

Overview: This experiment seeks to explore how competition between understory algae and benthic invertebrates drive ecosystem form and function on our coastal reefs. This sampling incorporates 7 sites (Arroyo Quemado, Naples, Isla Vista, Mohawk, Carpentaria, San Pedro Point, and Cavern Point) 5 of which are part of the SBC-LTER Benthic Monitoring Program with 2 new Santa Cruz Island sites being established as well. Sampling is conducted seasonally every 3 months. Two, 6 m circular plots (Figure 1) were deployed at all of the listed reefs, each consisting of 12: 5 cm thick, 50 x 70 cm rock plates bolted on all 4 corners, 12 adjacent control quads, 12 PVC recruitment plates (10 x 10 cm), 6 recruitment brushes, and one photosynthetically active radiation (PAR) light sensor. All plates were deployed across several months in 2020 and 2021 then cleaned and reset during initial sampling beginning in September 2021. The two circular plots lie 20-50 m apart at similar isobaths (~10 m) on each reef.

All BCE site plots labeled “2” are the giant kelp removal plots. Plot 1 (rock plates #1-12) represents the control plot with no giant kelp removal and plot 2 (rock plates #13-24) represents the kelp clearing plot where all giant kelp (recruits, holdfasts, and all fronds/stipes) are removed within an 8 m radius of the PAR stake which is located in the center of each of the rock plots. Giant kelp is removed seasonally during each sampling period. Divers work in pairs first reeling out an 8 m transect tape connected to the center bolt, they will swim in a circle removing any giant kelp plants growing on natural substrate within the 8 m arc created when swimming the tape around the center bolt. Divers should be able to simply pluck the plants by hand, but a dive knife can be used to remove larger plants. The goal is to remove the entire plant including the holdfast. Surface canopy blades encroaching inside the cleared circle will also be removed while at the surface at the end of the dive.

Understory algae is removed seasonally from half of the rock slates for both plots (Plot 1 rock plates #1-6 and Plot 2 rock plates #13-18). Because the rock plate numbers were randomly assigned, the locations of the understory removal were different among the sites/plots.

Table 1. Latitude and longitude coordinates for each of the two plots located in the seven reef sites. Dates, in the format yyyy-mm-dd, of the first data collection survey.

Reef	Plot	Latitude	Longitude	Date of First Survey
Arroyo Quemado (AQRK)	Plot 1	34.4669	-120.1175	2021-09-22
	Plot 2	34.466917	-120.11795	
Carpinteria (CPRK)	Plot 1	34.390167	-119.534933	2021-09-28
	Plot 2	34.389883	-119.535033	
Isla Vista (IVRK)	Plot 1	34.403967	-119.8622	2021-09-21
	Plot 2	34.403733	-119.862083	
Mohawk (MKRK)	Plot 1	34.3942	-119.729983	2021-09-23
	Plot 2	34.394217	-119.729483	
Naples (NPRK)	Plot 1	34.421833	-119.951467	2021-09-21
	Plot 2	34.4218	-119.951217	
Santa Cruz Island, Cavern Cove (CCRK)	Plot 1	34.053317	-119.56735	2021-10-01
	Plot 2	34.0536	-119.567217	
Santa Cruz Island, Pedro's Point (PPRK)	Plot 1	34.037717	-119.524617	2021-09-30
	Plot 2	34.037917	-119.524833	

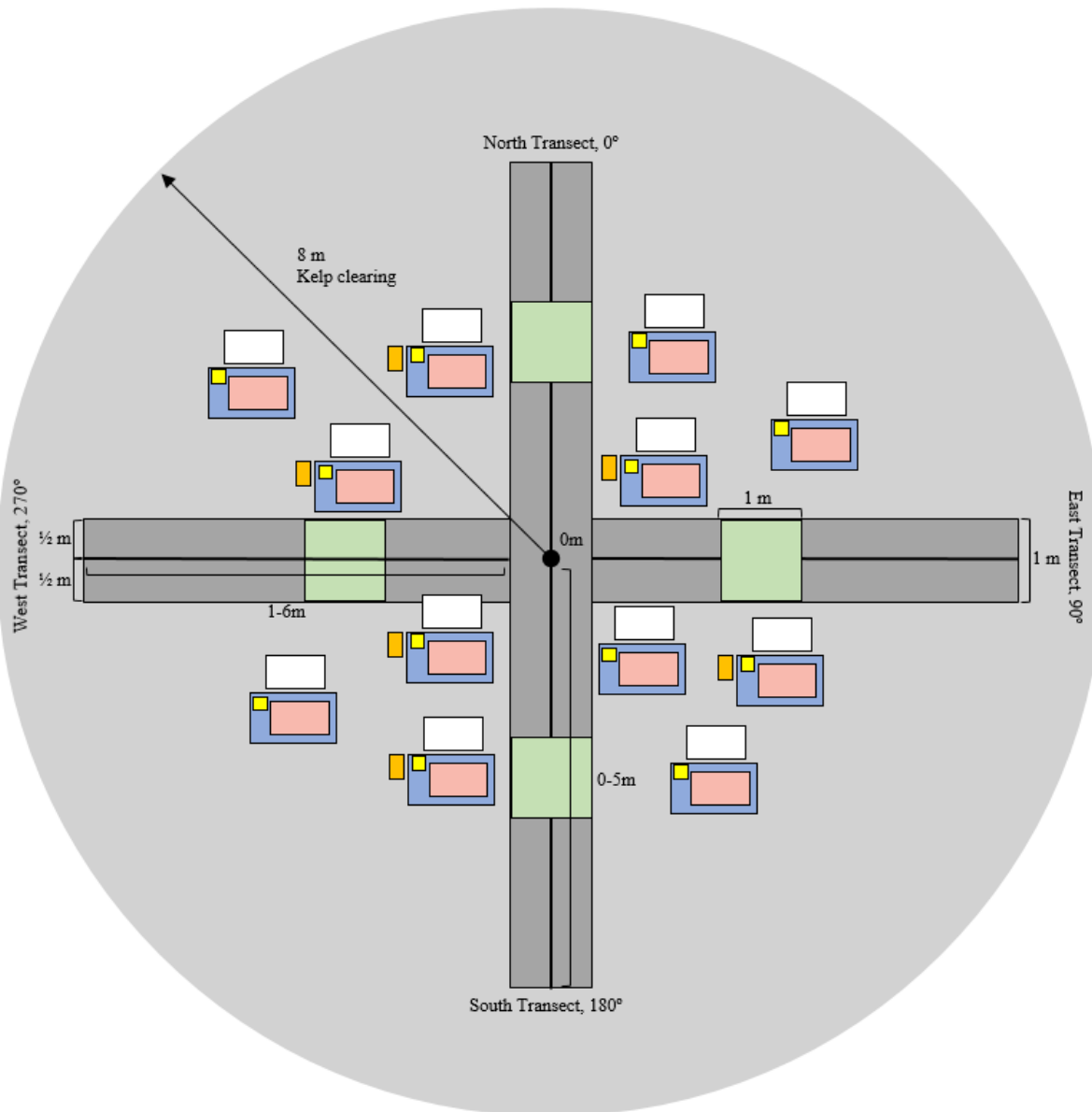
Methods:

Fish Sampling

Fish sampling consists of recording the size and abundance of common reef fish encountered on four 5 m transects, one in each cardinal direction away from the center plot marker (Figure 1). The lead diver descends first with the buddy behind them and begins the North transect immediately once the center stake is found. This diver records the number and size of any fish found within the 2x2x5 m transect (see species list below for details). Concurrently, the buddy

diver cleans and/or swaps the PAR logger on the center stake while the other diver completes these four transects.

Figure 1. Schematic diagram showing experimental layout for the plots within each of the seven sites. Within each plot are PAR light sensors (black circle) and four 5 m x 1 m transects running each cardinal direction (N, E, S, W) that get surveyed for benthic invertebrates, fish, and macroalgae. Within each plot there are 12 rock plates (blue rectangles). Photo quads are taken on each rock plate (pink rectangles), over the PVC recruitment plates (yellow squares) and control quad (white rectangles). Bristle brushes (orange rectangles) are randomly assigned to 6 rock plates. 1 m² urchin quads (green squares) are centered over the meter tape from 2-3 m. This diagram is not to scale and does not depict precise arrangement of rock plates which were haphazardly placed on the benthos. Note: the 8 m kelp clearing area is only done in the experimental plots at each site (plot #2), plot #1 will exclude this clearing.



Light measurements

PAR light sensors are deployed on the seafloor at the center of each BCE plot. An additional sensor is placed in air on the roof at the Marine Science Biotech building near Campus Point which acts as a calibration control when analyzing underwater light measurements. The data from these PAR sensors are used to model primary production by both giant kelp and understory algae in terms of light availability and to compare the differences between control and experimental plots cleared of giant kelp.

Kelp/Swath/Urchin Sampling

Kelp sampling consists of counting the number of fronds >1 m on each giant kelp plant (*Macrocystis pyrifera*) encountered on four 5x1 m transects, one in each cardinal direction away from the center plot marker. Swath sampling consists of counting and sizing common invertebrates and understory algae (See the species list for detail) encountered while searching the same four 5x1 m transects used for the kelp survey. A light is used during the swath sampling search. A 1 m² quad centered over the meter tape from 2-3m is used to create the sample area to count and size urchins. See the species list for codes that relate to the urchin sizes and species. The average size of each targeted species encountered was estimated for each 5 m x 1 m swath in each cardinal direction. East and West transects start 1 m from the center plot marker and survey to 6 m so there is no overlap in the transects near the center stake.

Photo Quad Sampling

A housed Canon 5DR SLR camera with a 16 mm lens, two strobes, and a 25x35 cm quadrat is used to document recruitment and percent cover on all 24 rock plates (R), 24 PVC recruitment plates (P), and 24 control quads (C). A diver first photographs the rock ID tag, the rock plate, recruitment plate, and control quad. These photographs are uploaded to a cloud-based image analysis platform (ViQi) and point contact analysis is conducted on them in the lab. See supplemental protocol for camera setup, downloading, and analysis. After all photographs have been taken, all recruitment plates are cleaned and all erect understory algae are removed seasonally from half of the rock plates for both plots (Plot 1 rock plates #1-6 and Plot 2 rock plates #13-18) to measure seasonal recruitment.

The images were evaluated in three categories: "100 points," representing the primary outcome of the point contact method, with a total of 100 points in the image; "kelp recruit," indicating additional points specifically focused on the recruitment of two algae; and "additional points" denoting the points on the plate treatment that were outside the 100-points category, which primarily document the species that were not appeared in the 100-points.

Recruitment Sampling

To quantify the richness and abundance of mobile invertebrates settling and recruiting to these experimental plots, 6 replicate brushes are swapped seasonally during each sampling period from each circle. Recruitment is measured as a proportion of the number of individuals found on the recruitment brushes (Figure 2). The 6 brushes are attached to randomly chosen rocks within each plot for a total of 12 bristle brushes collected from each site. These brushes are then brought back to the lab in labeled 100-micron plastic mesh bags and frozen until further processing. During processing, brushes are rinsed with fresh seawater to remove all organisms from within the bristles of each brush. All organisms from each individual brush are filtered through a 200-micron sieve and collected in a sample jar labeled with the site, collection date, and rock plate number it was obtained from. These samples are then sorted in the lab using microscopy to identify and enumerate all species found.

Figure 2. Wood-backed brush with polypropylene bristles measuring 20 x 7 cm (140 cm²) used for recruitment sampling of invertebrates.



Species list:

The full species list for the BCE benthic survey can be referred to:

<https://sbclter.msi.ucsb.edu/data/catalog/package/?package=knb-lter-sbc.120>

Specifically, see the survey methods for fish, swath, and urchin.

Supplemental Materials

BCE Photo Uploading and Labeling Protocol

All BCE Associated Experiment photos are stored in three places (DO NOT DELETE ANY IMAGES FROM THE FIELD - fun or experiment related)

1. SCB BON server
 1. Z:\internal\Photos\LTER_BCE
2. SBC LTER Google Drive
MSI SBCLTER Drive> BCE> Experiment Photos
3. BCE External Hard Drive (WD My Book - 12TB) - currently living in the Miller Lab 2004
SBCLTER>BCE

Open sbcbon server> internal> Photos> LTER_BCE and create a new folder labeled year and month, ex. 2021DEC and go into that folder. In that folder create a folder for each site (AQRK, CCRK, CPRK, IVRK, MKRK, NPRK, PPRK), a folder for “fun” and a folder for “tags and second takes.” In each site folder create a CR2 folder.

Remove the Lexar CF drive from the camera and connect it to the computer using an adaptor (Frankie has one on her desk) or a direct connection port if your computer has one.

Open up folders on your desktop and underneath LEXAR select DCIM then 100EOS5D. Select all files (.CR2 and .JPG) from the sampling dates you would like to transfer into the sbcbon server. Drag and drop these files into the correct date and month folder on the sbcbon server.

Label and separate all images into their correct folders on the sbcbon server first. Once all of the images have been labeled on the sbcbon server drag and drop each site folder into its corresponding year and month folder on the SBCLTER Drive. Drop the year and month folder into the BCE External Hard Drive. Label and separate all images into their correct folders on the sbcbon server first.

Labeling Images

Images are taken using the following protocol:

1. Image of the rock tag
2. Image of the rock
3. Image of the plate attached to the rock
4. Image of the control plot next to the rock

If the images need to be taken out of order a second image of the rock tag should be taken before the next image.

For example: Frankie took a photo of rock tag C10 then rock C10 and plate C10 but could not find control C10 so she moved on to rock tag C11. Before taking a photo of control plot C10 Frankie would take a photo of rock tag C10 and then control plot C10 to indicate that she went back to C10 to take an image of that control plot.

Images will be tagged using the following naming convention:

Site and Plot #	R/P/C and #	Image #	File type	Image label
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Isla Vista Plot 1	Rock 1	Image 3003	.CR2	IVRK1_R01_3003.CR2
Isla Vista Plot 1	Plate 1	Image 3004	.CR2	IVRK1_P01_3004.CR2
Isla Vista Plot 1	Control 1	Image 3005	.CR2	IVRK1_C01_3005.CR2
Isla Vista Plot 2	Rock 13	Image 4004	.JPG	IVRK2_R13_4004.JPG
Isla Vista Plot 2	Plate 13	Image 4005	.JPG	IVRK2_P13_4005.JPG
Isla Vista Plot 2	Plate 13	Image 4006	.JPG	IVRK2_C13_4006.JPG

BCE PAR Sensor Programming and Downloading Protocol

Programming

1. Plug the light sensor interface unit into a computer using the USB cord. Mount the instrument on the interface so that the groove near the bottom of the sensor fits into the ridge on the mount. Orient the sensor so that the serial number is facing up. When the sensor is mounted correctly, a red LED light is visible on the bottom side of the sensor.
2. Log into the Reef Tech computer with the username “reef_tech” and password “reef88”.
3. Open Google chrome and go to URL “<https://time.is/UTC>”. Check to see if the computer time on the bottom right corner is the same as the UTC time. If the computer time is off by more than a few seconds:
 - a. Click on clock located near the bottom of the screen
 - b. Click “change date and time”
 - c. Select the Internet time tab, then change settings
 - d. Uncheck the “Synchronize with an Internet time server” option
 - e. Go back to the Date and Time tab, select “change date and time”, then set the time and date to match the website
4. Once the computer has the correct UTC time, open the DEFI software on the computer. To begin programming the sensor, click the “Deployment” tab and press the “Read Information” tab. Click “Set from PC” so that the sensor’s internal clock matches the computer.
5. If the Memory section says “Data inside”, double check the sensor logs to see if the sensor data has been downloaded before clicking the “Clear memory” tab. (Steps to check the log will be detailed further down).
6. Once the Memory says “No data” and the Status says “Stop” check to make sure all the parameters are correct.
 - a. Calendar: should be today’s date and the time should be within a few seconds of the UTC time.
 - b. Start time: set it to today’s date and have the time be 2 minutes in the future so you are able to double check to make sure the sensor is recording data.
 - c. Interval: 1 minute
 - d. Density constant: 1.0250 (on the parameter screen it’ll show up as “- - -”)
 - e. Environment: Water (for LTE and BCE sensors) and Air (for the Marine Biotech roof sensor)
 - f. Set up LED: Nothing

7. When all of the parameters have been set and double-checked to ensure they are correct, click the “Complete” button at the bottom of the screen.
8. Once the sensor has been activated, double check to make sure it has been done correctly. To do this, take the sensor off the interface and wait for the start time that you set earlier in the parameters. Once it is that time, place the sensor back on and click “Read Information” again. This time, the sensor “Status” should say “Measuring” and the “Memory” should say “Data Inside”, which indicates that it is ready to be deployed.

Downloading

1. Open the DEFI Series Software. To begin downloading the sensor, click the “Retrieval” tab.
2. Click the “Stop” tab to end data collection. The sensor information should appear in the first row below. If not, readjust the orientation of the sensor to ensure the serial number is facing up. Make sure the serial number matches the instrument, the interval reads 60 sec, and that the start time and end time appear to be around 3 months apart.
3. Next, save the file to the appropriate folder, depending on what type of sensor you are downloading.
 - a. BCEs: “Internal” → “research” → “Reef” → “Working” → “Data” → “BCE” → “BCE Continuous Light” → “data archive” → “raw_data” → Either AIR or WATER → Choose which site and year the sensor has been collected from
 - b. Once the sensor has been saved to the appropriate folder, click the “Download” button.
 - c. After downloading has been completed, open the folder up in the server so that you can rename the file. Right click on the file name to rename it. Use the following template: “Four Digit Serial #_Year_Month_Day”. **This date should correspond to the day the sensor was deployed, not today’s date.**
4. Return to the DEFI software and click the “Open” tab. Find the file that you just renamed and double click to open it. Here, we are checking to see if the sensor had any malfunction in the three months it has been deployed.
 2. The blue lines on the graph represent seafloor irradiance. You are looking for oscillations in these points that portray the amount of light being recorded during the day and at night.
 3. The red lines on the graph represent battery life. This line should stay fairly consistent throughout the three months and not drop well below 1.6V. Double check the y-axis on the graph to see if there are significant fluctuations in battery life. Notify a technician if something seems off.
5. Once you have downloaded the data into the DEFI software, recorded the information into the deployment log, renamed the file on the server, and observed the graph to check for any sensor malfunctions

BCE Brush Rinsing and Invertebrate Sorting Protocols

Lab Rinsing

1. Obtain bristle brush from the large, white lab freezer in 3003
2. All rinsing takes place in the Seawater room (3001) next door to 3003

3. Remove brush and record label from mesh bag onto plastic tub



4. Clearly label the plastic jar found on the seawater room shelf using the marking tape and label with the same information found on each mesh bag tag including the date the sample was collected
 - a. Example: IVRK 23 6/22/2022
5. Rinse mesh bag into a plastic tub to remove all inverts using running seawater
6. Once the mesh bag has been thoroughly rinsed, place mesh bag on plastic clips to air dry
7. Rinse brush inside the same plastic tub using running seawater and a toothbrush/scrapper to remove and capture all invertebrates from within the bristles



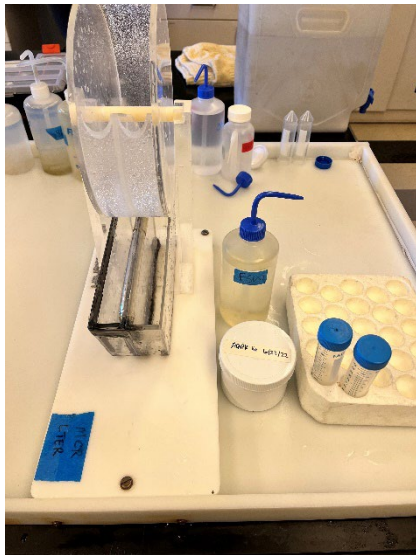
8. Periodically pour seawater contents (seawater + inverts) from plastic tub into the sieve to catch all the inverts while draining the seawater
9. Once all inverts have been thoroughly rinsed from the brush, transfer all inverts from sieve into your labelled plastic jar using the small metal scraper and minimal seawater
10. Limit the amount of seawater poured into the plastic jar (no more than 3/4 full)
11. Place sample in the same large, white freezer where sample was first obtained

Lab Sorting

1. Obtain frozen sample from freezer
2. Place sample plastic jar in Tupperware and immerse in hot water from the sink for approximately 30-45 minutes to completely thaw
3. If this is a full proportion sample, please continue with the following steps.

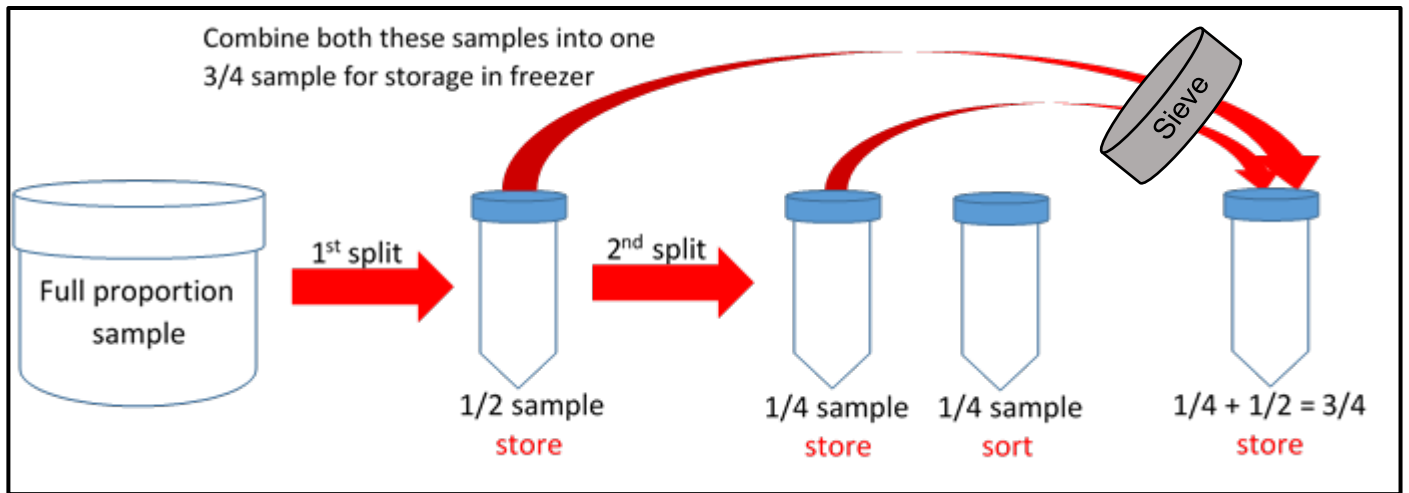
****If the sample has already been split into proportions (3/4, 1/4) skip to step #12****

4. Full proportion sample should be split down to one $\frac{1}{4}$ proportion and stored in the blue cap plastic vials held in the Styrofoam holders or sorted immediately
5. The leftover $\frac{3}{4}$ proportion should be stored in a separate blue cap plastic vial and frozen
6. Use the plankton splitter to equally split the full proportion sample using seawater **ONLY** to thoroughly rinse all organisms into appropriate plankton splitter sections. Make sure plankton splitter is level before splitting!
7. Quickly and carefully pour all plastic jar contents into splitter while using seawater and/or metal scraper to remove all organisms
8. Once all contents are in the plankton splitter, roll plankton splitter back and forth to evenly distribute sample within the plankton splitter
9. Quickly and carefully pour plankton splitter contents into plankton splitter containers/dividers

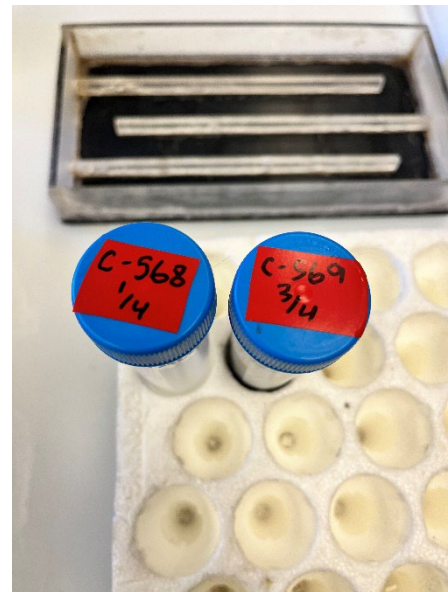


****Make sure not to spill contents behind the containers by rotating the plankton splitter too far****

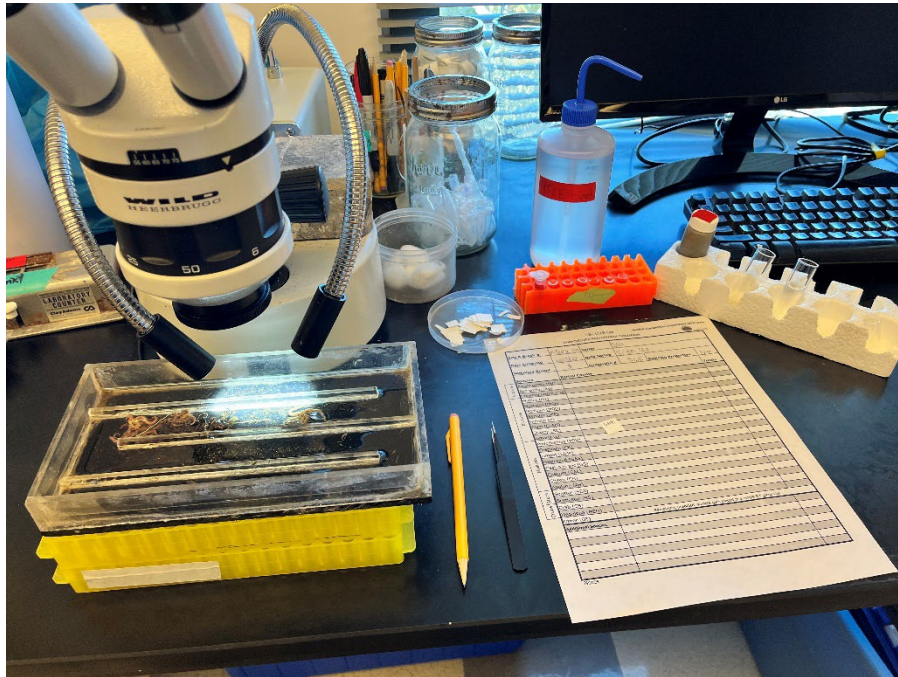
10. If there is too much seawater to fit into blue cap plastic vials, drain seawater through the sieve then transfer split proportion sample into blue cap plastic vial using a funnel



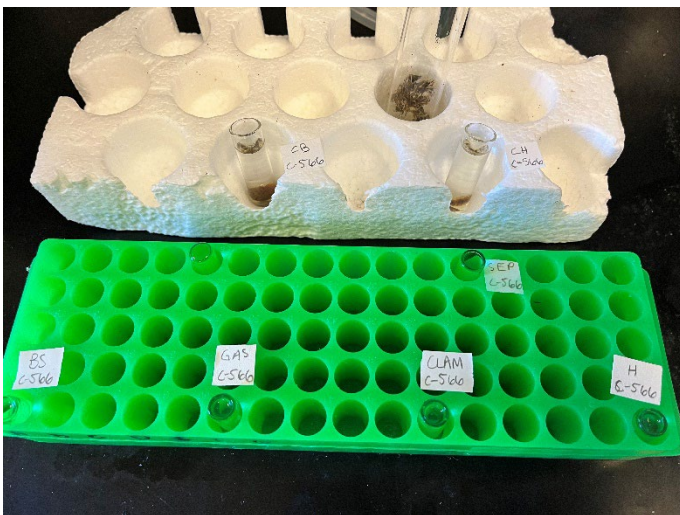
- Clearly label your split proportion vials with labelling tape on the side and top of each blue cap plastic vial and should include **composite #** from the data sheet **AND** the appropriate **splitting proportion** listed above



- Once you have a split proportion ready for sorting, dump contents into a sorting tray (zig-zag shaped tray) and spread out evenly using seawater rinse bottle
 - Remove and count large organisms (seen with naked eye) first
 - Place all organisms that are being sorting in glass vials
 - Make a label for each vial using the 100% cotton paper and the archival ink pen (or pencil) to record the **composite #** and **organism being sorted**
 - Fill glass vial 1/2 full with ethanol (ETOH) before placing first organism into vial for an easier transfer
- **Stop adding specimens to glass vial after 25 have been collected, but continue to count those organisms which will later be added to the Other (OT) vial at the end****



17. Once all organisms have been sorted, fill glass vials approximately 3/4 full with ethanol (ETOH), add the appropriate label (facing out), and plug with a tight fitting cotton ball that's been pre-soaked in ethanol so there is no air pocket in vial



18. Remaining organisms in sorting tray should be rinsed through the sieve using seawater
19. These remaining contents should be transferred to a separate glass vial (just like the sorted organisms in previous steps) and contain their own "OT" labelled cotton paper tag, filled with ethanol (ETOH), and plugged with a pre-soaked cotton ball
20. All glass vials from the same sample should be placed within a large glass jar with white lids and covered with ethanol (ETOH).
 - **Multiple samples from multiple sites may be added to a single, large, glass jar as long as all sorted glass vials fit properly****
21. Make sure to accurately add a label onto the large glass jar with the **composite #**



****Be sure to clean your work station and put everything back how you found it and replace microscope cover****