MORRO BAY NATIONAL ESTUARY PROGRAM COMMUNITY PROJECTS PROGRAM PROPOSAL: DEVELOPING A LONG-TERM MONITORING PROGRAM FOR MICROPLASTIC IN THE MORRO BAY NATIONAL ESTUARY

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Morro Bay National Estuary Program
Community Project Program

A. Project title: Developing a Long-Term Monitoring Program for Microplastics in Morro Bay National Estuary

B. Applicant organization: Department of Biological Sciences, California Polytechnic State University, San Luis Obispo (Cal Poly)

C. Organization type: California State University

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F. Amount requested: $6,551

G. Total project cost: over $17,000 (if including matching volunteer time)

H. Amount and source of other funding (if applicable): Our matching funds and items include in-kind time commitments for Dr. Adams and student volunteers from Cal Poly at an estimated amount of greater than $11,000. We anticipate this project will utilize 25 hrs of student time/mo ($15/hr) and 8 hrs of Dr. Adams's time/mo ($75/hr) for 12 months. Therefore, an estimate of matching time is ~ $11,700 for the year (students = $4,500, Adams= $7,200). In addition, we will borrow equipment (microscopes, filtering apparatus, and sampling gear) and supplies as necessary from the Adams lab to assure the project goals are met.

I. Project description

I.1. Main Objectives

1. Establish a long-term monitoring program for microplastics in the Morro Bay National Estuary using methods that align with other monitoring efforts along the coast of California.
2. Develop outreach opportunities to improve public engagement and understanding about the science of microplastics.

I.2. Background

Plastic pollution in the world's oceans was documented as early as the 1960s (Zettler et al., 2013). For example, marine organisms can ingest plastics causing feelings of false fullness and experience ecotoxicological effects from leachates in plastics. More recently, microplastics, fragments or fibers of plastic that are smaller than 5mm, have received special attention from researchers and policymakers because of the damage they inflict to marine organisms and humans. Although modern studies have helped us understand the impacts that both large plastics and some microplastics have on marine organisms and human health, there are many gaps in our full understanding of the pervasiveness and effects of microplastics in aquatic

environments. Long-term monitoring efforts that identify the prevalence and distribution of microplastics in time and space and among various habitats will increase our understanding of the current state of this issue, inform regulatory agencies on where and how to act, and will inform future studies on how these microplastics and fibers might impact marine organisms as well as how to prevent dispersal or remove these items from the environment.

Recent studies have documented the accumulation of microplastics in areas such as islands and public beaches. One recent study by Miller et al. (2018) found a variety of types of marine debris at every location they sampled, which included remote Channel Island sites and beaches along the California coastline in Ventura County. These investigators indicated that it was likely debris at their different types of sampling sites came from different sources. For example, a large portion of debris on the Channel Islands was from fishing gear and debris on the public beaches was largely plastic that could have come from storm runoff or direct dispersal onto the beaches. Studies like these have also demonstrated that even with little human contact, plastic can reach and pollute places we think of as isolated. Meanwhile, locations with a large urban population can have even higher amounts of plastic (Sutton et al., 2016).

Nevertheless, a thorough examination of plastic particles in estuaries where freshwater meets oceanic saltwater are few and far between (Sutton et al., 2016). Studies by Cannas et al. (2017) and Wessel et al. (2016) indicated that microplastics are found in the sediments in estuarine environments and there tend to be higher concentrations on the shoreline than in open waters. We hope to apply what has been learned from other coastal studies and current monitoring efforts to determine what microplastic types or concentration levels we may find at the different sites in the Morro Bay Estuary.

The Morro Bay Estuary is a Marine Protected Area, nestled between a tourist zone and residential area, which remains vulnerable to the influence of anthropogenic pollution, including microplastics. Its proximity to private housing, septic tanks, stream runoff, a busy downtown, recreational and economic use, along with frequent boaters make it susceptible to input of marine debris. The estuary is also located within a watershed that is heavily influenced by agricultural runoff and visitors to the surrounding areas. To this date, there is little published information about microplastic accumulation and prevalence in the estuary. We hope to apply the standard best practices and methodologies established by other investigators to establish a successful microplastic monitoring program and to better understand the impacts of microplastics in the Morro Bay Estuary.

I.3. Brief Overview of Plans

We plan to conduct a year-long survey assessing the presence and concentration of microplastic particles in the Morro Bay Estuary. Students will sample sediments for microplastics at three select locations within the estuary that are suspected of having varying microplastic concentrations. Samples will be analyzed for microplastics at Cal Poly, San Luis Obispo. Prior to the sampling process, students will receive special training on how to sample marine sediments and analyze samples from experts currently conducting research on microplastic in sediments.

Establishing a long-term monitoring program in a partnership with the Morro Bay National Estuary Program (MBNEP) is the next crucial step in understanding the dynamic and complex impact that microplastics have on the estuarine ecosystem, and human health. In establishing this long-term monitoring assessment of marine microplastics, we aim to establish a baseline for how much plastic is currently present in the estuary and later assess the mode which

plastic enters the bay by collaborating with other scientists and local agencies to understand the local currents and inputs to the bay, eventually allowing us to develop a comprehensive plan for the prevention of further pollution. Funding and approval for this project will allow us to perform our initial surveys, identify the best methods for long-term monitoring and expand the monitoring protocol to other local beaches and bays (and potentially the water column with collaborators) while using the data to educate the Morro Bay and Los Osos communities on the negative effects that plastic pollution can inflict on the estuary. In addition, we hope to align our efforts with other monitoring programs along the California coast, and add additional components of community involvement and outreach to this project in collaboration with others. This program will function as a unit that brings together the "Learn By Doing" motto from Cal Poly while educating the public and bringing together community members and tourists to become better ocean stewards.

We expect to find that some locations have an increased amount of plastic in comparison to others and hypothesize that our sampling locations closer to the mouth of the Bay, or near the open Pacific Ocean, and near areas with high human activities (i.e. heavier foot traffic) will have increased amounts of plastics like the results of Sutton et al. (2016). We also expect that our sampling location near the "back bay" where freshwater meets saltwater in the estuary will have lower concentrations or possibly different types of microplastics because of freshwater input and decreased human presence. Nevertheless, we recognize that there are areas of the back bay that are also influenced by stream input from other sources, a marina, and the community of Los Osos. We also hope this study can eventually grow to include more sampling sites, form collaboration with others to identify the concentrations and types of microplastics in the water column at various sites and determine whether concentrations of microplastics in the sediments may correlate with abiotic factors like wind-driven surface or tidal currents in the Bay.

While it is important to note the major differences between the study sites of San Luis Obispo (Morro Bay Estuary, CA) and in the previous studies in Ventura County (Channel Islands, sandy beaches) such as human population, isolation from human presence, and proximity to tourist locations it is also important to consider that both studies have the same goal: to assess and monitor abundance of marine pollution. Even if we do not find significant levels of microplastics in this initial study, our results (and methods) can serve as a baseline for continued monitoring efforts and can serve as a more pristine study-site to compare to other locations.

Information gained from this study will be used to compare microplastic concentrations to other locations in California and will be used as teaching and learning opportunities for community members in the Morro Bay-Los Osos area. Some engagement opportunities will primarily be targeted to improve opportunities for the students of Morro Bay High School. We also plan to extend engagement opportunities to the public by creating activities that can be shared through virtual opportunities as we navigate through the COVID-19 pandemic or, in the future, at in-person public events with proper precautions.

More specifically, we will:

1. Establish a long-term monitoring program for microplastics in the Morro Bay National Estuary using methods that align with other monitoring efforts along the coast of California. This will involve a team of at least four undergraduate students from Cal Poly. Students will participate in sampling and processing and analysis methods. This effort will result in a final report for the Morro Bay National Estuary Program, a detailed sampling protocol for future

sampling efforts and potentially at least one publication in a scientific journal. Students will present their results at scientific conferences and spread an awareness of this topic and the MBNEP to other scientists.

2. Develop outreach opportunities to improve student and public engagement and understanding about the science of microplastics. We hope to provide an updated protocol for sampling methods and outreach involving Morro Bay High School students as well as creating at least one adaptable table top/outreach activity informing the public about microplastics that can be used at public events in Morro Bay.

J. Project Tasks

We will collect samples twice a month at peak low tides to assess the presence of microplastics in the sediments of Morro Bay. We consulted oceanographer Dr. Ryan Walter, who has characterized current patterns and sediment types in the Bay to identify logical sample sites, After considering sediment type and current flow patterns, we tentatively propose the following sampling sites: Coleman Beach, a common area for SUP and kayak launching (an original site for MBNEP sampling with MBHS students), mudflats near the Morro Bay State Marina, and Pasadena Point in the back of the bay, Baywood (Fig. 1) at the high tide mark indicated by the separation of wet and dry sediment or the edge of foliage. We have visited all of these sites many times and understand the challenges, but feel these sites allow us to make robust comparisons and have reliable access.

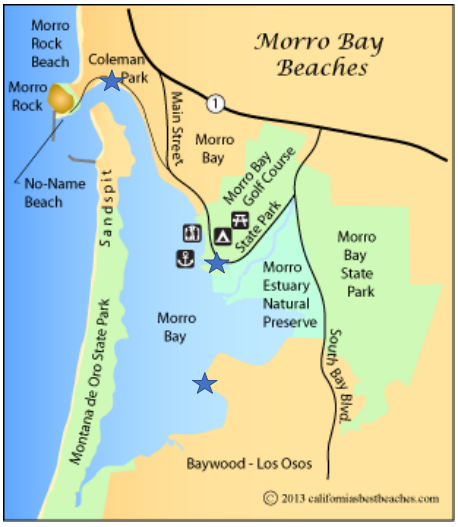

Figure 1. Proposed sampling site in Morro Bay. Original image by *California's Best Beaches*.

Prior to starting official sampling, we will receive hands-on training from a PhD student, Dorothy Horn, who is an expert at processing sediment samples and identifying microplastics in California and Oregon (as per her contributions to Miller et al, 2018). Using her guidance and additional protocols, we plan to use the methods outlined below.

J.1 Sediment Sampling

We will set up a transect composed of two 10-meter tapes that hit both dry and wet sediment at each sampling site. We will use a quadrat one square meter in area and a random number generator to determine where we will sample along the transect. Once we have the coordinates for sampling, we will align our quadrat with the top left corner of the transect hitting the numbered coordinates and use this one-meter area to dig 5cm into the sediment. We will determine the 5cm depth mark with a metal measuring tape. We will use a metal hand shovel (trowel) to scoop the sediment into metal buckets to avoid contamination from using a plastic containers. Any large items that are found in the samples, like pebbles, shells, marine algae, etc. will be rinsed with seawater over the sample container in a metal sieve (size) and discarded. Remaining sediments and plastic pieces will be transferred into a glass container for transport back to the lab at Cal Poly. The bucket will be rinsed with seawater in between samples. We will

label samples with their location (inside using waterproof paper and on the outside) repeat this step five times at each sampling site to get a total of 15 samples per visit to the estuary. At each sampling location, we will also have a glass jar open during the entire sediment collection to work as our control sample to account for any airborne plastic particles (as in Miller et al., 2018). Individuals taking samples will wear only 100% cotton clothing to avoid contamination of samples and to avoid the addition of microplastics in the study sites.

J.2. Sample Processing

J.2.a. Dissolving Organic Matter

The chances of entrapping marine organisms in sediment sampling is lower than that of skimming the surface of the water in an estuary in other studies (Valine et al., 2020). Nevertheless, to be safe we will utilize a method for dissolving organic matter in our sediment samples. We will use the technique described by Rochman et al. (2015) to complete this step. Briefly, we will transport field samples to the lab and incubate them in glass containers large enough to hold both the sample and three times the measured volume amount of 10% potassium hydroxide (KOH) to properly dissolve any existing organic matter in the samples. These samples will be incubated with constant stirring at 60 °C for 24hrs to ensure proper mixing and dissolution of any organic material. We will acid wash and rinse all glassware three times with milli-Q water between samples to avoid any potential cross contamination. Once all organics are dissolved, samples will be held at 4 °C (as per Rochman et al., 2015) until they can be examined. The research team will wear only 100% cotton clothing to avoid possible sample contamination and an open glass petri dish will be present on the workspace to account for any possible plastic contamination that may have occurred in the lab during sample preparation.

J.2.b Separation of Sediment and Microplastics

After dissolution of organic matter, we will begin the process of separating microplastics from the sediments collected. The most efficient way to separate the plastics from the sediment is to complete this step based on the density properties of plastics and sediments using the methods from Thompson et al. (2004) and Valine et al. (2020). We will filter the samples onto petri dishes with a copper 63 micron sieve. We will rehydrate samples in a glass container using 2L of milli-Q water 168.4g of kosher salt with an anti-caking agent to make a hypersaline solution (as per Valine et al., 2020). Once the brine solution and samples are combined, they will be shaken vigorously for 60 seconds to segregate sediment from plastic material.. According to Valine et al. (2020) and Crichton et al. (2017) there is a chance that denser plastic particles could be lost in the heavy sediments during this step. Therefore, we will vacuum filter samples using a glass fiber filter to extract the plastics to separate the sediments from the plastics in the saline solution, as advised by Dorothy Horn, a PhD. This will be done using an Erlenmeyer flask, rubber tube, Buchner funnel and vacuum spout in the fume hood. The vacuum will draw out the solution water and the plastic will be caught by the funnel and filter for microscope analysis and quantification. This entire process will be done with a control petri dish open on the workspace to account for any airborne plastic particles in the lab.

J.2.c. Quantifying Microplastics and Statistical Analysis

We will examine samples and quantify the concentrations and types of microplastics at 40X using a compound microscope at Cal Poly. First, we will record the characteristics and quantity of each plastic particle we encounter, specifically noting the fragment type (fiber,

fragment, microbead, etc.), the primary color, and their size in mm. We will perform this analysis after staining with Red Nile Dye as described in Valine et al. (2020). This stain specifically adheres to hydrophobic and lipophilic substances and is known to enhance the viewing of microplastics that would ordinarily go undetected (Erni-Cassola et al., 2017). After assessing the filters stained with Nile Red, we will also use the counterstain Calcofluor White on the filters. The counterstain method was developed by Maxwell et al. (2020) because of common misidentification of organic material as plastics in the staining process with Nile Red dye. Imploring the counterstain will ensure that our counts of plastic on the filters are accurate. Once the filters are dried with the new stain, they will undergo the same microscope analysis for the characteristics above. It is important to note that because Calcofluor White is a fluorescent dye, we will need to examine these samples using fluoresce microscopy. Our data will then be analyzed with the statistics program R Studio running multivariate and univariate tests.

J.3. Community Outreach Projects Program

We hope to partner with the MBNEP to engage community members and spark awareness of local priority issues through outreach and participation. Residents and visitors to the Morro Bay Estuary and San Luis Obispo County take special interest in the environmental impacts in our local area. This project has the potential to engage all members of the community through group presentations and activities. For this first year, we hope to help facilitate outreach through the following activities: 1. Helping the MBNEP continue to involve local schools in hands-on science activities, 2. Involve Cal Poly students in our research efforts to teach them about the Estuary and the issue of microplastics and 3. Create outreach (table top or virtual activities that can be shared with the public online or at events such as the Morro Bay Harbor Festival or the MBNEP outreach center.

First, we hope to learn from, integrate and update the methods established by the MBNEP to involve school-age students (K-12) in this effort. The only updates that may be required are altering the sampling and analysis methods we are proposing that will align more with other monitoring efforts. In the future, students can continue to learn about microplastics and how to be scientists and stewards by collecting raw data of sedimented microplastics on Coleman Beach in Morro Bay, CA in outreach events and planned field-trips and seeing how the Cal Poly students are analyzing these data. A future goal that is beyond the scope of the project this year, is to build on what the MBNEP has already created allowing students to have hands-on experiences learning how microplastics enter the bay, collecting samples with guidance of Cal Poly Students and individuals associated with MBNEP as well as learning lab techniques for microplastic analysis. Engaging young community members will teach responsibility and accountability for the impact that humans have on the ocean.

In addition to K-12 learners, this long-term monitoring program will also involve the participation of Cal Poly students in this project. We will involve a team of at least four students. Our sampling data can be used as a baseline for other senior projects and linked to other larger research collaborations. College students who choose to become involved could use what they learn to engage more community members and advocate for local ecosystem, economic, and human health.

Another way Cal Poly students will become involved is by helping create meaningful outreach activities in course at Cal Poly. Dr. Nikki Adams will be teaching the MSCI 440-Communicating Ocean Sciences to Informal Audiences (COSIA) this Fall. The COSIA course is specifically designed to teach students how to interact with the public to facilitate engagement

and learning of science and how to create engaging hands-on outreach activities that they lead. This Fall, at least one team of COSIA students can focus on designing an activity focusing on microplastics in the ocean and possible sources of microplastics that could be found in Morro Bay. Due to COVID-19 concerns, we are unlikely to be able to run face-to-face events currently, but these activities can be created to implement once concerns and restrictions are alleviated. Alternatively, some students can focus on partnering with the MBNEP to create online learning activities, events or materials that educate the public about microplastics.

Once we have tested and established the best methods for sampling and created impactful outreach activities, we hope to encourage residents and visitors to the Morro Bay Estuary and MBNEP office to engage in the data collection process in future years. By participating in citizen science, science and research conducted by non-professional scientists, individuals will have the opportunity to learn about pressing issues on the local-scale and how these issues impact not only our small-scale sites but the surrounding world.

K. Itemized Budget

Category	**Description**	**Estimated Cost**
Materials	Sampling, sample processing (filtering), imaging	$5,100
Specific Use Scientific Collecting Permit	MPA Individual Specifics sampling Permits (one for lead student and one for Dr. Adams to ensure continuity)	$473
Travel Costs	Student travel to field sites Visiting scientist (training)	$414 $164
Outreach Efforts	Materials and Supplies	$400
Total Cost		$6,551

L. Budget Justification

L.1. Supplies: The majority of the funds requested will be used to procure small equipment (vacuum pump, filtering manifolds, flasks, sieves, etc.) and consumables (filters, dyes, pipettes and glassware). Because we are just initiating this procedure and need to prevent contamination of our samples, we must purchase new equipment and supplies specifically for this project. Once the method is established, the sampling gear and filtering supplies will be available to continue the project and the costs associated with annual sampling will be much lower.

L.2. Collecting permit: We are required to purchase an individual lab Specific Use Scientific Collecting Permit to conduct the sampling in the Morro Bay Estuary Marine Protected area. We are working to procure a permit that can list both Dr. Adams and Ashley Adams on the permits at the primary permittees who will be present during each sampling event. Alternatively, we must procure two permits to assure one of us is always available during the sampling events. The California Fish and Wildlife Office charges a permitting fee and a separate application fee.

L.3. Travel: We are requesting a small amount of funds to offset the cost of student travel from Cal Poly to Morro Bay for collecting trips over the year of sampling. We estimated this at 24 trips 30 miles round trip x $0.575/ mile ($414). These estimates are low considering some students may need to drive separately due to COVID-19 restrictions. In addition, we have the opportunity to train with a PhD student at the University of Oregon, Dorothy Horn who has become an expert in sampling of microplastics from sediments. She will be in the Ventura area this Fall and has offered to come train our students on sampling and processing methods. We are hoping to be able to compensate her for her travel costs from Ventura (286 miles round trip=$164.45).

L.4. Outreach: We are requesting funds to purchase updated sampling gear for a Morro Bay High School program as well and materials and supplies to create at least one activity for outreach events.

M. Schedule

We propose the timeline outlined in Figure 2. Briefly, we will use September 2020 to procure the necessary supplies and equipment and assemble our team. In addition, we will initiate preliminary training for sampling and sample processing. This October 2020 we will commence training sampling of the three sites, process samples and summarize data. We will continue sampling, processing and analysis once per month during November 2020-September 2021.We will start comparing and summarizing our data analysis each month starting in November. We will submit a final report in October 2021.

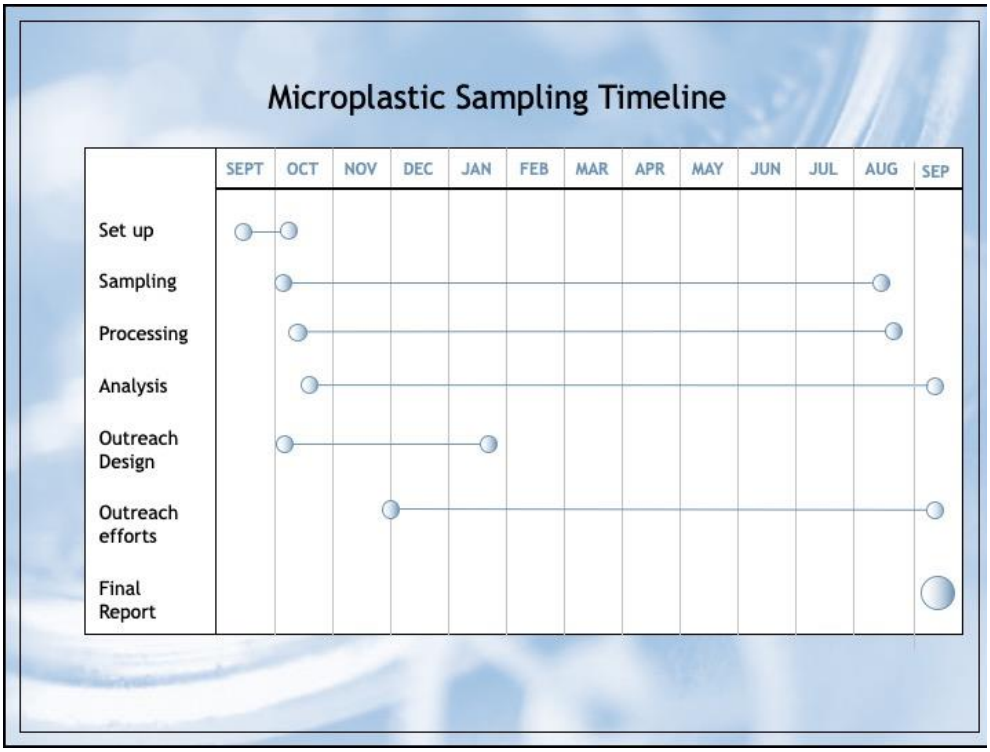

Figure 2. Project Timeline

N. Note about Feasibility with Partial Funding

Full funding of this proposal will allow the successful completion of one year of sampling and analysis of samples as well as the creation of outreach effects. We are required to have a separate specific use collecting permit for this project, so that cost is not negotiable.

Nevertheless, partial funding of this proposal will at least allow us to purchase the collecting permit, basic equipment and initial disposable supplies to initiate the project and meet the goal of identifying some of the best long-term microplastic monitoring methods

We have tried to keep our budget estimations to the minimum of scientific equipment and supplies necessary to conduct this project independently for a full year sampling cycle. We may be able to borrow a few larger equipment items from other labs for the first year of the project. This would allow us to reduce the amount of the materials cost to make to project doable. If necessary, we can decrease the amount focus on outreach efforts. In addition, we reduce our reimbursement for travel costs to decrease the budget expenditures.

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