PANAMEX: Pan American Experimental Initiative
Caging Experiment Protocol

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1. Objectives:

To assess latitudinal patterns in key demographic processes and community interactions, including recruitment, assemblage composition, and the influence of predation pressure on the development of fouling communities.

Settling plates serve as passive collectors for colonization of marine organisms, providing an easy and standardized method to assess the presence of key species and development of benthic communities. With your help, we will carry out a settling plate experiment at a series of locations throughout the Americas representing a (nearly) pole-to-pole latitudinal gradient in Atlantic and Pacific Oceans. We will assess changes in assemblage over time (repeated measures) and manipulate predation pressure using exclusion cages.

2. Hypotheses

1. Recruitment, organism growth, and percent cover will increase at a faster rate toward the equator
2. Assemblage diversity (richness & evenness) will be higher toward the equator
3. Predation pressure will be more intense, reducing biomass to a greater extent, toward the equator

3. Experimental design

At each location, partners will deploy 8 replicates of 4 treatments (32 panels total):

1. Control (open-panel)
2. Caged panel
3. Cage-control (half cage over panel)
4. Exposed caged panel (panel caged for duration then exposed (open) at the end to observe predator visitation and effect on mature community)

4. Site Selection:

Look for a nearby marina with floating pontoons that are ideally wooden sided (can hammer bent-nails into them), and not too high from the water. Sites should be at least 2m deep at low tide and marine, with minimal fresh-water influence (>25ppt). If you do not have access to such a site please contact us to discuss alternatives.

A straight pontoon of ~80m is ideal for deploying the plates (n=40) in a straight line with at least 2m in between adjacent plates. (40m can also work, with plates being deployed on either side)
Some areas require a collection permit to sample (collect) any marine life. This requires a brief explanation of the purpose and methods to be used. Permits are usually renewable on an annual or biennial basis, upon receipt of a brief report of past activity.

5. Timing:

For the northern hemisphere, the plates will be deployed on or about the 15th June and retrieved 3 months later (~15th September).

Every 2 weeks, cages are exchanged and photographs of each panel are taken. The cages that are removed will be cleaned for replacement at the next time period (2 full sets of cages will be provided, with only 1 set being in use at any time).

Time (weeks)	Activity	Photographs	Exchange cages	Wet weight without brick
0	Deploy experiment			
2		X	X	
4		X	X	
6		X	X	
8		X	X	
10	Do not replace exposure cages ****More details** **to follow****	X	X	
12	Retrieve all plates and equipment ****More details** **to follow****	X	X	X

6. Equipment

Plates are 15 × 15 cm and made of PVC. Plates are suspended horizontally with the experimental surface face down, 1m below the water-surface for a total deployment of 3 months.

Provided by SERC:

- 32 sanded PVC panels (+ 3 spares)
- 32 short + 32 long lengths of line (+ 3 spares)
- 40 Bent nails (fence staples)
- 48 snap-clips (2 per cage)
- Cable ties – short & long (5-6 per panel)
- 48 cage tops (2 sets of: 16 for Cage & Cage-Exposure; 8 for Cage-Control)
- 32 cage bottoms
- HOBO datalogger (set to record @ 30min interval)
- Photo-labels
- Deployment, monitoring & retrieval datasheets

Provided by site partner:

- 20 Cored bricks (broken in half using hammer)
- Hammer
- Camera
- Salinity probe/YSI
- Secchi disc
- Clipboard
- Clippers/Scissors
- Sharpie marker pen
- Battery-powered weighing scales (kitchen/mail-room quality)

7. Assembling the settlement plates

Step A: Lace a smaller (yellow in figures) cable tie from top of one hole in the plate, under (sanded) bottom of plate and up through second hole on the SAME side. Close cable tie so there is a loose loop. See Figure A.

Step B: Repeat on other side of plate.

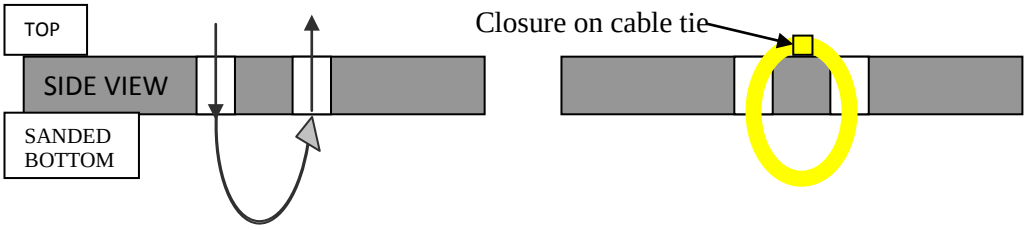


Figure A. Side views of PVC Plate Construction

Step C: Place half brick in middle of plate on top (smooth) side so the cable tie loops are on the sides of brick.

Step D: Lace a large cable tie (red in Figure B) through one loop, through the hole in the brick, through the other loop, around the side of the brick and back through the hole to the other side to close cable tie.

Step E: Tighten all cable ties.

Step F: Loop 2 large cable ties through the hole in the brick and for 24 bricks, through a snap clip on the top side (Figure B). The remaining 8 bricks will be Controls- and do not need the snap-clip.

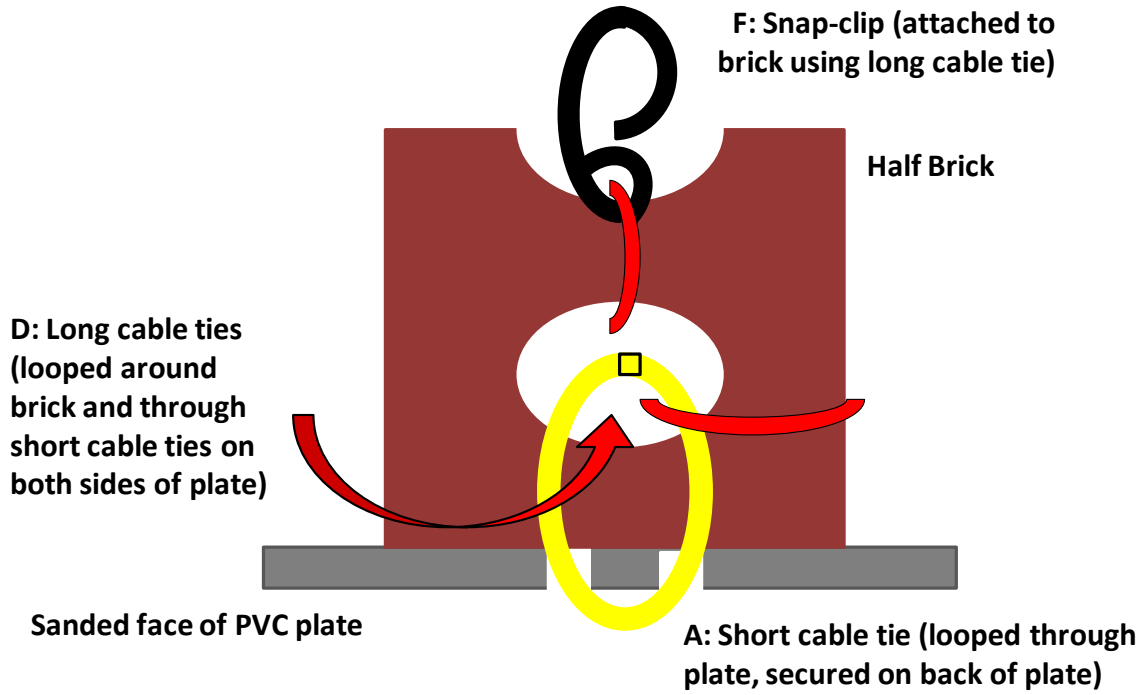
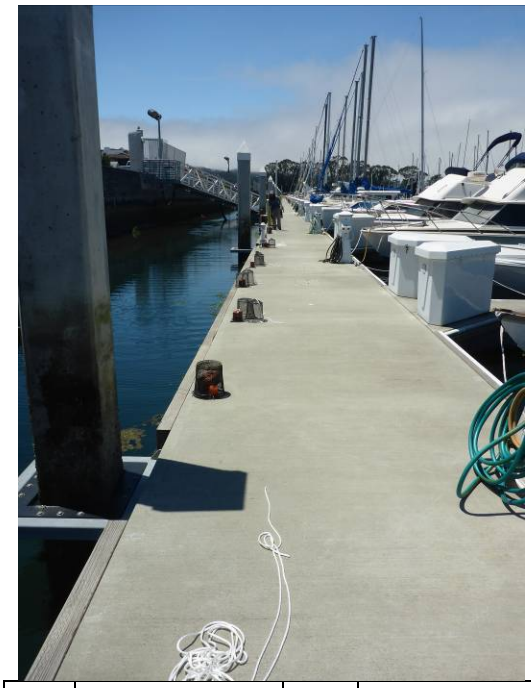


Figure B. Side views of brick attachment to PVC plate

8. Deployment

Settlement plates should be assembled to bricks prior to deployment. Having chosen the ~80m length of dock, hammer a bent nail into each location where a plate is to be hung. We recommend the front corners or backsides of boat slips- i.e. trying to avoid interference with docking boats. Plates should be spaced at least 2m apart. Lay out the plates & cages in the order they are to be deployed, and **label the back of each plate** using a black sharpie. Label opposite top & bottom corners- which will be used to identify the orientation of the plate for photographs.

Cages laid out in order along the dock for deployment in San Francisco



Deploy the panels in the below order (also included on waterproof paper). Attach the **datalogger** to the back of Panel 16 using an extra cable tie (***). An example from San Francisco of the plate locations is also shown.

#	**Treatment**	#	**Treatment**	#	**Treatment**	#	**Treatment**
1	Half cage	9	Cage	17	Control	25	Half cage
2	Cage (exposure)	10	Half cage	18	Cage	26	Control
3	Control	11	Cage (exposure)	19	Half cage	27	Cage (exposure)
4	Cage	12	Control	20	Cage (exposure)	28	Cage
5	Half cage	13	Cage (exposure)	21	Control	29	Cage
6	Cage (exposure)	14	Control	22	Cage	30	Cage (exposure)
7	Control	15	Cage	23	Half cage	31	Control
8	Cage	16	Half cage***	24	Cage (exposure)	32	Half cage

*1 Half-cage	*3 Control	*5 Half cage	*7 Control
*2 Cage (exposure)	*4 Cage	*6 Cage (exposure)	*8 Cage...

Example of deployment layout at San Francisco Marina. A straight dock length of >100m where panels can be deployed away from moving boats was selected. The first plate is not at the far right corner of the dock because the water depth is <2m at low tide there. The plates are deployed >2m apart. Bent nails are hammered into the dock at the asterisks.

To assemble the cages:

1. Secure plates inside the cage tops using the clip that's attached to the brick to clip to the loop that's hanging inside the cage top (x24) make sure the plates are labelled.
2. Attached the cage bottoms and secure closed using the safety pins (x16)
3. Tie Control plates to the longer lengths of lines using a **bowline** knot (www.netknots.com/rope_knots/bowline) (x8)
4. Attach the header line to the bent nail using a bowline knot (x32)
5. Adjust the length of the line attached to the dock (dockline) so that the panel will be suspended 1m below the water surface.
6. Lower plates into the water. Ensure that the bricks are pulled to the top of the cage tops (The plates may get stuck on the sides & need a quick shake.)

Plate clipped inside a cage top (left & middle) then secured onto a cage bottom (right).

Safety pin closure mechanism (only the short loop goes through the cage).

9. Two-week sampling

Every two weeks the cages will be exchanged for clean ones and a photograph of each plate will be taken. Image quality is paramount to the success of this study.

A. Environmental Data

Measure the temperature and salinity at 1m water depth at both ends of the dock (or at multiple docks if plates are spread out).

B. Cage removal

To remove the cage, bring the cage (half-cage or control panel) onto the dockside and lay it on its side- being careful not to drop the plate to the bottom/sides of the cage. Open the cage (if present) & slide the plate out of the cage, being careful not to touch/scrape the experimental surface (sanded side of the plate). At this point the cage can be unclipped from the dockline.

C. Photographing panels

Make sure the number on the back of the panel is the next number in succession and the correct treatment. (If not, one may be missing, make note of this.) Take a photograph of the panel with the appropriate plate-label in the foreground. Take a second photograph of just the panel. These photographs will be used to identify any organisms settling on the panel to the best of our ability. Take this into consideration when assessing the quality of the photo. The photo should be level to the panel with the surface area of the panel taking up most of the image and the bulk of organisms being in focus. As a guide, we suggest you have the camera ~30cm away from the panel. Do not use a digital zoom, move the camera towards/away from the panel. Multiple photographs of the same panel can be taken- see Picture Pointers at the end of this document.

Upload the images to the Dropbox for review by SERC scientists.

D. Replacing cages

Pass the line through the top of the new cage, draw the panel inside the cage & replace the bottom half of the cage (for a full-cage treatment). Clip the line from the top of the cage to the dockline & gently lower the panel back into the water.

E. Cleaning cages

Anytime within the 2 week window, the ‘old’ cages will need to be cleaned with a plastic scrubbing brush. Depending on the fouling you encounter, drying the cage prior to cleaning may be helpful (or not). If you encounter hard encrusting organisms (barnacles, bryozoans etc), ensure that all pieces of material are removed from the cage.

10. Penultimate sample point

At this time point (after 10 weeks), Cage-exposure panels will be exposed for the subsequent 2-week period.

**********GoPro Deployment**********

**********SquidPop Deployment**********

**********More details to follow**********

11. Final sample point

After 12 weeks in situ, all panels will be retrieved, photographed & analysed. The number on the back of the panels should still be in place, but just in case, keep the laminated panel label with the panel from this point so that it can be easily determined.

**********Metagenetics Sampling**********

**********More details to follow**********

Field Data

Take a photograph of the panel as described above.
Remove the panel from its brick by carefully clipping the two small cable-ties from the backside.
Place the panel in a plastic tub & take several photographs of the panel completely submerged. Place the panel in a zip-lock bag for return to the lab.
Collect the temperature logger. (Please send this back to SERC)

Lab Data

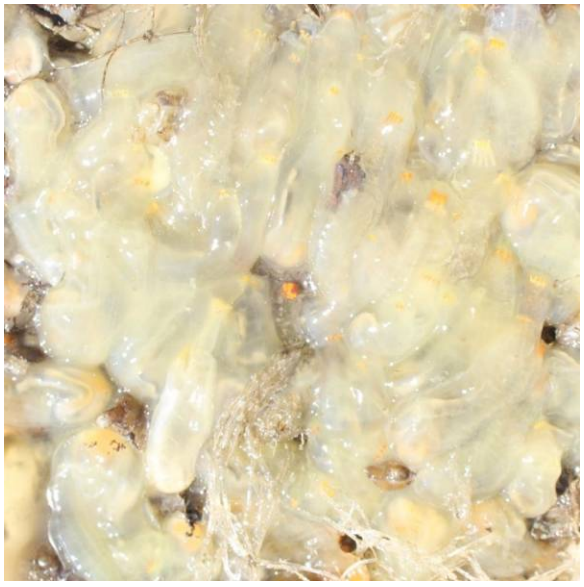
Wet weight: Place the whole plate onto a kitchen weighing scale. This can also be done in the field if the team is not taking any measurements at the lab.

PICTURE POINTERS

Taking pictures is the most important part of this project. Though an SLR camera takes very high quality pictures, cameras on most cell phones are more than adequate for this task!

- **Take pictures of the settlement plates OUTSIDE.** If it is too bright or there is a glare, take pictures in the shade or position your body to cast a shadow over the plate. Never take plate pictures under fluorescent lighting and take extra precaution to avoid "glare spots".
- **When in doubt, shade the plate when taking a picture.** When sunlight is diffused by clouds, trees, or buildings, there should still be plenty of ambient light from the sky to light the plate.
- **Take pictures of the plates both IN and OUT of the water** (in water using an appropriate sized bucket so that the surface of the panel & any organisms are submerged). By doing this, you give those using the pictures more options when analyzing photographs.
- **Do NOT rely on the cameras "Image Preview" to determine if you took a good picture.** Pictures that appear clear and crisp on the cameras preview setting may not appear the same way once downloaded. To be sure you are taking the best pictures possible, take multiple pictures of the same plate and regularly upload them to a desktop computer.
- **If something looks interesting on the plate, take a close-up picture of the object!**

Examples of BAD plate pictures:



Over-exposed with glare.
Unable to zoom in.

Glare on top of the water.
Organisms are in and out of water.
Unable to zoom in.

Examples of a GOOD plate picture:

Photo 1: Label clearly identifies plate.

Photo 2: Great resolution.
Able to zoom in to see smaller organisms.