Blue Ocean MARICULTURE

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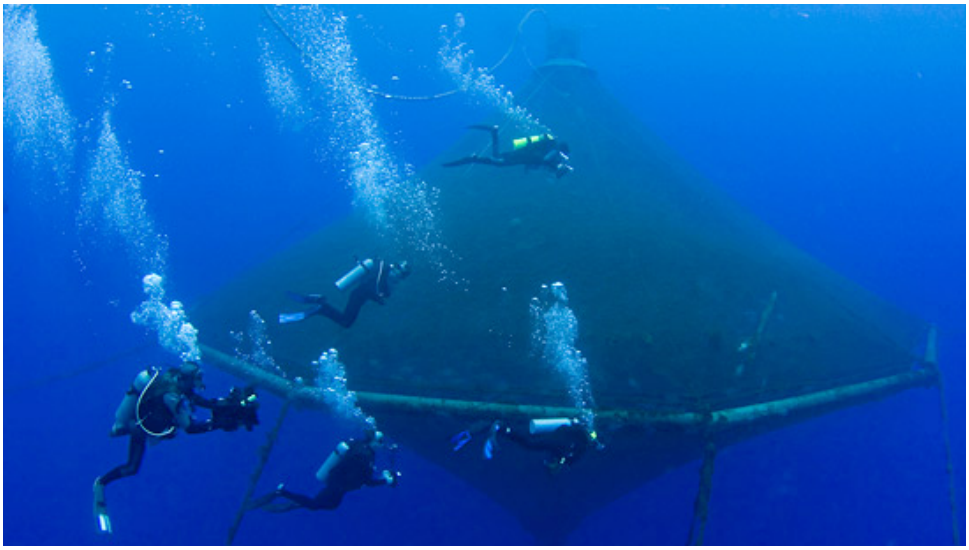

11/29/2023

Annual Benthic Monitoring Report – 2023

Prepared by

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BLUE OCEAN MARICULTURE

ANNUAL BENTHIC MONITORING REPORT – 2023

ANNUAL REPORT

For the Period

Year 2023

Subject Location

Open Ocean Mariculture Site

Authorized by

US Federal NPDES Permit HI 0021825

State of Hawaii CDUP HA-3497

Operated by

Blue Ocean Mariculture

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REPORT INFORMATION

Samples Collected at The Offshore Farm Site On:

November 23rd, 2023

By

PlanB Consultancy

Chief Scientist: Dr. John Burns

This report details the 2023 annual benthic monitoring for the Blue Ocean Mariculture fish pens off Unualoha Point on the Kona Coast of Hawaii Island. All samples were collected with a ponar grab sampler lowered to the benthic substrate. Samples were collected from five survey locations used for benthic monitoring (Figure 1). Two additional samples were collected at the Control and ZOM locations, but only the five historical locations are included in the temporal graphs for reporting consistency. The grab sampler was deployed and retrieved from a boat with an electric winch. Upon retrieval, the samples were transferred from the ponar grab sampler and placed in a receptacle along with the seawater collected with each sample.

The following parameters were assessed immediately for each sample:

- *General appearance*
- *Macro fauna*
- *Macro algae*
- *Oxidation/Reduction Potential (ORP) readings*
- *Odor (presence of H_2S)*

Sub-samples were collected from each sample in order to analyze the following parameters:

- *Total organic carbon (TOC)*
- *Benthic sand characterization*
- *Micromollusc characterization*
- *Copper, Zinc, Silver, Arsenic, Selenium, and Chromium concentration*

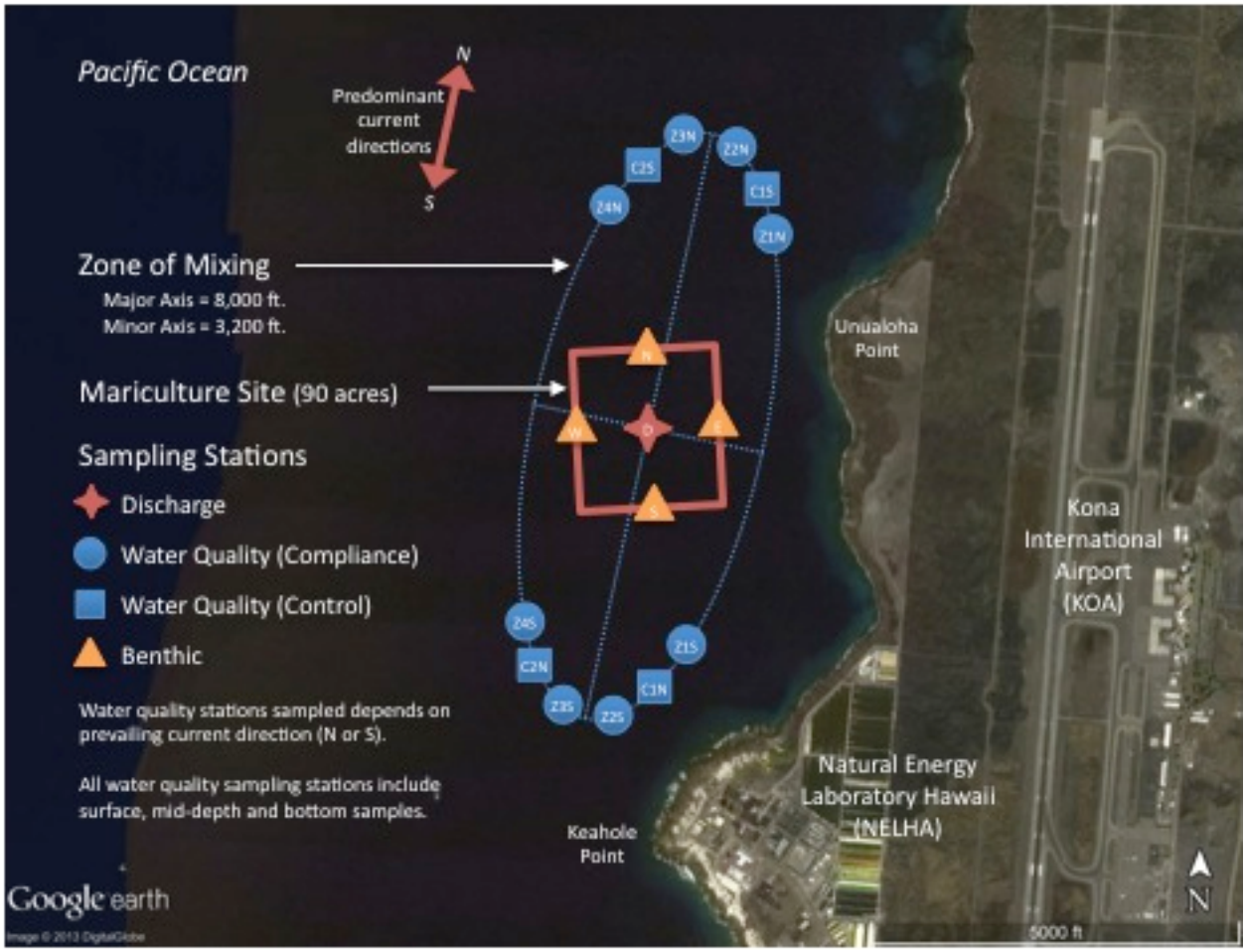


Figure 1. Site image of Blue Ocean Mariculture fish pens. Orange triangles and star represent the locations utilized for benthic sampling.

GENERAL *IN-SITU* SAMPLING OBSERVATIONS AND DATA DESCRIPTION

Samples were collected during a dropping tide (1.9ft – 0.4ft) influenced by a 59% waxing moon. Conditions consisted of 10-15kt Southwest winds that calmed to 4-6kt Southwest winds halfway through the sampling. There was a consistent 2-4ft Northwest swel. There was minimal VOG hovering over Kailua-Kona during the sampling with clear skies. There were visible rip lines with minimal visible particulates at all sampling locations. Baitfish and large predators (dolphins, monk seals, sharks) were present at the cages. A moderate north current was evident at all sites during the benthic sampling.

The water was initially rough and became calm halfway through sampling. The rough conditions were due to a storm that occurred overnight and passed in the morning.

The water was clear at each sampling location.. There was no evidence of high turbidity, discoloration, visible sheen, foam, solids, or floating debris at any location during the benthic sampling locations. There were some visible plankton and microorganisms at the surface water.

The current was moderate and thus the sampling was mostly successful and at most required 3 drops to successfully receive the sediment samples. The benthos was filmed at each location using an ROV as opposed to a stationary drop camera, which has been used in the past.

The five historical sites are reported below for consistency and analyses of temporal trends. Data for the additional sites (Control, ZOM) are available in the spreadsheet data for 2023.

North Anchor	**10:12**
Appearance:	Brown
Macro Fauna:	Polychaete spp.
Macro Algae:	*Cladophora laetevirens, Halimeda kanaloana, CCA*
ORP:	225.3
Odor:	None

South Anchor	**12:04**
Appearance:	Brown
Macro Fauna:	*Gastropod spp.*
Macro Algae:	*Cladophora laetevirens*
ORP:	212.3
Odor:	None

West Anchor	**10:35**
Appearance:	Light Brown
Macro Fauna:	*Polychaete spp.*
Macro Algae:	*Halimeda kanaloana, CCA*
ORP:	186.7
Odor:	None

East Anchor	**11:36**
Appearance:	Dark Brown
Macro Fauna:	*Crustacea spp.*
Macro Algae:	*Cladophora laetevirens, Halimeda kanaloana*
ORP:	162.4
Odor:	None

Under Cage	**11:12**
Appearance:	Dark Brown
Macro Fauna:	*Hermit crab*
Macro Algae:	*Cladophora laetevirens, Halimeda kanaloana, CCA*
ORP:	211.7
Odor:	None

BENTHIC SAND CHARACTERIZATION

Intro/Methods

Sediments play a significant role in the structure of benthic communities because many organisms have grain size preferences, thus changes in sediment composition can affect organisms occupying the benthic habitat. Furthermore, sediment characteristics can provide useful information about source materials, the depositional environment (amount of energy in waves and currents), and other physical and chemical factors.

Sediment samples were collected in order to assess sediment composition. Three sub-samples from each site (North, East, West, South, Under Cages) were washed with fresh water to remove salt and then sun-dried. Sand grain size analysis was conducted using a set of 7 US Standard sieves (5,10,35,60,120,230, and Dust). Samples were processed through the sieves using a motorized shaker to adequately separate sand grains based on physical size. An electronic balance was used to measure the mass of each sample proportion that was isolated in the individual sieves. The average retained weights, and percentage of total weights, are presented in the data table below. Additional samples were collected at Control and ZOM sites. The additional sample data is not included below to maintain temporal consistency in the report, and these data are available in the report spreadsheet.

Data/Results

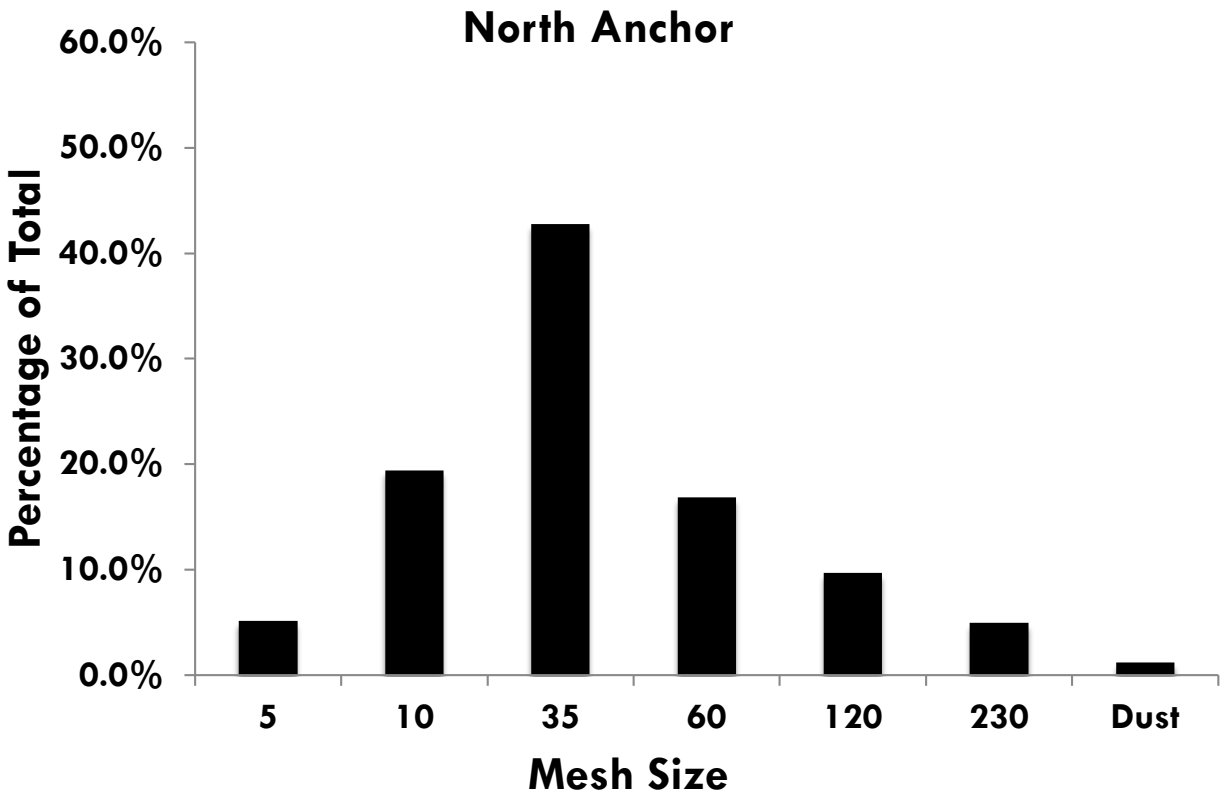
North Anchor		
Mesh Size	Mean Retained Weight (grams)	Mean Percentage of Total
5	11.34	5.1%
10	43.02	19.4%
35	94.67	42.7%
60	37.29	16.8%
120	21.47	9.7%
230	10.98	5.0%
Dust	2.72	1.2%
TOTAL	221.49	

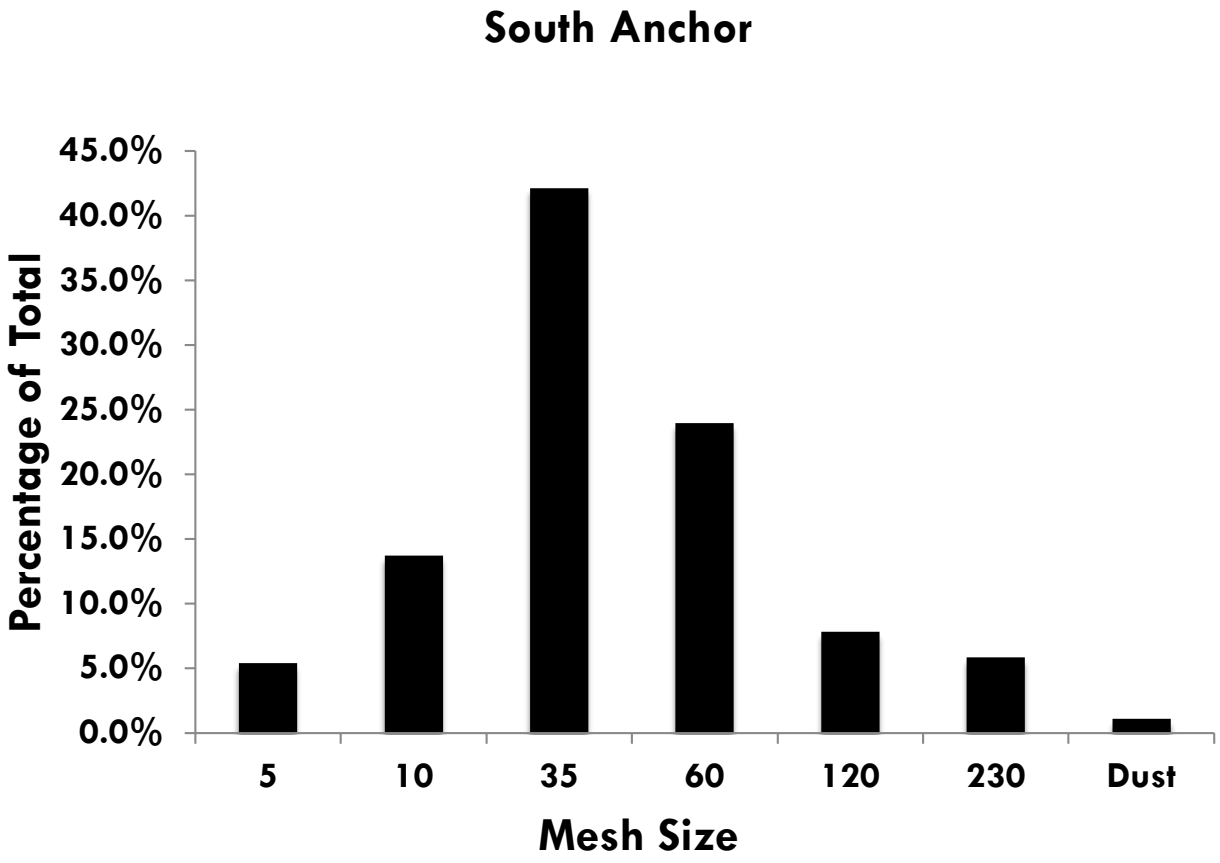
South Anchor		
Mesh Size	Mean Retained Weight (grams)	Mean Percentage of Total
5	12.69	5.4%
10	32.09	13.7%
35	98.72	42.1%
60	56.09	23.9%
120	18.35	7.8%
230	13.70	5.9%
Dust	2.60	1.1%
TOTAL	234.24	

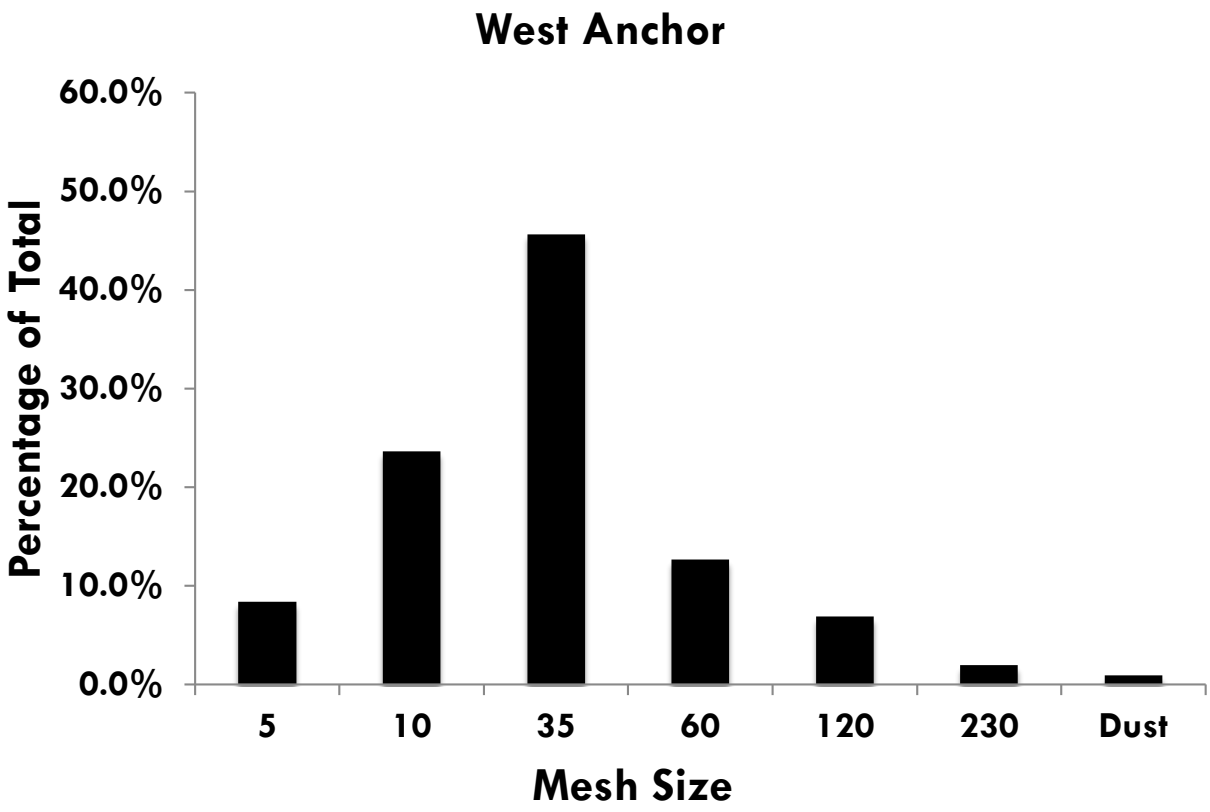
West Anchor		
Mesh Size	Mean Retained Weight (grams)	Mean Percentage of Total
5	21.03	8.4%
10	59.39	23.6%
35	114.57	45.6%
60	31.75	12.6%
120	17.26	6.9%
230	4.96	2.0%
Dust	2.34	0.9%
TOTAL	251.29	

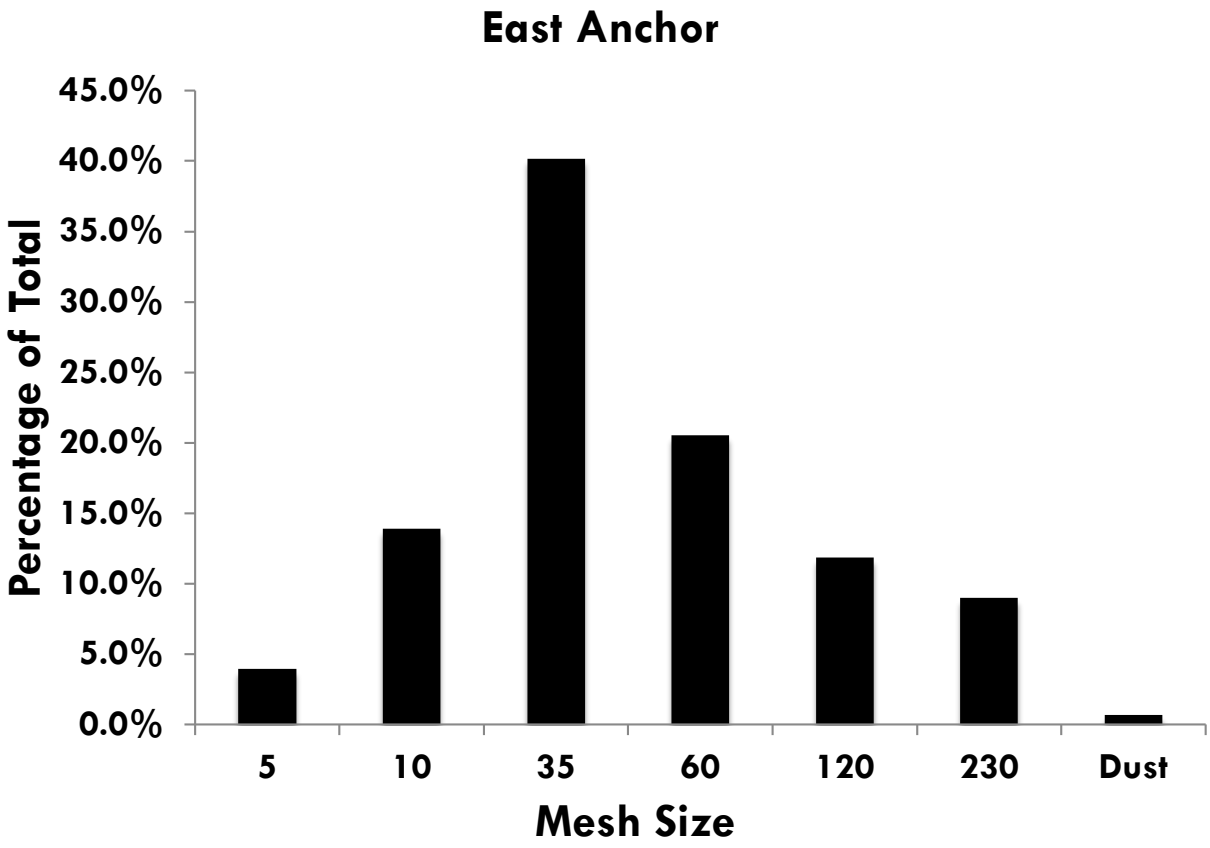
East Anchor		
Mesh Size	Mean Retained Weight (grams)	Mean Percentage of Total
5	8.10	3.9%
10	28.50	13.9%
35	82.34	40.1%
60	42.11	20.5%
120	24.27	11.8%
230	18.42	9.0%
Dust	1.36	0.7%
TOTAL	205.10	

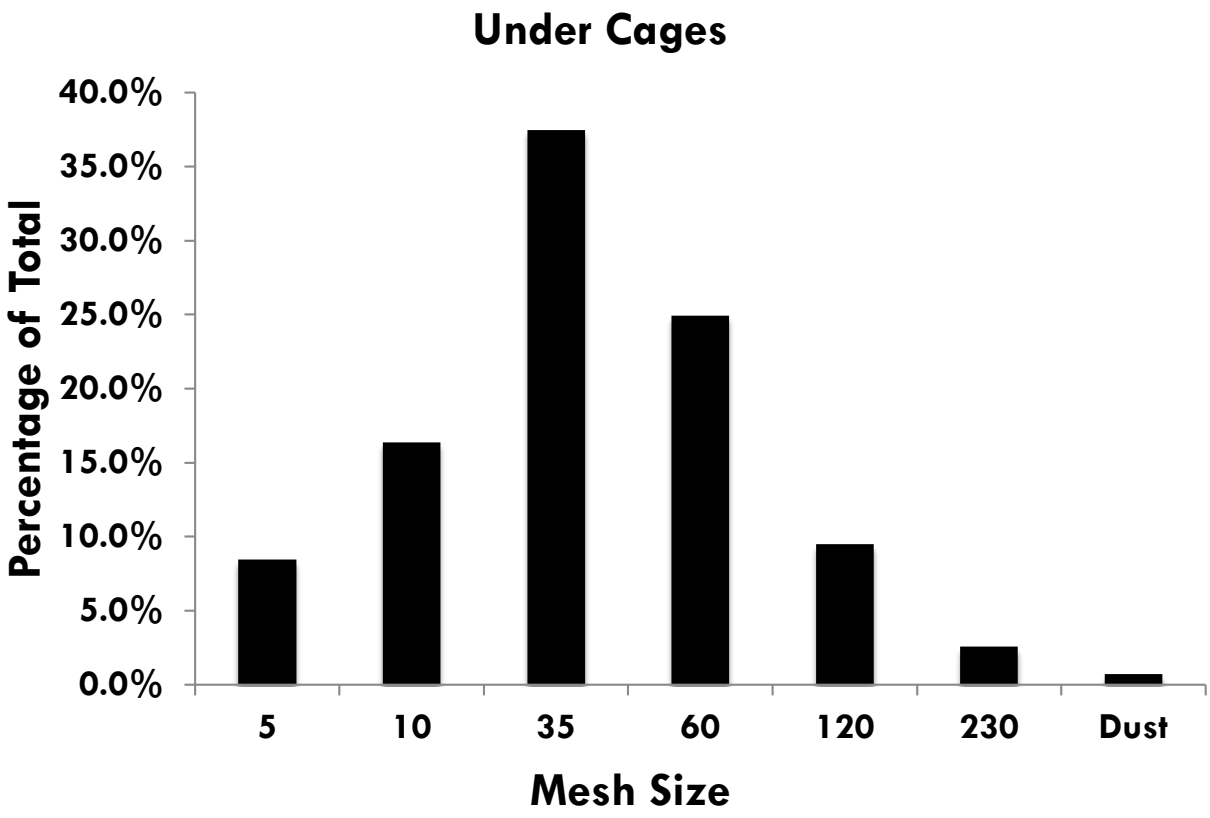
Under Cages		
Mesh Size	Mean Retained Weight (grams)	Mean Percentage of Total
5	17.58	8.5%
10	33.99	16.4%
35	77.80	37.5%
60	51.74	24.9%
120	19.71	9.5%
230	5.33	2.6%
Dust	1.52	0.7%
TOTAL	207.67	


North Anchor
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
Percentage of Total
5
10
35
60
120
230
Dust
Mesh Size


South Anchor
45.0%
40.0%
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
Percentage of Total
5
10
35
60
120
230
Dust
Mesh Size


West Anchor
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
Percentage of Total
5
10
35
60
120
230
Dust
Mesh Size


East Anchor
45.0%
40.0%
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
Percentage of Total
5
10
35
60
120
230
Dust
Mesh Size


Under Cages
40.0%
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
Percentage of Total
5
10
35
60
120
230
Dust
Mesh Size

Summary

The sediment compositions of the 2023 samples exhibited similar characteristics to the samples collected from previous years (Figure 2 and Figure 3). All samples were composed of primarily coarse sand with minimal silt. There was no evidence of live scleractinian (stony) coral or hard substrate from the collected samples, and this is to be expected considering the depth of the benthic environment at these monitoring sites (~60m). The observed macrofauna polychaete spp. at the North Anchor, West Anchor, and ZOM sites. Gastropod spp. were observed at the South Anchor Site. Crustacea spp. were observed at the East Anchor site. A hermit crab was observed at the Center Site. A lizard fish and leptoseris encrusting coral spp. were observed at the Control Site. The observe flora included *Cladophora laetevirens* and *Halimeda kanaloana,* which were present in nearly all samples. The *Halimeda kanaloana* alga is very abundant at the West Anchor site. It appears to that the calcified blades of this alga species are one of the primary components of the sediment at this location (mesh size 10 & 35, see West Anchor graph above). Small amounts of crustose coralline algae were observed at the North Anchor Site, West Anchor Site, Center Site and Control Site. Comparing these findings to those from previous years suggests the benthic sediment at this site has exhibited a similar sand and benthic composition over time, thus no effects associated with the presence of the fish cages are apparent (Figure 2 and Figure 3). The abundance and diversity of the observed fauna and flora indicate that the sediment composition is not having any detrimental impact on marine life. The currents in this area are known to run at speeds of up to 2 knots in various directions. The disruptive hydrology likely impedes any settlement of harmful detritus or debris and facilitates the consistently similar benthic composition that has been observed in the annual benthic monitoring of these sites. It is likely that any changes in composition observed among the monitored years is due to disturbances caused by the natural hydrological characteristics at this site.

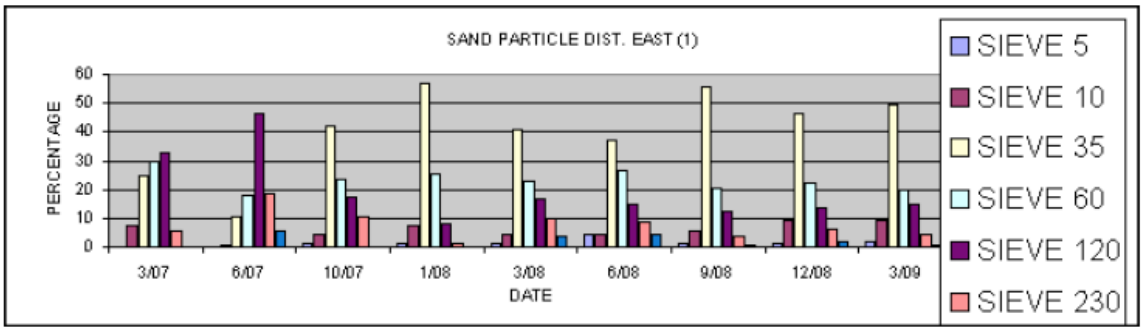


Figure 2. Sand characterization data from the East Anchor site (2007-2009). Note the similar composition to the 2023 samples.

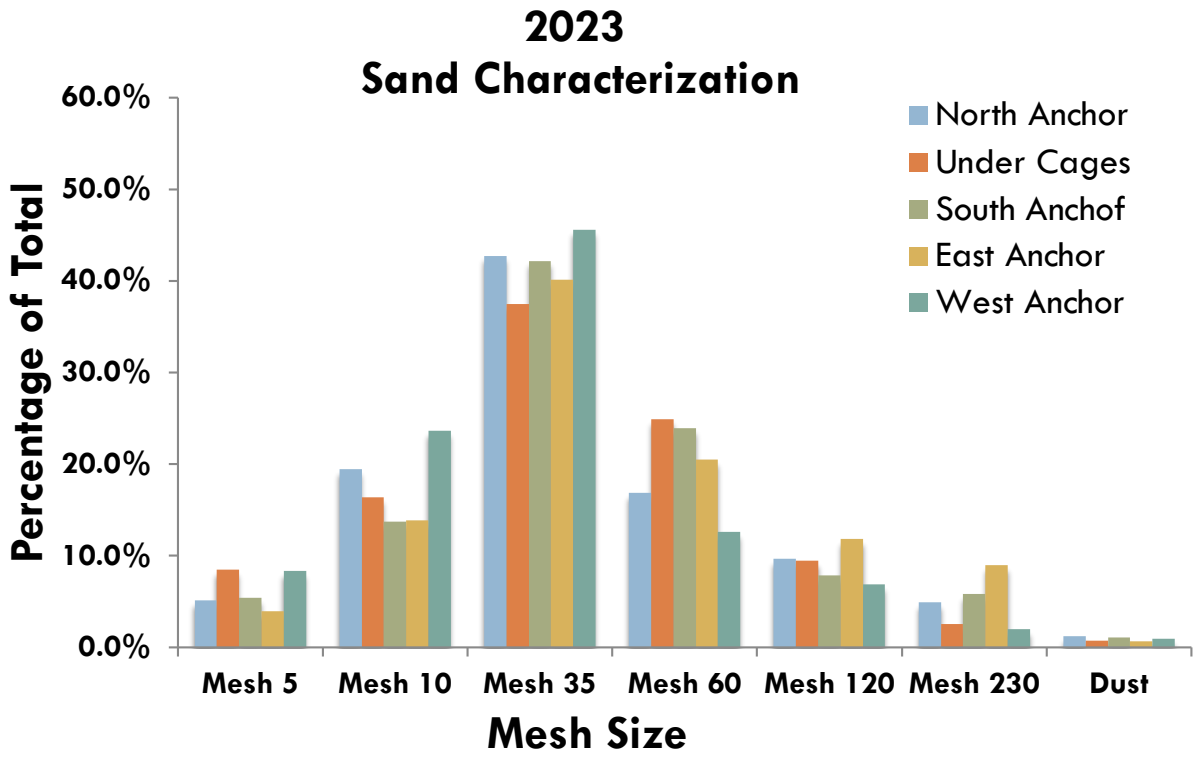


Figure 3. Sand characterization data for all sites from 2023 benthic analysis.

CHEMICAL ANALYSIS

Intro/Methods

Chemical analyses were performed on samples collected from each monitored location. Sub-samples were properly stored and transported to the UH-Hilo EPSCoR Analytical Laboratory for Total Organic Carbon (TOC) analysis. Two sub-samples were analyzed for each location, and the average TOC values are presented in the tables and figures below. Samples where weighed, acidified, weighed again, and then the percentage of organic carbon was determined by processing the samples through a CHN analyzer. TOC (represented as a percentage of total dry sample weight) was calculated by multiplying the percentage of organic carbon by the mass of the acidified sample, and then this value was divided by the mass of the pre-acidified sediment sample. Oxidation/Reduction Potential (ORP) was measured immediately with an ORP meter upon sample retrieval while in the boat.

Measuring the amount of TOC in benthic samples provides information on the amount of sample material that may be derived from decaying vegetation, bacterial growth, and metabolic activities of living organisms or chemicals. Levels of TOC can be indicative of contaminants from discharge, and therefore provides an important measure of the potential effects of industrial discharge on the environment and human health. TOC is a highly sensitive, and non-specific, measurement of all organic elements that are present in a sample. A low value of TOC can confirm the absence of potentially harmful organic chemicals in water exposed to any form of industrial discharge.

ORP provides an indirect method to evaluate the level of biological activity in a benthic sample. This analytical technique also provides a measure of chemical exchange between the substrate and the water column. Low ORP values indicate a high amount of biological activity and an insufficient exchange for maintaining aerobic conditions. Readings below the value of 0 indicate anaerobic conditions. Anaerobic conditions typically occur when

high levels of biological activity remove oxygen faster than it can be restored, thus creating an anaerobic environment. Aerobic and anaerobic conditions will determine the types of organisms that can inhabit the substrate and can cause a shift in the composition of the fauna and flora. Introduction of excess organic materials (fish feces, uneaten feed, or large amounts of bio-fouling material from cages) can be deposited on the substrate and result in anaerobic conditions. ORP therefore enables monitoring of the aerobic state of the substrate below the cages in order to ensure the fish farm is not affecting the biological composition of the sea floor.

Copper and Zinc concentrations were measured from the sediment samples to monitor the levels of these metals. Temporal monitoring will indicate if the copper cages installed in 2015 are affecting concentration levels of these metals in the sediments. For the new permit taking effect in 2021, Silver, Arsenic, Selenium, and Chromium were also added to the analyses of metal concentrations. All the metal concentration values are determined with inductively coupled plasma—mass spectrometry (ICP-MS). Analytical mass and instrumental parameters are selected to ensure accurate and precise determination of each metal by using known standards.

All values for the chemical and metal analyses are presented for the five historical sites to preserve temporal consistency in the report. Values for the additional sites, Control and ZOM, are provided in the report spreadsheet.

Data/Results

Site	ORP Value
North Anchor	225.3
South Anchor	212.3
West Anchor	186.7
East Anchor	162.4
Under Cages	211.7

Site	TOC Value
North Anchor	
South Anchor	
West Anchor	
East Anchor	
Under Cages	

Site	Cu concentration (mg/kg)
North Anchor	
South Anchor	
West Anchor	
East Anchor	
Under Cages	

Site	Zn concentration (mg/kg)
North Anchor	
South Anchor	
West Anchor	
East Anchor	
Under Cages	

Site	Ag concentration (mg/kg)
North Anchor	
South Anchor	
West Anchor	
East Anchor	
Under Cages	

Site	As concentration (mg/kg)
North Anchor	
South Anchor	
West Anchor	
East Anchor	
Under Cages	

Site	Se concentration (mg/kg)
North Anchor	
South Anchor	
West Anchor	
East Anchor	
Under Cages	

Site	Cr concentration (mg/kg)
North Anchor	
South Anchor	
West Anchor	
East Anchor	
Under Cages	

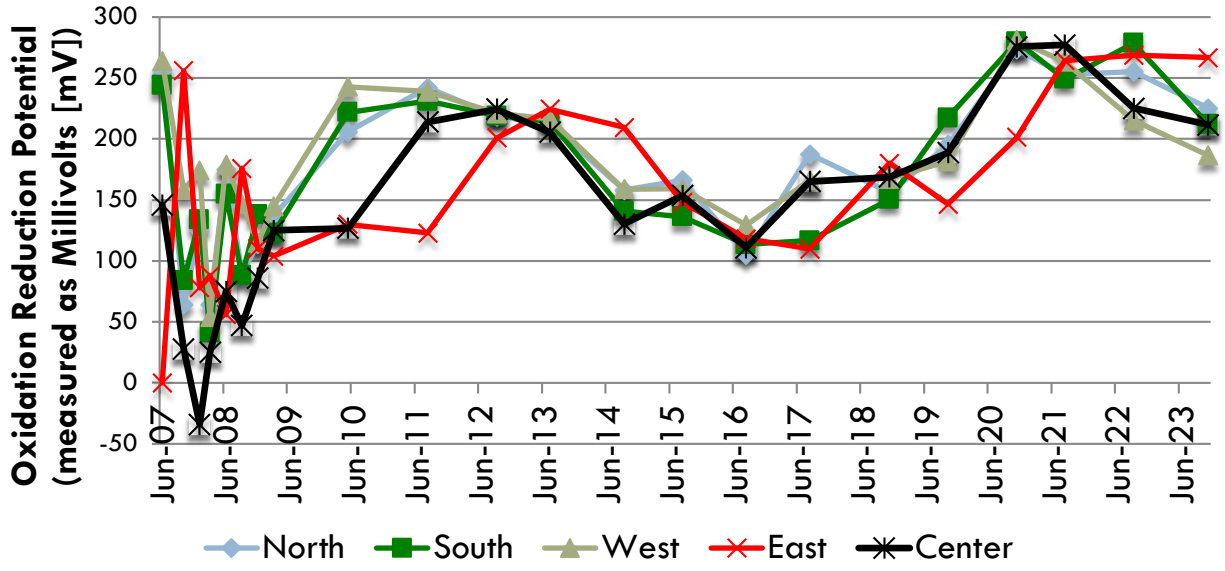
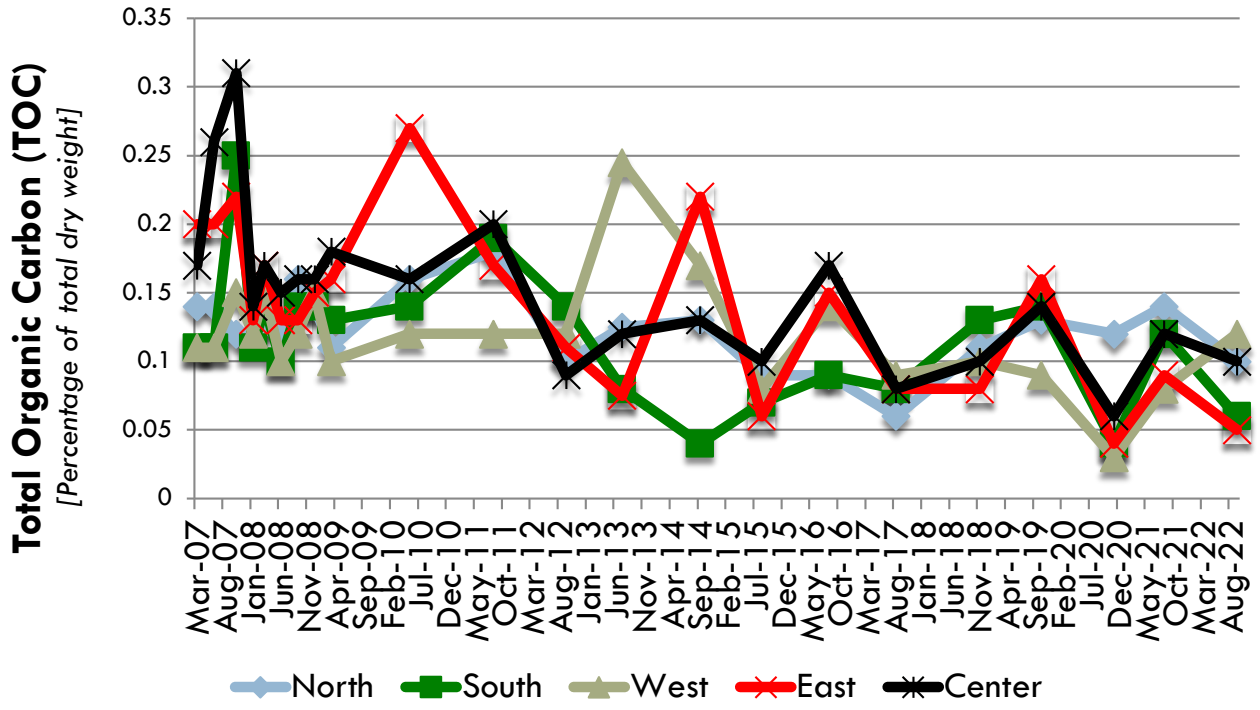


Figure 3. Temporal comparison of ORP values measured at each monitoring location (North, South, West, East, Under Cages).



PENDING 2023 DATA

Figure 4. Temporal comparison of TOC values measured at each monitoring location (North, South, West, East, Under Cages).

Summary

ORP readings taken over the last thirteen years have consistently yielded values over 100 Millivolts (Figure 3). Despite high variability and apparently one case of anoxic conditions, among measurements taken between 2007 and 2009, there appears to be no current evidence of anoxic conditions in the substrate below any of the monitored stations. This is reflected in the color, presence of macro algae and micromolluscs, and lack of odor from the samples monitored this year. These findings provide evidence that the benthic substrate below the cages is aerobic and there appears to be no influence of fish-cage discharge on the oxidation-reduction potential of the benthic substrate. This is likely due in part to the hydrologic mixing induced by the strong currents that affect the benthic substrate in this area.

TOC and metals are pending results for 2023.

MICROMOLLUSC DISTRIBUTION REPORT

CHAIN OF CUSTODY FORMS

Please call if you have any questions regarding the annual benthic monitoring report.

Sincerely,

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