Experimental Studies of Factors Affecting Coral Recruitment Success in La Parguera, Puerto Rico

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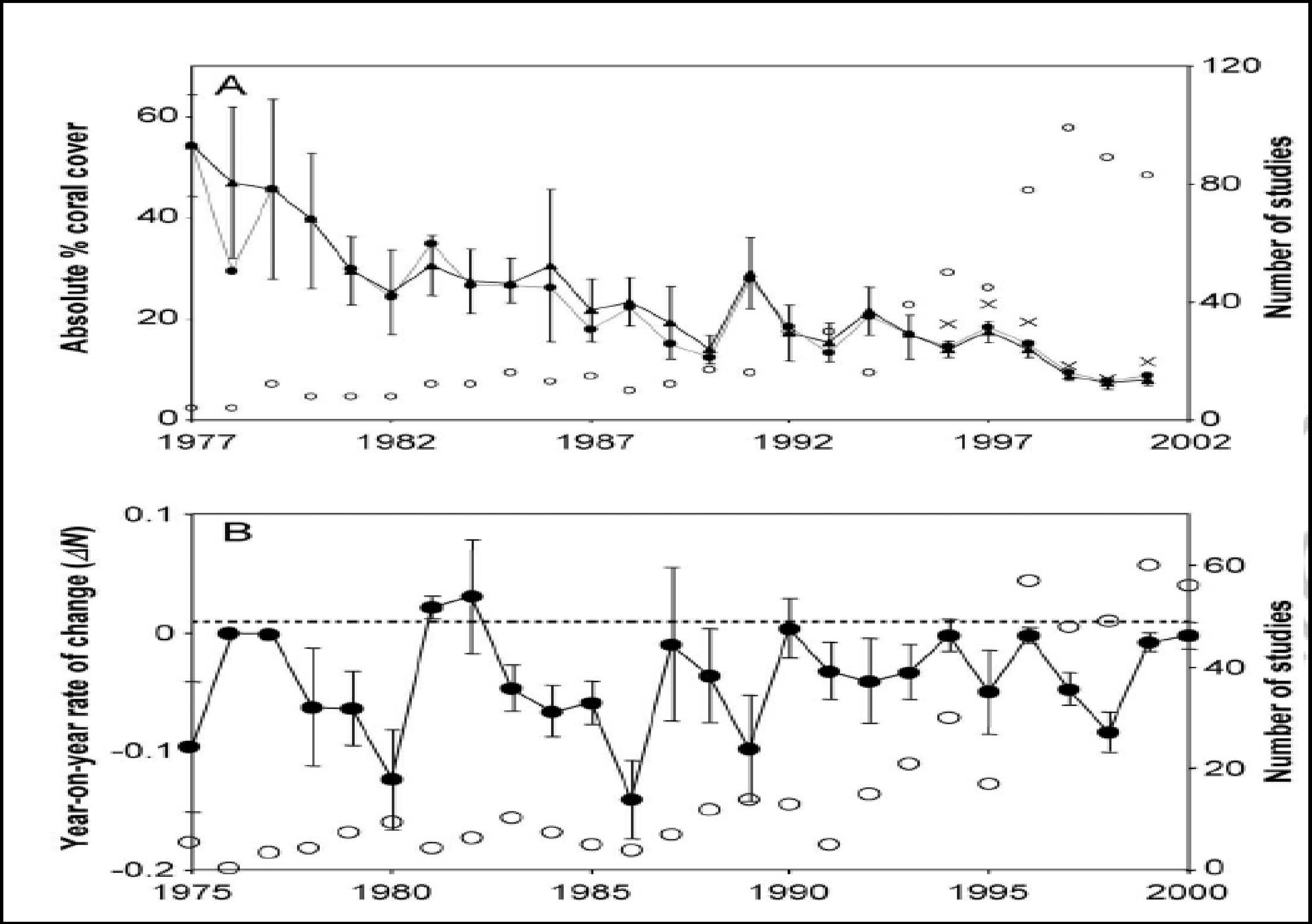
The Problem: Regional Decline in Caribbean coral cover based on published surveys



(Gardner et al 2003: Science)

Situation and Need:

- Most major reef building coral species are not recruiting well
- Anthropogenic activities could be affecting recruitment success
- We know little about the processes affecting coral recruitment, especially the early stages
- Research is needed to better understand conditions that are promoting or impeding coral recruitment
- We need to start at the beginning: *settlement and post-settlement survivorship*

The Whole Story...

Coral Recruitment will be determined or limited (concept of recruitment bottleneck) by one or all of the following:

1) Larval supply
2) Dispersal patterns of larvae
3) Substrate quality of receiving reef
4) "Nursery quality" of receiving reef

Larval Supply

- adult abundance
- adult fecundity
- fertilization success
- developmental success (% that reach competency)

Dispersal Distance/Pattern

- duration of larval period
- hydrographic conditions
- larval swimming behavior
- distance between reefs

Settlement Success
(once larvae arrive at reefs)

- **substrate characteristics**
  - CCA cover and species composition?
  - microbial community?
  - macroalgal cover
  - *sediment cover & type*

Affected by...

- grazer community composition
- weather, tides, storms
- *water quality conditions*

Corals must compete against many plants and animals that also want to occupy the reef substrate, and coral larvae are very small at time of settlement

Therefore, environmental factors that affect substrate composition, such as water quality and grazing community structure may be important determinants of coral settlement and recruitment

Research Objectives Summer 2005:

PRIMARY: Settle coral larvae of as many species as possible onto settlement plates pre-conditioned at 3 sites along a water quality gradient; map spat and follow survivorship

- Inshore: Pelotas
- Mid-shelf: Turrumote
- Offshore: Buoy shelf edge

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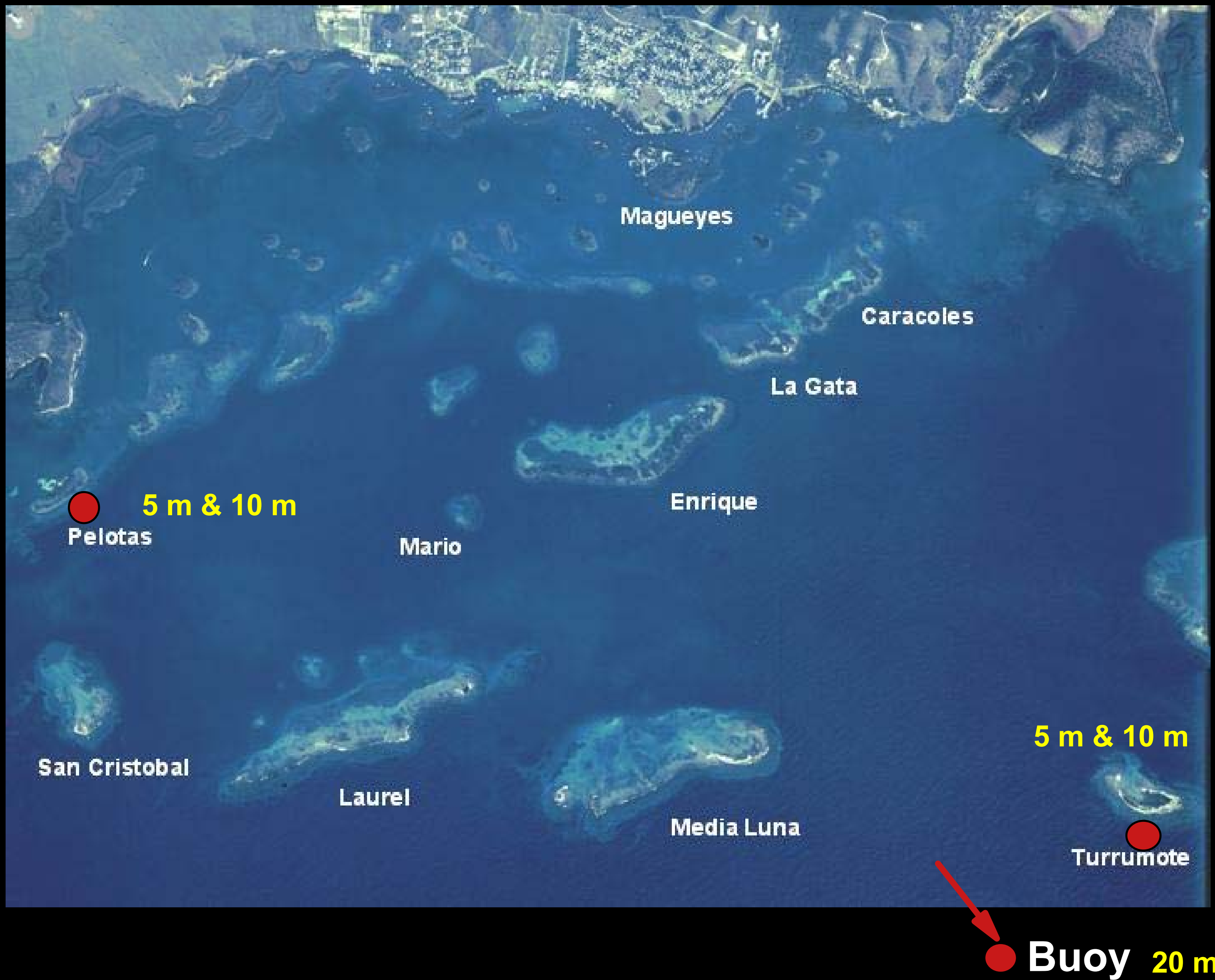
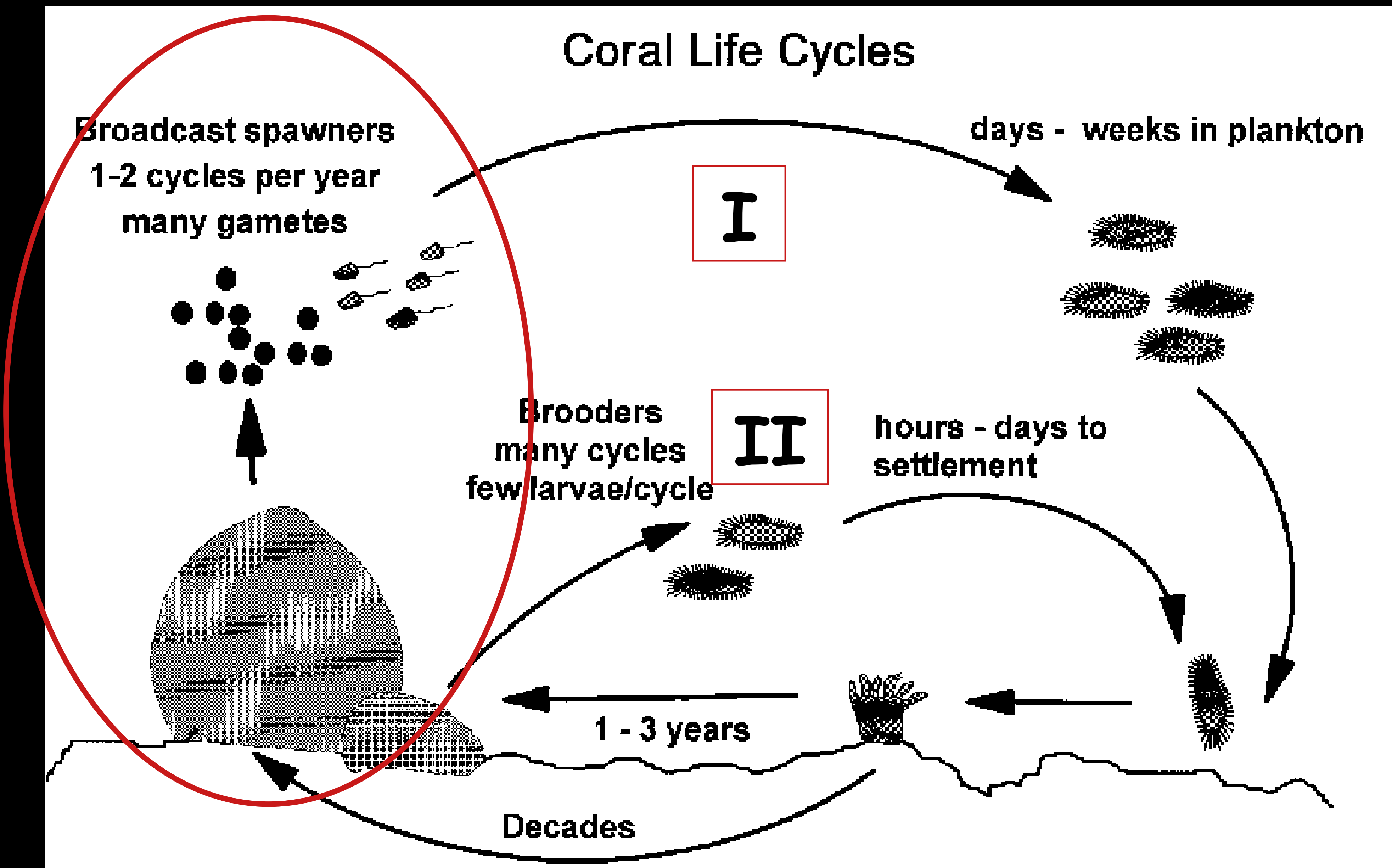
<u>SECONDARY</u>:

- Continue research on settlement cues and preferences
- Begin work on effects of feeding on survivorship
- Continue research on larval behavior
- Capacity building in this research topic

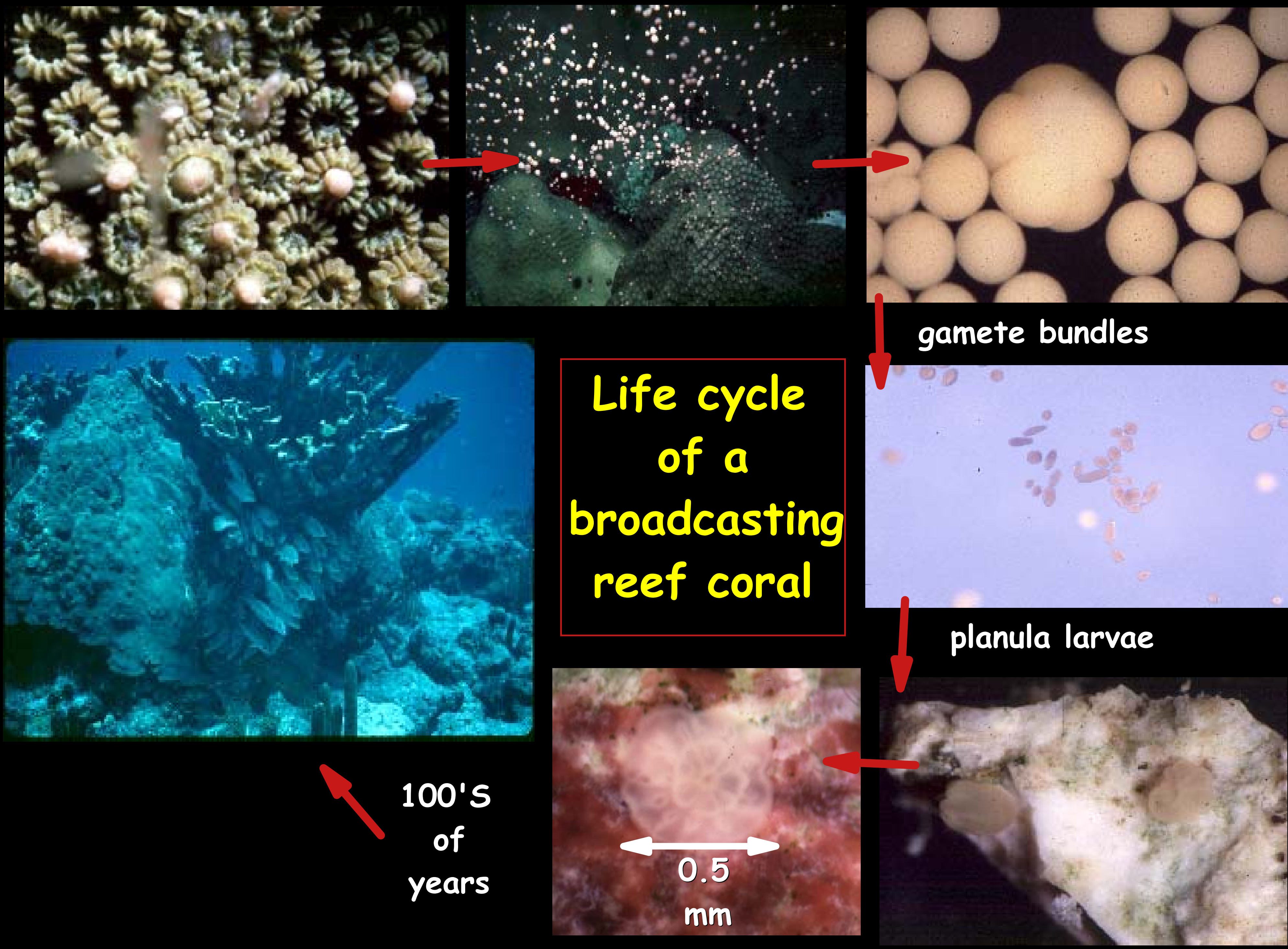
Study Sites: 3 sites, inshore to offshore



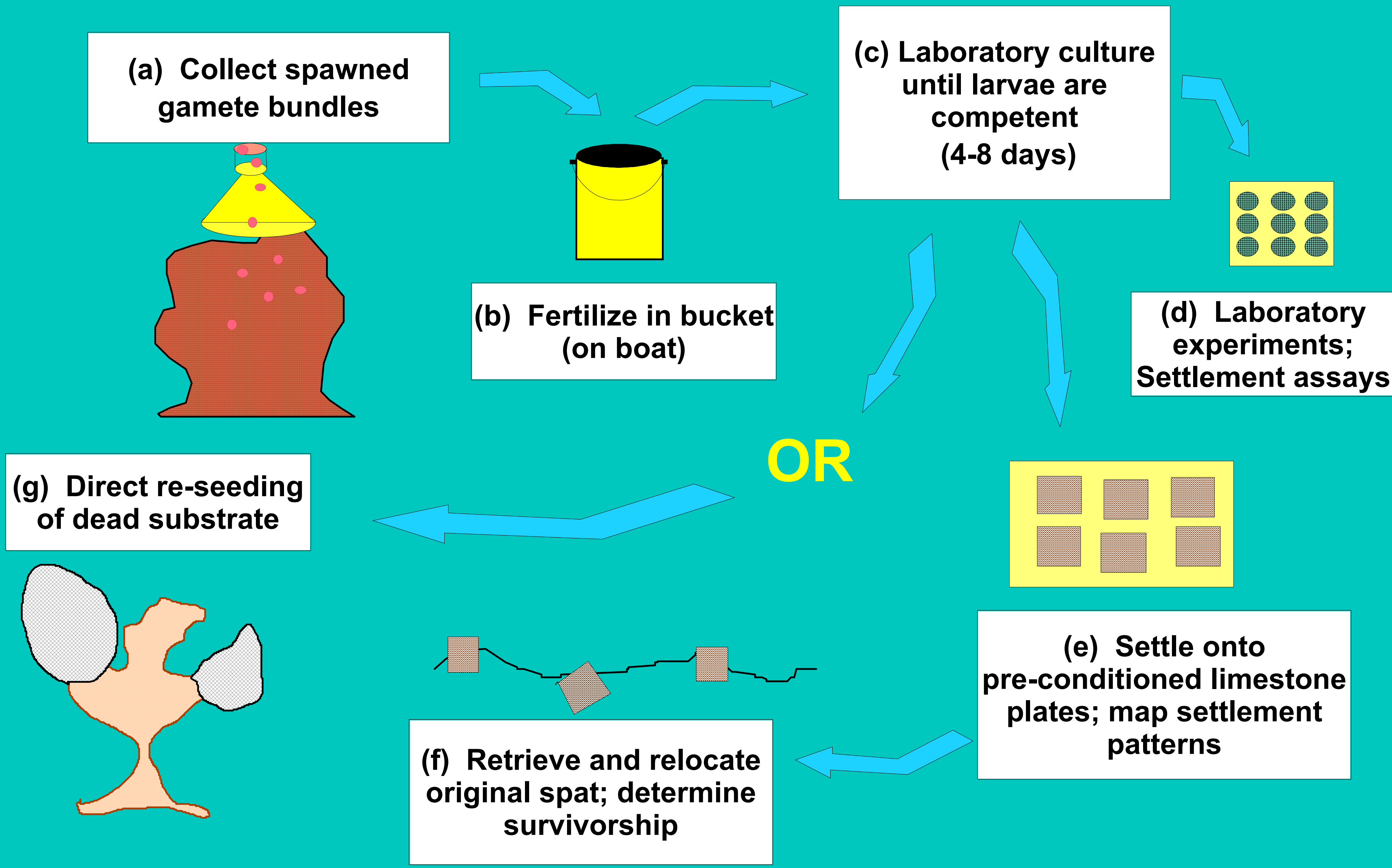
Coral Reproduction 101



Life cycle of a broadcasting reef coral



SUMMARY OF RESEARCH APPROACH



Settlement Behavior, Patterns and Cues

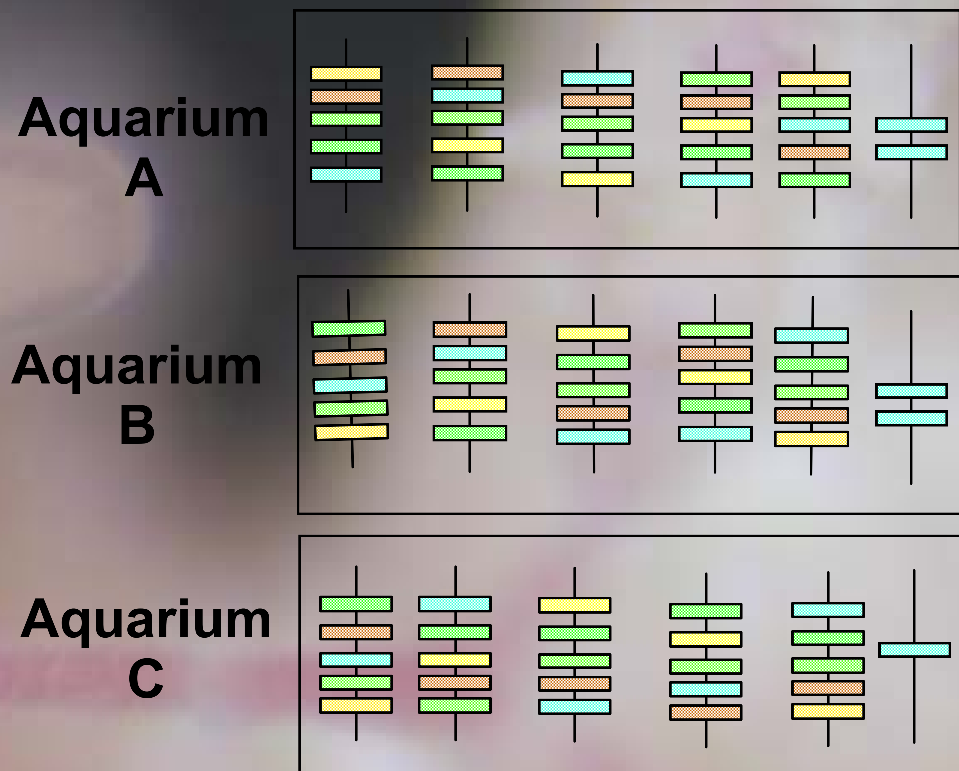
Larvae explore substrate before choosing a settlement site

Experimental Summary:

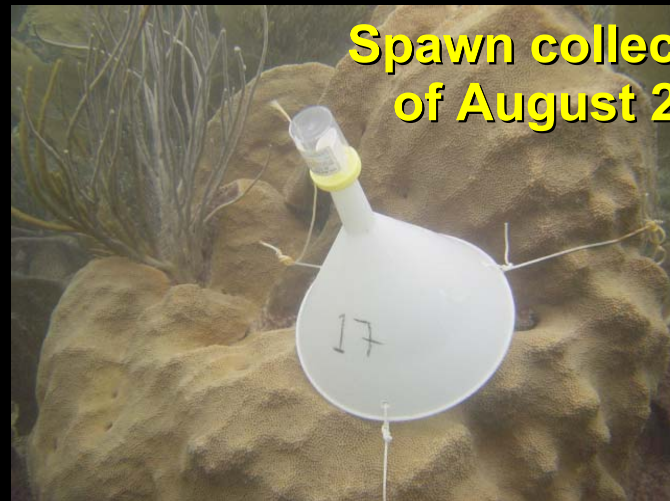
- 3 locations: Pelotas, Turrumote, Buoy
- 2 depths (5 and 10 m) at Pelotas and Turrumote
- 15 plates per depth for Pelotas and Turrumote
- 20 plates at Buoy

Settlement Procedure:

- Total of 80 plates randomly assigned in sets of one plate from each site/depth per rod (5 extras from Buoy on additional rods)
- Therefore 5 plates from each location in each of three aquaria
- 30,000 larvae of Montastraea faveolata added to each aquarium
- Larvae allowed to settle and attach for one week before mapping



Plates put out to condition June 2005

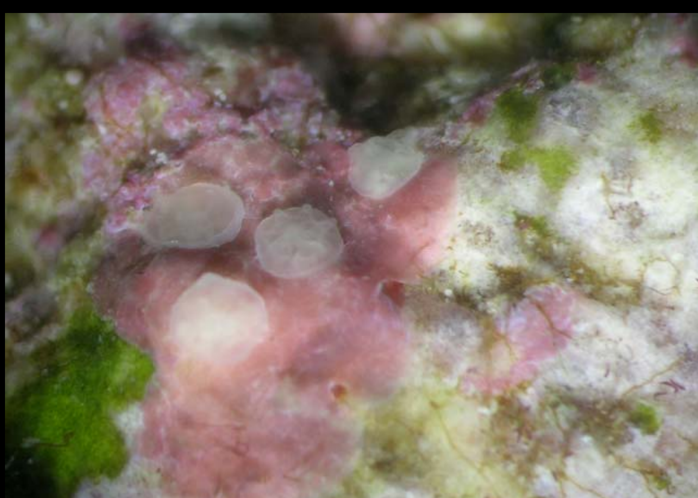


Spawn collected week of August 22, 2005

Plates mapped week of Sept 6

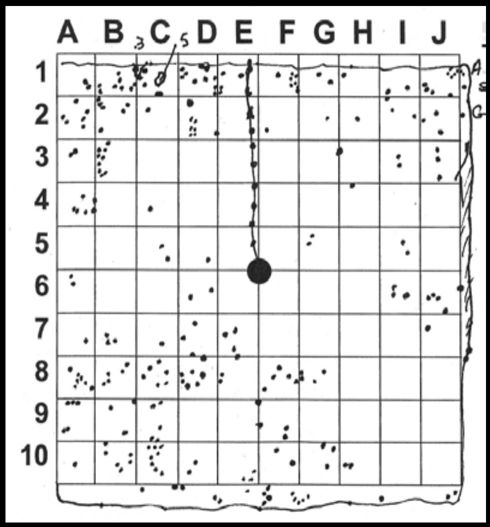
APPROACH

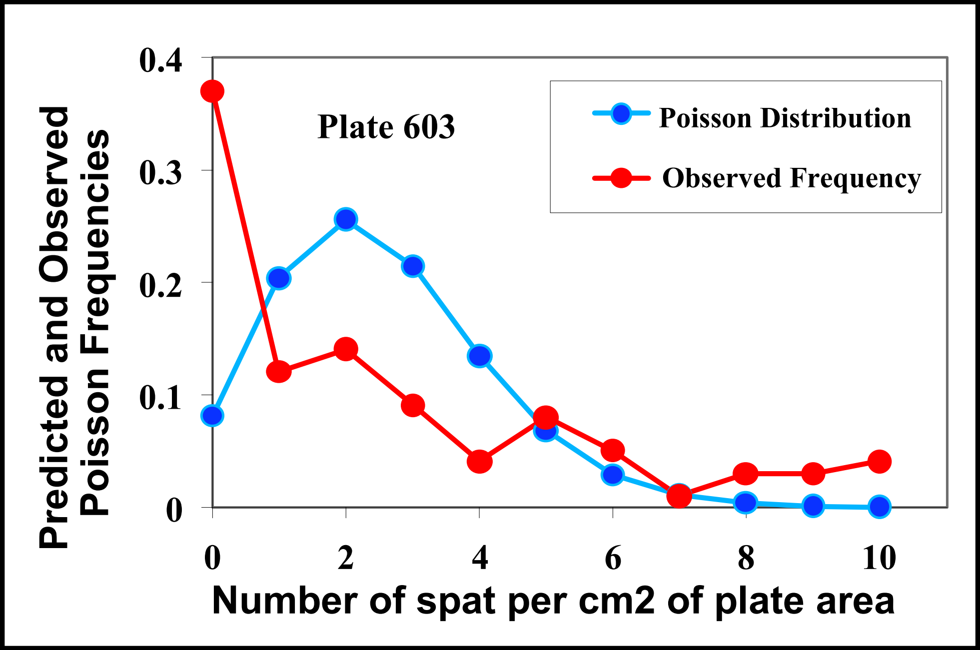
Plates redeployed to reefs week of Sept 10, 2005



One-month Survivorship being measured this week (weather permitting)

Example of a settlement map and statistical analysis of settlement patterns



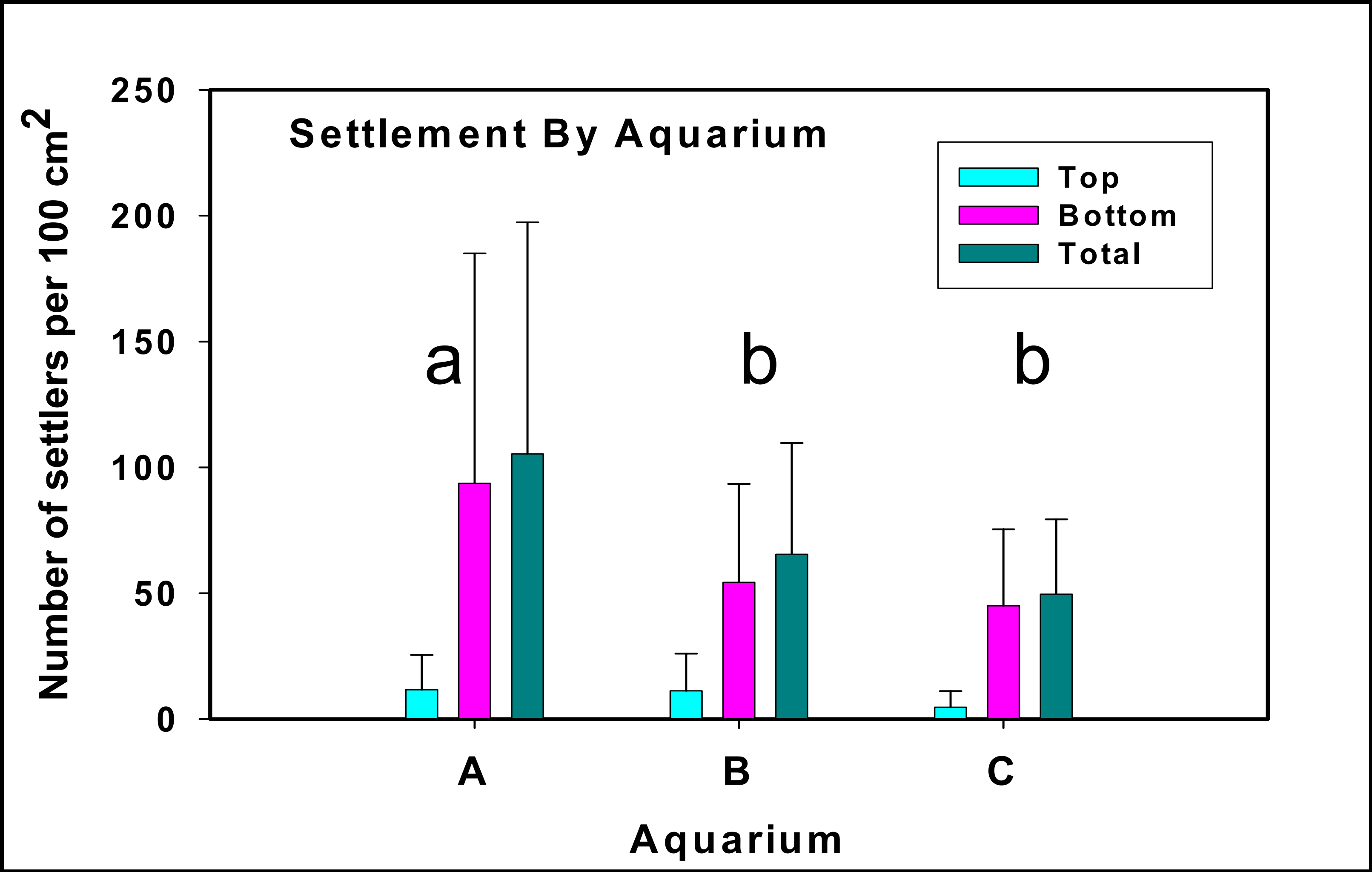


Settlers of Montastraea faveolata have a strongly aggregated pattern:

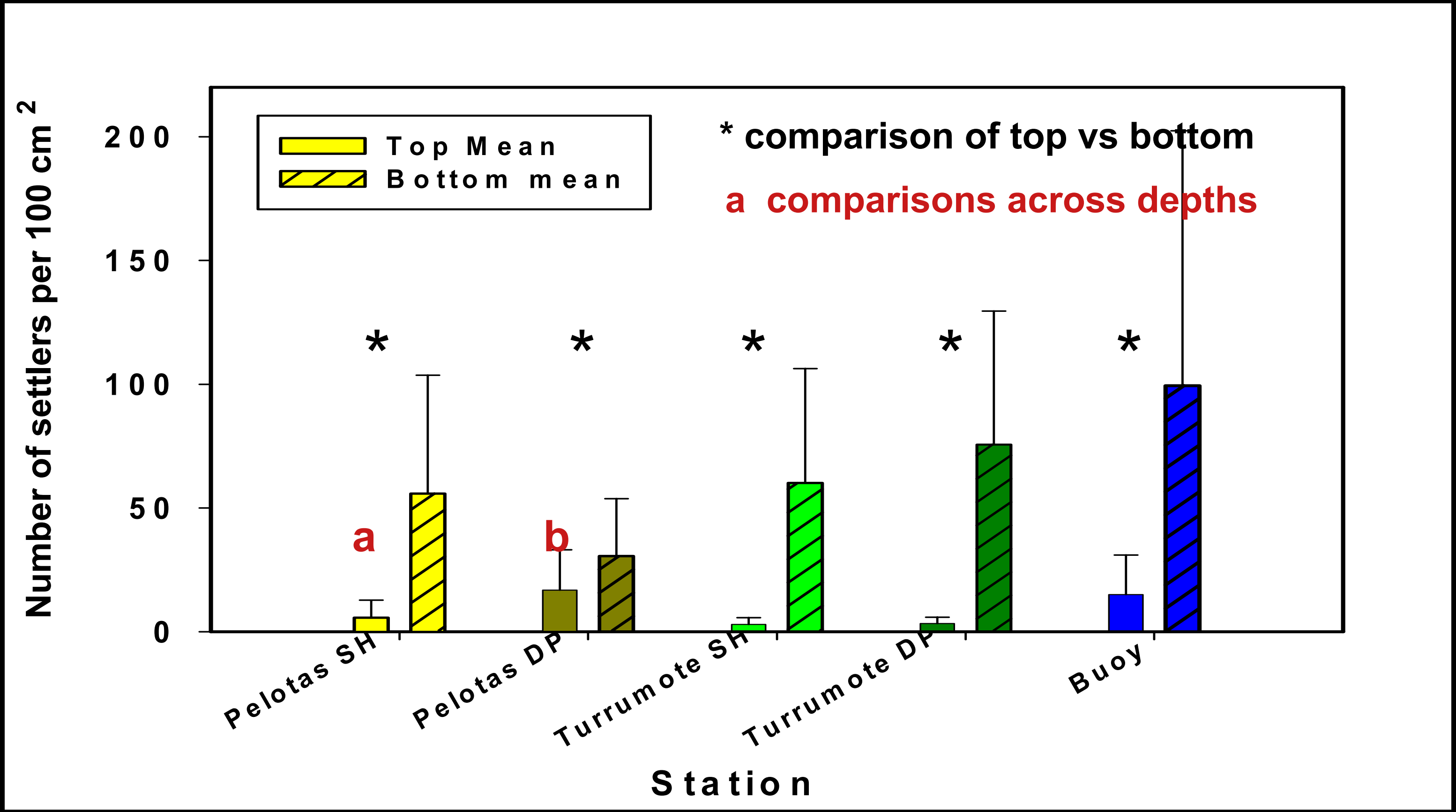
This suggests presence of settlement cues: crustose coralline algae or microbial films?

Larval settlement differed among aquaria:

- on average, plates in aquarium A had more settlers than plates in aquaria B and C
- very high variance among plates in each aquarium, rods, positions on rods and stations

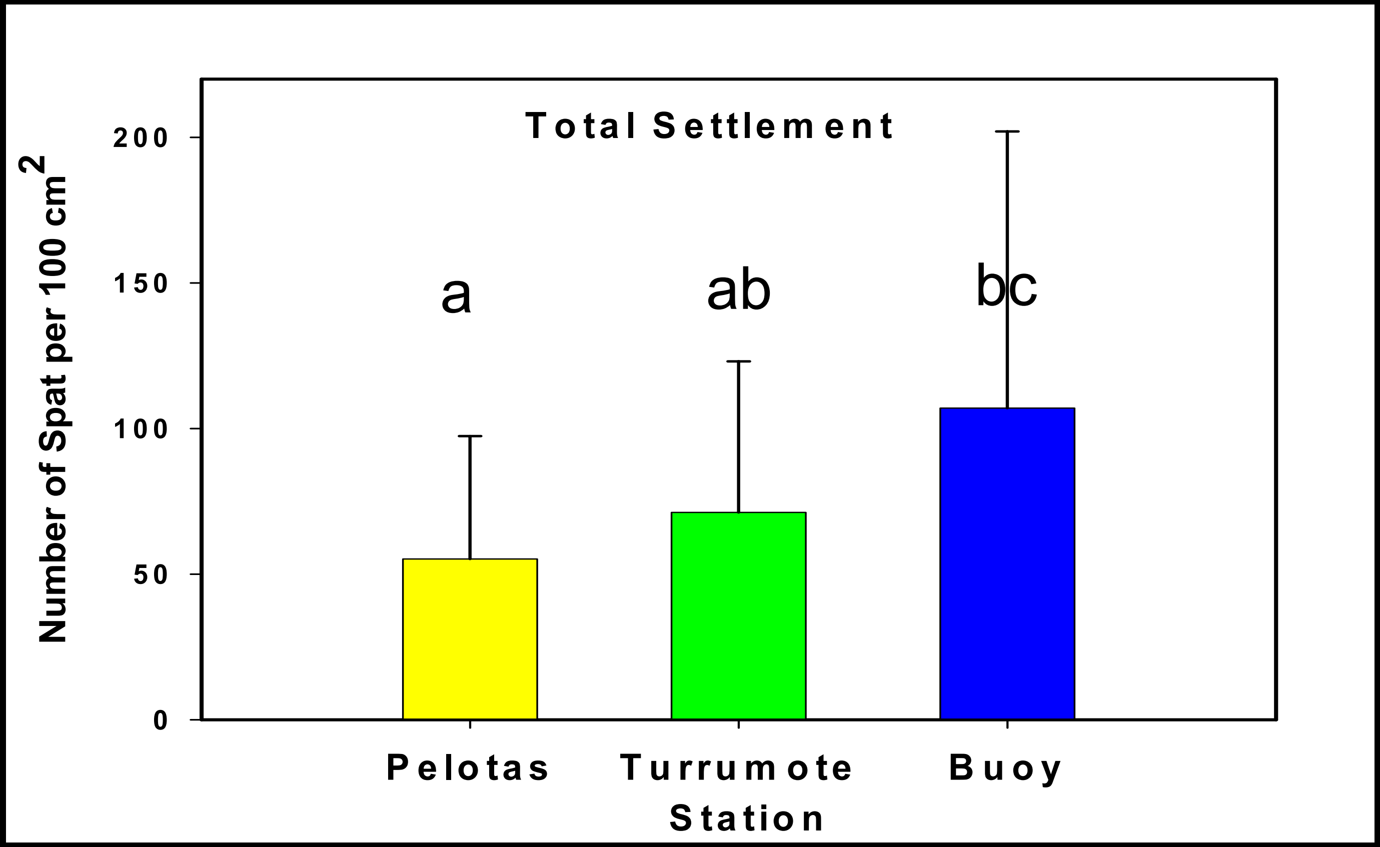


Tops vs Bottoms of Plates at Each Site



- Larval settlement was significantly greater on plate undersides than on tops for all stations (confirming previous findings)
- Pelotas Deep had significantly more settlers on plate tops than the other stations
- There were no differences between depths for Pelotas bottoms or for either surface at Turrumote

Depths Combined



- **Settlement was significantly higher on Buoy plates than on Pelotas plates**
- **Settlement was higher but not significantly different on Turrumote than on Pelotas**
- **Experimental settlement densities were in the order of 5,000 to 10,000 per m^2**

SUMMARY OF PRIMARY OBJECTIVE ACCOMPLISHMENTS

1) We were able to culture large numbers of *Montastraea faveolata* larvae (500K+), but only small numbers of *M. cavernosa*, *Acropora palmata* and *Diploria strigosa*

2) Therefore only *M. faveolata* was used for survivorship study (ca. 5500 *coral spat settled and mapped on 80 settlement plates)*

3) We got good settlement and evidence for an environmental gradient in substrate quality

4) Survivorship measurements are in progress:

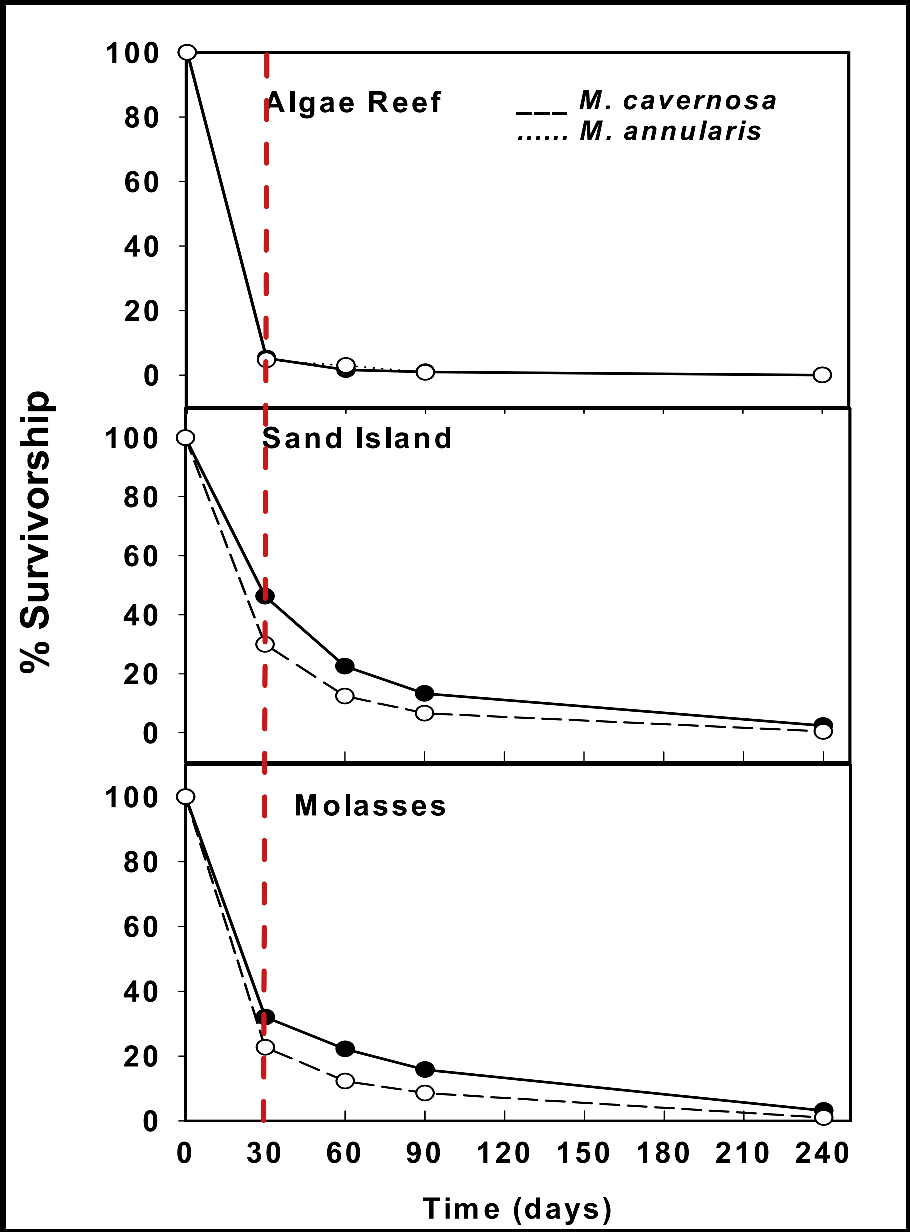
Buoy results after 30 days in the field:

Tops:	**4.7 ± 7.5 %**
Bottoms:	**3.3 ± 3.2 %**
Total:	**3.8 ± 3.5 %**

Final Density = 240 per m^2

5) Larvae of other species were used for experiments listed under the secondary objectives (settlement preferences and larval behavior), and an NSF funded genomics project

For Comparison: 2004 Survivorship in Florida Keys



mid-shore

offshore
low relief

offshore
high relief

Future Directions

- Complete analysis of substrate characteristics of plates from various sites (Aihnoa Leon; photoanalysis)
- Focus in on factors affecting substrate community structure that supports greater coral settlement (bottoms vs top phenomenon; inshore vs offshore
- Multifactorial experiment in which we vary environmental conditions (water flow; light; grazing) to which settlement plates are exposed during aging period
- More work on early survivorship and polyp feeding

SUMMARY OF PROBLEMS ENCOUNTERED

1) The seawater system has inadequate water pressure, and too long a residence time in the storage tank

2) Ambient and seawater temperatures were stressfully warm in our outside work area (> 31 oC)

3) Rainwater leaked through holes in roof into our cultures and killed them

4*)* We need to use a bigger support vessel to handle uncooperative weather conditions during spawn collection nights

For next year, we need a place to work where temperatures can be maintained < 30 oC, and with sufficient water supply

Research Objectives Summer 2005:

SECONDARY:

- Continue research on settlement cues and preferences
- Begin work on effects of feeding on survivorship
- Continue research on larval behavior
- Capacity building in this research topic

Coral settlement cues: Summary

Maggy Nugues UNCW

Background

- Coral larvae have been shown to settle in response to crustose coralline algae (CCA) (Morse et al.) and bacteria isolated from CCA (Negri et al).
- Many of the results have not been reproducible.
- We still have very little information on the natural inducers and associated chemicals driving settlement in Caribbean corals.

Research Objectives

Testing coral settlement response to:

1. **Extracts from several species of CCA**
2. **Bacteria isolated from the same CCA**
3. **Preconditioned glass slides (biofilm)**

Methods

1. Extracts preparation: Acetone, methanol and methylene-chloride extracts from three CCAs and one encrusting red alga
2. Bacterial isolation: Bacteria were isolated from fresh samples of CCA chips that induced coral settlement in Curaçao
3. Biofilm: Glass microscope slides were conditioned at Turrumote Reef for three weeks to acquire a biofilm
4. Bioassays: Assays were run in 6-well plates. Extracts were impregnated onto boileezers; isolated bacteria were cultured onto autoclaved limestone chips. One granule or chip plus 6-10 larvae were added to each well. Controls included control granule or chip and live CCAs. N = 3 replicates

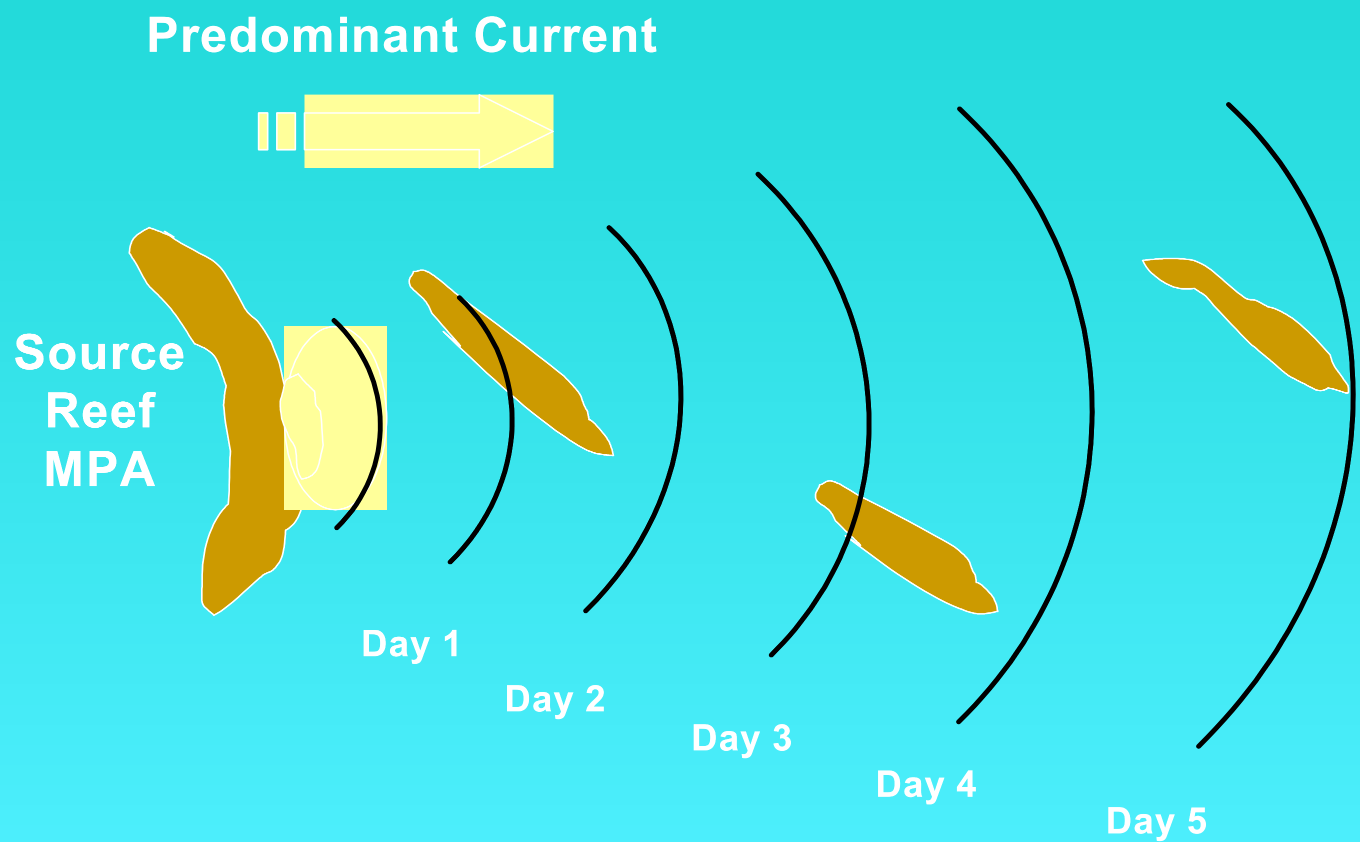
Results and Discussion

- None of the extracts induced settlement in 8-days old *Montastraea cavernosa* larvae.
- None of the bacterial films induced settlement in *M. cavernosa, M. faveolata* or *Favia fragrum*.
- Preconditioned microscopic slides induced 20 to 50 % settlement in 1- to 4-days old *F. fragrum* larvae (NB: clean slides did not).
- Microscopic observations of these slides revealed a diverse community of diatoms, blue-greens, filamentous algae and CCA. Microbial and algal community structure is currently being analysed using molecular techniques.

Future Directions

- Follow the successional development of the microbial and algal communities on the glass slides to determine when they begin to have larval induction properties.
- Test preconditioned slides exposed to specific inhibitors that will selectively modify the existing communities (e.g. antibiotics, GeO2).
- Identify, culture and test algae present on preconditioned slides.

Coral Connectivity: A function of larval development and behavior patterns



- Gametes are released as bundles of eggs and sperm
- Eggs are full of lipid
- Bundles rise to sea surface where they break up and form large slicks
- Eggs are fertilized in surface layer where they float around while they develop into planula larvae

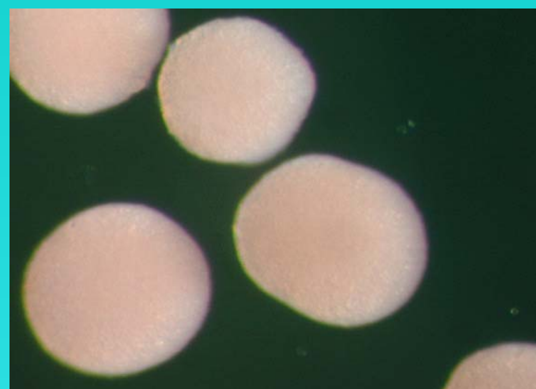
Q: How long can larvae be treated as passive particles?

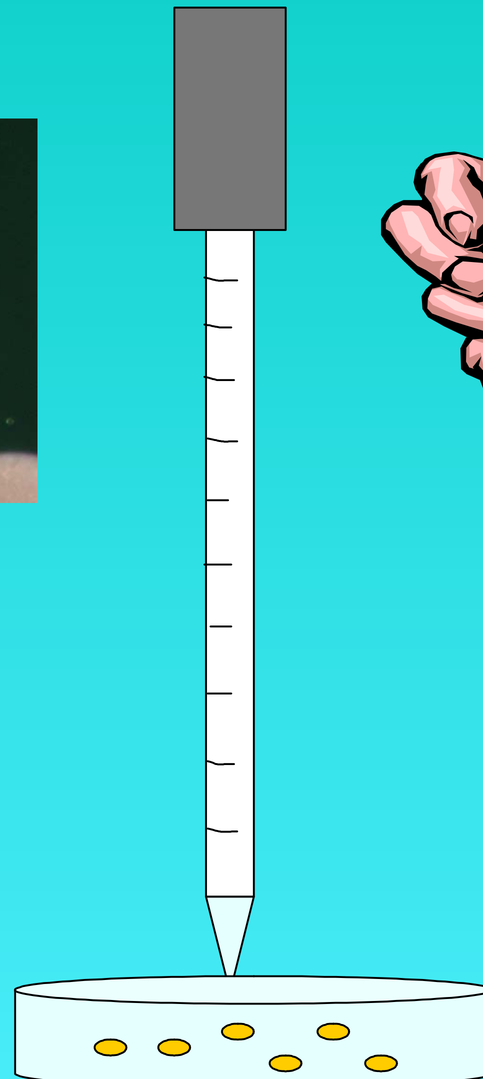
WE NEED TO KNOW:
Larval behavior over time

- Time-course of 'particle behavior'**
- Development of swimming ability**
- Time course of reaching competency

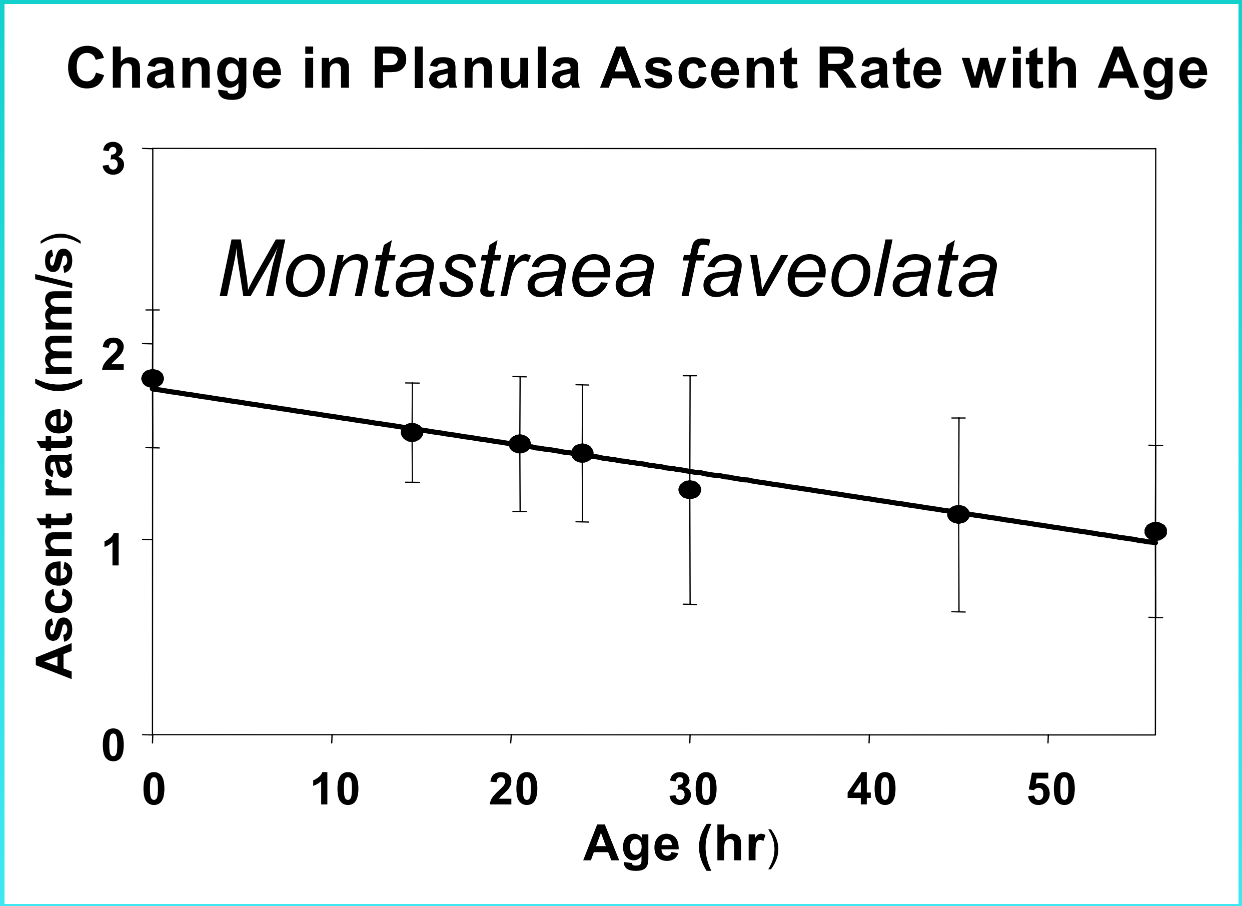
Miguel A. Ruiz Zarate and Aihnoa Leon Zubillaga worked on these topics with *Diploria strigosa*

Buoyancy Measurement Apparatus





Buoyancy decreases as embryos use up lipid reserves

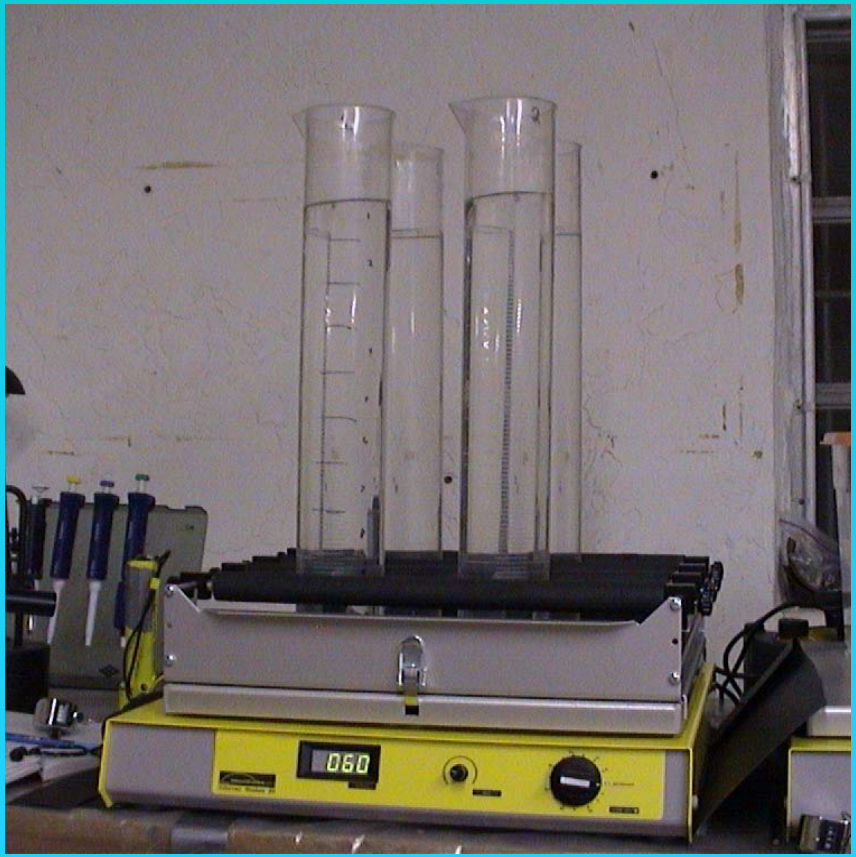


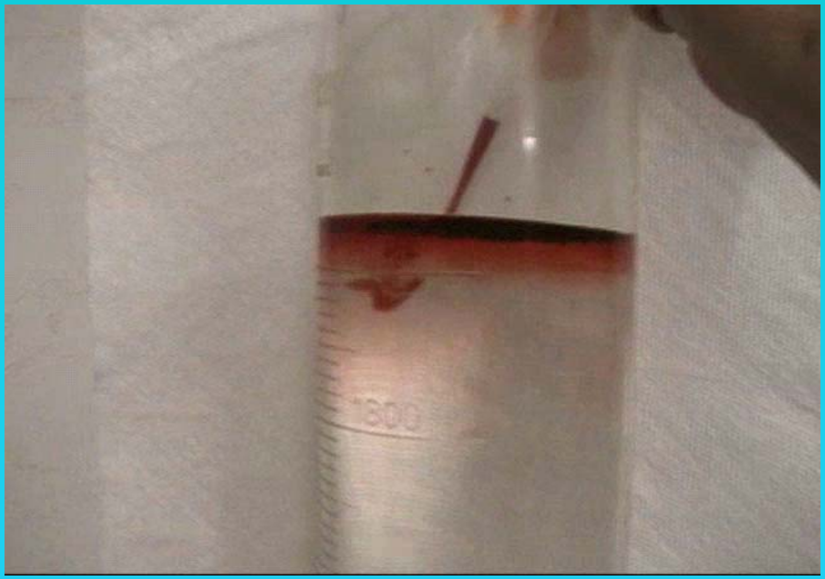
- **<u>56 hrs:</u> 20% neutrally buoyant or swimming**
- **<u>63.5 hrs:</u> 67% neutral or swimming**
- **<u>70 hrs:</u> 73% neutral or swimming**
- **<u>78 hrs:</u> 100% swimming**

In 2004: Similar results for *M. annularis* and *M. cavernosa,* but *Acropora palmata* **took longer to swim**

Question 2: When do planulae begin to exhibit significant downwards swimming behaviors?

EXPERIMENTAL APPARATUS: Vertical mixing in system is weak; not enough to mix dye or particles downwards

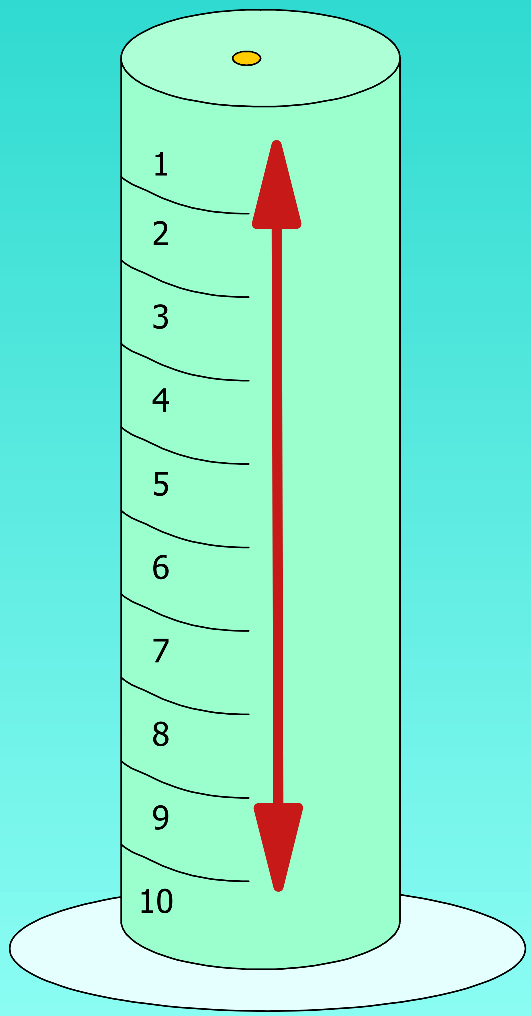




We covered shakers at night to keep system in the dark

Eventually larvae develop ability to swim and over-come any residual buoyancy

Vertical Movement Pattern

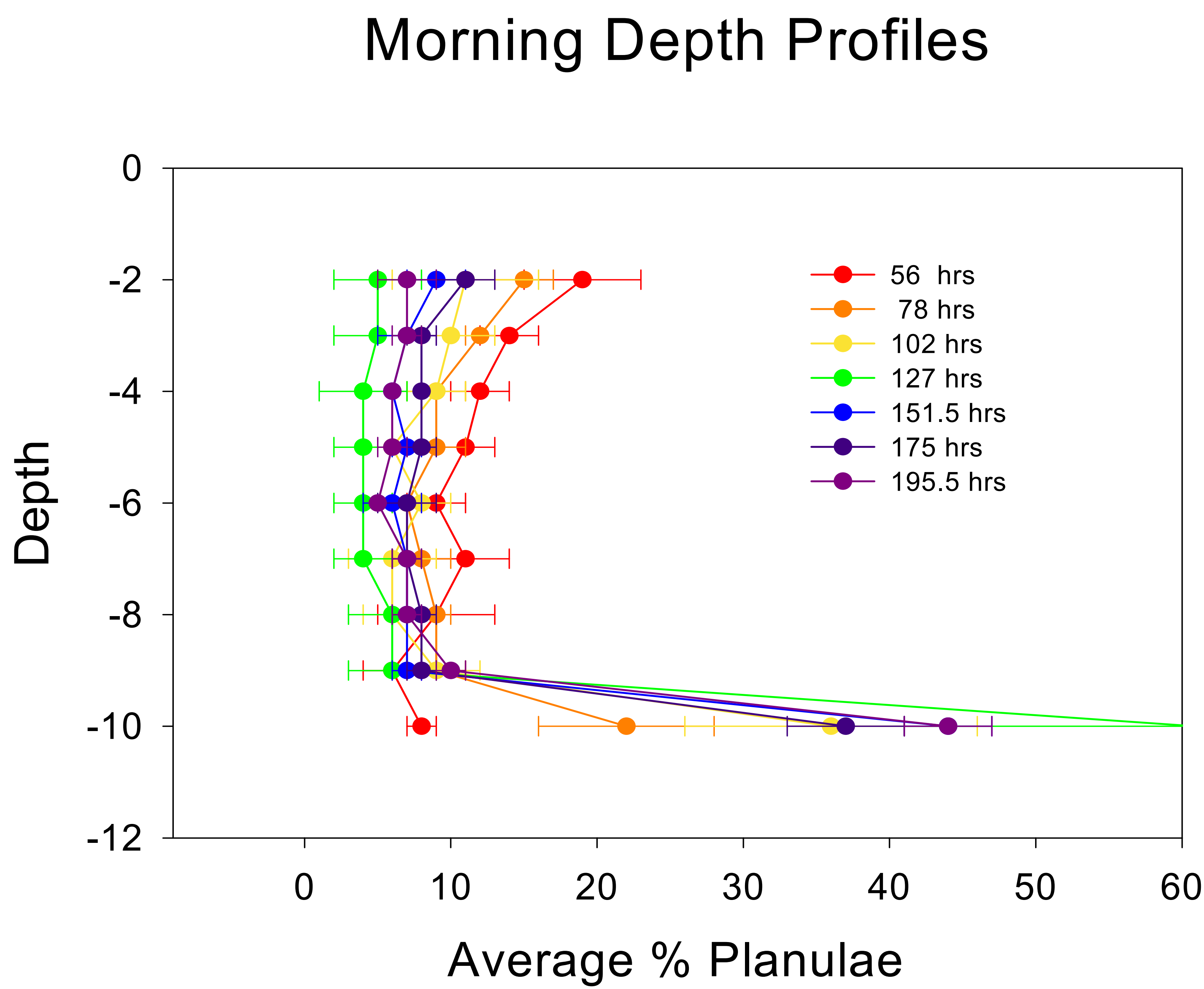


Days 1-4

Day 5 Dark

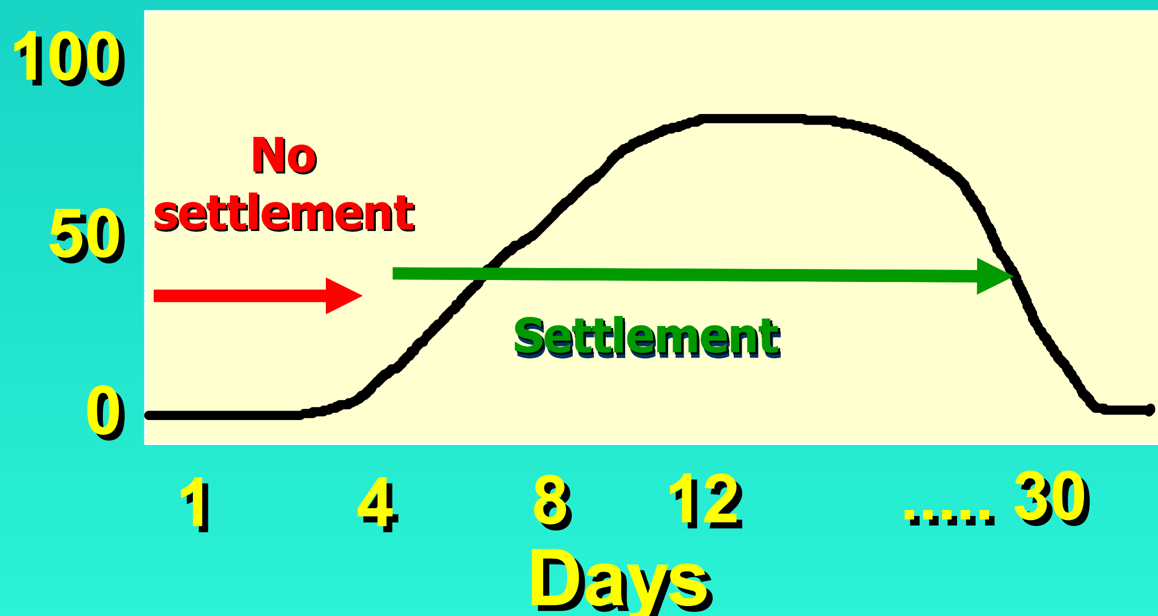
Day 5 Light

Day 6 Dark


Morning Depth Profiles
Depth
Average % Planulae
0
-2
-4
-6
-8
-10
-12
0
10
20
30
40
50
60
56 hrs
78 hrs
102 hrs
127 hrs
151.5 hrs
175 hrs
195.5 hrs

CONCLUSIONS WITH REGARD TO POTENTIAL DISPERSAL OF *Montastraea faveolata*

- Dispersal distance from source reef will depend on weather, local hydrography and distribution of reefs



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Dr. Weil and Szmant's groups celebrating the end of spawning and plate reading!