Name of the organization: North San Diego County Watershed Monitoring Program (NSDCWMP) created by Preserve Calavera, a non-profit volunteer-based organization whose mission is to preserve the natural resources of the coastal San Diego North County.

Introduction - In the spring of 2019, Preserve Calavera created the North San Diego County Watershed Monitoring Program (NSDCWMP) to continue the decade-long work of San Diego Coastkeeper (SDCK) of assessing the health of local surface waters. The water quality in three coastal watersheds (i.e., Buena Vista Creek, Agua Hedionda and Batiquitos Lagoons), all of which are part of the Carlsbad Hydrologic Unit (Fig. 1) is evaluated by sampling water at multiple locations on a bimonthly basis and measuring basic physical (conductivity, turbidity), chemical (pH, dissolved oxygen, nutrient, and ammonia), and biological (total and pathogenic coliform bacteria) parameters. In addition, the team has initiated sampling for the analysis of microfibers in conjunction with Dr. Dimitri Deheyn's laboratory at UC San Diego (Marine Biology Research Division, Scripps Institution of Oceanography).

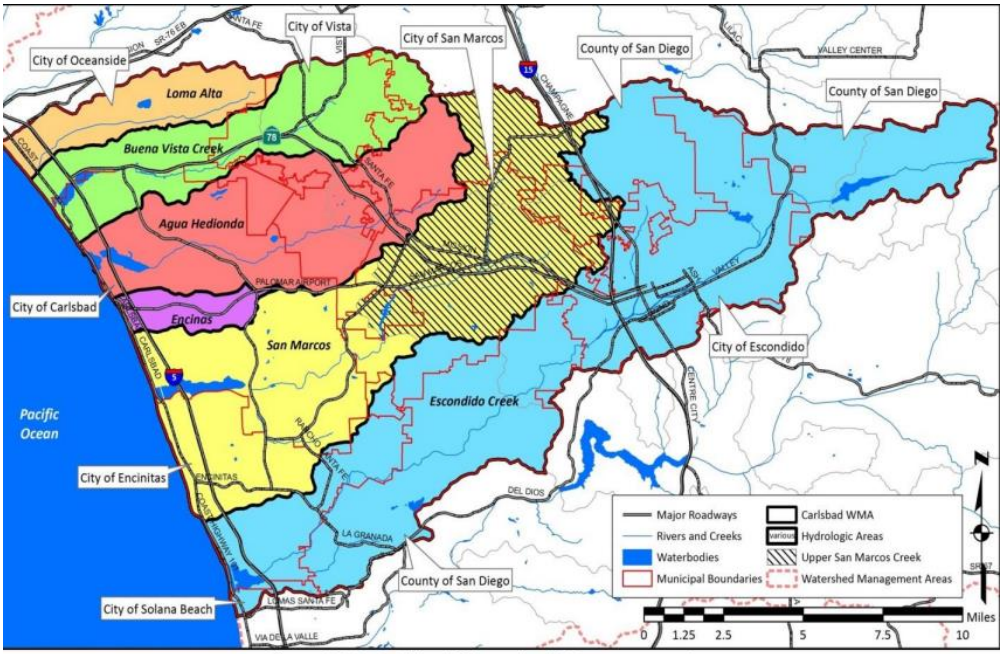


Figure ES-1: Carlsbad Watershed Management Area

NSDCWMP is an all-volunteer citizen science effort with a leadership management team comprised of two Preserve Calavera board members (also leaders of the Buena Vista Creek and Batiquitos Lagoon monitoring teams). Technical advisors from the California Water Resources Control Board as well as the San Diego Regional Water Quality Control Board (SDRWCB) provide guidance to the NSDCWMP. Data is posted at www.preservecalavera.org and on the CEDEN website and shared with SDRWCB and the cities of Carlsbad, Oceanside, San Marcos, and Vista as needed.

Methodology:

Sampling sites: Water samples are collected by trained volunteers at three (3) sites at each sampling event (six events per year) for the three (3) coastal watersheds (e.g., Buena Vista, Agua Hedionda, and Batiquitos).

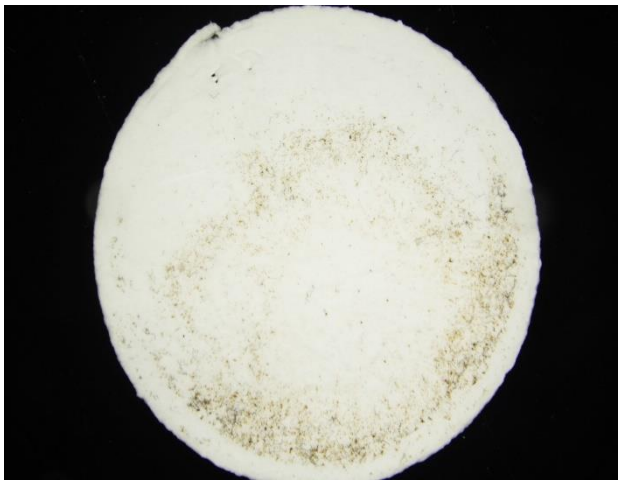
Measures and Material:

Parameter	Type	Definition	Method
Temperature	F (teams)		
Dissolved oxygen	F (teams)	A measure of how much oxygen is dissolved in the water - the amount of oxygen available to living aquatic organisms.	Hach HQ40d Luminescent Dissolved Oxygen
Conductivity	F (teams)	**The degree to which a specified material conducts electricity**, calculated as the ratio of the current density in the material to the electric field that causes the flow of current. It is the reciprocal of the resistivity.	Hach HQ40d Conductivity probe
pH	F (teams)	**A measure of how acidic/basic water is**. The range goes from 0 - 14, with 7 being neutral. pHs of less than 7 indicate acidity, whereas a pH of greater than 7 indicates a base. pH is really a measure of the relative amount of free hydrogen and hydroxyl ions	Oakton pHTestr20
Nitrates	L (Preserve Calavera)	Nitrates are a **set of compounds that involve nitrogen and oxygen molecules.** While they are often associated with cured meats, green, leafy vegetables are actually much richer in nitrates. Nitrogen is essential for all living things, but high levels of nitrate in drinking water can be dangerous to health, especially for infants	Hach 10206 (TNT 835)
Phosphorus (total) and reactive phosphorus	L (Preserve Calavera)	Phosphorus is **a mineral that naturally occurs in many foods** and is also available as a supplement. It plays multiple roles in the body. It is a key element of bones, teeth, and cell membranes. It helps to activate enzymes, and keeps blood pH within a normal range. Reactive phosphorousis inorganic and is the ionic form.	Hach 10210 (TNT 843)
Ammonium	L (Preserve Calavera)	**Ammonium is ionized, and has the formula NH_4^+**. Ammonia is un-ionized, and has the formula NH_3. The major factor that determines the proportion of ammonia or ammonium in water is water pH. The activity of ammonia also is influenced by temperature and ionic strength.	Hach 10205 (TNT 830)
Total coliform	L (Preserve Calavera)	Total coliforms include **bacteria that are found in the soil, in water that has been influenced by surface water, and in human or animal waste**.	IDEXX Colilert 18
E. coli.	L (Preserve Calavera)	*Escherichia coli* (abbreviated as *E. coli*) are **bacteria found in the environment, foods, and intestines of people and animals.** *E. coli* are a large and diverse group of bacteria. Although most strains of *E. coli* are harmless, others can make you sick. Some kinds of *E. coli* can cause diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses.	IDEXX Colilert 18

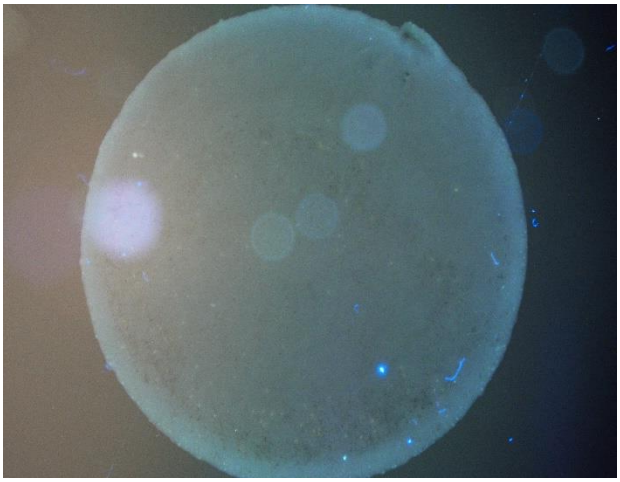
To those measures, counts of **microfibers** have been recently added (2022-23). Microfibers are of anthropogenic origin. They are flexible, fibrous particles that originate from clothing/textiles. They are a specific type of microplastics (usually between 1μm and 5mm), yet microfibers are usually 10μm in diameter and up to 100μm in length. And as opposed to other microplastics, microfibers can have a natural origin (cotton, hemp, or wool textile) but most abundantly also be synthetic (polyester, nylon, spandex), which is found increasingly in clothes.

Three water samples (50 mL each) for each site are collected and are filtered at our laboratory in Carlsbad according to the methodology described in “Protocol for Microfiber Sampling”

developed by Deheyn's laboratory research team (2021). Each filter is then photographed in a brightfield and fluorescent view using a Nikon SMZ 1800 stereomicroscope equipped with brightfield and epifluorescence modules and Nikon digital imaging camera. Trained volunteers/research assistants use an Image Manipulation Program (GIMP) allowing high resolution to identify microfibers, as defined earlier, that they observe on both views. They report their counts in a shared Excel file for comparison between observers.



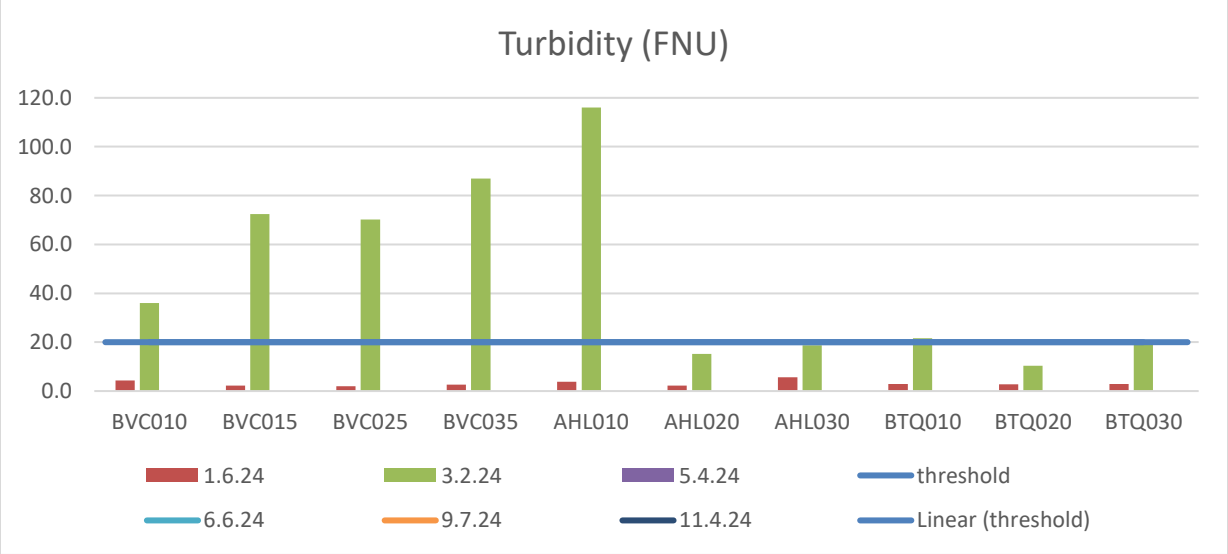
Brightfield View for Agua Hedionda (AHL020)



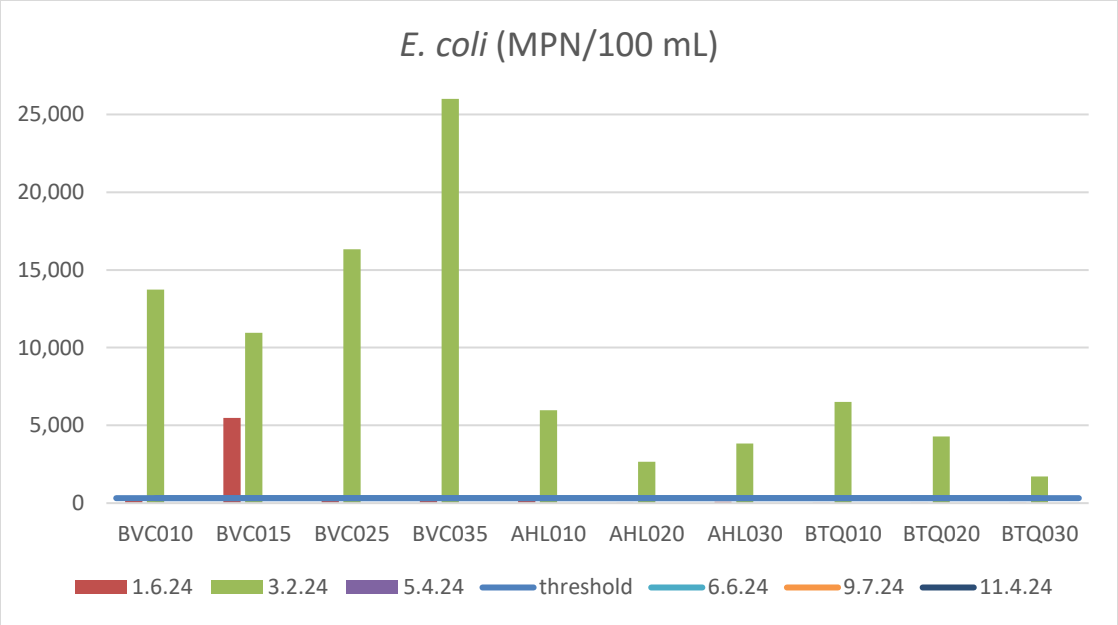
Fluorescent View for Agua Hedionda (AHL020)

Latest Findings:

Field and lab measures are analyzed following the protocol determined by SDCK. Here are the findings for Turbidity for the ten sites from the latest sampling event on March 2nd, 2024, as an example. This bar graph shows the increase in turbidity above threshold from the prior event, mostly due to heavy runoff within 72 hours before the event.



Of interest are the findings for *E. coli* in correspondence with Turbidity. The bar graph below shows that *E. coli* is this time way above threshold.



Microfiber counting from filters is performed following the instructions/training used by the Deheyn's laboratory research team and described in "Microfiber Counting Protocol."
When fibers fluoresce, they are likely to be of chemical (plastic/synthetic) origin, revealing the presence of microplastics in the watershed and the potential for toxicity in the food chain.
As an example, and to provide a reference base, in the fluorescent view above, up to 12 microfibers were detected from a water sample of only 50 ml. The brightfield view shows over thousands of fibers more likely of natural origin that are further analyzed using infrared spectroscopy. This technique (commonly referred to as FTIR) can determine the nature of small particles, and thus identify whether natural or synthetic.

This data is reported to several local and regional governmental agencies which may use them to make informed regulatory decisions regarding the protection and management of the rivers and streams in our local watershed.

References

1. Sunanda Mishra, Chandi Charan Rath, Alok Prasad Das (2019). *Marine microfiber pollution: A review on present status and future challenges.* Marine Pollution Bulletin, Vol. 140, 188-197.
2. N. Hodkovicova, A., Hollerova, Z., Svobodova, M. Faldyna, C., Faggio C., (2022). *Effects of plastic particles on aquatic invertebrates and fish – A review.* Environmental Toxicology and Pharmacology, 96, 104013.
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