PANAMEX: Pan American Experimental Initiative:

Additional (opt-in) Protocols

Additional details for weeks 10 & 12

Dear Colleagues,

Thank you for your time & contributions so far. We have the predation experiment deployed at 19 sites in the northern hemisphere! As we near week 10 we have finalised the additional details for activities to be conducted during the last two time points. The value of the experiment to all will be maximized if you are able to opt in to as many of the 4 options below as possible. A brief summary of the 4 tasks is provided below, with further details on following pages. Once you opt in, we will send out supplies ahead of your week 10 and 12 sampling dates. The activities below are in addition to the wet weight measurements that are already required in the protocols (wet weights without bricks, measured on the dock).

Please email Dr Gail Ashton (ashtong@si.edu) to confirm your ability to perform these extra tasks at your site.

Week 10

1) **Predator assessment** (GoPro Deployment)

Aim: Visual quantification and identification of predators
Time estimate: Regular field time + 20 mins
Overview: Attach a GoPro to 2 of the exposure treatments whilst recording week 10 photos

2) **Predator activity** (SquidPops)

Aim: Alternative measure of predation in the vicinity of the panels
Time estimate: Regular field time + 1.5hrs
Overview: Attach squid bait assemblies (to be provided) to the ropes above the cages and monitor after 1 hr to quantify predator strikes in the area

Week 12

3) **Fouling community composition** (Point Counts in the lab)

Aim: Live assessment of community settled on panels
Time estimate: 4hrs
Overview: Taxonomic analysis of species cover at 25 points on each panel.

3b) **Fouling community composition** (Point Counts in the field—if you have limited capacity to transport panels)

4) **High-resolution biodiversity surveys** (Sample collection for Metagenetics)

Aim: Collect samples for metagenetic barcoding
Time estimate: 2 people, 3 days in the lab (multiple trips to the field will be necessary)
Overview: Biological community from each panel will be scraped from the panel, blended, and preserved for metagenetic analyses.

- **If you are able and willing to take some metagenetics samples, but not able to dedicate the personpower necessary for all samples, please let us know and we will help to prioritise the samples.**

Predator assessment (GoPro Deployment)

You provide:

GoPro (3 or later)

Waterproof housing

GoPro bolt

Internal battery (charged)

External battery (charged; battery 'backpack')

64GB microSD card (empty)

Timer/watch

We provide (in original shipment):

Aluminum bar cage attachment

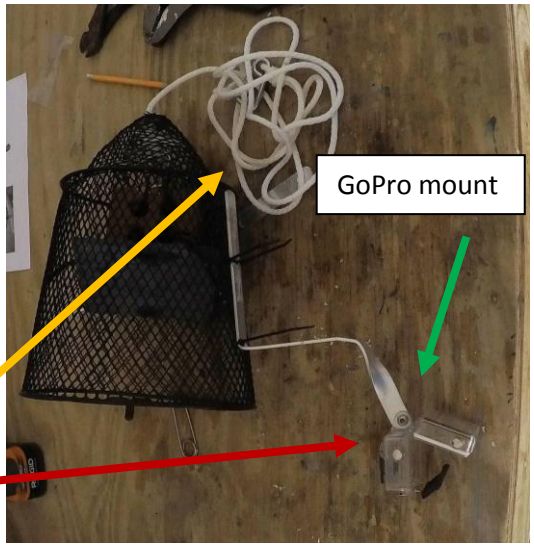
Cable ties

Protocol:

Before field day

Charge both batteries & format SD card.

Set GoPro to record **1 frame per second on timelapse** 12Megapixel Wide. Assemble GoPro in waterproof housing & attach to aluminum bar using bolt provided with GoPro. Attach aluminum bar to the outside of a clean cage top using 2 cable ties. One end of the aluminum should be in line with the upper loop of the cage. The GoPro should be pointed back towards the opening of the cage- where the panel will hang (Figure to the right).



In the field

- When you get to the first 'Cage Exposure' panel (should be panel #2), take the photos of that panel as you have previously.
- Clip the panel inside the cage top with the GoPro attached to the outside (prepared the day before); press Go on the GoPro & re-deploy the panel (without a cage bottom).
- Set a timer for 2hrs.
- Leave the GoPro in place & continue taking photos of the next panels.
- With subsequent Cage Exposure panels (#6, #11, #13...), do not place a bottom on the cage after recording panel photos—the panel should remain exposed for the last 2 weeks.
- When the timer goes off, remove the GoPro from its initial panel and stop recording.
- Attach the GoPro to a new cage top using new cable ties.
- When you get to the next Cage Exposure panel, after taking photos of that panel, use the cage top with the GoPro attached to deploy the panel (without a cage bottom). Press Go on deployment & start the timer for 2hrs.
- Continue taking photos of the rest of the panels (leaving all Cage Exposures open)
- When the timer goes off, retrieve the GoPro.

Predator activity (Squidpops)

You provide:

We provide:

Cable ties

Fishing line with a piece of squid attached

Electrical tape

Protocol:

In the field

- At the first panel, once you have taken the photo and are ready to redeploy, attach the cable tie above the knot in the line & close it tight on the line.
- Attach the loop in the fishing line to the end of the cable tie using electrical tape, so that it is held away from the line and can move independently.
- Note the time on the datasheet. (Set another timer if desired)
- Repeat this procedure with all subsequent panels, photographing & attaching squid as you go.
- One hour after the first squidpop deployment, return to the first panel & score the squid as either present, partially missing, or absent (entirely missing).

- **Fouling community composition** (Point Counts in the lab/field)

You provide:	**We provide:**
1 Gallon Ziploc bags	Point count grid
Microscope	Data sheets
Tray/container large enough to hold panel under microscope	
Cooler for transport of the panels	
Buckets for transport of water	
Weighing scale (kitchen/similar)	

Protocol:

In the field

- Retrieve the first panel, scrape the back and sides of the panel so only the organisms growing on the front (downward facing) side remain.
- Place the panel on the weighing scale & record the wet weight.
- Place the panel in a Ziploc bag with some seawater and the photograph label so that you can identify which panel is which. ***
- Place the plate, in its Ziploc bag, in the cooler for return (gently) to the lab
- Repeat for all remaining panels

In the lab

- Place the first panel in a container and add enough sea water so that it is just covered
- Place the point count grid over the panel
- Looking through a dissecting microscope, record the organism attached directly under each point (crosshair). Record to the highest taxonomic resolution as you are able, and also assign the organism to one of the categories that we have provided (e.g. Erect bryozoan, *Bugula neritina*)
- If there is an organism laying over the substrate (not directly attached, e.g. erect bryozoans, algae or some solitary tunicates), record this as 'canopy'.
- Each panel should have 25 points recorded before moving on to the next panel.
- Repeat for all remaining panels.

*** If you are not able to transport the panels to a lab, please consider doing a point count in the field- using the same method but without a microscope.

- **High-resolution biodiversity surveys** (Metagenetics)

You provide:

1-Gallon Ziploc bags

Cooler for transport of the panels

Laboratory gloves

Tray bigger than panel

Paint scraper (newer is better)

Squeeze bottle for seawater

Squeeze bottle for DMSO

Food blender capable of blending ice

Spare carafe for above is useful

2 spatulas (bleached)

Lab tape

Marker

We provide:

4L DMSO + EDTA (preservative)

65 Falcon tubes (2 per panel)

Parafilm

Labels

Protocol:

Before field day

- Clean all supplies and work bench thoroughly with bleach.
- Label falcon tubes and prepare internal labels

In the field

- When retrieving the panels, scrape the back and sides of the panel so only the organisms growing on the front (downward facing) side remain. Place each panel in a Ziploc bag with some seawater and the photograph label so that you can identify which panel is which.
- Place the plates in the cooler for return to the lab. Plates should not be left in bags overnight, so it will be necessary to make multiple trips if you are able to dedicate the time.

In the lab

- If doing point counts for fouling community composition (see above); do that first.
- Using fresh gloves, place the first panel in the clean tray, without water, and scrape the front face into the tray.
- Transfer the content of the tray carefully into the blender and blend at full strength for 15 seconds or until the fraction is well homogenized (if sea water is necessary, only use a small amount).
- Place ~10 ml of the slurry from the blender (avoiding any froth) into each of the two falcon tubes, add internal label and fill with DMSO. Shake vigorously to homogenize.
- Seal closed tubes with parafilm. Place in the freezer.
- Before starting a new panel, ALL equipment needs to be soaked in 10% bleach solution for 10 minutes & rinsed thoroughly in fresh water.

**** As mentioned on the front page, if you are not able to process all panels, we can prioritise panels to fit your time availability.**