Benthic, Oceanographic, & Water Quality Monitoring Plan for The Nordic Aquafarms California, LLC Outfall in Samoa, California

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Abbreviated Terms

Key terms and acronyms used throughout this document are defined here.

ADCP	Acoustic Doppler current profiler
ASCII	American Standard Code for Information Interchange
C	(Degrees) Celsius
CCC	California Coastal Commission
CEDEN	California Environmental Data Exchange Network
CMECS	Coastal and Marine Ecological Classification Standard
CeNCOOS	Central and Northern California ocean observing system
Cm	Centimeters
CTD	Conductivity, temperature, depth (recording device)
DNA	Deoxyribonucleic acid
DTSS	Delphis Technical Support and Solutions
eDNA	Environmental deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assays
EPA	Environmental Protection Agency
F	(Degrees) Fahrenheit
FGDC	Federal Geographic Data Committee
ft	Feet
GBIF	Global Biodiversity Information Facility
GPM	Gallons per minute
HAB	Harmful algal bloom
in	Inches
ISO	International Organization for Standards
L	Liters
km	Kilometers
m	Meters
MLML	Moss Landing Marine Laboratories
NAFC	Nordic Aquafarms California LLC
NCBI	National Center for Biotechnology Information
nMDS	Non-metric multidimensional scaling
NOAA	National Oceanic and Atmospheric Administration
NPDES	National Pollutant Discharge Elimination Systems
oz	Ounces
PCR	Polymerase chain reaction
ppb	Parts per billion
psu	Practical salinity units
QA/QC	Quality assurance and quality control
RMT II	Redwood Marine Terminal II
ROV	Remotely operated vehicle
RV	Research vessel
RWQCB	Regional Water Quality Control Board
SCCWRP	Southern California Coastal Water Research Project
SWAMP	Surface Water Ambient Monitoring Program
TN	Total nitrogen
TOC	Total organic carbon
TSS	Total suspended solids

Nordic Aquafarms California LLC (NAFC) plans to construct a land-based aquaculture facility to cultivate Yellowtail kingfish (*Seriola lalandi*) in Samoa, California, located in the northern peninsula of Humboldt Bay. The facility will be constructed at the site of a former pulp mill and will utilize the existing Redwood Marine Terminal II (RMT II) intake structure, ocean outfall and multiport diffuser to discharge water from the facility to the ocean. The outfall is composed of a 36 in (91.4 cm) diameter pipe that extends 1.55 miles (2.49 km) into the ocean and ends in an 852 ft (259.7 m) multiport diffuser. The multiport diffuser contains 144 ports paired on either side of the diffuser that are 2.4 in (6.1 cm) diameter, spaced 12 ft (3.7 m) apart (Figure 1). NAFC plans to open an additional 50-60 ports along the diffuser, and the facility will discharge an average of 8,681 GPM with a salinity of ~31 psu and temperature of ~68°F (20°C). Before the water is discharged through the RMT II outfall, the effluent will be treated to decrease nutrients and organic suspended solids (GHD 2021). First discharge is planned for 2027. Table 1 provides expected discharge velocities under all proposed discharge volumes.

Table 1. Expected discharge velocities under all proposed discharge volumes. (Taken from GHD 2023).

% Decrease of Port Exit Velocity	Plume Velocity (ft/s)	NE Horizontal Port Angle		SE Horizontal Port Angle	
		Plume Travel Distance (ft)	Plume Volume (ft^3)	Plume Travel Distance (ft)	Plume Volume (ft^3)
100%	10	0	0	0	0
95%	9.5	0.013	0.00015	0.013	0.00015
90%	9	0.060	0.00076	0.061	0.00076
85%	8.5	0.11	0.0015	0.11	0.0015
80%	8	0.13	0.0021	0.13	0.0021
75%	7.5	0.18	0.0033	0.18	0.0033
70%	7	0.23	0.0047	0.24	0.0051
65%	6.5	0.29	0.0070	0.31	0.0075
60%	6	0.36	0.010	0.40	0.011
55%	5.5	0.45	0.015	0.46	0.016
50%	5	0.53	0.021	0.57	0.023
45%	4.5	0.63	0.031	0.69	0.036
40%	4	0.78	0.048	0.84	0.054
35%	3.5	0.93	0.073	1.0	0.086
30%	3	1.1	0.13	1.2	0.14
25%	2.5	1.4	0.21	1.5	0.25
20%	2	1.8	0.41	2.0	0.52
15%	1.5	2.3	0.96	2.7	1.3
10%	1	3.2	2.9	4.1	4.7
5%	0.5	5.2	18	8.0	46

This document details NAFC's Marine Monitoring Survey Plan that is intended to assess potential changes to oceanographic conditions, water quality, and benthic habitat and biota near the outfall to uphold the National Pollutant Discharge Elimination Systems (NPDES) Permit (ORDER NO: R1-2023-0019; NPDES NO: CA1000003) from the Regional Water Quality Control Board (RWQCB) and Coastal Consistency permit (Permit Application Number: 9-20-0488) from the California Coastal Commission (CCC) that were issued for the Project. Monitoring is scheduled to begin in February 2025 and will continue for up to five years.

Key to the design, implementation, and management of this monitoring program is the understanding and acknowledgement that the oceanographic and weather conditions around the outfall are unique and challenging to work in relative to much of the rest of the California coastline. As a result, this monitoring program has been designed to address the stated environmental concerns and potential effects of discharge to the marine environment. Additionally, this monitoring program must be sufficiently adaptive in nature to address unexpected and unanticipated events and findings. As such, changes may be necessary to continue to assess potential marine effects of the NAFC outfall discharge on the marine environment. Any substantive changes in program design will be made with the full concurrence of the RWQCB and CCC staffs prior to implementation.

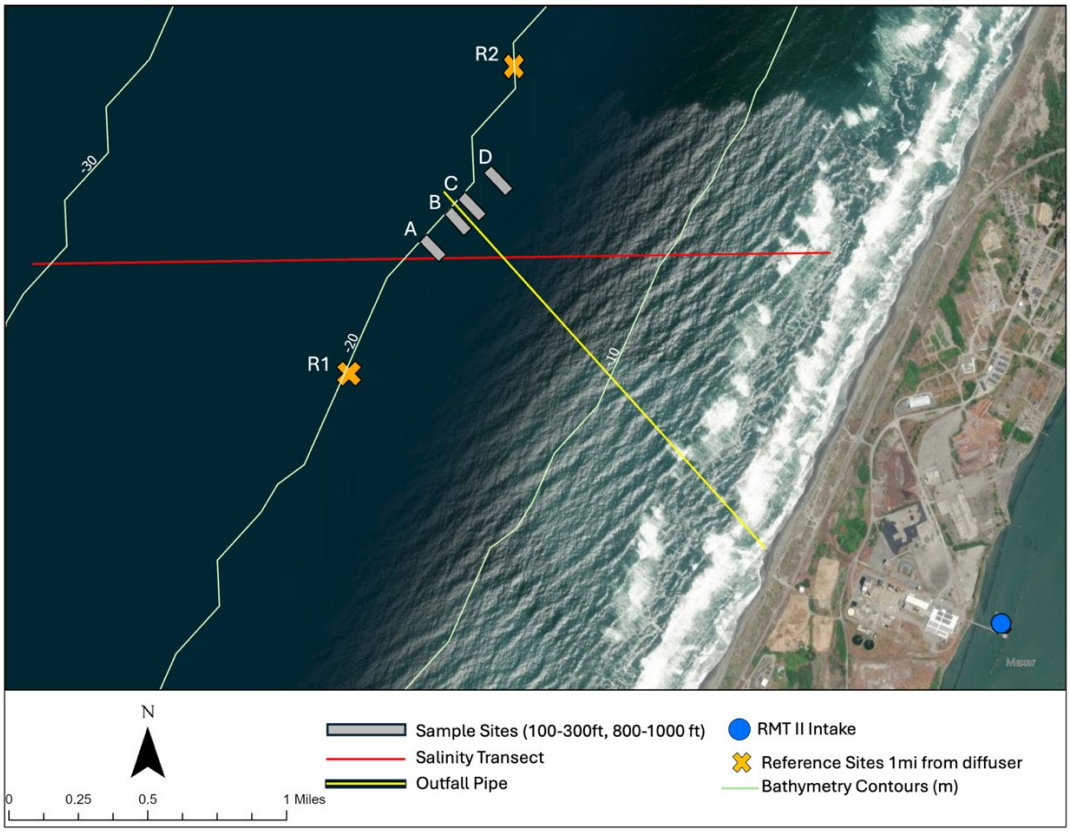

Figure 1. Map of the proposed study location. Gray boxes labeled A-D represent sampling stations.

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The objectives of this Monitoring Plan are to:

1. Obtain water column current data that can be used to further support and confirm the results of a previously run dilution model for the outfall and evaluate outfall sheer stress effects on plankton.
2. Examine water column profiles to assess turbidity, temperature, conductivity, salinity, and pH.
3. Collect water samples to quantify nutrients and suspended solids.
4. Document the occurrence of harmful algal bloom (HAB) causing taxa in the receiving water.
5. Assess any potential changes to the marine benthic community and water column nutrient levels that may be occurring within seasons, between seasons, and between years from the outfall's combined discharge.

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The NAFC component of the combined outfall discharge has no substantial effect on the epibenthic communities and water quality within the zone of influence surrounding the outfall[1].

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Sampling will be conducted by marine scientists from Applied Marine Sciences (AMS) using Cal Poly Humboldt University's RV Coral Sea, during daylight hours. Monitoring will occur four times annually during two different sampling seasons: February-April and July-September. Sampling efforts within the same season will be conducted a minimum of four weeks apart to capture any within season variability. Sampling will be conducted for two years prior to NAFC's first discharge and will continue for three years following the first discharge. This monitoring effort will focus on collecting data and gaining local information pertaining to three main areas: local current patterns, water quality, and benthic habitat and biota (Figure 2).

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2.1.1 Current Profiling

In 2021, GHD conducted a dilution study near the outfall to create a model of local current patterns (GHD 2021). Additionally, H.T. Harvey & Associates has been conducting modelling of the potential effects of shear stress on plankton from the outfall discharge. All dispersion and sheer stress modeling conducted to date has used regional current profile data. To verify the results of this modeling, actual site-specific current profiling data will be collected. Applied Marine Sciences will work with Delphis Technical Support & Solutions, LLC. (DTSS) in addition to Cal Poly Humboldt to deploy one Nortek Signature 1000 series Acoustic Doppler Current Profiler (ADCP) roughly 0.5 miles (0.8 km) down current of the diffuser at a similar depth in fulfilment of NAFC's NPDES (ORDER NO: R1-2023-0019; NPDES NO: CA1000003) and Coastal Consistency (Permit Application Number: 9-20-0488) Permit requirements. Typically, a one-month deployment is sufficient to assess tidal harmonics and provide relatively accurate predictions of future currents based on those harmonics. In this case, two separate, 60-day deployments

[1] The combined outfall can have up to three component discharges. NAFC, DG Fairhaven Power, LLC, and the Samoa Wastewater Treatment Plant are all permitted to discharge through the combined outfall.

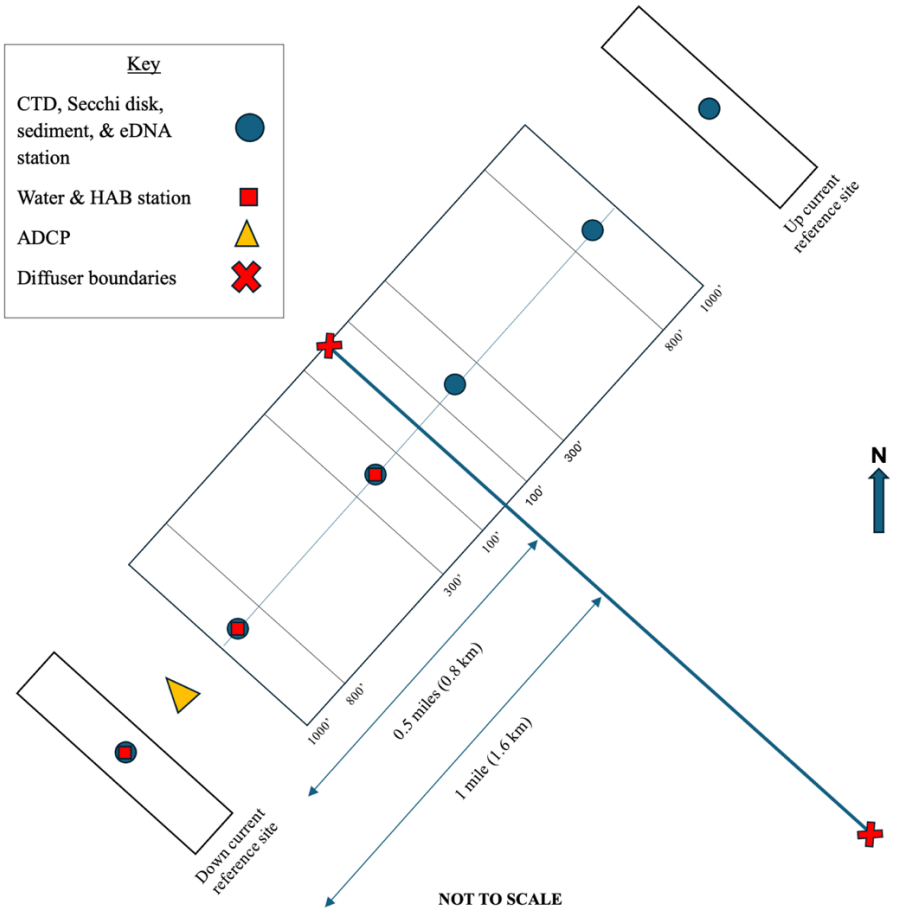

Figure 2. Visual representation of where each sampling effort will be performed. (Image not to scale).

are planned: February 1 to April 30 and July 1 to September 30. The ADCP will be deployed oriented downward from a surface buoy. Sampling profile rates and bin size will be set to cross compare with other data sets from sources such as the National Oceanic and Atmospheric Administration (NOAA) for model comparison.

2.1.2 Currents

Nortek Signature 1000 (ADCP) (Figure 3) vertical current profiles will be collected at a maximum rate of a 3-minute sample period beginning every 10 minutes. Following download of measured data, processing is performed on the data to remove poor quality or erroneous data. Poor quality data are typically a result of environmental conditions which cause poor acoustic signal return or instrument orientation problems which prohibit referencing the current data to a known datum. Erroneous data are those that are known to be collected beyond the physical water boundary or loss of bottom track boundary (e.g., insufficient range or number of cells to seafloor).

Each recorded vertical profile of current consists of velocity measurements in a fixed number of cells (bins) below the instrument, e.g., spaced at 0.5-2.0 m (1.6-6.6 ft) intervals. The first cell begins 0.5 m (1.6 ft) below the instrument and the most distant (bottom) cell is programmed during instrument setup using

Nortek Signature Series Deployment Software to be below the seafloor at high tide with high wave conditions. Thus, the current velocity recorded for a cell location that is physically below the seafloor at a given tide is presumed to be erroneous. The number of cells measuring invalid velocity data changes with the water surface elevation fluctuations and those erroneous data points must be removed from the mean value of measurements to obtain a valid depth-average velocity. The direction of the currents measured during each velocity profile are relative to magnetic north; it is desirable to correct this data to a true north direction during processing.

Current processing is performed with Nortek *SignatureViewer64* processing software for the ADCP. Additional processing will be accomplished using in-house proprietary DTSS software that has been previously used successfully for the same applications. The mean water depth during each current profile will be used to eliminate values from those cells that are below the seafloor (correcting for tide). In addition, any near-bottom cells which may be biased by bottom boundary effects will be removed from the data prior to computing a depth average current. Values in the remaining cells are termed "valid" current data.

Following data collection and download, the Team will identify data dropouts or bad data within each instrument dataset as part of our QA/QC procedures and record where errors occur. If part of a data record is not acceptable, it will be noted as such and render the remaining data record usable in terms of maintaining serial continuity with other data sets. Data will be exported and saved as American Standard Code for Information Interchange (ASCII) data files following general conventions to permit maximum accessibility and utility to each data record and retain the ability to merge and append data records in terms of a common reference time. The ASCII file will be provided in Microsoft Excel .xlsx format. A final report shall be provided electronically to NAFC upon completion of the project and after review of the interim data. The report will document project data collection inventory and physical units, data structure, sample data plots, and summary interpretation of data quality and noteworthy data.

Figure 3. Nortek Signature 1000 ADCP.

2.1.3 Deployment Location

The current meter will be deployed at a single location for approximately 60 days (weather dependent). The deployment location will be within 0.5 mile (0.8 km) of the terminus of the outfall as illustrated in Figure 4. The water depth is expected to be between 20-30 m (65.6-98.4 ft) relative to the mean lower low

water. Actual coordinates of the deployment will be provided following each deployment and reported to the United States Coast Guard for *Notice-to-Mariners* publication.

2.1.4 Instruments

The proposed instrumentation to meet the scope of work requirements includes:

- Nortek Signature 1000 kHz down-looking ADCP profiler with AHRS (movement compensation).
- Medium duty surface buoy (Nexsens CB450 or above) equipped with surface beacon and radar reflector. The buoy will support additional, external power to current profiler.
- Extreme-conditions compliant mooring with anchor and compliant sections. Two sections required to maintain unobstructed profiles from mooring lines as shown in Figure 5.

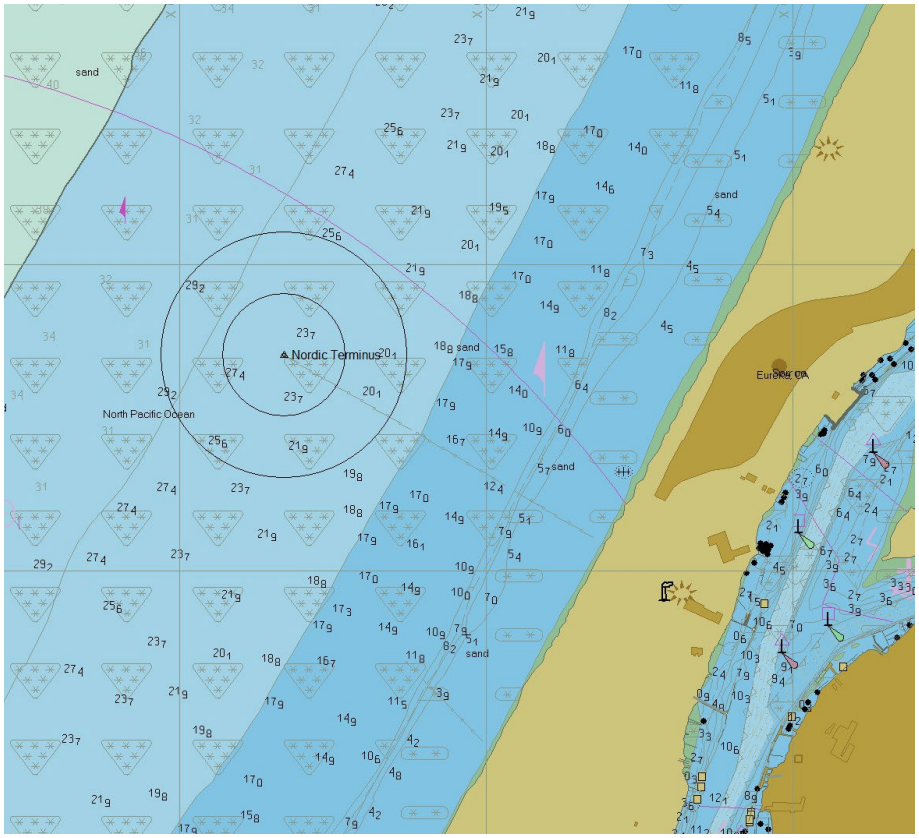

Figure 4. Proposed deployment area for the surface mounted current meter (rings at 0.25 mile [0.4 km] radius).

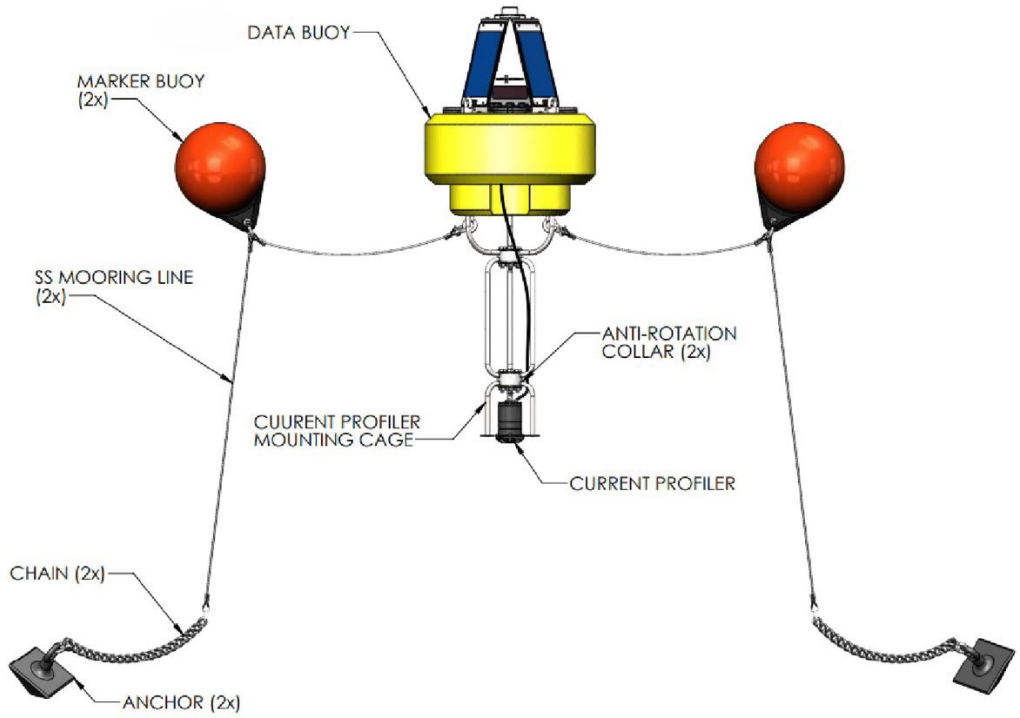

Figure 5. Proposed downward oriented ADCP on surface buoy.

2.1.5 Conductivity, Temperature and Depth (CTD) Profiles

Water column profiles will be sampled using a Eureka Manta+35 (Figure 6) water quality multiprobe at six stations: 100-300 ft (30.5-91.4 m) down and up current of the diffuser, 800-1,000 ft (243.8-304.8 m) down and up current of the diffuser, and at two reference sites roughly a mile (1.6 km) down and up current of the diffuser. These stations will be sampled four times per year, twice between February and April and twice between July and September. Sampling events within seasons will occur no less than four weeks apart. The Manta+35 CTD measures depth, conductivity (salinity), temperature, pH, dissolved oxygen, and turbidity at two second intervals. Water column profiles will be conducted from the surface to approximately 1 m (3.3 ft) above the seafloor. Conductivity, pH, turbidity, and dissolved oxygen probes will be calibrated no sooner than 24 hours before each sampling event using standard calibration solutions. Prior to every water column profile, the CTD will be lowered to the surface and allowed to acclimate to the current station. After three minutes, the CTD will descend at an approximate rate of 17 m/minute (55 ft/minute) until it is approximately 1 m (3.3 ft) above the seafloor. The CTD will be attached to a mini rosette water sampler which will collect water grab samples on its ascent.

Turbidity will be measured using an optical sensor probe within the Manta+35 according to the International Organization for Standards (ISO) 7027 standard (ISO, 1990), which measures the amount of light scattered by suspended particles at 90 degrees to a beam of infrared light. A Secchi disk (Figure 7) will also be used to assess water clarity in addition to the Manta+35 measurement of turbidity. Secchi disk procedures will follow California Surface Water Ambient Monitoring Program (SWAMP) standard operating procedures (SWRCB, 2023). Secchi disk transparency depth will be measured at all CTD stations.

Figure 6. Eureka Manta+35 CTD: On the left is a Eureka Manta+35 CTD which measures conductivity (salinity), temperature, depth, pH, dissolved oxygen, and turbidity. On the right is an image depicting deployment/retrieval of a Eureka Manta+35.

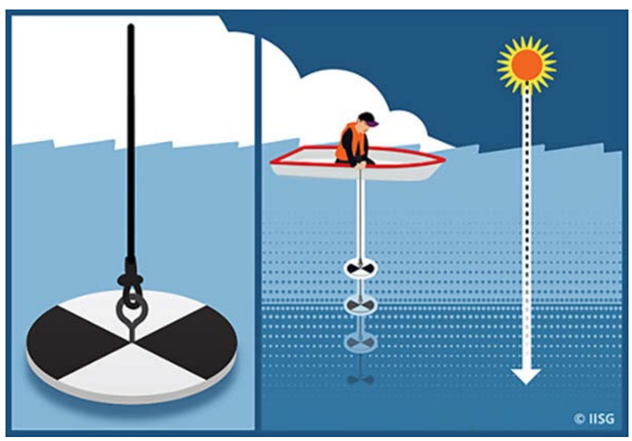

Figure 7. Depiction of a Secchi disk deployment.

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The mini rosette water sampler (General Oceanics, model 1018; Figure 8) will be equipped with six 5 L (1.3 gal) Niskin or Go-Flow sampling bottles that can be pre-programmed to sample at specified water depths. Water samples will be collected from near bottom, within 3-5 ft (0.9-1.5 m) of the seafloor, and near surface, within 1-3 ft (0.3-0.9 m) of the sea surface at all down current CTD stations. Two to three bottles will collect water samples at each depth to ensure enough water is collected for all analytes simultaneously. The Manta+35 CTD multiprobe will be attached to the mini rosette frame and profile the water column during descent. The multiprobe will also collect data on the ascent but stops will be made to collect water samples at the near bottom and near surface locations.

Figure 8. Mini Six position 5 L (1.3 gal) Rosette Mode Ml1018 General Oceanics. The Eureka Manta+35 will be positioned on the frame where the white probe is in this photo.

2.2.3 Nutrient Samples

The NPDES Permit (ORDER NO: R1-2023-0019; NPDES NO: CA1000003) issued to NAFC by the RWQCB requires that several nitrogen-based nutrients as well as suspended solids be monitored. Ammonia, nitrate, nitrite, total nitrogen (TN), and total suspended solids (TSS) samples will be collected at the down current CTD stations with the rosette water sampler during each survey. Water column profiles of turbidity as discussed above will be collected with the CTD multiprobe (ISO, 1990). Grab water samples will be collected near surface within the top 1-3 ft (0.3-0.9 m) of the water column and near bottom, within 3-5 ft (0.9-1.5 m) of the seafloor (depending on sea conditions) with the General Oceanics mini rosette water sampling system comprised of six 5 L (1.3 gal) Niskin or Go-Flow bottles. Chlorophyll-a samples will be analyzed from the near surface water samples at all water quality stations during the July-September events. All samples will be stored appropriately per analysis type and shipped/delivered to State approved laboratories (Table 2).

Table 2. Summary of the State approved laboratories and analyses that will be used to assess the analytes collected in the water and sediment grab samples.

Analyte	Analysis	State Approved Laboratory
Ammonia	The Lachat Autoanalyzer which is NPDES accepted or equivalent to EPA method 350.1 (EPA, 1993; Lachat Instruments, 2018).	Moss Landing Marine Laboratories (Moss Landing, CA)
Nitrate	The Lachat Autoanalyzer which is NPDES accepted or equivalent to EPA method 353.2 (EPA, 1993; Lachat Instruments, 2018).	Moss Landing Marine Laboratories (Moss Landing, CA)
Nitrite	The Lachat Autoanalyzer which is NPDES accepted or equivalent to EPA method 353.2 (EPA, 1993; Lachat Instruments, 2018).	Moss Landing Marine Laboratories (Moss Landing, CA)
TN	Teledyne Tekmar Torch Combustion TOC/TN Analyzer ISO 20236	Moss Landing Marine Laboratories (Moss Landing, CA)
TOC	EPA Method 9060M	Physis Environmental Laboratories, Inc. (Anaheim, CA)
Grain size	EPA Method SM2560	Physis Environmental Laboratories, Inc. (Anaheim, CA)
TSS	Standard SM2540-D-2011 method (SM 2540).	Alpha Analytical Laboratories, Inc. (Ukiah, CA)
Chlorophyll-a	Standard in vitro methods as described in EPA method 445.0 (EPA, 1997) Bend Genetics (Sacramento, CA)	Bend Genetics (Sacramento, CA)
HAB	Marine algal toxins will be assessed using enzyme–linked immunosorbent assays (ELISA; EPA method 546; EPA, 2016) as well as microscope cell enumeration (cells/mL and biovolume) of potentially toxic genera.	Bend Genetics (Sacramento, CA)
eDNA	Polymerase chain reaction (PCR).	NatureMetrics (Guelph, ON, Canada)

2.2.4 Harmful Agal Bloom Samples

Harmful algal blooms (HABs) are caused by outbreaks of toxin-producing phytoplankton such as dinoflagellates and diatoms. High nutrient loads coupled with warming waters occurring as a result of climate change have increased the incidence of HABs globally. These events can negatively impact nutrient cycles, marine species, fisheries, economies, and human health (Fu et al. 2012; Gobler 2020). Harmful algal blooms from taxa such as *Pseudo-nitzschia* spp., which produces the neurotoxin domoic acid, have become an increasing concern in Humboldt Bay. Humboldt Bay is located between Cape Mendocino, California and Cape Blanco, Oregon which have been described as "hot spots" for *Pseudo-nitzschia* spp. (Trainer et al. 2009; Winnacott 2023). This area experiences significant upwelling in the spring which brings cool, nutrient rich waters from the deep to the surface that support primary productivity. This is followed by a relaxation period in the summer characterized by lower winds and warmer temperatures (Garcia-Reyes and Largier 2012). Additionally, the bathymetric features of Cape Mendocino and Cape Blanco promote eddies that can trap HAB producing species (Largier et al. 1993; Barth et al. 2000; Trainer et al. 2009; Winnacott 2023). Therefore, HABs that might occur inside Humboldt Bay would likely be oceanic in origin and would be heavily dependent on tidal changes, temperature, and light levels within the Bay (Winnacott 2023).

The CCC's conditional approval (Permit Application Number: 9-20-0488) and the NPDES Permit (ORDER NO: R1-2023-0019; NPDES NO: CA1000003) require monitoring for HABs. Surface water

samples will be collected at all water stations down current of the discharge outfall to determine presence of HAB cells and toxins during summer sampling efforts (July-September). Harmful algal bloom monitoring will include cell enumeration (cells/mL) and biovolume of potentially toxic genera. Marine algal toxin concentrations will also be assessed (Table 3).

Table 3. Limits of detection per marine algal toxin from Bend Genetics (Sacramento, California). *ppb = parts per billion.

Algal Toxin	Analysis	Limit of Detection
Brevetoxins	Enzyme–linked immunosorbent assay (ELISA)	0.05 ppb in water
Cyclic imines, including spirolides and pinnatoxins	ELISA	8 ppb in water
Domoic acid	ELISA	6 ppb in water
Saxitoxins	ELISA	0.015 ppb in water

2.2.5 Laboratory Analysis

Dissolved nutrients in sea water will be analyzed colormetrically using the Lachat Quickchem 8000 Flow Injection Analyzer with an autosampler. The Lachat Autoanalyzer is a five-channel system where all analytes can be measured in one sample aliquot simultaneously. Ammonia, nitrate, and nitrite will be measured using this technique by Moss Landing Marine Laboratories' (MLML) Analytical Nutrient Lab. The nitrogen methods used by the Lachat Quickchem autoanalyzer are NPDES equivalent to Environmental Protection Agency (EPA) method 353.2 (EPA, 1993a; Lachat Instruments, 2018). Ammonia determined by the autoanalyzer is NPDES equivalent to EPA method 350.1 (EPA, 1993b; Lachat Instruments, 2018). Total nitrogen will be analyzed using a Teledyne Tekmar Torch Combustion TOC/TN Analyzer also by MLML's Analytical Nutrient Lab. Total nitrogen determined by the Torch analyzer uses the most recent international method standard ISO 20236 (IS0 2021). MLML Analytical Nutrient Lab follows the California SWAMP Quality Assurance Program Plan to the best of their ability. Total suspended solids will be analyzed by Alpha Analytical Laboratories, Inc. in Ukiah, California (California Accreditation, cert #: 1551) using the standard SM2540-D-2011 method (SM 2540).

Chlorophyll-a and HAB analyses will be conducted by Bend Genetics (Sacramento, California). Chlorophyll-a analysis will follow standard *in vitro* methods as described in EPA method 445.0 (EPA, 1997). Analysis of HABs will be conducted two ways, marine algal toxins (Table 2) will be assessed using enzyme–linked immunosorbent assays (ELISA; EPA method 546; EPA, 2016) as well as microscope cell enumeration (cells/mL and biovolume) of potentially toxic genera. The proper QA/QC methodologies including matrix spikes, duplicates, lab blanks and STD checks for ammonia, nitrate, and nitrite, matrix spikes, duplicates, lab blanks, and standard deviation checks for TN, duplicates for TSS, Duplicates and standard deviation checks for chlorophyll-a and turbidity. A summary of the State approved laboratories and analyses that will be used to assess the analytes collected in the water and sediment grab samples is provided in Table 2.

2.2.6 Data Analysis

Both CTD and water grab sample nutrient results will be used to assess differences between areas near the discharge outfall and reference sites. These water quality parameters will be used in multivariate statistical methods to help explain potential differences between sampling areas. Additionally, these parameters may be used to explore drivers of variation in epibenthic communities in the study area.

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2.3.1 Sediment Grab Samples: Total Organic Carbon and Grain Size

The composition of soft sediment marine environments strongly influences the biological communities that may be present in an area (Soto et al. 2016), and subtle differences may result in highly localized differences in communities. To assess how benthic species composition may be influenced by sediment characteristics, total organic carbon (TOC) and sediment grain size will be analyzed once per season at all sites using a Shipek or Van Veen sediment grab with a 0.1 square meter (0.3 ft^2) capacity (Figure 9). Approximately 100 g (3.5 oz) of sediment from the top 2 in (5 cm) of sample will be scooped from several locations in the grab and placed into a labeled amber 8 oz (227 g) glass sample jar for analysis of TOC. Another set of scoops from the top 2 in (5 cm) will be collected and placed into a zipper storage bag for analysis of grain size. All samples for laboratory analysis will be initially stored in coolers with either wet or blue ice, and subsequently transferred to refrigeration at 4˚C (39.2˚F), prior to shipment for laboratory analysis. Samples will be shipped to Physis Environmental Laboratories, Inc in Anaheim, California for analysis. Grain size analysis will be conducted using standard method 2560 (SM 2560). Total organic carbon is assessed by EPA method 9060A (EPA 2004) (Table 2).

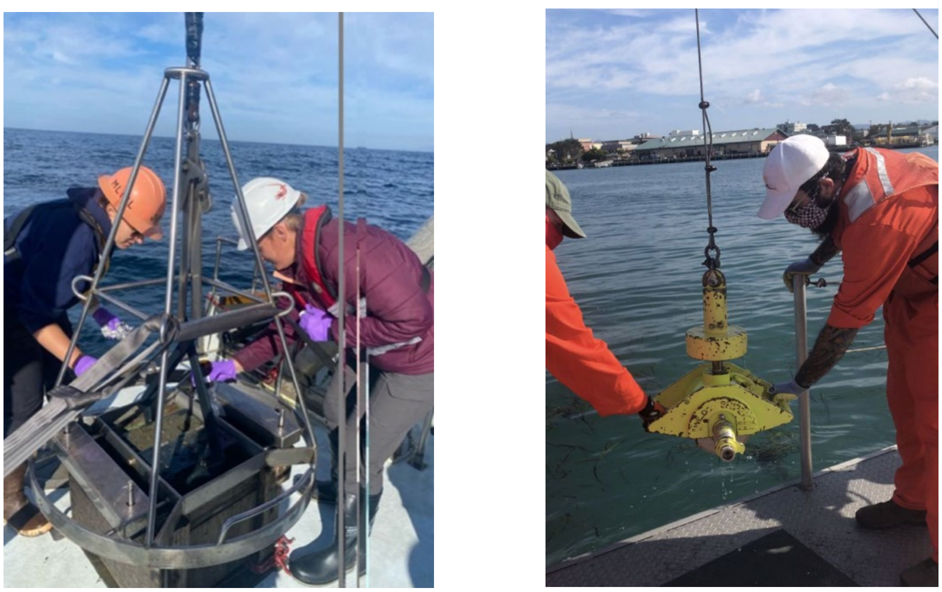

Figure 9. Sediment grabs: On the left is a Van Veen sediment grab with a 0.1 m^2 (0.3 ft^2) capacity, and on the right is a Shipek grab sampler.

2.3.2 Epibenthic Community Monitoring Considerations

Collecting meaningful information about the macrobenthic and fish communities along the North Coast of California is extremely challenging due to the highly dynamic weather and sea conditions and consistently turbid water column. The NPDES (ORDER NO: R1-2023-0019; NPDES NO: CA1000003) and Coastal Consistency Permits (Permit Application Number: 9-20-0488) both outline the use of more traditional visual survey methods utilizing either drop cameras or remotely operated vehicles (ROVs) to collect qualitative data, which can be valuable under optimal light, sea, and weather conditions. The monitoring stations established in the permits are located in a highly dynamic area at the edge of the surf zone that regularly experiences unusual and unpredictable sea conditions as well as high turbidity (Houle 2015). These unfavorable conditions are anticipated to result in very blurry and unusable benthic images or video footage. In 2022, H.T. Harvey & Associates conducted drop camera surveys near the RMT-II

outfall and noted that turbidity seriously impeded their ability to accurately identify and quantify species in their images (H.T. Harvey 2022).

Drop camera and ROV surveys are also quite time consuming, and weather windows that will allow for safe boating conditions to facilitate this sampling approach may be too short to complete planned survey efforts within the study area. This is especially likely during the February-April sampling period, which experiences some of the worst sea and weather conditions in the region. Additionally, the February-April sampling period overlaps with the Dungeness crab fishery season, and towing/deploying an ROV or drop camera poses a risk for entanglement with crab pots. These concerns make using an ROV or drop camera to survey the benthic community offshore Humboldt Bay problematic at best.

To further complicate the scientific data gathering approach, there are known biases associated with using image-based surveys. Fishes and other mobile organisms can sense the pressure waves that occur as a camera is lowered or moves above the seafloor. This can prompt a flight response causing species to flee the area or hide or bury themselves (Koslow et al. 1995; Laidig et al. 2012). Regardless, the organisms are not visible in the image or video footage, resulting in the taxa being wrongfully identified as absent from the area and study results. Similarly, this methodology tends to over account for non-mobile and slow-moving taxa.

The lights associated with drop cameras and ROVs are also known to scare or attract fishes, which can compromise fish counts (Stoner et al. 2008; Rooper et al. 2015). Additionally, noise from the survey vessel or the camera itself can cause fishes to leave the area (Vabo et al. 2002; Handegard et al. 2003; Stoner et al. 2008). These occurrences result in inaccurate and biased data. For safety consideration, surveys will also only be conducted during daylight hours, which means that utilizing ROVs or drop cameras will not capture taxa that are more active at night; again, introducing bias into study findings and results. Additionally, despite best efforts and professional expertise, there remains potential for reviewers to misidentify or fail to detect taxa that may be present because of poor image quality during the image processing stage (Durden et al. 2016).

The severe limitations of the photogrammetry-based monitoring approach in the Project study area prompted an examination of other available and relevant methods of sampling benthic communities which could provide consistent and better-quality data on the epibenthic, and fish communities that could be potentially affected by NAFC's outfall discharge.

Environmental DNA (eDNA) is non-invasive and utilizes the DNA shed by organisms into the environment. This includes genetic material found in skin, scales, hair, gametes, and feces that can be used to monitor the presence of organisms in an ecosystem (Diaz-Ferguson and Moyer 2014; Rees et al. 2014; Ip et al. 2022). This material can be collected via sediment, air, or water samples (Thomsen et al. 2012; Jeunen et al. 2018; Sakata et al. 2020; Clare et al. 2022). Environmental DNA methods utilize genetic markers unique to individual species that can be extracted to determine the presence of single or multiple species and estimate relative abundance and populations density (Lacoursiere-Roussel et al. 2016; Fediajevaite et al. 2021; Bradley et al. 2022)

Environmental DNA sampling is not a novel practice. Microbial DNA was first extracted from sediments in the 1980s (Ogram et al. 1987). In the 1990s, eDNA was used to monitor phytoplankton blooms (Bailiff and Karl 1991). Environmental DNA was first used to study multicellular organisms in 1998 by Paget et al. to determine the length of time that genetically modified tobacco DNA could remain in the soil (Paget et al.1998). This was followed by its application in the field of paleoecology to reconstruct ancient plant, fungi, and protist communities in Northern Greenland (Willerslev et al. 1999). The first analysis of multicellular organisms in water samples occurred in 2005 (Martellini et al. 2005). Since then, eDNA has been used to sample a wide variety of vertebrate, invertebrate, bacterial, and plant communities (Thomsen

et al. 2012; Kraaijeveld et al. 2014; Port et al. 2015; Andruszkiewicz et al. 2017; Everett and Park 2018; Jeunen et al. 2018; Lafferty et al. 2018; Closek et al. 2019; Leduk et al. 2019; Djurhuus et al. 2020; Gold 2020; Sakata et al. 2020; Piggott et al. 2021; Clare et al. 2022; Ip et al. 2022; Miya et al. 2022; Mac Loughlin et al. 2024). One of the most common uses is sampling marine and aquatic fishes (Thomsen et al. 2012; Port et al. 2015; Andruszkiewicz et al. 2017; Jeunen et al. 2018; Lafferty et al. 2018; Closek et al. 2019; Gold 2020; Sakata et al. 2020; Piggott et al. 2021; Miya et al. 2022; Mac Loughlin et al. 2024).

Environmental DNA methods are comparable to or outperform more traditional environmental monitoring strategies such as scuba, snorkel, trawling, plankton pumps, plankton tows, gillnets, fish pots, fyke-nets, beach seining, electrofishing, lure fishing, Van Veen grabs, sediment cores, and push netting (Thomsen et al. 2012; Closek et al. 2019; Leduc et al. 2019; Gold 2020; Piggott et al. 2021; Bradley et al. 2022; Ip et al. 2022). It has become increasingly popular as a non-invasive sampling strategy for agencies around the country, and around the world. Environmental DNA methods have been implemented by NOAA to study deep sea corals on the West Coast; the U.S. Fish and Wildlife Service to monitor endangered species, and the U.S. Army Corps of Engineers to detect invasive species in the Great Lakes (Everett and Park 2018; Laschever et al. 2023).

One of the major concerns with eDNA methods in the United States has been a lack of standardization for implementing ecosystem management practices (Kelly et al. 2023). Canada has already adopted a set of national standards (Gagné et al. 2021), and nations like Australia, New Zealand, and Finland have made serious strides toward establishing their own national standards (Norros et al. 2022; De Brauwer et al. 2023). However, a recent White House-led task force has established the National Aquatic eDNA Strategy to provide a clearer pathway for federal agencies to adopt widely accepted eDNA standards that protect, support, and better understand the Nation's biological resources (Gold et al. 2024). In California, the Southern California Coastal Water Research Project (SCCWRP) and its partners are preparing to lead the State in implementing the program. Environmental DNA methods have already been used by SCCWRP partners including the statewide Estuary Marine Protected Areas Monitoring Program and the Southern California Bight Regional Monitoring Program to assess California's aquatic resources (SCCWRP 2024).

As discussed above, there are some limitations to using eDNA, as with the drop camera and ROV methods. However, eDNA will undoubtedly provide much more information about what species are present near the outfall and reference sites which will aid in answering resource management questions about potential changes to the benthic community that could be occurring due to the discharging of wastewater from NAFC's aquaculture facility. An additional comparison of eDNA approaches and ROV and drop camera survey methodologies is provided in Table 4.

Table 4. Comparison of eDNA methods with drop camera and ROV surveys for benthic community surveys.

Sampling Concern	Drop Camera & ROV Surveys	eDNA
Weather and sea conditions	Drop camera and ROV surveys require significantly calmer conditions to ensure stability of the camera and safe operations.	eDNA samples can be collected in any safe boating conditions because all that is required is a water sample.
Turbidity/light levels	Drop camera and ROV surveys are challenging to complete in low light/high turbidity locations.	eDNA samples can be collected in low light/high turbidity locations. However, high turbidity may increase the sample filtration time and require the use of a pump.
Inclusion and identification of species	Species identification is limited to the organisms that happen to be present at the moment the images or video footage is captured, and image quality has a significant effect on accurate species identification. Additionally, vessel noise and the pressure waves caused by the lowering of the instrument may startle and cause highly mobile species to vacate the area.	eDNA can identify all species that have occurred in the area even days after they appeared. eDNA samples will also better detect new or invasive species in very low abundances within the study area.
Size of organisms	Drop camera and ROV surveys will under detect small, microscopic, and low abundance organisms.	eDNA will detect small and microscopic organisms but may over account for larger taxa that shed more DNA.
Field time	More field time is required for drop camera or ROV operations making them more challenging to conduct due to rapidly changing weather and sea conditions in the study area.	eDNA is much more efficient as it only requires additional water samples which are the priority of this sampling effort. Additionally, sample filtration can be completed at a later time.
Post-processing time	It can be very time consuming to process images and identify species in an image or video footage	PCR is very efficient.
Contamination	Contamination is not a concern in drop camera or ROV surveys.	Sample contamination may occur due to improperly cleaned sample bottles, during sample filtration or handling, or during sample processing in the lab. Contamination can introduce DNA from species that are not present at the sample location resulting in a false positive. The eDNA lab, NatureMetrics, has well established QA/QC and operational procedures to make contamination and the reporting of false positives in samples a very low concern.

2.3.3 Environmental DNA Sampling

Environmental DNA in marine environments is very dilute. Therefore, sample volume should be maximized to better represent the environment. Good results have been obtained with 2-5 L (0.79-1.3 gal) samples (Bruce et al. 2021). The General Oceanics mini rosette contains six, 5 L (1.3 gal) Niskin/Go-Flow bottles, and three bottles will be used to collect eDNA samples near the seafloor within four sampling areas within the zone of influence: 100 to 300 ft (30.5-91.4 m) down current of the diffusers, 800 to 1,000 ft (243.8-305 m) down current of the diffusers, 100 to 300 ft (30.5-91.4 m) up current of the diffusers, and 800 to 1,000 ft (243.8-305 m) up current of the diffusers, and at two sampling areas outside the zone of influence: at a reference site 1 mile (1.6 km) down current of the diffusers and at a reference site 1 mile (1.6 km) up current of the diffusers.

Collecting near seafloor eDNA samples should be sufficient to assess the epibenthic and fish communities near the RMT-II outfall. Nichols and Marko (2018) demonstrated that diver surveys were highly correlated with the abundance of coral collected in water samples 1 m (3.3 ft) above the reef. Jeunen et al. (2019) observed that marine environments display permanent vertical zonation and therefore little vertical mixing. Haloclines and other oceanographic conditions have been reported to restrict vertical migration of eDNA (Jeunen et al. 2019). The surge induced turbidity reported occurring near the seafloor offshore Humboldt Bay is extremely high (H.T. Harvey 2022) and is anticipated to act as a barrier to vertical migration of eDNA present near the seafloor.
Environmental DNA samples are highly sensitive to contamination. To avoid contamination between stations, the Niskin/Go-Flow bottles in the General Oceanics mini rosette will be cleaned with a 10% bleach solution, followed by deionized water and methanol rinses after each sample collection. Field controls will also be used to ensure quality. The sample laboratory, NatureMetrics (Table 2), conducts rigorous quality control testing and follows strict procedures to prevent contamination from the lab.

Following sample collection, the water will be filtered through laboratory supplied sterile capsule filters with 0.8 µm pore size to capture the eDNA from the seawater. Due to the turbid nature of the near seafloor waters in Humboldt Bay, this process could be lengthy (Bruce et al. 2021) and may require the use of a pump to push the sample through the filter. However, if there is not ample time or sea/weather conditions do not allow for filtration on the vessel, samples can be filtered upon return to dock. After filtration, the samples will be carefully packaged in lab supplied buffer solutions and sent to NatureMetrics for analysis. Laboratory analysis consists of the following steps:

- DNA extraction.
- Quality control testing to ensure extracted DNA meets required thresholds.
- Inhibition testing and additional purification of samples where required.
- Polymerase chain reaction (PCR) amplification, carried out in 12 replicate reactions for each water sample. Analyzing replicates for the water samples helps to compensate for amplification stochasticity due to low concentrations of target DNA and increases the chances of detecting rare species. Amplified DNA is checked using gel electrophoresis and replicates for each sample pooled for downstream analysis.
- Library preparation using dual-indexed tags.
- Quality control testing to ensure libraries meet required thresholds.
- Purification, quantification, and normalization of libraries.
- Sequencing on the Illumina MiSeq platform with a target sequencing depth of approximately 100,000 sequences per sample.
- Bioinformatics processing to quality filter, denoise, cluster and assign taxonomy to the sequences.
- Ecological statistics and data visualizations

DNA sequences will be referenced to the National Center for Biotechnology Information (NCBI) and Global Biodiversity Information Facility (GBIF) databases.

2.3.4 Data analysis

The number of organisms identified to the lowest taxonomic division possible will be used to determine species richness. The proportion of each unique genetic fragment at each site will be used to determine the relative abundance of each identified taxon.

To investigate whether the assemblages differ between years, seasons, and sites, multiple indices will be used including species richness, evolutionary diversity, stepwise regression and cluster analysis and if possible, non-metric multidimensional scaling (nMDS) (Closek et al. 2019). Different indices will be used to evaluate within season, between season, between years and before and after operations, as appropriate and supported by the data.

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The monitoring stations are located in a highly dynamic area at the edge of the surf zone that regularly experiences extreme weather and sea conditions. Strong winds during the spring months (April-June) promote coastal upwelling which transports cold, nutrient-rich waters from the deep ocean toward the surface, which supports primary productivity. Weaker winds and reduced storm activity in the summer months (July-September) results in a relaxation season when ocean conditions are calmer (Garcia-Reyes and Largier 2012). Therefore, it is possible that entire sampling events or seasons, especially the February-April season may be challenging to complete due to a lack of safe operating conditions, or an inability to space within seasons sampling events at least four weeks apart. Utilizing the eDNA approach for sampling benthic epifauna is best able to accommodate these logistic challenges since collection of water quality and CTD samples has been identified by both RWQCB and CCC staffs as the highest priority samples to be collected. Additionally, the deployment of the Nortek 1000 ADCP unit for the duration or as close to the duration of each sampling season as safely possible will be prioritized.

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A report will be submitted to the agencies following each sampling season to document results and from the oceanographic, sediment, water quality, HAB, and biological surveys. An annual report will also be submitted at the end of each year documenting intra-annual variability. The first two years of reporting will focus on baseline data prior to NAFC's discharge. The third year of reporting will begin to capture conditions during discharge.

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The data collected from these sampling efforts will be analyzed in accordance with and entered into existing datasets to build on knowledge of the marine environment in Humboldt County. The Coastal and Marine Ecological Classification Standard (CMECS) will be employed to describe ecological and benthic habitats encountered during the surveys. The CMECS is a hierarchical catalogue of ecological terms to define and interpret coastal and marine communities and habitats as well as streamline various sensors and platforms of data collection (FGDC 2012). The language follows a federal standard implemented by the Federal Geographic Data Committee (FGDC) allowing for universal classification standard for data sharing. Benthic survey reports and analyses will use CMECS terminology and data will be submitted to contribute to this repository. Oceanographic data such as temperature, salinity, conductivity, pH, and Chlorophyll-a will be entered into the Central & Northern California Ocean Observing system (CeNCOOS) database. Additionally, nutrient data from the water quality sampling efforts will be added to the California Environmental Data Exchange Network (CEDEN) database.

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