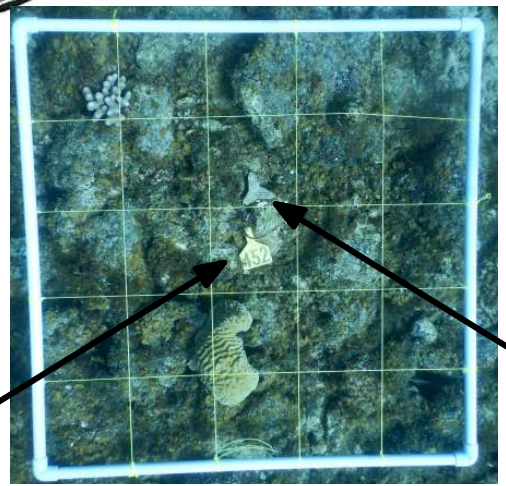


Photo credit: N. Hurtado

Tripod with settlers, marked with a cable-tie to facilitate handling

# Monitoring settlers

**1.** Do a basic assessment of the reef quality at your outplanting site (CPCe analysis of 1 x 1 m photo-quadrats of each outplant location will do!; n ≥ 15)



**2.** Properly mark and map each outplanting location


**3.** Just before outplanting, assess the number of settlers present on a subset of substrates (n ≥ 15 substrates per outplanting site).

**4.** Outplant one substrate within each of the monitored quadrants (if you outplant more than 1 substrate per quadrant, ensure the substrates you are monitoring are labeled accordingly)

**5.** Regularly monitor the survival of outplanted settlers (common first time points are 1 mo, 3 mo, 6 mo, 1 yr, 2 yr…)

***If you outplant, be sure you monitor! Our field needs those data…***

# Final caveat: Practice matters, location matters

- It's VERY difficult to get everything right the first time
- Your best bet is to learn methods in person from an established spawning research group
- Let us know if we can help troubleshoot: CALL US, EMAIL US, BEFORE, DURING AND AFTER! (We're doing spawning too so we'll be awake…)
- Despite your best efforts, there are many ways things can go wrong; **don't be discouraged** if things go wrong the first time… or second time…
- Consider where you set up shop. You will have MUCH greater success if you start with relatively healthy coral populations in **clean water**. Some examples:
    - M. Vermeij scooped up a few gamete bundles during coral spawning at the Flower Garden Banks. He left them in a coffee cup… and they developed fine!
    - In Curacao, we've seen the highest larval survivorship when gametes were collected from a remote, healthy reef at Westpoint. In fact, so few larvae died that it nearly ruined the stats in the survivorship experiments…
    - We've taught groups how to succeed in Curacao only to hear that their settlers died back in Florida or their larvae died the next year in Panama - we can't fully detangle the group's approach from the location, but we know that BOTH MATTER.

# State of the Science

## And where to go next

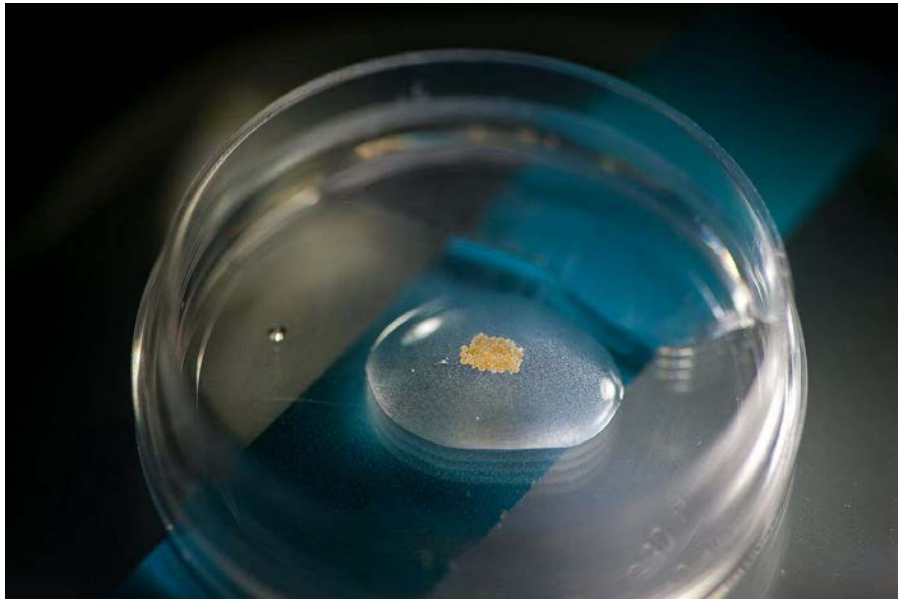


Photo credit: P. Selvaggio

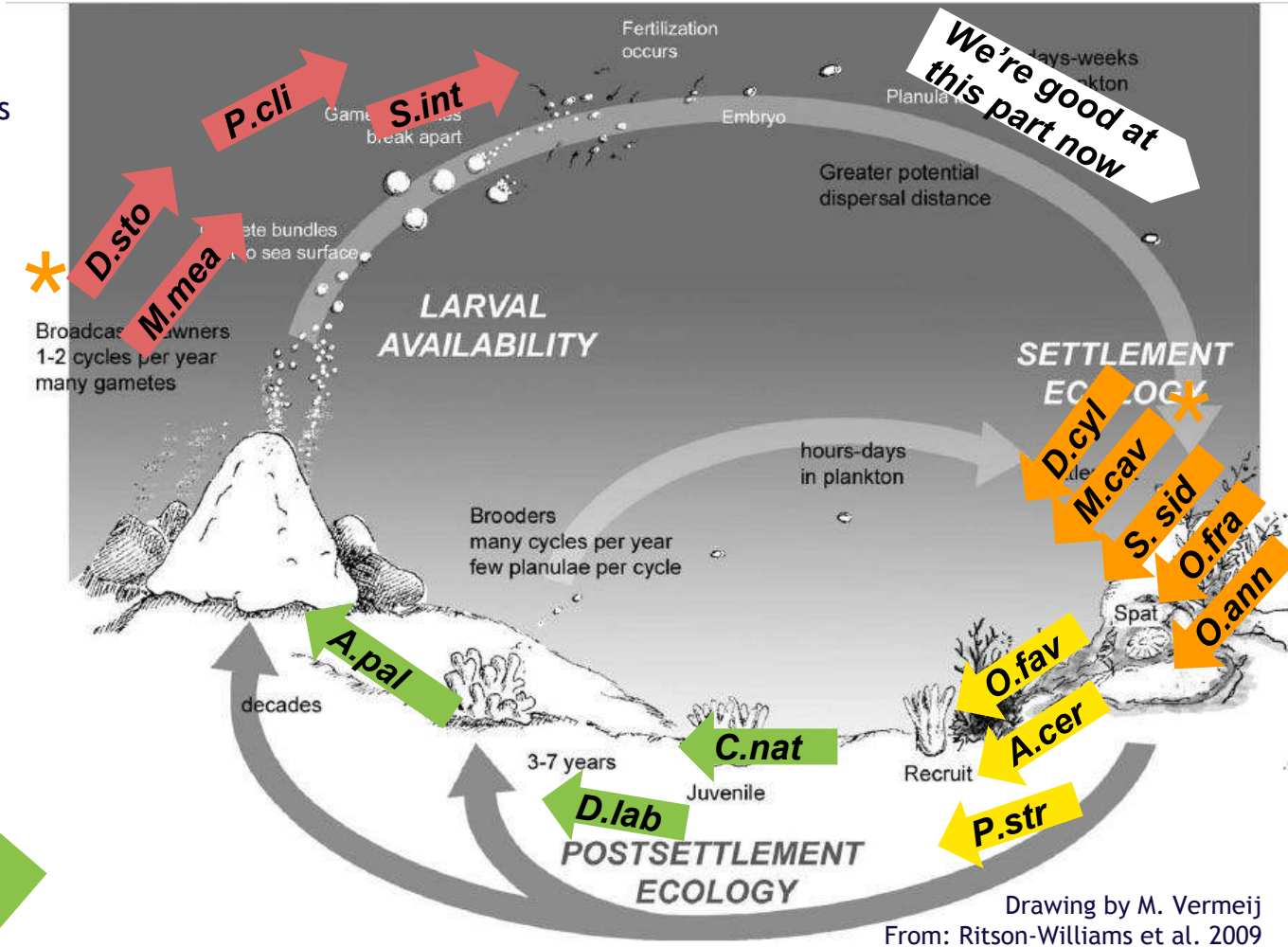
# Where are we?

- We can now get many species to the settlement or post-settlement stage BUT…
- In many cases we have just **barely** reached these stages
- For each species, there are still barriers left to crack
- At each bottleneck, there is more optimization to do

*Species in the early stages of research* *

*Species at the settlement bottleneck* *

*Species we can raise to dividing recruits*




Drawing by M. Vermeij
From: Ritson-Williams et al. 2009
Smith Contrib Mar Sci 38:437-457.

# State of the science: Acroporids

BASIC RESEARCH

- Spawning times and spawning variability (Williams, Fogarty, Levitan, Szmant, Soong, Steiner, de Graaf, Vargas-Angel, Van Veghel, Vermeij, Petersen, … )
- Fertilization / sperm choice / hybridization (Levitan, Fogarty, Baums, … )
- Developmental series / embryogenesis (Randall and Szmant, … )
- Symbiont uptake (Baums, Medina, Baker, Coffroth, and many more)
- Larval biology / survival / behavior - salinity, temp, microbes (Szmant, Vermeij, Baums, Medina, Ritson-Williams, Miller, Fogarty, Chamberland, Sneed, … )
- Settlement preferences / CCA / artificial substrates (Ritson-Williams, Paul, Vermeij, Chamberland, Sneed, Paul, … )

RESTORATION

- *A. palmata* LIFE CYCLE COMPLETED! Four-year-old SECORE outplants are now spawning!
- Outplanting methods and timing fairly well worked out (SECORE, Chamberland, Vermeij, Miller, Szmant, …)
- Microbial cues for settlement under development (Sharp, Paul, Marhaver, … )
- Broodstock: In 2016, SECORE found it worked well to use CRF's mid-water nurseries for *A. cervicornis* spawning broodstock in Curacao!

Photo credit: P. Selvaggio

Photo credit: P. Bosch, CRF

# Research needed for Acroporids

BASIC RESEARCH

- Detailed embryogenesis publication with histology for Carib species
- Molecular determinants of timing of spawning (and variability of spawning)

RESTORATION/APPLIED RESEARCH

- How to improve symbiont uptake for better settler survival
- Optimizing surfaces for settlement
- Post-settlement survival, competition, ecology
- Ideal timing of settler outplanting, especially on more degraded reefs
- Role/use of hybrid (*A. prolifera*) in restoration
- Prevalence of chimeras in natural populations vs. outplanted settlers, implication of using chimerism in restoration (good or bad? short term vs long term benefits?)

Photo credit: P. Selvaggio

Photo credit: P. Bosch, CRF

# State of the science: *Orbicella* complex

BASIC RESEARCH

- Spawning times / coordination between colonies (Szmant, Van Veghel, de Graaf, Steiner, Gittings, Soong, Wyers, Miller, Knowlton, Levitan, Fogarty, Sanchez, Vize, Croquer, Bastidas … )
- Species boundaries (Knowlton, Levitan, Fogarty, Szmant, Medina, … )
- Fertilization / sperm choice (Levitan, Fogarty, … )
- Developmental series / embryogenesis
- Symbiont uptake (Baums, Medina, Coffroth, Vollmer, many more!)
- Larval biology / survival / behavior - salinity, temp, microbes (Szmant, Vermeij, Ritson-Williams, Miller, Fogarty, Hartmann, Marhaver, Chamberland, … )

RESTORATION

- Settlement preferences / CCA / artificial substrates (Ritson-Williams, Paul, Sneed, Marhaver, … )
- Microbial cues for settlement (Marhaver, … )
- Settlement and outplanting (Vermeij, Miller, Szmant, Chamberland, SECORE, … )

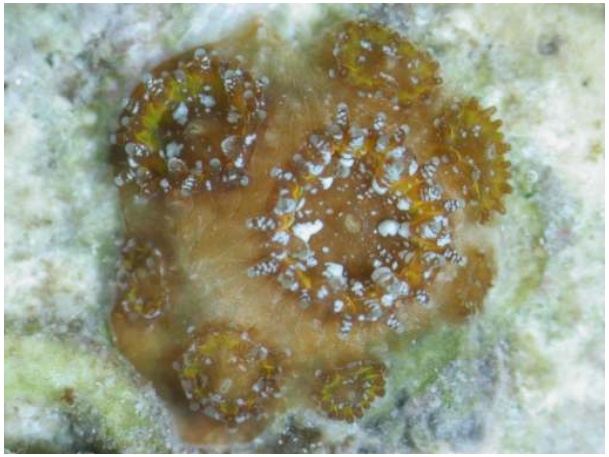


Photo credit: N. Fogarty

Budding, lab-reared,
*O. faveolata* ~8 months old!

# Research needed for *Orbicella* spp.

BASIC RESEARCH

- Are there even more species? Do they spawn together or separately?
- *Where* are the naturally-occurring recruits? What do they need in order to recruit in nature?

RESTORATION

- How to overcome fragileness, pickiness, unpredictability at settlement stage
- How to achieve good survival (or ANY survival) at outplanting stage
- How to successfully scale-up settlement and outplanting methods?

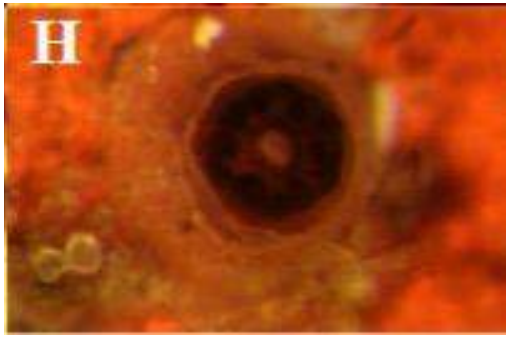



Photo credit: J. Gijsbers

Settled *O. faveolata* polyp on a terra cotta substrate

Photo credit: K. Marhaver

A rare *Orbicella* juvenile living in front of CARMABI… possibly from all the larvae we tossed off the pier?

# State of the science: Brain corals and gonochores

BRAIN CORALS (*C. natans*, *P. strigosa*, *P. clivosa*, *D. labyrinthiformis*)

- KNOWN: Spawning times, (Szmant, Vize, Weil, Hagman, Steiner, Boland, Duerden, Wyers, de Graaf, Gittings, Alvarado, Bastidas, Croquer, Vermeij, Muller, Chamberland, Snowden, Marhaver, … )
- Embryogenesis, settlement & outplanting, outplanting with high survivorship even at degraded sites (Vermeij, Chamberland, … )
- TO DO: Figure out regional differences in spawning times (some places have two spawning peaks/year), scaling up methods for large-scale outplanting

*Montastraea cavernosa*

- KNOWN: Spawning times, development/larval biology, some settlement biology (Vize, Soong, Szmant, Wyers, Gittings, Steiner, Acosta, Hagman, de Graaf, Beaver, Vermeij, Chamberland, Figueiredo, … )
- TO DO: Improve methods to collect gametes, methods for settlement enhancement (they're picky and give up quick!), improve post-settlement survival (they pretty much all die). Conduct first outplanting.

*Dendrogyra cylindrus*

- KNOWN: Spawning time, some developmental biology, some settlement biology (Szmant, Miller, Acosta, Neely, Lewis, Baums, Marhaver, … )
- TO DO: Improve methods to collect gametes, determine ideal fertilization and settlement methods, determine methods for successful outplanting and post-settlement survival

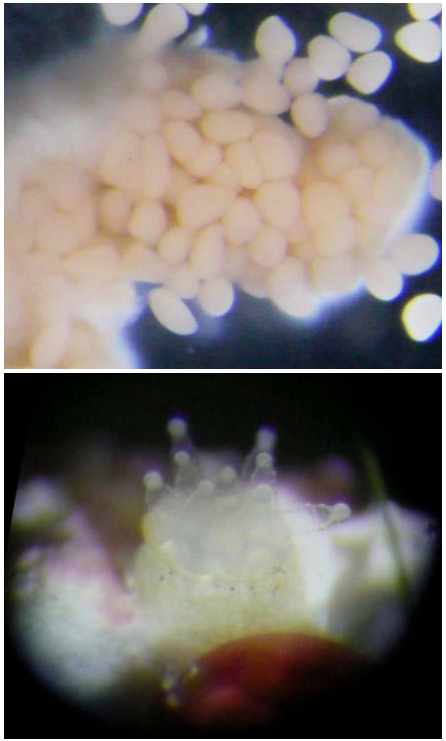


Photo credits: K. Marhaver

*C. natans* gamete bundle (above)
*D. cylindrus* settler (below)

# Calls for info: Your turn to give us the scoop

- SPAWNING TIMES: Nikki is collecting data on spawning times in the Caribbean: **please** send her your data for all observation dives and all species… and stay tuned for the Jordan and Fogarty publication later this year
- GONOCHORES: Kristen is working on a review of gonochoric spawners: please send any spawning observations of *Meandrina*, *Dichocoenia*, *Stephanocoenia*, and any reports from groups working to rear larvae of gonochoric species
- SETTLEMENT + OUTPLANTING: If you 'crack the code' for a specific species (e.g., ideal settlement surface, outplant depth, whatever) *please* consider sharing; we would love to get this information to restoration groups
- MONITORING OUTPLANTS: Our field **really** needs good data on post-settlement survival; we encourage you to collect data and conduct monitoring ANY time you outplant settlers

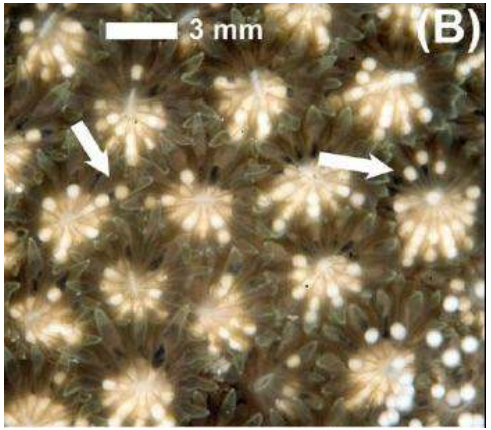
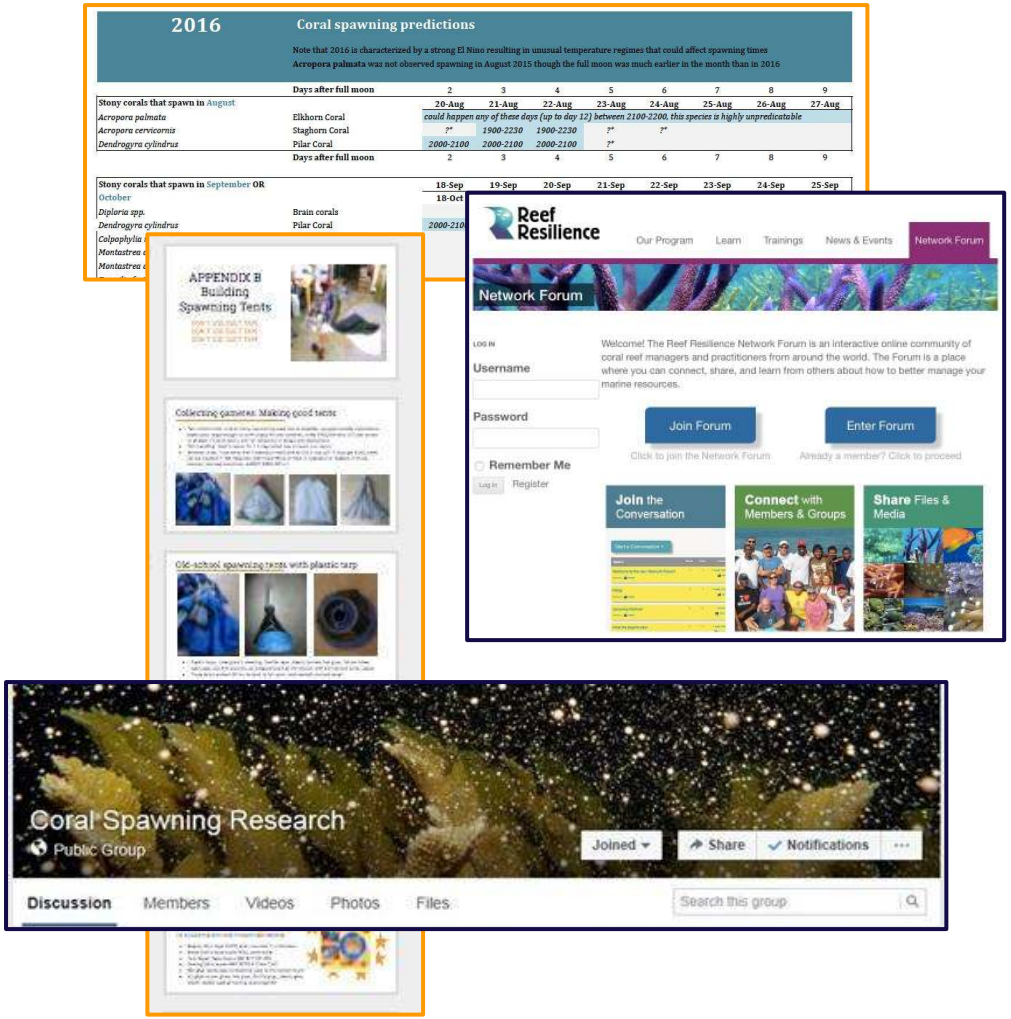



Photo: Vermeij et al. Coral Reefs 2010

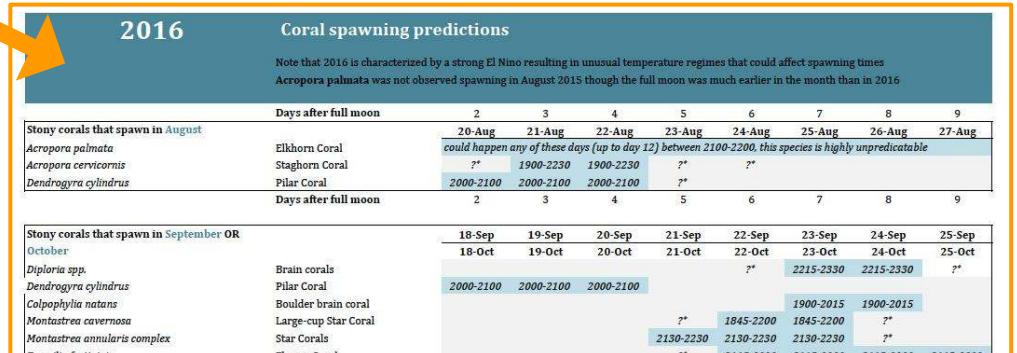
Spawning by a humble little *S. intersepta*

# Resources + Reference Materials



# Where to learn more + stay connected

- **Visit an active spawning program** e.g., CARMABI, STRI, MOTE, UNAM, NOVA SE…
- **Attend a workshop**, e.g., SECORE Workshops in Curacao, Mexico, USVI, Bahamas www.SECORE.org (contact Valérie: chamberland.f.valerie@gmail.com)
- **Download this presentation**
  - We've prepared appendices with details on building spawning tents, purchasing materials, and setting up the lab
  - PDF will be posted to Reef Resilience forum
- **Useful references on coral reproduction**: Fadlallah 1983, Szmant 1986, Richmond & Hunter 1990, Hirose et al. 2001, Levitan 2004, Marlow & Martindale 2007, Baird et al. 2009, Omori 2011, Ritson-Williams et al. 2011
- **Spawning Predictions**: CARMABI issues annual predictions for the southern Caribbean http://www.researchstationcarmabi.org/category/carmabi-news/ow

**2016** **Coral spawning predictions**

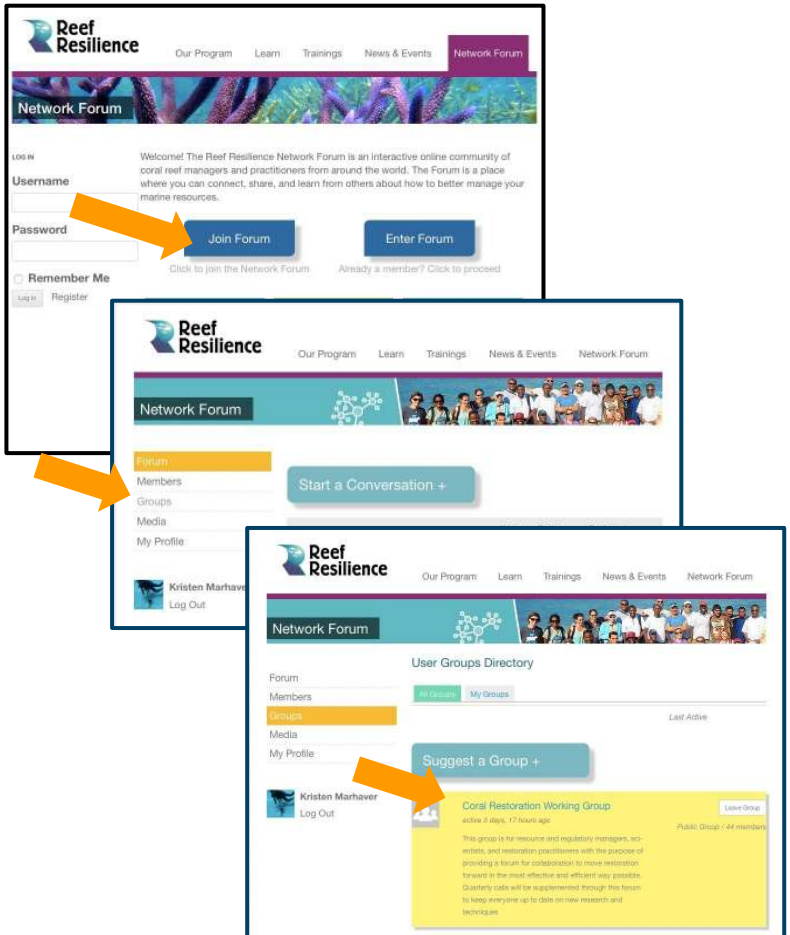
Note that 2016 is characterized by a strong El Nino resulting in unusual temperature regimes that could affect spawning times
**Acropora palmata** was not observed spawning in August 2015 though the full moon was much earlier in the month than in 2016

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Days after full moon** | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| **Stony corals that spawn in August** | | 20-Aug | 21-Aug | 22-Aug | 23-Aug | 24-Aug | 25-Aug | 26-Aug | 27-Aug |
| *Acropora palmata* | Elkhorn Coral | *could happen any of these days (up to day 12) between 2100-2200, this species is highly unpredictable* | | | | | | | |
| *Acropora cervicornis* | Staghorn Coral | *?** | *1900-2230* | *1900-2230* | *?** | *?** | | | |
| *Dendrogyra cylindrus* | Pilar Coral | *2000-2100* | *2000-2100* | *2000-2100* | *?** | | | | |
| | **Days after full moon** | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Stony corals that spawn in September OR October** | | 18-Sep / 18-Oct | 19-Sep / 19-Oct | 20-Sep / 20-Oct | 21-Sep / 21-Oct | 22-Sep / 22-Oct | 23-Sep / 23-Oct | 24-Sep / 24-Oct | 25-Sep / 25-Oct |
| *Diploria spp.* | Brain corals | | | | | *?** | *2215-2330* | *2215-2330* | *?** |
| *Dendrogyra cylindrus* | Pilar Coral | *2000-2100* | *2000-2100* | *2000-2100* | | | | | |
| *Colpophylia natans* | Boulder brain coral | | | | | | *1900-2015* | *1900-2015* | |
| *Montastrea cavernosa* | Large-cup Star Coral | | | | *?** | *1845-2200* | *1845-2200* | *?** | |
| *Montastrea annularis complex* | Star Corals | | | | *2130-2230* | *2130-2230* | *2130-2230* | *?** | |

# Where to learn more + stay connected

- **Caribbean Coral Restoration Consortium** (being formed now)
- **NOAA/TNC Coral Restoration Webinar Series**
  - Next up is Iliana Baums on May 10th 2017
- **New Online Forum at TNC Reef Resilience!**
  - **JOIN FORUM** at www.reefresilience.org/network
  - Navigate to **GROUPS**
  - **CHOOSE Coral Restoration Working Group**
- **Facebook Groups**
  - **Coral Spawning Research** (1000 members) https://www.facebook.com/groups/270783472935805/
  - **Coral Restoration Coordination** (150 members) https://www.facebook.com/groups/coral.nursery
  - **Captive Coral Spawning Research** (600 members) https://www.facebook.com/groups/420196901490017/




# Questions + Answers

Can we help un-confuse you?

- During the webinar: Enter your questions into the GoToWebinar panel under QUESTIONS. We will answer your typed question or un-mute you to ask for more details about your question
- Aim for questions that are informative for the full group
- Focus on technical, practical, methodological aspects
- Speak up! If you are struggling with a certain step, it’s probably true for others as well

# 10 Minute Break

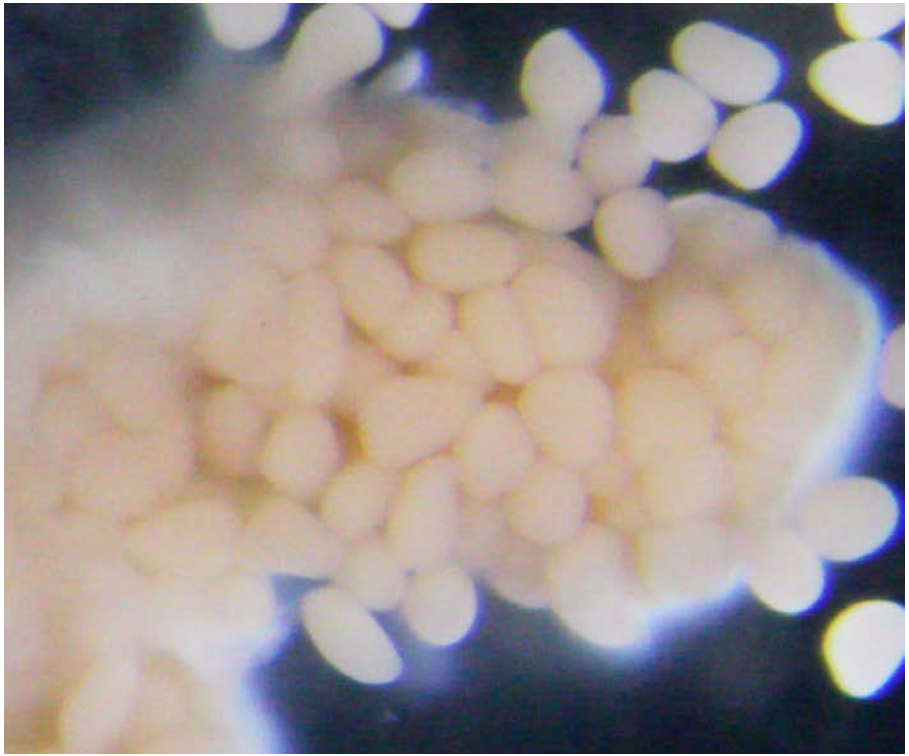


Photo credit: K. Marhaver

*C. natans* gamete bundle...
also taking a break

# Group Discussion

- Priorities for our field as a whole
- Plans for upcoming seasons + beyond


Photo credit: P. Selvaggio

Haploid *Orbicella* individuals interact with one another

# WEBINAR FOOTNOTES

- Items with footnotes are marked in the slides above with a *
- This additional information was provided by colleagues and webinar participants during and after the webinar

# Footnotes to the webinar

- Slide 32: Setting (aka staging) looks different for each coral species and it is **not** always visible or obvious for every species. *Orbicella* and *Acropora* are pretty 'honest' about their plans. Brain corals may not display many or any bundles on the surface. Gonochores may have puffy tissues with eggs visible inside… or not!
- Slide 35: *C. natans* can spawn as late as November in Curacao, perhaps as late as October in the northern Caribbean, especially if the full moon is early/late in the month
- Slide 42: Always dive your study site at least once during daylight hours before beginning a spawning project. It is also useful to dive during the day to set out underwater navigation aids, such as glow sticks to mark entries and exits. Also consider diving once to set out gear on the reef, then again to monitor for spawning. At CARMABI, we often do this for *Orbicella* spawning; we carry everything out to the reef, set tents *next to* good-looking colonies, then swap out dive tanks on shore. This reduces stress and air consumption concerns, and thus dramatically increases safety, especially with small teams and/or relatively new divers. It also creates extra time for photography and natural history observation during both dives
- Slide 48: Goopy gametes are often the result of spawning tents rubbing against coral tissue, which causes the release of mucus. Regardless of cause, whenever a tube of gametes looks slimy, goopy, or clumpy, THROW IT OUT. Do not use this material in a fertilization batch, it will cause you many headaches and may kill your whole batch
- Slide 48: When fertilizing, try to mix similar volumes of material from each parent - don't let one prolific parent dominate the entire gamete pool, unless you are very, very short on material
- Slide 48: *A. palmata* populations can be highly clonal. M. Miller reports that even *Orbicella* populations in Florida are highly clonal. Finding separate genotypes and achieving good fertilization is a challenge in these populations. When fertilizing, make an effort to mix gametes from different genotypes. If you don't have genetic data, try to collect from corals that are far apart, or even distribute your dive team across two different reefs

# Footnotes to the webinar

- Slide 52: If a subset of bundles do not break apart with the others, remove each clumpy bundle from the container with a transfer pipette and discard it… sticky bundles will **not** fertilize well and can cause problems later on
- Slide 54: *Orbicella* and *Acropora* gametes can stay viable for up to two hours in closed tubes; we **don't** recommend waiting this long, but keep this in mind to plan the pace of your work. In one case, a batch of *Orbicella* died almost instantly… we mixed leftover gametes 90 mins later and they developed well!
- Slide 69: Regardless of the stage you're at, STAY AHEAD ON THE DE-SCUZZING. If you have time, do some de-scuzzing. Even when it looks good, things can go bad FAST
- Slide 77: Isn't plastic a bad thing to put in the ocean? We don't worry about this right now. It's teaching us a lot, and it may be the secret to helping some species. Over the long term, biodegradable and natural materials are certainly going to be preferred
- Slide 78: M. Miller notes that she finds that *O. faveolata* and *A. palmata* settlement is often higher on heavily-colonized substrates compared to cleaner substrates, but per-settler survivorship is often lower on the heavily-colonized substrates; we are only starting to test conditions for long term survival at this stage
- Slide 88: M. Vermeij notes that when outplanting, look for places with lots of coral settlers and recruits, even if these are other species; abundant *Agaricia* settlers are a sign that baby corals have a chance at that site. M. Miller notes that parrotfish are attracted to novel elements, like outplanted corals! Watch out for their behavior especially at sites with high grazing. She also notes that outplanting at very degraded reefs may fail. We may want to consider multi-stage restoration, e.g., outplanting fragments to jump-start recovery before outplanting settlers
- Slide 92: J. Figueiredo has 5-month-old *M. cavernosa* settlers growing in a closed system at NOVA Southeastern! They are fed rotifers raised on microalgae. L. MacLaughlin reported that she has *D. stokesii* spawning observations from Florida! We plan to incorporate this info into CARMABI's 2017 spawning predictions

# THANKS!

**To contact the webinar hosts + authors:**
Kristen: kristen@marhaverlab.com
Valerie: chamberland.f.valerie@gmail.com
Nikki: nicole.fogarty@nova.edu

**To contact additional photographers:**
Mark Vermeij: carmabilog@gmail.com
Paul Selvaggio: photomanps@aol.com
Natalia Hurtado: natihurtadolopez@gmail.com
Benjamin Mueller: muellerb@ymail.com

Photo credit: K. Marhaver

# Appendices

Appendix A: Preparing materials + preparing the lab

Appendix B: Building spawning tents

# Appendix A: Preparing materials + preparing the lab

Important care and paranoia now saves you major pain later

Photo credit: K. Marhaver

# Critical laboratory paranoia: Chemicals and fumes



Photo credit: K. Marhaver

- AVOID insect repellent, greasy lotion/sunscreen on your hands/arms/face, any kind of perfume or perfumed beauty products… they can kill your larvae!
- If possible, avoid all insect repellent during spawning season; pants are best. If you MUST use it, use DEET (not essential oils/citronella) and apply only after leaving the lab for the final time that day
- Clean the lab with ONLY nontoxic lab soap and water; lab surfaces can be sterilized with Ethanol or Isopropanol and GOOD VENTILATION
- THESE ARE NEVER ALLOWED IN THE LAB: bleach, formaldehyde, any/all fixatives, chloroform, mercaptans, cleaning products, sprays of ANY KIND

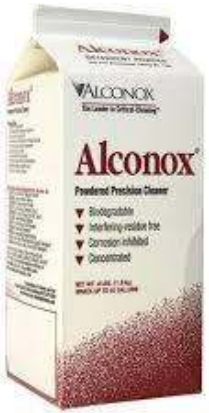
# Spawning shopping: Buying all that stuff

- Big vendors like Fisher, Corning, VWR give amazing discounts to universities but are cost prohibitive for small groups, NGOs, field stations etc… There are a few items you are best off purchasing through a colleague at a university (e.g., disc filters)
- USA Scientific sells directly to customers and has good prices on conical tubes, petris, and transfer pipettes (We don't love their conical tubes, but they DO work)
- Equipment: For peristaltic pumps, vacuum pumps, etc, try looking on LABX, eBay, University Surplus Stores, and similar sites for used equipment (be sure to check the Volts and Hertz of your spawning location); you can often find used pumps for 50-75% off
- Mc-Master Carr is a GREAT source for grommets, nuts, bolts, zip ties, and other construction materials, plus they ship very fast and for a reasonable cost
- Amazon is a good source for kitchen items, gravy boats, fabric shower curtains, and some sciencey stuff like Petris, Kimwipes, and transfer pipettes, but DO watch out; Amazon sometimes has steep price increases compared to scientific supply companies
- Restaurant Supply Stores are good for polystyrene deli containers - it's better to order these by mail than to visit each store in your local urban area (trust us, we know…), and NEVER trust last year's store to still carry these same critical items next year

# Spawning shopping: Obscure materials

| THING | Specifications | Brands | Example URL |
|---|---|---|---|
| **Filter holders** | 47 mm, inline filter holder with adaptors and tubing to fit your peristaltic pump OR 47 mm filter holder for vacuum pumps | Pall, Nalgene, Cole Parmer, others | http://www.pall.com/main/laboratory/product.page?id=20096 |
| **Water filters: 0.45 µm** | 0.45 µm HPWP impact filter, 47 mm diameter, pack of 100, polyethersulfone, CAT# HPWP04700 | Millipore | https://www.emdmillipore.com/US/en/product/Millipore-Express-PLUS-Membrane-Filter,-polyethersulfone,-Hydrophilic,-0.45%C2%A0µm,-47%C2%A0mm,MM_NF-HPWP04700 |
| **Water filters: GF/F (approx 0.7 µm)** | GF/F glass fiber filters, 47 mm diameter | Whatman, Fisherbrand | https://www.coleparmer.com/i/whatman-1825-047-gf-f-glass-microfiber-filters-0-7um-4-7cm-100-box/0664891? |
| **Polystyrene deli containers** | 8” or 9”, clear, clamshell, single compartment, deli/takeout containers. NOTE: AVOID PET, polypropylene, and white polystyrene foam | Dart, Durable Packaging | http://www.cooksdirect.com/product/durable-packaging-pxt-900/carry-out-supplies |
| **Fat separators** | Volume of at least 4 cups/~1000 ml, plastic, no ‘funny’ gadgets or attachments, straight walls | Norpro | https://www.amazon.com/Norpro-Gravy-Separator-Juice-Measurer/dp/B0000VLXYG |
| **Sodium thiosulfate pentahydrate** | Sold in granules form by chemical suppiers, also sold as Chlorine Neutralizer but be sure this contains only sodium thiosulfate with NO ADDITIVES | Fisher, Sigma, The Science Company, Pool Supply stores | https://www.sciencecompany.com/Sodium-Thiosulfate-Pentahydrate-500g-P6376.aspx |

# Lab prep: Cleaning and bleaching supplies

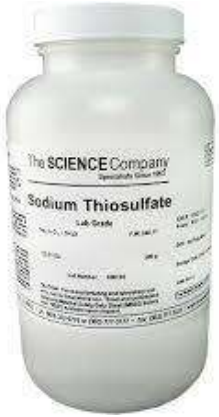




SOAPY STUFF

- NON-TOXIC laboratory glassware soap, e.g., Alconox or Sparkleen; importantly, this soap is is biodegradable, foams up well, and rinses very quickly and completely
- Use very soft, new sponges (never use the rough scrubby side)


BLEACH

- Regular household strength chlorine bleach
- Usually sold as 5.25% strength, 8.25% is also OK
- Avoid bleach with perfumes, “splashless” bleach with thickeners, or any kind of “bleach alternatives”





CHLORINE NEUTRALIZER

- Sodium thiosulfate pentahydrate (sold as large, pea-sized crystals/granules)
- Sold by scientific suppliers e.g., Fisher, Sigma, The Science Company
- Also available from pool supply stores under the name “Chlorine Neutralizer” - be SURE there are no other added ingredients!
- This chemical is hydrophillic - keep containers tightly closed

A CLEAN SURFACE

- We prefer clean paper towels or clean cloth towels; use to place underneath wet items and to drape over drying items to block dust
- A clean set of drying racks or a large sterile countertop are OK

# Lab prep: Cleaning and bleaching procedure


DE-FAT
Wash slimy things with lab soap, rinse well in freshwater

BLEACH
250-500 ml bleach in ~50 L freshwater, refresh 2-3x/week

DE-BLEACH
Sodium thiosulfate pentahydrate (one big handful) in ~50L freshwater, stir to dissolve, refresh 2-3x/week

RINSE
Clean or filtered tap water, changed daily

DRY
Place upside down in clean, low-dust area

- Set up a cleaning + bleaching station **AWAY** from all larval rearing areas; e.g., in an outdoor wet lab or separate room
- Large rubber/plastic bins (e.g., dive gear bins) are useful
- **ANY** containers that held larvae or scuzz should first be washed with **non-toxic** lab soap (e.g., Alconox, Sparkleen) and a SOFT sponge, then rinsed in fresh water. Never use the scrubbing side of the sponge; it will scratch plastics.
- ***All*** plastic and glassware items are then cleaned as follows:
  - BLEACH: 10-minute to 24-hour soak in diluted bleach, the solution should be 5-10% of the pre-bottled strength
  - DE-BLEACH: 5-second dunk in dilute sodium thiosulfate solution; can leave items for up to 20 mins, but **not overnight!** A slimy film will form.
  - RINSE: 5-second dunk in freshwater; again a 20 min soak is OK, but do not leave overnight!
  - DRY: Place upside down in a clean area; Avoid letting dust or rain fall on items

PROTIP: Use the de-bleach solution to neutralize the bleach solution before discarding it… and be sure to bleach the other two bins 1x/week!

# Lab prep: Setting up the lab for spawning

Photo credit: P. Selvaggio

CLEAN SURFACES

- Clean all laboratory surfaces and floors with a wet rag or with very dilute, non-toxic soap to ensure you remove as much dust as possible
- Disinfect counters with 70% isopropanol or 70% ethanol and let the room air out for several hours (up to a day) before unpacking and setting up any of your coral spawning materials
- We cover our benches with thick, sturdy black garbage bags to ensure no chemical residues remaining on the counters will contact with the larval cultures. The black background also contrasts very well with coral larvae and makes them much easier to see in the transparent deli containers

TEMPERATURE

- Several days before coral spawning, adjust the lab temperature to ambient sea surface temperature (SST); check thermometers often
- Keep doors and windows shut as much as possible to keep the temperature constant (and to keep out unwanted guests, e.g., insects!)
- While working in the lab with a large team, keep an eye on the temperature; the more people working, the warmer it gets!
- Avoid keeping larval cultures directly in the airflow of the air conditioner as temperature will be lower in that area

# Water prep: Supplies for water filtering

Photo credit: K. Marhaver

A valiant peristaltic pump staying ahead of the game

CONTAINERS: Purchase a dedicated set of new plastic jugs or cubitainers for FSW; these are often sold at camping, hardware, or automotive supply stores… NEVER use them for any other purpose!

PREFILTERS: Construct a nylon prefilter (Nitex 35-50 µm mesh) with a PVC tube and/or funnel; this is used to prefilter seawater when filling source bins

PUMPS: Use peristaltic or vacuum pump systems to minimize contact with the water

- Brands include Masterflex, Cole Parmer, Pall Corporation, Nalgene, Millipore
- Multiple small, parallel systems are better than one big huge filter
- Habe a backup: Don't trust ONE pump to last an entire spawning season!
- Watch out for differences in 110/220V and 50/60Hz between countries
- Keep pump up and away from salty splashes, puddles, and people

FILTERS: Small-pore filters are annoying, but clean water really helps your larvae

- We've had success with 0.22 µm (Sterivex GP polyethersulfone) and 0.45 µm (Millipore HPWP polyethersulfone) filters for *Orbicella*, and GF/F glass fiber filters (Whatman; approx 0.7 µm) for Acroporids and brain corals
- These materials don't appear to leach any toxins or inhibitors into the seawater
- Use any other filter materials (such as cellulose) AT YOUR OWN RISK!

# Water prep: Rules for water filtering


Photo credit: V. Chamberland

100 liters and counting…

LABELS: Mark all containers with CONTENTS and DATE (e.g., GF/F FSW 9/15, 35 µm FSW 9/15, 0.22 µm FSW 9/15)

STERILIZING PARTS: All filter parts (e.g., tubes, filter holders, fittings) that touch the seawater should be bleached, debleached, and rinsed before use

- Repeat this for all pieces every 1-2 days during larval rearing
- Consider buying spare parts to keep them cycling through the bleach

PROPER RINSING PARANOIA: Pre-rinse ALL jugs ANY TIME you fill them!

- Before first use and when you are using up water slowly: bleach, debleach, and rinse the inside of each container 3x with fresh water
- Before ANY use, first rinse the container with 500-1000 ml of whatever it is about to be filled with (whether 35 µm or 0.22 µm FSW)
- When using up water quickly, you can rinse containers between fillings with 1000 ml fresh water to osmotically shock bacteria, then with 500-1000 ml FSW

PREFILTERING: When filling source jugs, pre-filter water with the Nitex nylon mesh to remove sediments, algae, and large plankton

VOLUMES: Aim to have AT LEAST 100 l of filtered seawater (FSW) for dive night 1… more is better. For large operations, aim for at least 160 liters

STORAGE: Keep water in the lab, at ambient seawater temp, for up to 2-3 days max; Yes, some water will get dumped out

MANNERS: Be nice to your water filter crew and buy them extra beers

# Appendix B: Building spawning tents

DON’T USE DUCT TAPE
DON’T USE DUCT TAPE
DON’T USE DUCT TAPE

Photo credit: K. Marhaver

# Spawning tent styles and keys to success

- Tent construction: Use as many non-rusting materials as possible, use **good quality** components, make tents large enough to fit comfortably over coral colonies, make **enough** tents (3-5 per person or at least 15-20 in total), aim for simplicity in design and deployment
- Tent handling: Soak in water for 1-2 days when new to leach out toxins
- Between dives, rinse tents with freshwater and allow to dry in the sun. If they get slimy, tents can be cleaned in 10% bleach and rinsed well in fresh or seawater, or washed in dilute, nontoxic lab soap (e.g., Alconox). **Always rinse well!**

TYPE 0

Photo credit: K. Marhaver

TYPE 1

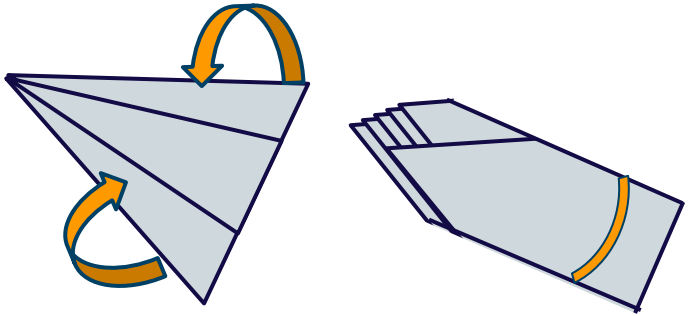
TYPE 2

Photo credits: R.-J. van der Houwen

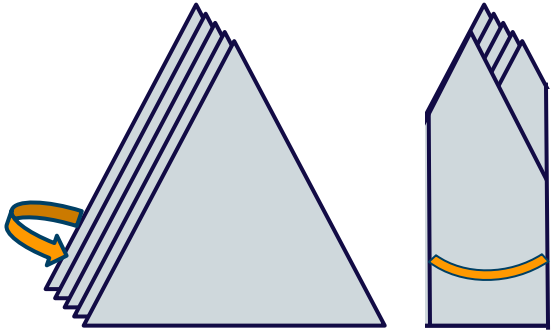
TYPE 3

# Tent types 0, 1, 2: Handling them neatly and safely

Above water: Fold tents into thirds, stack them, then bungee together near the bottom



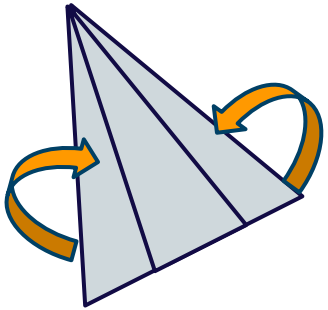
Underwater: Roll tents horizontally into a standing burrito, then bungee together

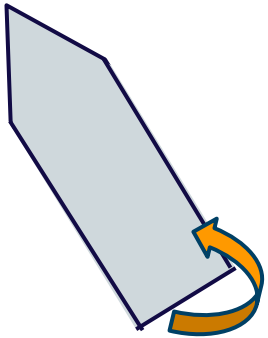


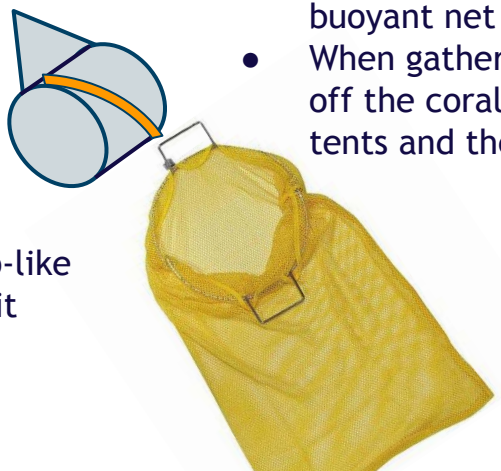
- Prior to diving, fold tents into thirds/fourths, then stack in piles of 3-6, then bundle the pile together with 1-2 rubber bungee cords. One un-folded tent can be put at the bottom of each pile and wrapped up over the other tents to make a neater bundle.
- WARNING: A large bundle of tents can be **heavy** and difficult to swim with, especially in rough conditions
- Lift bags can be used for very heavy tents, but should only be used by those with training and practice
- When swimming, achieve neutral or slightly negative buoyancy while holding the tents. At the ocean floor, make sure you are negatively buoyant before releasing the tents
- Restrict new divers to carrying and working with 1-3 tents, or assign new divers to help with spotting, flashlight holding, and handling bags, tubes, and caps
- When gathering tents underwater, gather 3-6 standing tents together and flatten each (like file folders in a row), grab the first tent at the bottom, and roll horizontally around all the other tents; you'll end up with a big, vertically-standing tent burrito. Tie with a bungee cord near the bottom for easier transport. We like to tie bungees in knots and not use the hooks.

# Tent type 3: Handling them neatly and safely

Above water: Fold tents lengthwise, roll up into a burrito, then secure with rubber band and place in a mesh bag







Underwater: Gather tent into a burrito-like shape, rubber band if possible, smush it back into your mesh bag

- Prior to diving, fold tents into thirds/fourths if needed, then roll from the bottom up, secure with a rubber band
- Carry nets in a mesh dive bag with a secure closure
- Underwater, one diver deploys a net while the dive buddy holds a flashlight
- Try to keep net open and relatively taught
- Try to orient funnel straight up, regardless of the orientation of the coral
- Try to keep the fabric up and off the coral tissue
- If nets do not contain weights, be extra careful: a buoyant net can float to the surface very quickly!
- When gathering tents underwater, carefully lift fabric off the coral surface: pulling or tugging damages the tents and the corals!

# Type 0 tents: An old-school mess of tape + plastic tarp



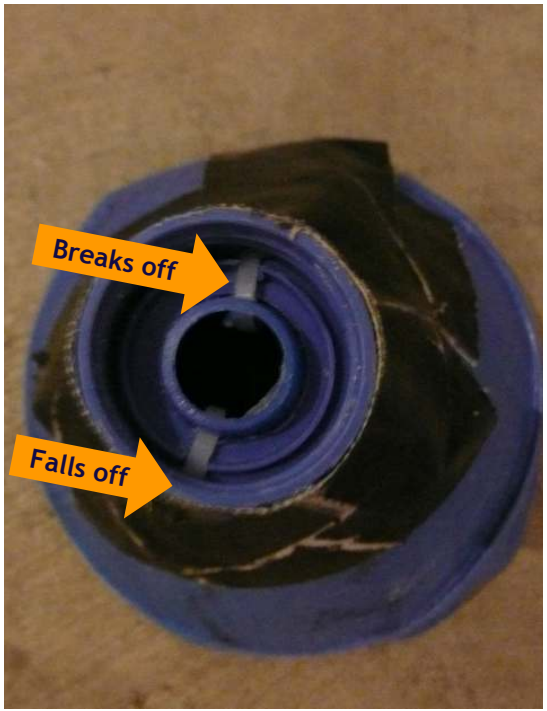



Photo credits: K. Marhaver, strong disliker of soggy duct tape

- These were made of thin plastic tarps, clear plastic sheeting, Duct tape, then Gorilla tape, plastic funnels, skinny zip ties, hot glue, generic 50 ml conical tubes, and tube caps, all weighed down at the bottom with zip ties, more tape (to prevent rips), and coral rubble that was too smooth
- These tents worked kind of OK, but they needed constant repair and reinforcement

# Type 1 tents: Sturdier models from plastic tarp

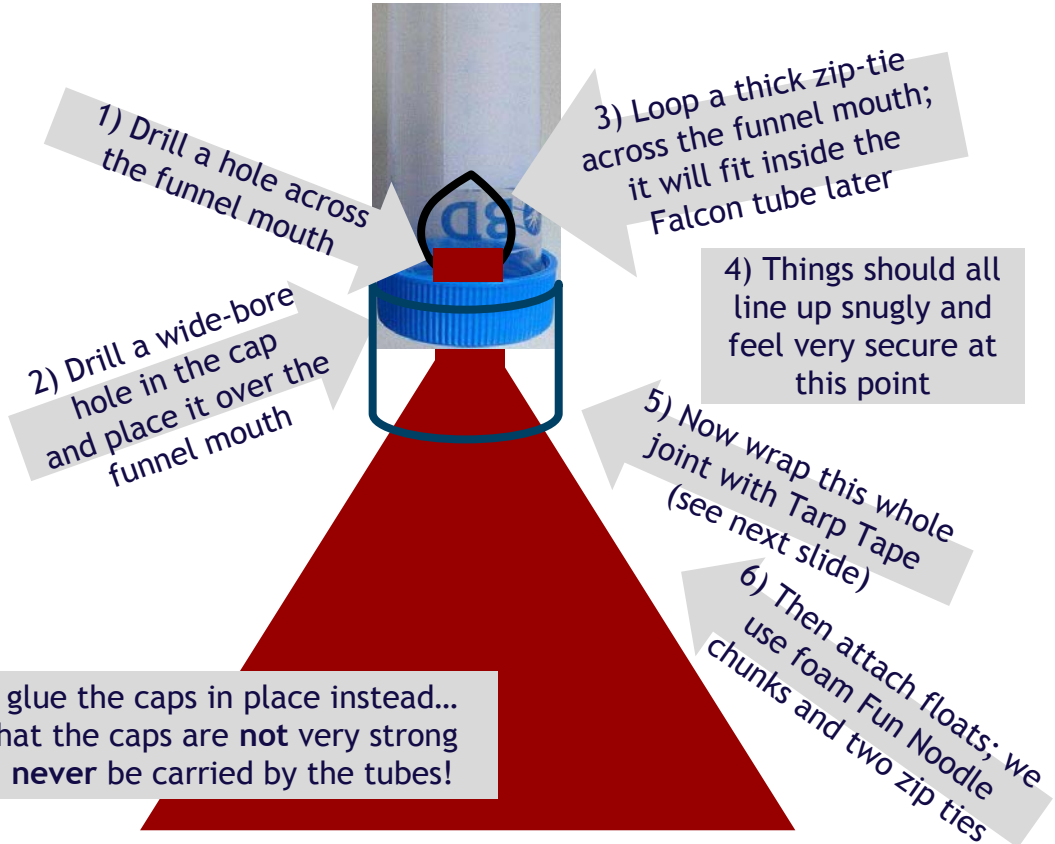
Photo credit: R.-J. van der Houwen

- HEAVY DUTY plastic tarp material, not regular tarps!
- Tarp is secured at the seam with TARP REPAIR TAPE and/or by sewing the material
- NO WINDOWS… they tear open and aren't that important or useful anyway
- 2" segments of thick steel reinforcement bar (REBAR) is ziptied to the bottom (it does rust a little)
- GORILLA TAPE or TARP TAPE is placed is on tarp before cutting any holes at the bottom, to prevent ripping
- A single HEAVY-DUTY funnel is attached to the tarp with four nylon hex nuts and bolts. There ARE gaps between funnel and tarp; some bundles do escape, esp. in surge
- Reflective tape on funnels helps locate them in the dark
- A chunk of hollow foam Fun Noodle is wrapped around funnel neck and secured with zip ties around it
- Tube cap is locked down with one big zip tie straight across the funnel mouth (no zip ties through the tube cap)
- Cap is reinforced all the way around with Tarp Repair Tape

# Spawning tent secrets: Caps that don't fall off

Perhaps that last slide didn't quite explain everything that we were talking about...


...so here's how to really keep those tubes on those tents:




Some groups hot glue the caps in place instead... just remember that the caps are **not** very strong and tents should **never** be carried by the tubes!

# Type 1 tents: Critical attachment intel



For all spawning tents made of plastic tarps/sheeting:

- Regular duct tape **sucks** when you take it underwater
- Black Gorilla Tape works pretty okay underwater
- Tarp Repair Tape does a **way better job** than those other tapes
- Sewing the plastic together also works well if you have the skills
- Hot glue works okay to hold the tube to the funnel mouth
- But, **all glues** suck at holding two pieces of plastic tarp together; this includes super glues, hot glue, Gorilla glue, plastic glue, GOOP, E6000, … believe us, **we know**
- We promise we are not sponsored by Tarp Tape but we wish we were

# Type 1 tents: Materials for construction


Forstner drill bit for tube caps



Fat rebar cut into small chunks, or other weighting system


Plus: A drill, drill bits that match the nuts+bolts, scissors, sewing machine and thread (if sewing tarps together)

# Type 2 tents: Shower curtain fabric

Photo credit: R.-J. van der Houwen

- Based on previous hassles, Kristen aimed to design a tent for Team CARMABI with no glue, no tape, no metal, and therefore no rust… In the end, we used a small piece of tarp repair tape to hold the tube cap in place
- Waterproof shower curtain liners are lightweight and can be easily sewn into desired shape (e.g., InterDesign brand)
- Fabric can be removed and washed in the washing machine after spawning season ends
- We put 8 plastic grommets (McMaster-Carr) into the bottom of the tent to prevent tearing/ripping (alternatively: sew buttonholes)
- We zip-tied pieces of coral rubble through the grommets - not an elegant solution, but it's free, it sinks, and it doesn't rust
- A piece of foam Fun Noodle + two zip ties keeps the funnel afloat; CAREFUL: the foam will compress on the first dive and may escape
- These tents are easy to clean and easy to carry underwater
- However, these tents can be floppy in surge or current, in which case the fabric can get slimy and/or interfere with bundles. Heavy weights and floaty floats can help prevent that. Test in advance!

# Type 2 tents: Materials for construction


Forstner drill bit for tube caps



InterDesign Waterproof Polyester Shower Curtain/Liner, Gray


Easy-Install Two-Piece Grommets

No setting tools required—snap the two parts togeth sold separately). Grommets are plastic and can b durometer. Temperature range for grommet with 1/4" I grommets with 1/2" ID is 30° to 180° F.

Grommets

| ID | OD | Ht. | Max. Material Thick. | Color | Pkg. Qty. |
|---|---|---|---|---|---|
| 1/4" | 1 1/8" | 13/32" | 0.203" | Black | 10 |
| 1/2" | 1 1/8" | 15/64" | 0.025" | Black | 25 |
| 1/2" | 1 1/8" | 15/64" | 0.025" | White | 50 |

Plus: A sewing machine + thick thread, fat *Acropora* rubble or other heavy sturdy weights, drill and drill bits to match the nuts/bolts, scissors, a knife or saw to cut funnels, a hammer for grommets

# Type 2 tents: The handy funnel sandwich

- The outer funnel is left intact and a second interior funnel is trimmed on top and bottom (with a sturdy, sharp utility knife or fine saw) so that it fits flush inside the first funnel with the tent in between.
- Holes are drilled through **both** funnels at the same time; drill two sets of holes across from each other. Keep track of which funnel pairs go together, or else you'll never line them up correctly again… we know!
- The fabric is secured between the funnels using nylon (6/6) hex nuts and screws, finger tight is ok, washers optional
- Fabric stays in place without gaps or wrinkles on the inside, where bundles can otherwise escape or get stuck
- Nylon fittings won't rust! And they're cheap.
- Reflective tape is placed on the funnel in four places to make them easier to find from long distances in the dark.

(Pretend that hole is much higher up; a zip tie goes across to lock down the tube cap)

Drill two of these holes; drill through both funnels at once

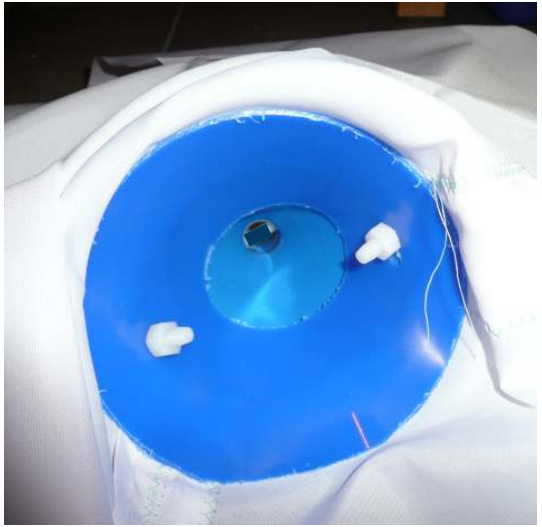


Photo credits: K. Marhaver

# Type 3 tents: Nylon mesh, for *Acropora* spawning

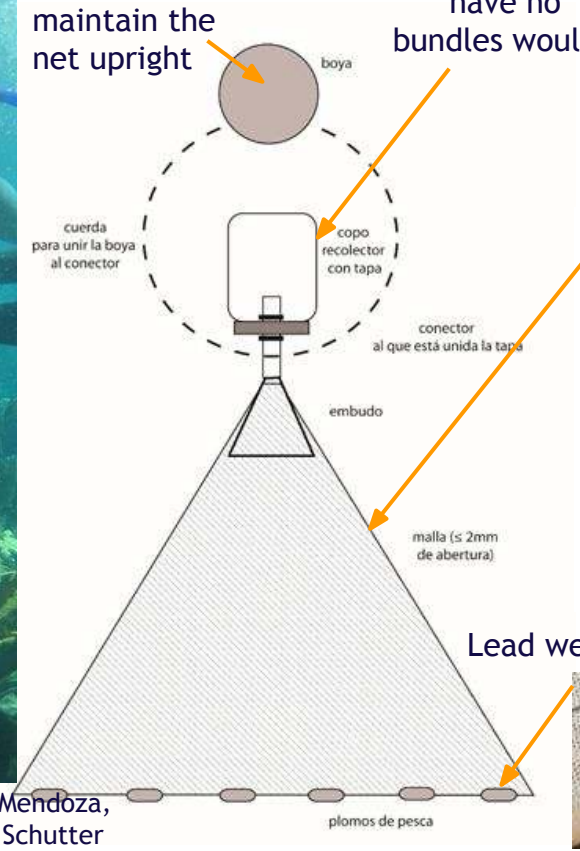
Photo credit: R.-J. van der Houwen

- Rip-stop nylon “mesh” that allows water to flow through
- Mesh is sewn into shape with a sewing machine
- Can be constructed as cones for large colonies, or long tubes or sleeves for single *Acropora* branches… it’s useful to make a variety of sizes to accommodate various colony shapes and sizes
- Mesh fabric is hot glued to a single funnel, or secured in the middle of a “funnel sandwich”
- The funnel+cap+tube connection here is similar to that in other net styles described above
- Lead line is sewn all the way around the bottom of the net, or weights are zip-tied around the bottom of the net
- Alternatively, nylon cord and stoppers can be sewn into the bottom of the tent, but tents will be POSITIVELY BUOYANT overall; these can escape and create a diving hazard
- Mesh can be washed with lab soap between uses
- Rips can be repaired with thread or a sewing machine

# Type 3 tents: Construction instructions

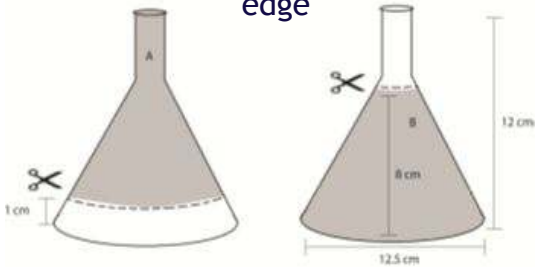
Kindly provided by Anastazia Banaszak

Buoy used to maintain the net upright

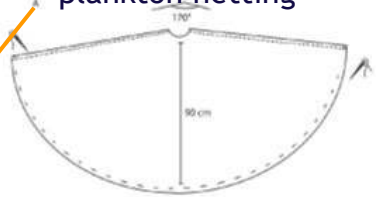


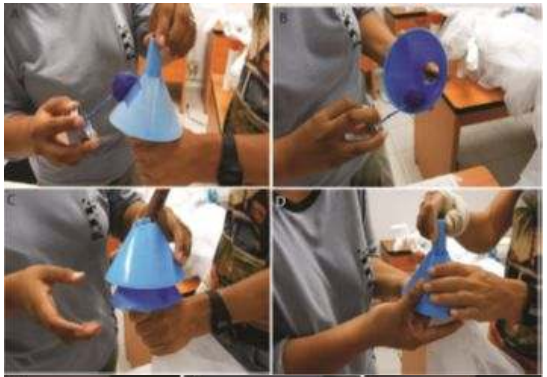

Collecting cups should have no "lip" where bundles would get stuck

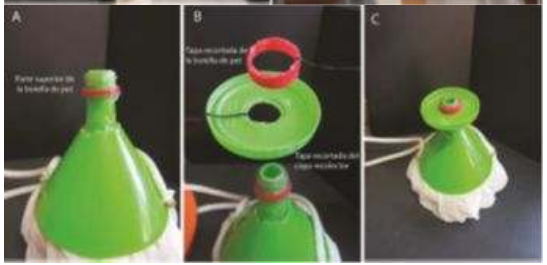
A "funnel sandwich" is used so that bundles don't accumulate at the funnel's edge




Fabric is Nylon mesh or plankton netting






Lead weights hold the net in place

Photo credits: C. Voolstra, S. Mendoza, S. Guendulain; Schematic: M. Schutter

# Caring for spawning tents

Photo credit: K. Marhaver

- Soak all NEW tents in seawater for at least a day before use to leach chemicals from plastics, then clean and rinse in freshwater
- ALWAYS hold/carry by the fabric - never by the funnel, cap, or tube!
- After each dive night, thoroughly rinse tents with fresh water and dry overnight
- To dry; hang over railings, hang on a clothesline, or place in the sun
- If tents are scummy/slimy after touching corals, wash in non-toxic lab soap or dilute bleach, then rinse VERY VERY well in freshwater and/or seawater
- Note: If left on the ground overnight, crawling animals might use tents as a home. Watch for scorpions!
- During the offseason, store tents in a flat pile in a controlled climate

Care for your nets and they will last for many years

# GOOD LUCK!

Here’s to a future with baby corals everywhere