SANTA BARBARA STREAMS AND ESTUARIES BIOASSESSMENT PROGRAM

2021 REPORT

Prepared for:

City of Santa Barbara,
Creeks Division

County of Santa Barbara,
Project Clean Water

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Executive Summary

Introduction

This report summarizes the results of the 2021 Santa Barbara Streams and Estuaries Bioassessment Program, an effort funded by the City of Santa Barbara and County of Santa Barbara. Ecology Consultants, Inc. (Ecology) prepared this report, and serves as the City's and County's consultant for the Program, which began in 2000. The purpose of the Program is to assess and monitor the biological integrity of study streams and estuaries as they respond through time to natural and human influences. The Program involves annual collection and analyses of benthic macroinvertebrate (BMI) samples and other pertinent physiochemical and biological data at study streams and estuaries. BMI samples are analyzed in the laboratory to determine BMI abundance, composition, and diversity. Study sites have included the range available along a disturbance gradient, from "reference" sites that are fairly intact in form with little human disturbance in their watersheds to "disturbed" sites that have been substantially altered in form and drain highly developed watersheds.

A BMI based Index of Biological Integrity (IBI) has been developed by Ecology for local streams, which have been studied since 2000. The IBI yields a numeric score and classifies the biological integrity of a given stream as Very Poor, Poor, Fair, Good, or Excellent based on the contents of the BMI sample collected from the stream. By condensing complex biological data into an easily understood score and classification of biological integrity, the IBI serves as an effective tool for the City and County in monitoring the condition of local streams through time, and their responses to natural and human influences. The IBI is updated ever 5 years or so to allow the inclusion of data from study streams as they are affected through time by changing environmental conditions.

Local estuaries have been studied since 2012. Due to extreme fluctuations in water chemistry (e.g., salinity), determining the impacts of natural physiochemical variability and human land uses on the BMI communities in local estuaries has proven to be more difficult compared to streams. Nonetheless, a goal of studying local estuaries has been to develop an assessment tool similar to the streams IBI that measures and classifies the biological integrity of study estuaries. In 2018 a Scale of Biological Integrity was developed to serve as an indicator of biological health for study estuaries. Like the streams IBI, the estuary Scale of Biological Integrity has been refined in subsequent years to consider new data collected each year.

Study Area

The study area encompasses approximately 80 km of the Santa Barbara County coast from the Rincon Creek watershed at the Santa Barbara/Ventura County line to Jalama Creek, which is just north of Point Conception. A few streams further inland in the Santa Ynez River watershed, and Orcutt Creek near Santa Maria have also been studied. More than 60 different stream study reaches have been surveyed on one or more occasions during the 22 years of the Program, while 13 different estuaries have been studied once or more in 10 years of study.

Results

Over the past 22 years, the Program has provided a wealth of information regarding the physiochemical habitat conditions and biota, and in particular the BMI communities, present in local streams. The influences of natural physiochemical and climatic variability and human development on local stream communities have been extensively studied. The following statements can be made based on the research completed thus far:

- Negative impacts of human development on local stream communities (particularly BMIs) have been documented with highly significant statistical test results. Degradation of stream communities (e.g., lower IBI scores and loss of sensitive species) and physiochemical habitat conditions has increased linearly with increased watershed development. Urban development has been shown to have greater impacts on stream communities than has agricultural development.
- The IBI is highly effective as an indicator of biological integrity, as it has highly significant relationships with indicies of human disturbance. The IBI differentiates between REF, MOD DIST, and HIGH DIST groups with a high degree of accuracy and consistency.
- Major episodic disturbances including extreme stream flows, prolonged drought, and wildfires have been definitively shown, through rigorous statistical analyses, to negatively impact stream communities at REF and MOD DIST sites, as evidenced by lower IBI scores and loss or significant reduction of sensitive BMI taxa following such events. In past years, local stream BMI communities were resilient following floods and fires, typically showing dramatic recovery within 2 or 3 years. A string of events in recent years, starting with a prolonged drought (2013 to 2017), followed by the catastrophic Thomas Fire and extreme stream flows during a very wet winter (2018-2019), caused a prolonged trend of depressed overall IBI scores at REF and MOD DIST sites for several years. Rainfall and stream flows have been more consistent over the past few years, and IBI scores at non-fire affected REF and MOD DIST sites have generally improved. Continued monitoring will be needed to determine future trends in the BMI community of local streams.
- Due to a combination of wildfires, floods, and drought over an approximately 10 year period from 2008-2018, Rainbow trout appeared to be greatly reduced or eliminated in many study area streams including Mission Creek, Montecito Creek, Carpinteria Creek, and Rincon Creek and their tributaries. In today's world, it is inherently more difficult for southern steelhead trout to re-populate local streams impacted by these types of events. This is due in large part to their small numbers (i.e., Federally endangered), and also the presence of fish passage barriers and/or degraded habitat conditions in the lower reaches of all local streams. This year, trout were observed at M4 and MONT3 for the first time in several years. Their reappearance in these streams provides a ray of hope for the species locally, and perhaps suggests that numerous efforts to mitigate fish passage barriers and improve degraded stream habitat in the lower reaches of these streams are working to some degree. Another bright spot for the species locally is Arroyo Hondo (study reaches AH0 and AH1), where juvenile and adult steelhead/rainbow trout have been observed consistently over the past 20 years.
- Stream habitat restoration sites M2 and AB5 have shown improved habitat conditions, but significant improvements in the BMI community have not been observed 10 to 15 years post-restoration. Channel and riparian restoration at these sites did not address larger scale impairments in hydrology, geomorphology, water quality, and habitat continuity and connectivity that have resulted from drastic human alteration of the respective watersheds. Although much of this watershed-scale impairment cannot be undone from a practical

sense, there are opportunities to restore hydrology and water quality on a larger scale. Whether or not current and future restoration efforts at these and other stream habitat restoration sites (e.g., AB9, AB1, and AB2a) will improve the BMI community in local streams can only be evaluated via continued monitoring.

Based on the 10 years of data available for estuaries, the following can be stated:

- Determining the impacts of human land use and natural physiochemical variability to the BMI communities in local estuaries has proven to be more difficult compared with streams. One reason is there are fewer estuaries in the study area compared with streams, particularly in the REF category. Also, the wide salinity fluctuations that occur through time makes estuaries harsher environments where a relatively small number of BMI taxa can survive when compared with streams. However, we have identified several taxa that are more abundant in the REF estuaries, which are the basis of the metric % sensitive BMIs. This metric appears so far to be a fairly reliable indicator of estuary condition, and was used to produce the Scale of Biological Integrity for local estuaries. The Scale of Biological Integrity has been 87% accurate in classifying study estuaries based on their *a priori* disturbance category, including 91% for REF and 94% HIGH DIST sites.
- Salinity has been shown to influence the composition of the BMI community (i.e., the specific taxa present) in local estuaries. Mean % sensitive BMIs, the basis for the estuarine Scale of Biological Integrity, also appears to be influenced by salinity. This metric was intermediate in the Low salinity class (32%), highest in the Moderate salinity class (46%), and lowest in the High salinity class (11%) with significant statistical test results. It may prove advantageous to develop separate scales of biological integrity based on salinity class. More replication of sites, particularly in the High salinity class, will be needed to evaluate this possibility.

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I. Introduction

This report summarizes the results of the 2021 Santa Barbara Streams and Estuaries Bioassessment Program, an effort funded by the City of Santa Barbara and County of Santa Barbara. Ecology Consultants, Inc. (Ecology) prepared this report, and serves as the City's and County's consultant for the Program, which began in 2000. The purpose of the Program is to assess and monitor the biological integrity of study streams and estuaries as they respond through time to natural and human influences. The Program involves annual collection and analyses of benthic macroinvertebrates (BMIs) and other pertinent physiochemical and biological data at study streams and estuaries using methodology based on United States Environmental Protection Agency (USEPA) rapid bioassessment protocols. BMIs are aquatic invertebrates (insects, crustaceans, mollusks, worms, etc.) of a half-millimeter (0.5mm) in size or greater that inhabit the bottom substrata of streams, lakes, ponds, estuaries, ocean waters, and other water bodies for at least part of their life cycles. BMI samples are analyzed in the laboratory to determine BMI abundance, composition, and diversity. Study sites have included the range available along a disturbance gradient, from "reference" sites that are fairly intact in form with little human disturbance in their watersheds to "disturbed" sites that have been substantially altered in form and drain highly developed watersheds. Scores and classifications of biological integrity are determined for study streams using the BMI based Index of Biological Integrity (IBI) developed by Ecology. The IBI was initially developed in 2004, and was updated in 2009, 2014, and 2019. In 2018 the Scale of Biological Integrity was developed to serve as an indicator of biological health for study estuaries. The Scale of Biological Integrity has been refined in subsequent years to consider new data from study estuaries.

What is "Biological Integrity"? What is "Bioassessment"?

"Biological Integrity" can be defined as "the ability (of a water body) to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitat of the region." (Miller et al., 1988). Natural perturbations such as heavy floods, droughts, and wildfires, as well as human disturbances to hydrology, geomorphology, water chemistry, and stream habitat have been shown to negatively impact the biological integrity of waters locally and around the world.

"Bioassessment" is the science of determining, or measuring, the biological integrity of water bodies by evaluating the composition of the biological communities that inhabit them. The origins of bioassessment in the United States and Europe date back to the late 1800's. Within the last few decades, the incorporation of bioassessment into water monitoring programs has increased dramatically throughout the United States because of the development of rapid, cost-effective assessment and data analysis techniques (Rosenberg and Resh, 1993). Currently, bioassessment is used throughout the U.S. and the world to assess, monitor, and manage the integrity of streams, rivers, lakes, ponds, estuaries, and coastal marine waters.

How does Bioassessment Work?

Bioassessment is based on the fact that individual aquatic species have varying habitat requirements and abilities to withstand natural and human disturbances. Thus, the composition of the biological community, or the species present and their relative abundances, provides a valuable indication of overall ecosystem integrity (i.e., health). The disturbance sensitivity of each unique species depends on their physiology, size, habitat requirements, survival strategy (i.e., primary producer, filter feeder, grazer, predator, etc.), and the nature and intensity of the disturbance(s).

Rainbow trout photographed in Rattlesnake Creek, 2004

As an example, the presence of viable native steelhead/rainbow trout populations in coastal California streams generally indicates good biological integrity. To thrive, trout require cool, clean, well-oxygenated water, clean cobble/gravel beds for spawning, deep pools for cover from predators, and an adequate aquatic invertebrate and vertebrate prey base. Trout are especially sensitive to increased fine sediment loads, higher stream temperatures, low dissolved oxygen levels, water pollutants, and other habitat modifications such as the construction of dams and other migration barriers that typically occur in areas with intensive human development. While species such as trout that are sensitive to habitat disturbances are typically reduced or eliminated in highly disturbed water bodies, disturbance tolerant species may persist or even flourish. Disturbed waters typically have lower overall taxonomic richness (i.e., number of species) compared to more natural, pristine waters.

Measurements of the biological community, or "biological metrics", relating to abundance, richness, proportion of disturbance sensitive species, and trophic structure have been shown to be reliable indicators of biological integrity in hundreds of bioassessment studies around the world. The reliability of such metrics as ecological indicators depends on the strength and predictability of their relationships with indicies of habitat disturbance.

Southerly view of Rattlesnake Canyon from East Camino Cielo above

Human development impact patterns in local watersheds

The study area primarily encompasses the southern slopes of the Santa Ynez mountains from the Santa Barbara/Ventura County line past Point Conception. In general, human development is minimal in the northern mountainous areas, with some grazing, orchards and rural residential uses in the foothills, transitioning to more intensive agriculture and urban development further southward where there are extensive coastal plains. The majority of development is concentrated in the cities of Santa Barbara, Goleta, and Carpinteria. Disturbance is limited mostly to orchards, grazing, and rural residential uses west of Goleta to Point Conception.

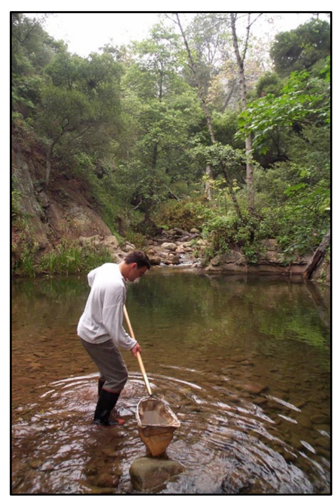
Generally, the nature and magnitude of disturbance to local streams and estuaries is proportional to the cumulative extent and intensity of human development in their watersheds. Plates 1 and 2 provide examples of two stream study reaches: (1) a relatively pristine stream in the undeveloped mountains, and (2) a disturbed stream on the urbanized coastal plain. The plates show the positions of these two stream reaches in their respective watersheds, surrounding land uses, and photographs of stream habitat conditions and aquatic species. Plate 2 illustrates some common forms of human disturbance, which include:

- Altered hydrology and geomorphology due to water diversions, urban and agricultural land development, and flood control projects.
- Burial of stream substrate due to increased deposition of fine sediments from eroding agricultural fields and stream banks.
- Loss of riparian and upland habitat essential to terrestrial stages of many aquatic species.
- Loss of habitat complexity, algal blooms, elevated water temperatures, wider fluctuations in dissolved oxygen, and loss of energy inputs due to stream channelization and removal of riparian vegetation.
- Degraded water quality due to inputs of fertilizers, pesticides, petroleum hydrocarbons, heavy metals, and other pollutants.
- Habitat fragmentation and barriers to species movement and migration due to the construction of in-stream barriers such as dams, road crossings, bridges, and culverts.
- Introductions of invasive, non-native plants and animals, which can outcompete and threaten the long-term viability of native species.
- Disturbances to vegetation and/or wildlife associated with trampling, noise, lighting, air pollution, and predation by domestic pets.

What is the Streams IBI? What does it tell us?

An Index of Biological Integrity (IBI) is a multimetric tool that provides a standardized, integrative, and readily understandable scale for measuring the biological integrity of specified waterbodies. The term multimetric refers to an IBI being constructed by combining several individual biological metrics into a single index. Because biological assemblages vary in response to natural physical and chemical gradients that occur through geographic space, IBIs are calculated for specific regions and water body types (i.e., streams, lakes, estuaries, etc.).

The Streams IBI incorporates 4 "core metrics" calculated using BMI data from local stream reaches. The core metrics are all highly sensitive to human disturbance as determined through rigorous



BMI sampling in Gobernador Creek

statistical analyses, and collectively represent different aspects of BMI community structure including relative abundances of disturbance sensitive taxa, taxonomic richness, and trophic structure. Values for each core metric at a study stream are scored on a dimensionless numeric scale (e.g., from 0 to 10) relative to the known distribution of values for sites along a human disturbance gradient. Higher scores (e.g., a 10) represent the conditions at relatively pristine reference sites, whereas lower scores indicate greater departure from reference conditions (i.e., highly disturbed sites). Scores assigned to the individual core metrics are equally weighted and combined into an overall score. The IBI classifies the biological integrity of a given stream as Very Poor, Poor, Fair, Good, or Excellent based on the based on the contents of the BMI sample collected from the stream. By condensing complex biological data into an easily understood score and classification of biological integrity, the IBI serves as an effective tool for the City and County in monitoring the condition of local streams as they respond to natural and human perturbations, devising and prioritizing watershed management actions, and evaluating their benefits or consequences.

Why use BMIs?

There are several reasons why BMIs are useful as biological indicators. First, BMIs are a critical component of aquatic ecosystems, often representing a large proportion of community biomass, performing important functions in the cycling of nutrients and energy, and constituting food sources for vertebrate predators such as fish and amphibians. Major changes in BMI assemblages have profound ramifications for aquatic ecosystems. Secondly, the responsiveness of BMIs to environmental perturbations, including human impacts, is well documented. Information is available on the life histories, distributions, habitat requirements, and disturbance tolerances of most BMIs. In the case of local streams, BMIs also are far more abundant and diverse compared to aquatic vertebrates (e.g., fish and amphibians), and are relatively easy to collect.

Stonefly (above) beetle (middle) and dragonfly (below) from Birabent Creek

Estuaries

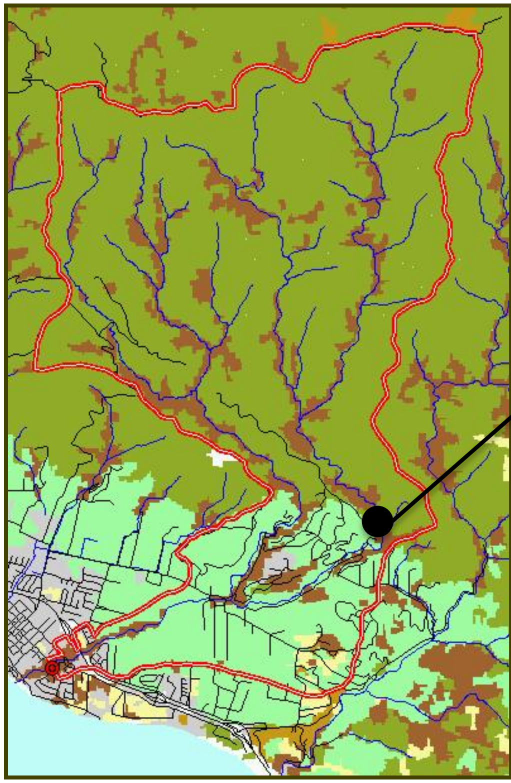
Estuaries are open water bodies where a freshwater stream meets and mixes with saltwater from the ocean, creating brackish water conditions with salinities that vary depending on fluctuating seasonal inputs from the stream and ocean. Similar to streams, study estuaries have included the range available along a disturbance gradient, from reference sites that are fairly intact in form with little urbanization in their watersheds to disturbed sites that have been substantially altered in form and drain highly urbanized watersheds.

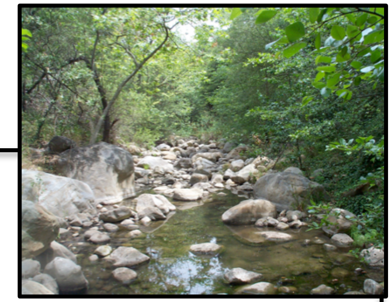
Jalama Creek estuary (low disturbance)

Determining the impacts of human land use on the BMI communities in local estuaries has proven to be more difficult compared to streams. One reason is there are fewer estuaries in the study area compared with streams, particularly in the REF category. Also, the wide salinity fluctuations that occur through time makes estuaries harsher environments where a relatively small number of BMI taxa can survive when compared with streams. Nonetheless, in 2018 the Scale of Biological Integrity was developed for study estuaries using the available data. Like the streams IBI, the estuary Scale of Biological Integrity has been refined in subsequent years to consider new data from study estuaries.

Mission Creek estuary (high disturbance)

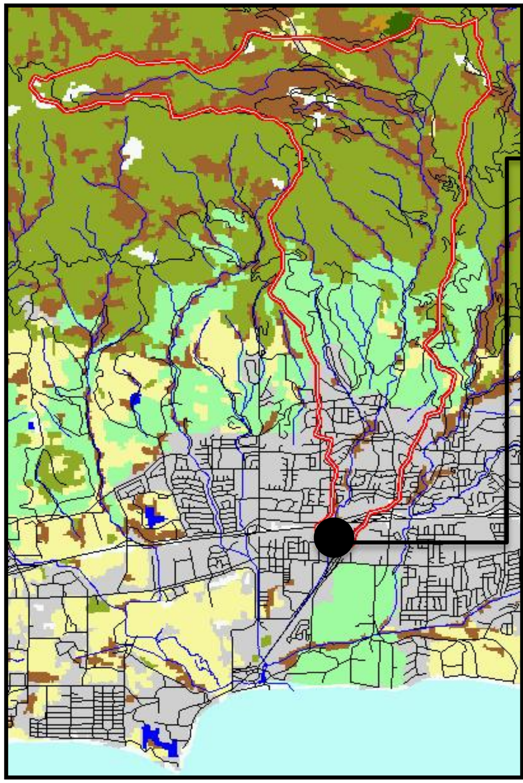
Plate 1: Reference Stream Reach Example





Stream reach location marked on map (left) by black dot. Upstream watershed drains wilderness lands (olive green and brown in map). Downstream agricultural (light green) and urban (grey) lands do not affect this stream reach. Stream has unaltered hydrology and form, with natural bed and banks, alternating riffles and pools, boulder and cobble beds, and intact mostly native riparian vegetation with mature canopy trees. Stream habitat is optimal for a variety of aquatic and riparian species, including a diverse BMI community and several sensitive aquatic vertebrates such as rainbow/steelhead trout, California newt, and southwestern pond turtle.

Plate 2: Disturbed Stream Reach Example



Stream reach location marked on map (left) by black dot. Stream drains urban (grey), agricultural (light green) and wilderness lands (olive green/brown). Impervious surfaces (urban), channelization, and increased fine sediment loads (agriculture) have altered stream hydrology and form, and water pollutants (e.g., nutrients, pesticides, hydrocarbons) are present. Stream banks have been largely denuded of native vegetation, resulting in unstable, eroding banks, establishment of invasive non-native plants (e.g., *Arundo donax* below), algal blooms, and wide fluctuations in water temperature and dissolved oxygen. Fine sediments largely smother boulder, cobble, and gravel that would provide stable aquatic habitat. Sensitive BMIs and aquatic vertebrates are largely absent due to habitat degradation.

II. Study Area

The study area encompasses approximately 50 miles of the southern Santa Barbara coast from the Rincon Creek watershed at the Santa Barbara/Ventura County line west to the Jalama Creek watershed, which is just north of Point Conception (see Figure 1). This year, 19 stream reaches and 9 estuaries were surveyed (see Table 1).

Table 1: 2021 Study Reaches

Study Reach	Location
RIN1	Rincon Creek just upstream of Highway 150/ Gobernador Canyon Rd.
C3	Gobernador Creek, approx. 0.25 mi. upstream of County detention basin
MONT3	Cold Springs Creek just upstream of Mountain Road.
SY2	Sycamore Creek 1,000′ below Hwy. 192 crossing and Coyote/Sycamore confluence
SY4	Sycamore Creek just upstream of Mountain Dr.
M0	Mission Creek just downstream of Cota St.
M3	Mission Creek at upstream end of Rocky Nook Park
M4	Rattlesnake Creek, approx. 0.5 mi. upstream of Las Canovas Rd. crossing
AB1	Arroyo Burro at upstream end of Alan Rd.
AB2	Arroyo Burro just downstream of Torino Rd.
AB2a	Arroyo Burro approximately 300′ upstream of Torino Rd.
AB3	San Roque Creek approx. 0.25 mi. upstream of Foothill Rd.
AB5	Mesa Creek just upstream of Arroyo Burro estuary.
AH1	Arroyo Hondo, approx. 1 mi. upstream of U.S. 101.
SO1	San Onofre Creek just downstream of U.S. 101.
GAV1	Gaviota Creek at State Beach/Park just upstream of access road crossing
SAL1	Salsipuedes Creek just downstream of Jalama Rd. crossing
AL1	Alamo Pintado Creek 0.25 mile downstream of Highway 246
OR1	Orcutt Creek just upstream of Blosser Ave.
Estuary Study Reaches	
ACe	Andria Clark Bird Refuge estuary
SYe	Sycamore Creek estuary
Me	Mission Creek estuary
ABe	Arroyo Burro estuary
Te	Tecolote Creek estuary
LFe	Las Flores Canyon Creek estuary
Re	Refugio Creek estuary
GAVe	Gaviota Creek estuary
Je	Jalama Creek estuary

Figure 1 shows an overall map of the study area, and Figures 2 and 3 provide detailed maps for the western and eastern study areas, showing locations of all stream and estuary study reaches that have been surveyed over the years. The Orcutt Creek study reach is well north of the other study sites, its approximate location shown in Figure 1.

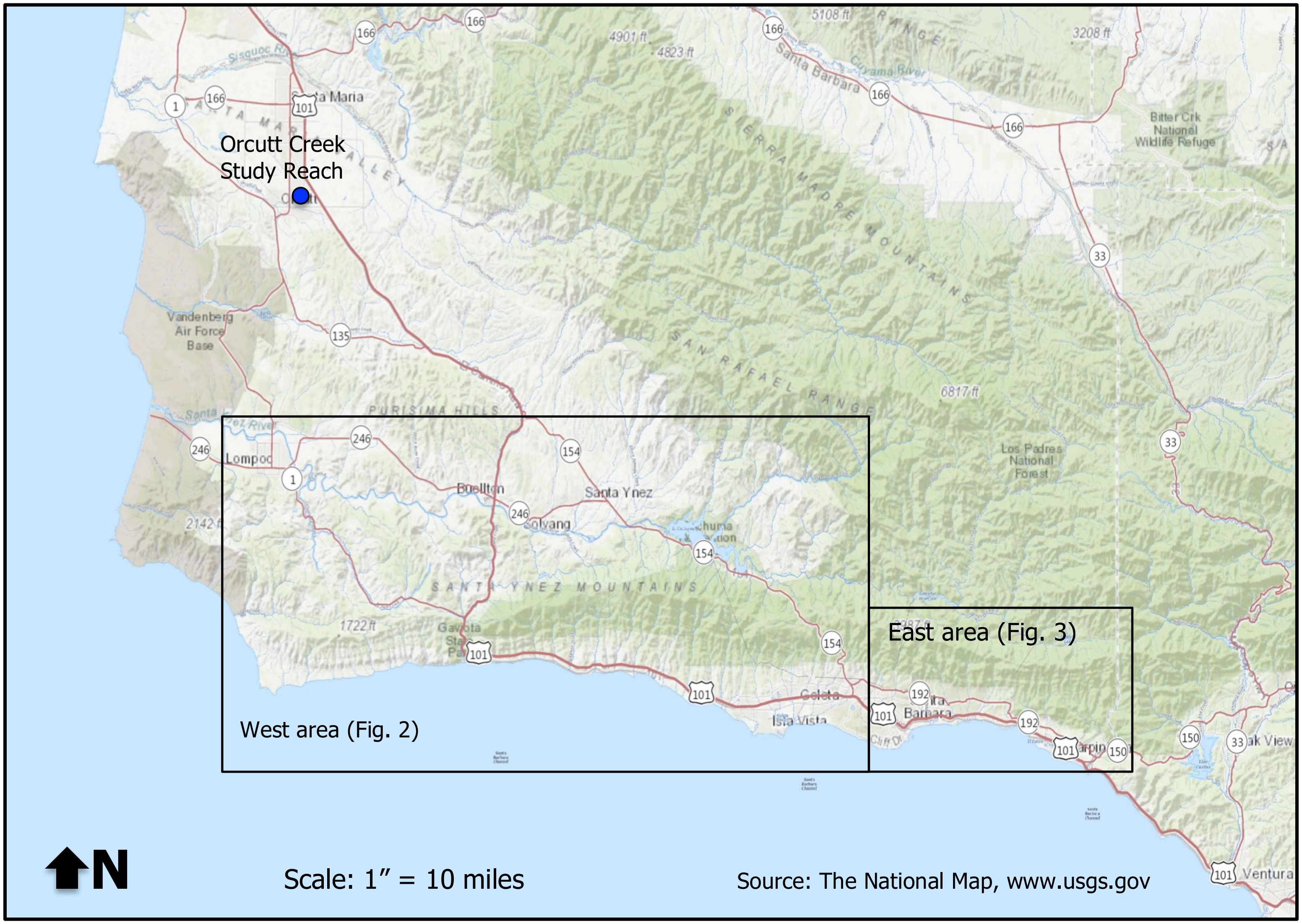


Figure 1: Study Area Map

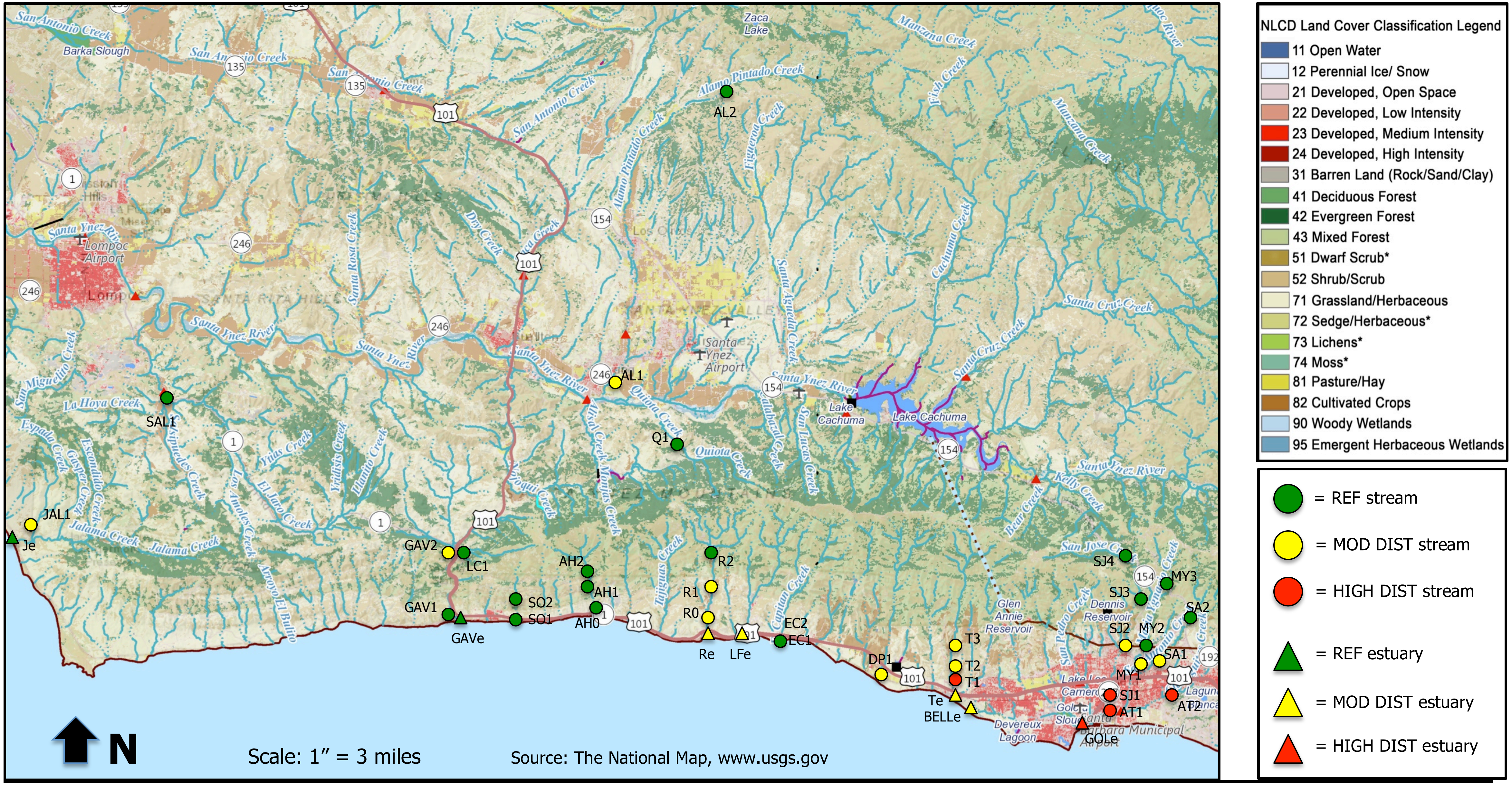


Figure 2: West Area Study Sites

Figure 3: East Area Study Sites

III. Methods

A. Field Surveys

1. Streams

Stream surveys involve annual collection of BMI samples and other pertinent physiochemical and biological data at study streams and estuaries using rapid bioassessment methodology. Our sampling methodology has been largely consistent since 2000. As in previous years, field surveys were conducted in the spring during base stream flow conditions (i.e., low flows). Sampling in the spring during base flow conditions provides consistency in the sampling from year to year, as the local stream biota is known to undergo seasonal succession (Cooper et al., 1986). The following were completed during each field survey:

- General observations were recorded on a standardized field data sheet, including location, date, time, weather, stream flow conditions, water clarity, and human impacts.
- A 100-meter study reach was delineated along the stream. Stream habitat units (i.e., riffles, runs, pools, etc.) within the study reach were mapped and quantified as a percentage of the total reach length.
- Stream wetted and channel bottom width were measured at three transects in the study reach. The three transects were established at the 25, 50 and 75 meter marks. Wetted perimeter width is the cross-sectional distance of streambed that is inundated with surface water. Channel bottom width is the cross-sectional distance between the bottoms of the stream banks.
- Riparian canopy cover was estimated in the center of the stream channel at the three transects using a spherical densitometer.
- Plant and wildlife species observed in the stream and riparian zone were recorded.
- Water temperature, conductance, pH, and dissolved oxygen concentration were measured in the field using YSI and Oakton handheld meters. Two measurements of each parameter were made, one in a riffle and the other in a pool, and the two values were averaged.
- One composite BMI sample was collected from each study reach based on the "multi-habitat" approach described in the USEPA's Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers (Barbour et al., 1999). Each sample represents approximately one square meter of stream bottom, collected from 10 individual, 0.1-square meter locations (each an approximately 30 cm square). The 10 locations that constituted the sample were selected based on the relative area each stream habitat (i.e., riffles, pools, falls, etc.) covered in the section of stream sampled. For example, if a stream reach contained approximately 50 percent riffles and 50 percent pools, five locations in riffles and five in pools were selected and sampled. Samples were collected using a D-frame net with 500 μm mesh. In locations with flowing water (e.g., riffles and runs), the net was held upright against the stream bottom, and substrata immediately upstream within the 0.1-square meter area was scraped and stirred up for approximately 15 seconds using feet and hands. Dislodged BMIs and stream bottom materials were carried into the net by the stream current. In areas with little or no current (e.g., pools), stream bottom material was stirred up by foot, followed by a quick sweep of the net through the water column to capture dislodged BMIs. This was repeated three times in each pool sampling location.
- After each BMI sample was collected, it was rinsed with water in a 500 μm sieve to wash out fine sediments, transferred to a plastic container, and preserved in 70 percent ethanol.

- A visually-based stream habitat assessment was completed. The protocol is based on a generic template from the USEPA, which has been modified by Ecology to suit local streams. Scoring was based on consideration of nine habitat components. Stream path and form was scored from 0 to 20, while a score from 0 to 10 was given for the other eight components: habitat diversity, habitat connectivity, hydrology, water column depth/velocity/quality, substrate/erosion/sedimentation, riparian vegetation cover/composition, riparian/upland buffer, and foot traffic/noise/lighting. Scores for each component were added for a total score of 0 to 100. Scoring criteria for each habitat component reflect the range of conditions present in the study area.
- Quality control measures were incorporated into the field surveys to insure accurate and consistent data gathering. Water monitoring equipment was calibrated regularly. Ecology's Principal Ecologist was present at all field surveys to ensure proper measurements, BMI sample collection, and stream habitat assessment scoring.

2. Estuaries

Ecology conducted a rapid bioassessment survey in each study estuary in the fall (early October). Methodology was based on the Tier 1 approach described in Estuarine and Coastal Marine Waters: Bioassessment and Biocriteria Technical Guidance (Bowman et al., 2000). The Tier 1 approach is intended to provide an assessment of coastal wetland habitats based on sampling of one or more biological assemblages (e.g., algae, invertebrates, fish, etc.) and collecting data on water chemistry and bottom characteristics. The following was completed:

- General observations were recorded, including study reach location, date, time, weather, water clarity, sediment composition, vegetation, hydrologic condition (i.e., estuary open or closed to ocean), tide conditions, and sources of human disturbance.
- Measurements of water temperature, pH, dissolved oxygen concentration, conductance, and salinity were made using YSI and Oakton handheld meters. Measurements were made at the downstream end of the estuary.
- BMI samples were collected at the downstream end of the estuary. Two separate samples were collected at each monitoring station; (1) an infaunal sample consisting of approximately the top 15 cm of sediments from two approximately 10 cm diameter areas of the estuary bottom collected in 0.3 to 0.6 m of water using a core sampler, and (2) an epibenthic sample consisting of material collected in sweeps in 5 locations with a D-net similar to the pool sampling method for streams (i.e., 0.1 m^2 per sweep). After collection, each sample was drained through a 0.5-millimeter mesh sieve to wash out fine sediments, and the remaining material was placed into a plastic bottle filled with 70% ethanol solution for preservation. In total, approximately 0.5 m^2 of bottom area was sampled at each site.
- A visually-based estuary habitat assessment was completed using a protocol developed by Ecology. Scoring was based on consideration of nine habitat components. Estuary form and perimeter was scored from 0 to 20, while a score from 0 to 10 was given for the other eight components: habitat diversity, connectivity, hydrology, substrate, water column, aquatic vegetation, riparian/upland buffer, and foot traffic/noise/lighting. Scores for each component were added for a total score of 0 to 100. Scoring criteria for each habitat component were carefully developed using our knowledge of local estuaries and the range of conditions present in the study area. Scoring for each estuary was based on field observations and measurements, and review of aerial photography.
- Quality control measures were incorporated into the field surveys to insure accurate and consistent data gathering. Water monitoring equipment was calibrated regularly. Ecology's

Principal Ecologist was present at all field surveys to ensure proper measurements, BMI sample collection, and estuary habitat assessment scoring.

B. Laboratory Analyses

BMI samples were processed in the laboratory to determine BMI community composition (i.e., taxa present and relative abundance) and overall BMI density (i.e., number of BMIs/m^2). Each BMI sample was strained through a 500 μm mesh sieve and washed with water to remove ethanol and fine sediments. The sample was placed in a plastic tray marked with equally-sized squares in a grid pattern. The entire sample was spread out evenly across the squares. Squares of material were randomly selected, and sorted one at a time under a dissecting microscope (7X to 50X magnification) until the targeted number of BMIs were located and picked out. The proportion of the sample sorted was noted. For streams, 300 BMIs were picked from each sample for identification. A target of 150 BMIs was set for each estuary sampling site. The infaunal sample was sorted through first, and up to 75 BMIs were picked and identified. Next, the epibenthic sample was sorted, and the remaining number of BMIs were picked and identified to reach 150. The targeted number of BMIs were achieved for most but not all samples.

BMIs were identified with the aid of taxonomic references including Merritt and Cummings (2008) and Smith and Carlton (1975). Insect taxa were identified to the family level. Non-insect taxa (e.g., oligochaetes, crustaceans, etc.) were typically identified to order or class. BMI sample processing methods were clearly established and strictly followed to ensure random selection and accurate enumeration and identification of BMIs. All BMI identifications were verified by Ecology's Principal Ecologist.

C. GIS Analyses

Geographic Resources Analyses Support System (GRASS), an open source GIS program, was used to calculate upstream watershed area and watershed land use coverages for each study reach. Watershed areas were calculated based on watershed boundaries generated in GRASS using the 3D Elevation Program (3DEP), a 1/3 arc-second (approximately 10 meter) resolution digital elevation model (DEM) downloaded from the USGS National Map website. Watershed land uses and percent cover for each study reach were calculated in GRASS by superimposing DEM-generated watershed boundaries over a digital land cover GIS layer for the region, the National Land Cover Database (NLCD). The NLCD, also downloaded from the USGS National Map website, was last updated in 2016. The NLCD classifies the land area of the continental U.S. at a spatial resolution of 30 meters into 21 land cover classes using Landsat data along with ancillary data sources, such as topography, census and agricultural statistics, soil characteristics, wetlands, and other land cover maps. Land cover classes present in the study area included the following:

- Open water
- Developed, Open Space
- Developed, Low Intensity
- Developed, Medium Intensity
- Developed, High Intensity
- Barren Land (Rock, Sand, Clay)
- Deciduous Forest
- Evergreen Forest
- Mixed Forest

- Shrub/Scrub
- Grasslands/Herbaceous
- Sedge/Herbaceous
- Pasture/Hay
- Cultivated Crops
- Woody Wetlands
- Emergent Herbaceous Wetlands

Recent aerial photographs of the region available on Google Earth were reviewed to refine the GIS land use layer as needed. The percentage of watershed disturbance was calculated for each study reach by using the following equation:

% watershed disturbed = % Developed Medium and High Intensity +
(0.75) (% Developed Low Intensity + % Cultivated Crops) +
(0.5) (% Developed Open Space + % Pasture/Hay)

Low intensity developed (e.g., rural residential), developed open space (e.g., golf courses, parks, and open fields) and crop and pasture areas were not counted as completely disturbed lands to reflect the fact that they retain some habitat value and hydrologic function (ground water infiltration, storm water filtration, etc.).

D. Review of Topographic Maps

USGS 7.5-minute quadrangle topographic maps (1:24,000 scale) for the study area were reviewed to determine stream order, elevation, and gradient for each study reach. Gradient was determined by dividing the elevation change between topographic contours immediately upstream and downstream of the study reach by the stream length between the contours. Stream length was determined by tracing a map wheel over the stream path.

E. Study Reach Grouping

Stream and estuary study reaches were separated into three different groups based on their level of human disturbance. These disturbance groups were assigned to study reaches *a priori*, or before the analyses of biological data, based on (1) physical habitat assessment scores, and (2) the percentage of upstream watershed disturbance. This approach allowed both reach and watershed scale impacts to be considered in the *a priori* assessment of habitat condition, both of which have been shown to be important predictors of BMI community composition in this and many other bioassessment studies. The following criteria are used to classify study reaches:

REF = Reaches that are in a "reference condition", or are minimally to lightly disturbed by human activities. Habitat assessment score is 75/100 or greater, and no more than 5 percent of the upstream watershed is developed through a combination of urban, agricultural and/or pasture lands.

MOD DIST = Reaches that are moderately disturbed by human activities. Habitat assessment score is 50/100 or greater, and no more than 20 percent of the upstream watershed is developed through a combination of urban, agricultural and/or pasture lands.

HIGH DIST= Reaches that are heavily disturbed by human activities. Habitat assessment score is less than 50 and/or greater than 20 percent of the upstream watershed is developed through a combination of urban, agricultural and/or pasture lands.

Previous analyses show that MOD DIST and REF study stream reaches affected by wildfires that burned 50% of greater of their upstream watersheds have almost exclusively exhibited Very Poor to Poor IBI scores in the first two years following fire. Similarly, the few MOD DIST and REF sites having no flow (i.e., pools only) at time of the survey have scored in the Very Poor range of the IBI. If taken out of context, IBI scores at these sites could lead to erroneous conclusions regarding their overall (i.e., long-term) biological integrity. To allow for proper consideration of this subset of MOD DIST and REF stream reaches, they have been labeled separately as the M/R F/P group. To date, a total of 20 study reaches fit into this group.

F. Calculation of Core Metrics for Streams

The 4 core metrics were calculated for each stream study reach for use in determining IBI scores and classifications of biological integrity. The core metrics are among the most sensitive to human disturbance as determined by rigorous statistical analyses (Ecology Consultants, Inc., 2019). Collectively, the core metrics are diversified in that they represent different aspects of community structure including richness, disturbance sensitivity, and trophic structure. Each of the core metrics and their methods of calculation are discussed below.

Number of Insect Families was determined by summing the number of insect families found in the sample.

Tolerance value average and **percent sensitive BMIs** were calculated using disturbance tolerance values for individual BMI taxa of between 0 and 10 based on their ability to withstand human disturbance. A tolerance value of 0 indicates that a BMI is extremely intolerant of human disturbance, with increasing scores indicating greater tolerances to human disturbance. **Tolerance value average** was determined by summing the tolerance values of all the individual BMIs in the sample, and dividing by the total number of BMIs in the sample. **Percent sensitive BMIs** was determined by summing the individuals with a tolerance value of 3 or less, dividing by the total number of BMIs in the sample, and multiplying by 100.

Tolerance values and sensitivity designations for individual BMI taxa are provided in Table A-1 of Appendix A. Tolerance values have been assigned to BMI taxa based on statistical analyses of BMI data collected in study area streams from 2000 to 2019. See the 2019 Report for details on methodology used to determine tolerance values.

Percent predators + shredders was determined by summing individual BMIs with a predator or shedder functional feeding group designation, dividing by the total number of BMIs in the sample, and multiplying by 100. Functional feeding group designations were obtained from An Introduction to the Aquatic Insects of North America (Merritt and Cummins, 2008).

G. Core Metric Scoring Ranges for Streams

The IBI provides scoring ranges of between 0 and 10 for each of the 4 core metrics (see Table 2). See the 2019 Report for discussion of how core metric scoring ranges were determined. For core metrics that decrease with increasing human disturbance (e.g., # insect families), higher values corresponded with higher scores. For core metrics that increase with increasing human disturbance (e.g., tolerance value average), lower values corresponded with higher scores.

Table 2 Core Metric Scoring Ranges				
Score	**% sens BMIs**	**# I fams**	**TV Avg.**	**% sh+pred**
10	60+	28+	3.81 or lower	17+
9	47 to 59	26,27	3.82 to 4.59	13 to 16
8	36 to 46	24, 25	4.60 to 5.01	11, 12
7	25 to 35	22, 23	5.02 to 5.42	10
6	13 to 24	20, 21	5.43 to 5.83	9
5	11 to 13	17 to 19	5.84 to 6.16	7, 8
4	8 to 10	14 to 16	6.17 to 6.50	6
3	5 to 7	12, 13	6.51 to 6.84	5
2	2 to 4	10, 11	6.85 to 7.18	4
1	1	7 to 9	7.19 to 8.08	2, 3
0	0	0 to 6	8.09+	0, 1

H. IBI Classifications of Biological Integrity and Scoring Ranges for Streams

Individual scores for the 4 core metrics are summed to provide a total score of between 0 and 40 for the study reach. The IBI provides 5 classifications of biological integrity based on the total score: Excellent, Good, Fair, Poor, and Very Poor. IBI classifications and scoring ranges are provided in Table 3. See the 2019 Report for discussion of how ranges were determined for the 5 classifications of biological integrity.

Table 3 Streams Classifications of Biological Integrity	
Category	**Scoring Range**
Excellent	34 to 40
Good	24 to 33
Fair	19 to 23
Poor	8 to 18
Very Poor	0 to 7

I. Additional Data Analyses for Streams

Oneway Analysis of Variance (ANOVA) were completed to evaluate mean values for IBI score between the disturbance groups (REF, MOD DIST, M/R I/F, and HIGH DIST), both overall, and year-to-year. These tests were completed to evaluate relationships between disturbance, both human and natural episodic, and the stream BMI community, and to evaluate trends through time.

An ANOVA is a statistical test that compares the means and distributions of a given metric among multiple sampling groups, and indicates the probability that the means for the groups are the same. The probability that the means are the same is expressed as p, which is between

0 and 1. The lower the *p*, the lower the probability that the group means are the same. A *p* of 0.05 (i.e., 5%) or less is generally accepted as indicating a statistically significant difference between group means.

J. Data Analyses for Estuaries

1. Evaluating Estuary BMI Taxa for Disturbance Sensitivity

ANOVAs were completed to compare mean abundances of individual BMI taxa among the REF, MOD DIST, and HIGH DIST groups. This was done to evaluate disturbance sensitivity of the individual taxa, and identify taxa that could be useful in developing BMI community metrics (i.e., % sensitive BMIs) that can serve as indicators of biological integrity. Taxa with higher mean abundance at REF sites compared to HIGH DIST sites with significant or near significant results were labeled as "sensitive". Taxa with higher mean abundance at HIGH DIST sites compared to REF sites with significant or near significant results would be labeled as "tolerant". Taxa that did not meet either of these criteria were labeled as moderately tolerant of human disturbance.

2. Calculation and Evaluation of BMI Metrics

9 BMI metrics were calculated for the study estuaries, including measures of abundance, diversity, disturbance sensitivity, and trophic structure (see Table 4). Many of the metrics calculated are the same or similar to those used effectively as indicators of biological condition in other estuarine studies conducted throughout the nation.

Table 4: BMI Metrics Calculated for Study Estuaries

BMI Metric	Abbreviation	Units of Measurement	Method of Calculation
BMI density	None	# per m^2	Lab
# of taxa	# taxa	None	Lab
# of sensitive taxa	# sens taxa	None	Lab
% sensitive BMIs	% sens BMIs	%	Lab
% insects	None	%	Lab
% dominant taxon	None	%	Lab
% 2 dominant taxa	None	%	Lab
% predators	% pred	%	Lab
% collector-gatherers	% cg	%	Lab

BMI density (individuals/m^2) was calculated by dividing the number of specimens picked out of the sample by the subsampled area. Richness parameters were determined by counting the number of specified taxa identified in each sample. % sensitive BMIs was calculated by adding the number of BMIs in the sample labeled as "sensitive" to human disturbance, dividing by the total number of individuals in the sample, and multiplying by 100. Functional feeding group parameters (e.g., percent collector-gatherers, % predators) were determined using functional feeding group designations for individual taxa provided in Merritt and Cummins (2008). % dominant taxon and % 2 dominant taxa were determined as the percentage of the sample represented by the most common and two most common taxa, respectively.

ANOVAs were completed to compare mean values for the 9 BMI metrics among the REF, MOD DIST, and HIGH DIST groups to evaluate their disturbance sensitivity. BMI metrics with significant differences in means between REF and HIGH DIST groups were considered sensitive to human disturbance.

3. Scale of Biological Integrity for Estuaries

The estuarine Scale of Biological Integrity is based on a single metric: % sens BMIs, and its distributions at REF and HIGH DIST sites. Past analyses showed that % sens BMIs was the only metric sensitive enough to human disturbance to be used in the estuarine Scale. Values of % sens BMIs are partitioned into three categories of biological integrity: Good, Fair, and Poor (see Table 5).

Table 5: Estuarine Scale of Biological Integrity

Classification of Biological Integrity	Scoring Criteria Based on % Sensitive BMIs	Scoring Range (% Sensitive BMIs)
Good	25th percentile of REF group and above	16-100%
Fair	From just above 75th percentile of HIGH DIST group to just below 25th percentile of REF group	7-15%
Poor	75th percentile of HIGH DIST group and below	0-6%

4. Evaluating Salinity Effects on Estuary BMI Taxa

An ANOVA was performed to evaluate the relationship between % sens BMIs and salinity in three classes: Low (less than 5 ppt), Moderate (5 to 18 ppt) and High (greater than 18 ppt). HIGH DIST sites were excluded from the analysis in an effort to minimize human disturbance confluences. Understanding the influences of natural physiochemical variability is an important part of the process of screening potential BMI indicator metrics. Based on the data collected thus far, salinity is the most important natural physiochemical factor affecting BMI composition in local estuaries. Other physiochemical parameters may be considered in the future as the data set grows.

IV. Results and Discussion

A. Physiochemical and Biological Data

Table A-1 in the Appendix provides physiochemical data collected at the streams this year and in previous years of study. Table A-1 also lists BMI taxa and abundances for each stream studied, as well as BMI density, core metric values, and IBI score. Tolerance values and functional feeding groups are provided for individual BMI taxa. Table A-2 provides a list of the plant species observed at each stream site studied this year, and the number and percentage of native vs. introduced plant species observed. Table A-3 provides a list of vertebrate species observed at this year's study streams. For streams that have been surveyed multiple times, plant and vertebrate species observations are combined. Table A-4 provides a list of the plant species observed at each estuary studied this year, and the number and percentage of native vs. introduced plant species observed. Table A-5 provides a list of vertebrate species observed at the study estuaries. For estuaries that have been surveyed multiple times, plant and vertebrate species observations are combined. Table A-6 provides physiochemical and BMI data and metrics for study estuaries. Site photographs for study streams and estuaries are also provided in the Appendix.

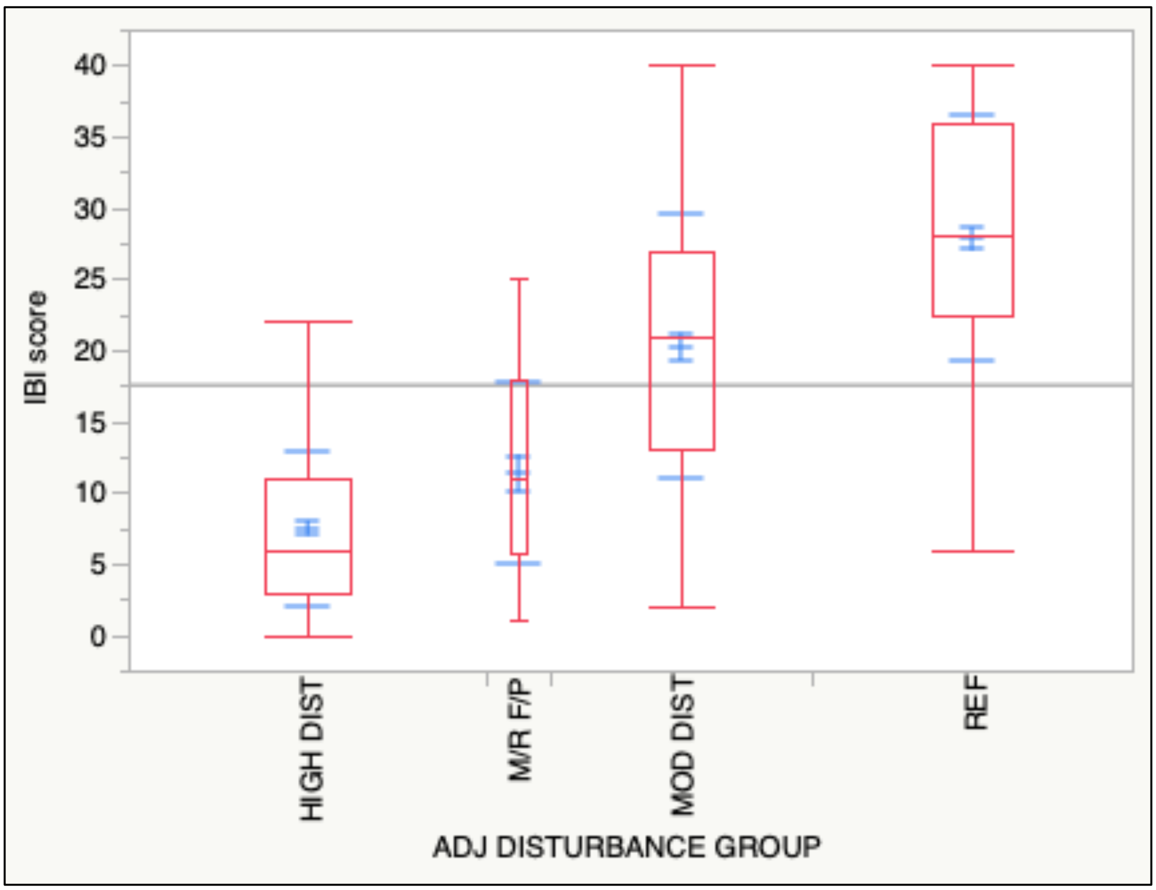
B. Data Analyses for Streams

1. IBI Scores by Disturbance Group, All years

ANOVA was used to evaluate differences in mean IBI score between the study reach groups using all study reaches from all years (i.e., 2000 to 2021) as replicates (n=417). ANOVA results are illustrated in Figure 4. The ANOVA shows that the IBI has a very strong negative response to increasing human disturbance, with highly significant declines in mean IBI score from REF (28) to MOD DIST (20) to HIGH DIST (7) groups ($p<0.0001$, $r^2=0.57$). Mean IBI score for the M/R F/P group (i.e., fire-impacted or pools only) was 11 (n=20), or intermediate between the MOD DIST and HIGH DIST groups.

Figure 4: Distribution of IBI Scores by Disturbance Group, all Streams Study Reaches (2000-2021)

Box plots of IBI score for the HIGH DIST, M/R F/P, MOD DIST, and REF groups (n=417) are shown below. Red boxes (i.e., box plots) represent 25th percentile (bottom), median (center line), and 75th percentile (top), with bottom and top bars representing 2.5th and 97.5th percentiles, respectively for each disturbance group. Blue bars represent means (centerline) standard error (inner bars), and standard deviation (outer bars). The ANOVA showed highly significant differences in mean IBI scores between disturbance groups, increasing in order from HIGH DIST, M/R F/P, MOD DIST and REF groups (r^2=0.57, p<0.0001). The p value is for the ANOVA where IBI score is the dependent variable and disturbance category is the independent variable. The r^2 is the proportion of variation in IBI score accounted for by disturbance category.



2. IBI Scores by Disturbance Group, Year to Year

Figure 5 illustrates mean IBI score by year (i.e., from 2000 to 2021) for the HIGH DIST, MOD DIST, and REF groups (M/R F/P sites excluded from analyses). ANOVA results for these comparisons are summarized in Table A-7.

The HIGH DIST group showed no significant differences in yearly mean IBI score, which has ranged from 3 to 11 amongst the 21 years studied ($p=0.09$, $r^2 = 0.19$, $n=149$). The MOD DIST group had wider fluctuations in mean yearly IBI score (11 to 30), with some statistically significant differences between years ($p=0.02$, $r^2 = 0.30$, $n=109$). Years in which mean IBI score was relatively low in the MOD DIST group include 2001, 2005, and 2018.

The REF group is less confounded by variable human disturbance impacts compared to the MOD DIST and HIGH DIST groups, and thus should yield the most meaningful information regarding natural yearly trends in IBI scores. Yearly mean IBI score for the REF group has ranged from 19 to 38 in 21 years of study. Statistically significant differences in mean IBI score for the REF group have occurred between several higher scoring years (2000-2003, 2006, 2007, 2009, 2010, and 2013) and several lower scoring years including 2005 and 2014 to 2018 ($p<0.0001$, $r^2 = 0.55$, $n=133$). Figure 5 illustrates the general pattern that has occurred at the REF sites. In general, mean IBI score was higher at the REF sites from 2000 to 2013 (over 30 in most years), a period where there were several average to high rainfall years. The exception in this period was 2005, when mean REF IBI score was 19. That year the surveys followed one of the wettest winters years on record when extreme stream flows largely scoured out stream communities. The effects of a prolonged drought were evidenced by low mean IBI scores in the REF group from 2014 through 2018 (19, 22, 18, 19, and 19, respectively). Mean REF IBI scores recovered somewhat in 2019 (23) and 2020 (27), likely in response to more stable stream flows caused by a very wet winter in 2017-18 and above average rainfall in winter 2019-20. This year's surveys followed a very dry winter, and mean REF IBI score was 26.

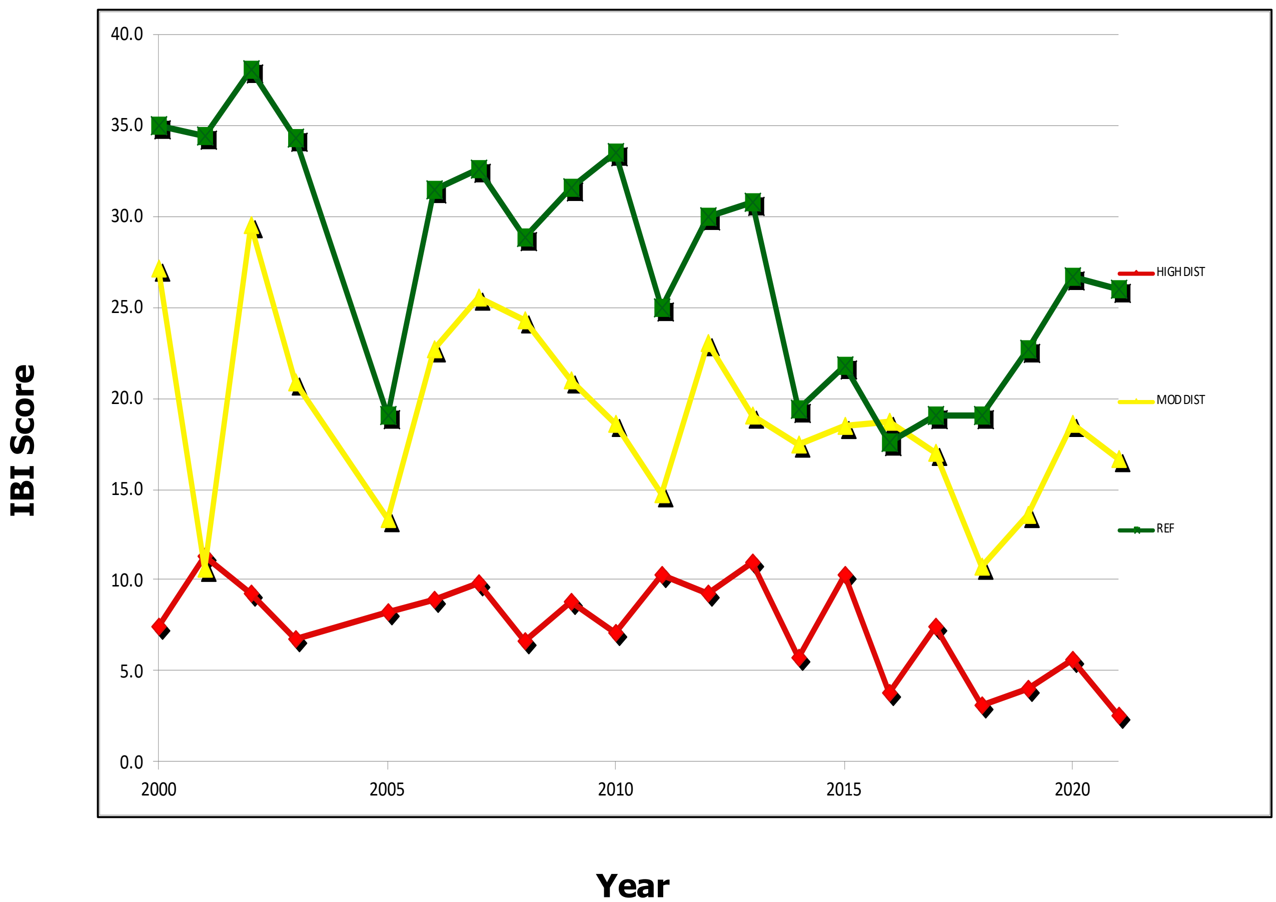
Figure 5: Mean IBI Score for Study Reach Groups (HIGH DIST, MOD DIST, and REF) by Year

Mean IBI score by study reach group (HIGH DIST, MOD DIST, and REF) and year are shown below. Dots are the mean value for each group (color coded, see legend) and year. ANOVA results are provided below. The *p* value and r^2 are provided below for each of the disturbance groups, comparing IBI score within each group by year. The *p* value is for the ANOVA where IBI score is the dependent variable and year is the independent variable. r^2 is the proportion of variation in IBI score accounted for by year.

REF group (GREEN): ($p<0.0001$, $r^2 = 0.55$, n=133)

MOD DIST group (YELLOW): ($p=0.02$, $r^2 = 0.30$, n=109)

HIGH DIST group (RED): ($p=0.09$, $r^2 = 0.19$, n=149).



3. 2021 Streams Results

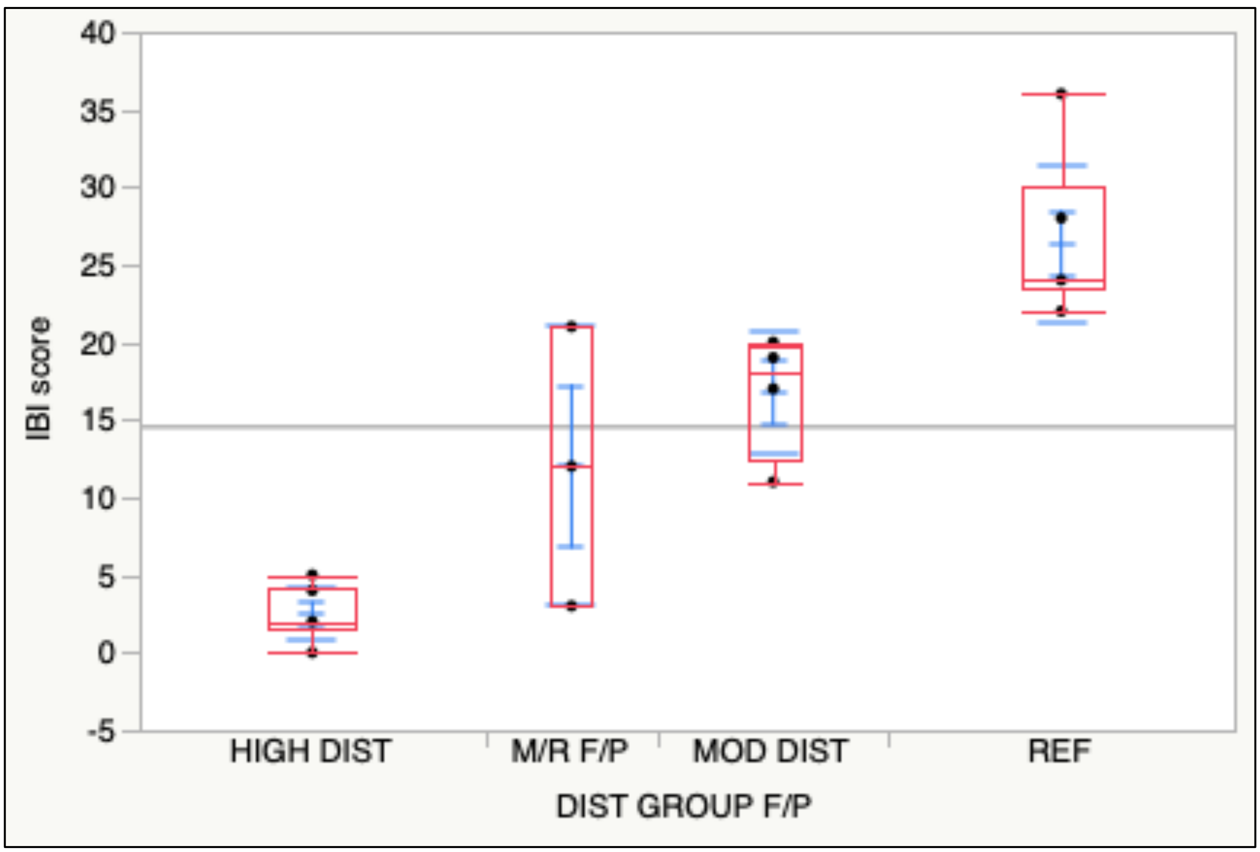
Data for individual streams study reaches in 2021 (and previous years) is provided in Appendix Tables A-1, A-2, and A-3. Site photographs for study stream reaches are also provided in the Appendix. Table 6 provides IBI scores for individual study reaches for this year from lowest to highest score by disturbance group. Figure 6 illustrates ANOVA results of IBI scores by study reach group for this year (2021). The IBI appropriately separated between disturbance groups with a high level of significance (p<0.0001, r^2 = 0.83, n=19), with mean IBI score increasing from HIGH DIST (3) to M/R F/P (12) to MOD DIST (17) to REF (26) groups.

Table 6
IBI Scores for this Year's Stream Study Reaches

Study Reach	Disturbance Group	IBI Score	Classification
M0	HIGH DIST	0	Very Poor
AB2a	HIGH DIST	2	Very Poor
AB5	HIGH DIST	2	Very Poor
OR1	HIGH DIST	2	Very Poor
AB1	HIGH DIST	4	Very Poor
AB2	HIGH DIST	5	Very Poor
AL1	M/R F/P	3	Very Poor
SY4	M/R F/P	12	Poor
AB3	M/R F/P	21	Fair
SY2	MOD DIST	11	Poor
RIN1	MOD DIST	17	Poor
M3	MOD DIST	19	Fair
MONT3	MOD DIST	20	Fair
SO1	REF	22	Fair
C3	REF	24	Good
GAV1	REF	24	Good
SAL1	REF	24	Good
M4	REF	28	Good
AH1	REF	36	Excellent

Figure 6: Oneway ANOVA of IBI Score by Disturbance Group, 2021

Box plots of IBI score for the HIGH DIST, M/R F/P, MOD DIST and REF groups (n=19) are shown below. Red boxes (i.e., box plots) represent 25th percentile (bottom), median (center line), and 75th percentile (top), with bottom and top bars representing 2.5th and 97.5th percentiles, respectively for each disturbance group. Blue bars represent means (centerline) standard error (inner bars), and standard deviation (outer bars). The ANOVA showed highly significant differences in mean IBI scores between disturbance groups (r^2=0.83, p<0.0001). The p value is for the ANOVA where IBI score is the dependent variable and disturbance category is the independent variable. The r^2 is the proportion of variation in the IBI score accounted for by the disturbance categories.



REF Group

The REF group included 6 study reaches: AH1 (Arroyo Hondo), C3 (Gobernador Creek), Gaviota Creek (GAV1), M4 (Rattlesnake Creek), SAL1 (Salsipuedes Creek), and San Onofre Creek (SO1). IBI score at REF sites ranged from 22 (Fair) at SO1 to 36 (Excellent) at AH1, with a mean score of 26. C3 reached the bottom end of the Good range (24). C3 was in the Poor Range the first year (2018) following the Thomas Fire of December 2017, and increased to Fair in 2019 and 2020. Although habitat conditions and IBI scores have steadily improved at C3 post-fire, the effects of the fire and ensuing extreme scouring stream flows were still evident 3 ½ years later as evidenced by relatively low riparian canopy cover, elevated stream temperature (21.7 °C), and a higher sand component in the stream bottom due to increases in runoff and erosion. GAV1 and SAL1 also scored at the bottom of the Good range (24). These are lower gradient streams with relatively large watersheds, wide channels, low riparian canopy cover, and higher average stream temperature. REF streams of this type typically score in the Fair to Good range. Study reach M4 (Rattlesnake Creek) had an IBI score of 28 (Good), while AH1 (Arroyo Hondo) scored 36 (Excellent). Higher gradient, shady, cold-water mountain REF streams such as these two typically score in the Good to Excellent range of the IBI.

Young of the year rainbow trout of between 1-3" in length were observed at AH1 during the spring surveys this year. Other native aquatic vertebrates observed at the Arroyo Hondo study reaches over the years include red-legged frog, California newt, southwestern pond turtle, two-striped garter snake, and California tree frog.

Two rainbow trout/steelhead of 10-12" in length were observed in the fish passage weir at SAL1 last year (2020). Southwestern pond turtle and three-spine stickleback have also been observed at SAL1.

Large numbers of California newts were observed at C3 (Gobernador Creek) this year, as they have for the last several years, both before and after the Thomas Fire of 2017. California and Pacific tree frogs, and two-striped garter snakes have also been observed at C3 over the years. Rainbow trout were regularly observed in large numbers at C3 in past years, but have not been observed since 2014, presumably due to the effects of prolonged drought of 2013 to 2017 followed by the Thomas Fire.

California newts were observed at M4 (Rattlesnake Creek) this year as in the past, as have California and Pacific tree frogs. A lone rainbow trout approximately 6" in length was observed in a plunge pool at M4 this year. This was the first trout sighting at M4 since 2009. Trout were regularly observed in large numbers at M4 and throughout Rattlesnake Creek and Upper Mission Creek prior to the destructive Jesusita Fire in 2009.

Several native aquatic vertebrates have been observed over the years at GAV1 including three-spine stickleback, western toad, pacific tree frog, and southwestern pond turtle. Arroyo chub, native further south in California but introduced in the Santa Barbara area, has also been observed. Steelhead/rainbow trout have been observed in lower Gaviota Creek in other studies.

SO1 was surveyed for the second time, and first time in almost 20 years. Pacific tree frog is the only aquatic vertebrate species observed.

MOD DIST Group

The MOD DIST group included 4 study reaches: RIN1 (Rincon Creek) and MONT3 (Cold Springs Creek), both impacted by the Thomas Fire in 2017, M3 (Mission Creek), and SY2 (Sycamore Creek). IBI scores were 11 (Poor) at SY2, 17 (Poor) at RIN1, 19 (Fair) at M3, and 20 (Fair) at

MONT3, with a mean score of 16. MONT3 and RIN1 showed continuing recovery from the Thomas Fire.

California and Pacific tree frogs have been observed in recent years at MONT3. Pacific tree frogs and western toads have been observed at M3. Three-spine stickleback and Pacific tree frog have been observed recently at RIN1. Rainbow trout were periodically observed at M3, MONT3, and RIN1 in the early 2000s, but had not been seen in many years following a regime of fires and drought beginning in 2009. This year, two rainbow trout of approximately 4" and 7" were observed in a large pool at MONT3.

M/R F/P Group

There were 3 study reaches in M/R I/F group this year, all of which lacked surface flow and had only standing pools: AB3 (San Roque Creek), SY4 (Sycamore Creek), and AL1 (Alamo Pintado Creek). IBI scores at these sites were 3 (Very Poor) at AL1, 12 (Poor) at SY4, and 21 (Fair) at AB3, with a mean score of 12. Pacific tree frogs have been observed at all M/R F/P sites. California newt has been observed at AB3, and three-spine stickleback at AL1.

HIGH DIST Group

The HIGH DIST group (n=6) included lower Arroyo Burro study reaches AB1, AB2, and AB2a, AB5 (Mesa Creek), M0 (lower Mission Creek), SY0 (lower Sycamore Creek), and OR1 (Orcutt Creek). IBI scores were Very Poor at all these sites, ranging from 0 to 5. Native aquatic vertebrate sightings at the HIGH DIST study reaches have been limited to Pacific tree frog, Western toad, and/or three-spine stickleback.

City of Santa Barbara Stream Study Reaches

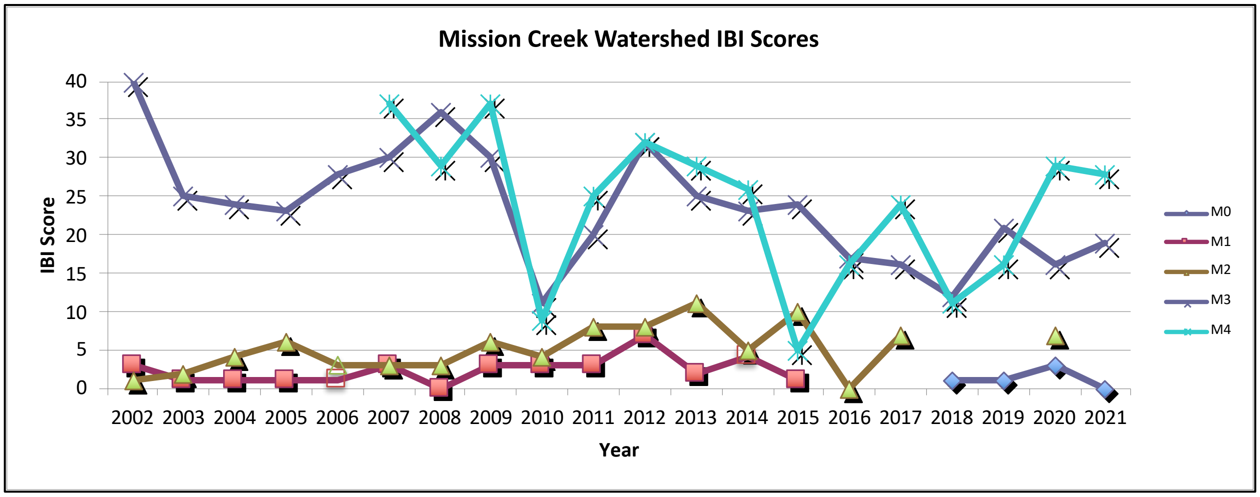
Figure 7 illustrates IBI scores by year for all City of Santa Barbara stream study reaches, inclusive of the Sycamore Creek, Mission Creek, and Arroyo Burro watersheds. HIGH DIST sites across the 3 watersheds (i.e., M0, M1, M2, AB1, AB2, AB5, SY0, SY1) have consistently been Very Poor to Poor over the years. MOD DIST and M/R F/P sites M3, SY2, SY4, AB3, and AB9 have typically fluctuated from Poor to Good over the years. REF sites M4 and AB3 have typically scored in the Good to Excellent range during periods of year-round flow, but both have scored in the Poor to Fair range of the IBI in years when stream flow was intermittent.

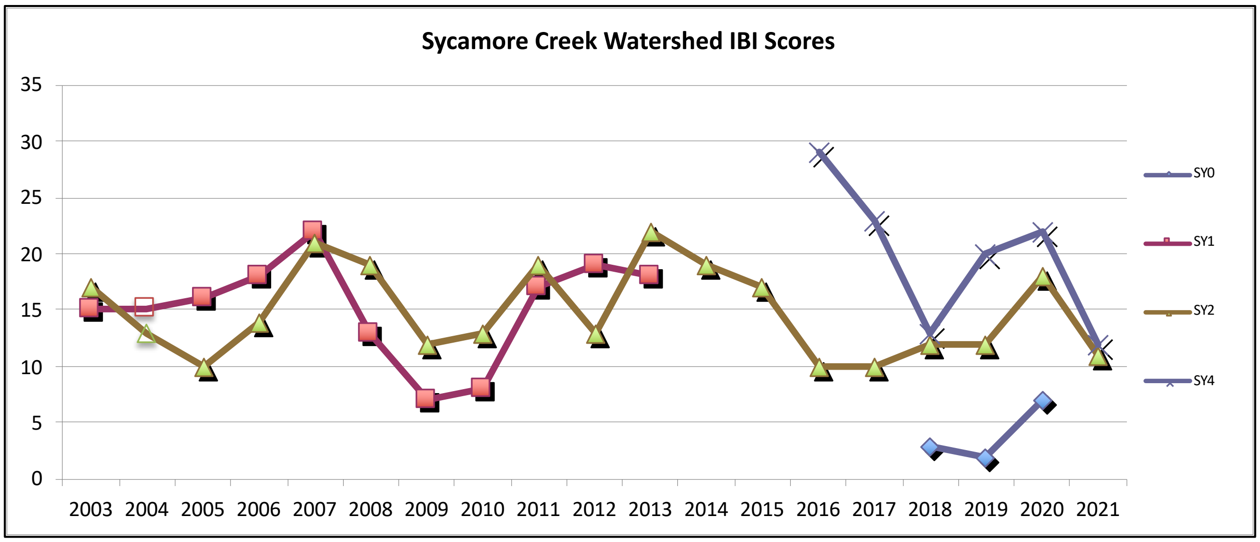
The City of Santa Barbara has undertaken stream habitat restoration projects at several sites in the Arroyo Burro and Mission Creek watersheds. Restoration efforts at these sites have involved re-shaping the stream channel and banks (or complete reconstruction in the case of AB5), improvement of storm water infiltration and filtering using bioswales, removal of existing non-native vegetation, and replanting and establishment of native riparian vegetation. Two of these restoration projects, M2 (Old Mission Creek) and AB5 (Mesa Creek), were initiated several years ago (M2 in 2002 and AB5 in 2007). Three other projects were initiated more recently, in 2016 at AB9 (Barger Canyon Creek), and in 2018 at AB1 (lower Arroyo Burro Creek) and AB2a (Arroyo Burro upstream of Torino Rd.).

Long term restoration sites M2, AB5, and more recently AB9, have shown improvements in habitat conditions over time, as reflected by improved Habitat Assessment score. Improved Habitat Assessment scores at M2, AB5, and AB9 owe largely to restored streamside, riparian vegetation and channel morphology due to initial restoration efforts and long-term maintenance. Native riparian plant cover and riparian canopy cover have greatly improved at all three of these restoration sites, presumably improving habitat conditions for riparian birds and other terrestrial vertebrate and invertebrate species. The improved riparian habitat at these sites has also improved bank stability, and has presumably resulted in lower average stream temperatures, reduced algal blooms, and more stable dissolved oxygen levels. The restoration projects at AB1 and AB2a were initiated in 2018, and these sites will require more time for visible improvements to occur.

Figure 8 shows IBI score by year post restoration at M2, AB5, AB9, AB2a, and AB1. Pre-restoration bioassessment data was collected for many years at AB1, and for one year at M2 and AB2a. There has not been a consistent upward trend in IBI scores at any of the sites through time, nor marked improvement when compared with pre-restoration averages, where available. In all cases IBI scores as these 5 sites have been in the Very Poor to Poor range. Despite measured improvements in habitat conditions at the restoration sites, as of yet there have not been notable improvements in the aquatic community as measured by IBI scores, nor have sensitive aquatic vertebrates been observed at any of the sites before or after restoration. Restoration efforts at these sites did not address larger scale impairments in hydrology, geomorphology, water quality, and habitat continuity and connectivity that have resulted from drastic human alteration of their respective watersheds. While much of this impairment cannot be undone from a practical sense, watershed-scale projects aimed at reducing pollution inputs, improving storm water filtration, and restoring freshwater inputs and hydrology (e.g., groundwater recharge) could in theory improve the overall health of local stream ecosystems. Whether or not current and future restoration efforts at these and other stream habitat restoration sites will measurably improve the aquatic community in local streams can only be evaluated via continued monitoring.

Figure 7: IBI Scores by Year for City of Santa Barbara Study Reaches





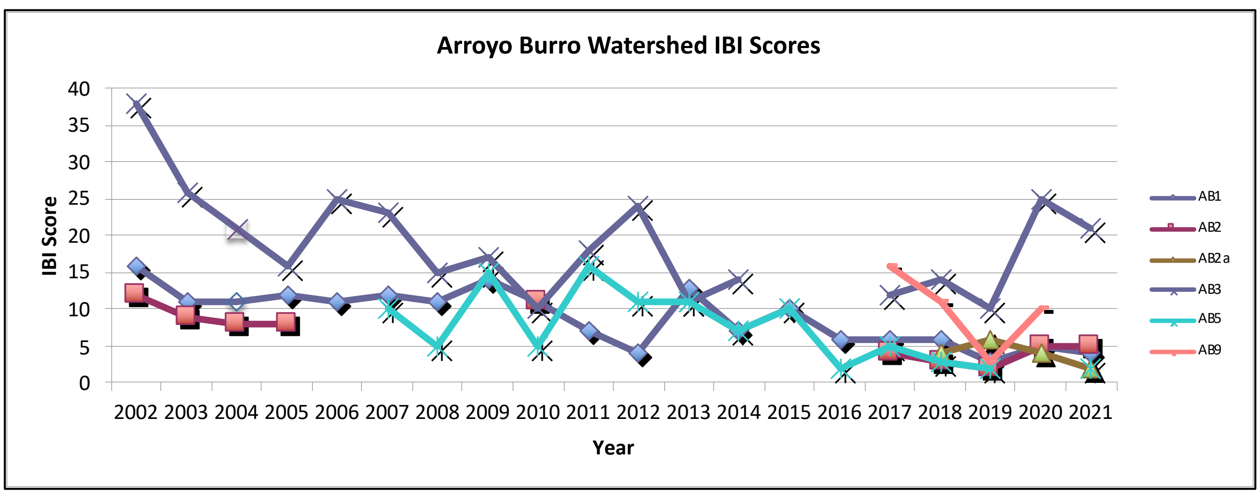
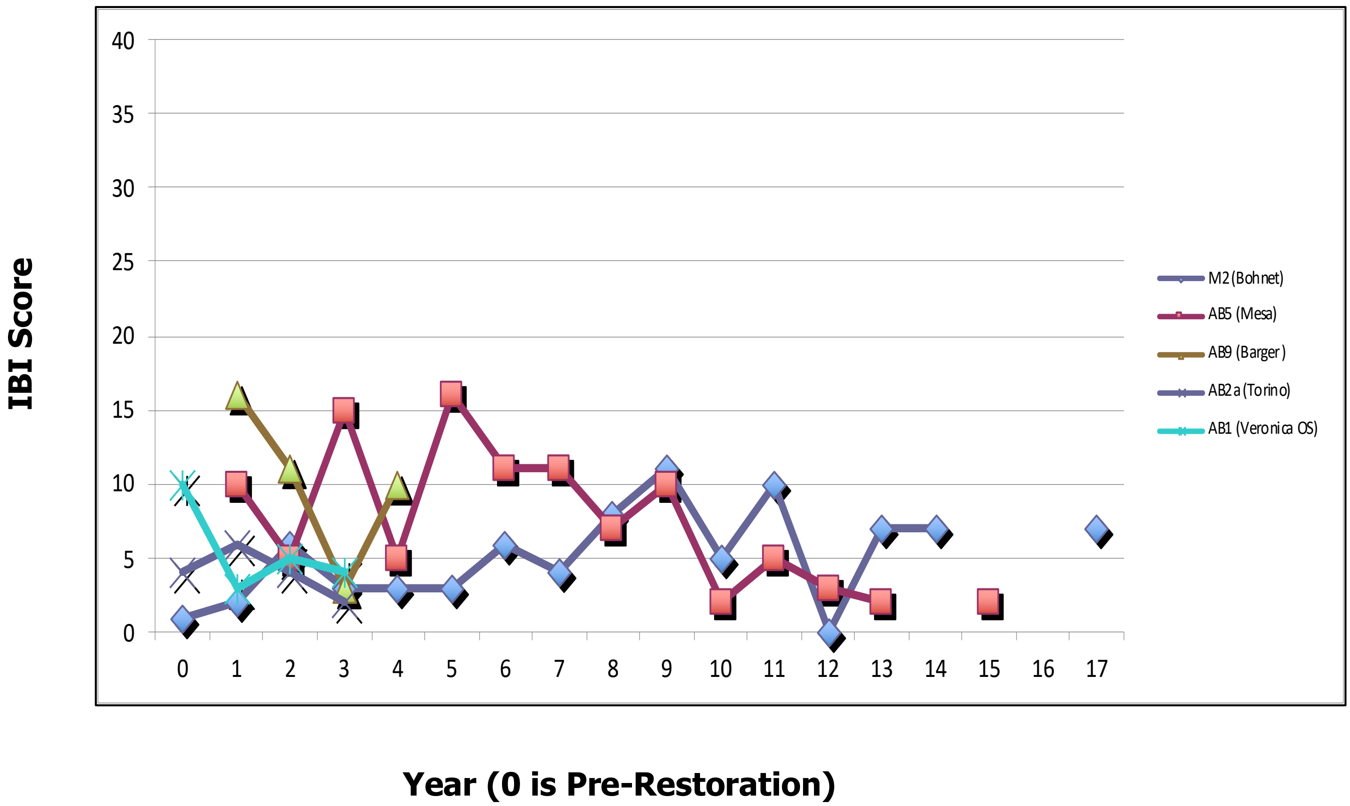


Figure 8: IBI Scores by Year at Stream Restoration Sites



C. Data Analyses for Estuaries

1. Estuary BMI Indicator Taxa and Metrics

Results of the ANOVAs of individual BMI taxa by disturbance group are summarized in Table A-8. Disturbance sensitive taxa, or those with significantly higher mean abundance at REF sites compared to HIGH DIST sites, were Baetidae (p=0.001, r^2 = 0.16), Aeshnidae (p=0.04, r^2 = 0.09), Acari (p=0.0004, r^2 = 0.19), Gammaridae (p=0.02, r^2 = 0.11), Mystidacea (p=0.002, r^2 = 0.14), and Isopoda (p=0.002, r^2 = 0.15). Coenagrionidae (p=0.06, r^2 = 0.08), Veliidae (p=0.21, r^2 = 0.04) and Notonectidae (p=0.22, r^2 = 0.04) also had noticeably higher mean abundance at REF sites compared to HIGH DIST sites with near-significant results. These taxa are designated as disturbance sensitive. Chironomidae had significantly highest mean abundance at MOD DIST sites (p=0.002, r^2 = 0.16), as did Ceratopogonidae (p=0.02, r^2 = 0.1). Corophidae had significantly highest abundance at HIGH DIST sites (p=0.02, r^2 = 0.11), and is designated as tolerant. Corixidae, Ostracoda, Cladocera, Oligochaeta, and Polychaeta all had higher mean abundances at HIGH DIST compared to REF sites, although none of the ANOVAs for these taxa had significant statistical results.

Results of the BMI metric ANOVAs by disturbance group are summarized in Table A-9. % sens BMIs significantly decreased in mean value from REF to MOD DIST to HIGH DIST with impressive *p* (<0.0001) and r^2 (0.43) (see Figure 9). # sens taxa was highest at REF sites (p<0.0001, r^2 = 0.29), compared to HIGH DIST and MOD DIST sites, but would be impractical to use as an indicator metric due to the small differences in mean values between groups (i.e., 3.1 for REF, 1.9 and 1.4 for MOD DIST and HIGH DIST, respectively). Metrics with weaker albeit significant relationships with disturbance included # taxa (negative relationship, p=0.001, r^2 = 0.14), % dominant taxon (positive relationship, p=0.001, r^2 = 0.14) and % 2 dominant taxa (positive relationship, p=0.003, r^2 = 0.12). Relationships between these metrics and disturbance are not strong enough for them to be effectively used as indicators of biological integrity in local estuaries, as evidenced by low r^2 values. % insects was significantly higher for the MOD DIST group compared to the REF and HIGH DIST groups (p=0.006, r^2 = 0.11), which were not significantly different from one another. BMI density, % predators and % collector-gatherers did not have significant differences in mean value among disturbance groups.

2. Salinity Effects

An ANOVA was performed to evaluate the relationship between % sens BMIs and salinity in three classes: Low (less than 5 ppt), Moderate (5 to 18 ppt) and High (greater than 18 ppt). HIGH DIST sites were excluded from the analysis in an effort to minimize human disturbance confluences. The REF and MOD DIST sites (n=44) had a salinity range of 0.8 to 31.5 ppt. The ANOVA shows an interesting pattern: mean % sensitive BMIs was intermediate in the Low salinity class (32%, n=27), highest in the Moderate salinity class (46%, n=12), and lowest in the High salinity class (11%, n=6) with significant results (p=0.02, r^2 = 0.16) (see Figure 10).

Figure 9: Oneway ANOVA of % Sensitive BMIs in Estuaries by Disturbance Group

Box plots of % sensitive BMIs for each of the REF, MOD DIST, and HIGH DIST disturbance groups are shown below. Red boxes (i.e., box plots) represent 25th percentile (bottom), median (center line), and 75th percentile (top), with bottom and top bars representing 2.5th and 97.5th percentiles, respectively for each disturbance group. Blue bars represent means (centerline) standard error (inner bars), and standard deviation (outer bars). The ANOVA showed highly significant differences in % sensitive BMIs between disturbance groups (r^2=0.43, p<0.0001, n=92). The p value is for the ANOVA where % sensitive BMIs is the dependent variable and disturbance category is the independent variable. The r^2 is the proportion of variation in % sensitive BMIs accounted for by the disturbance categories.

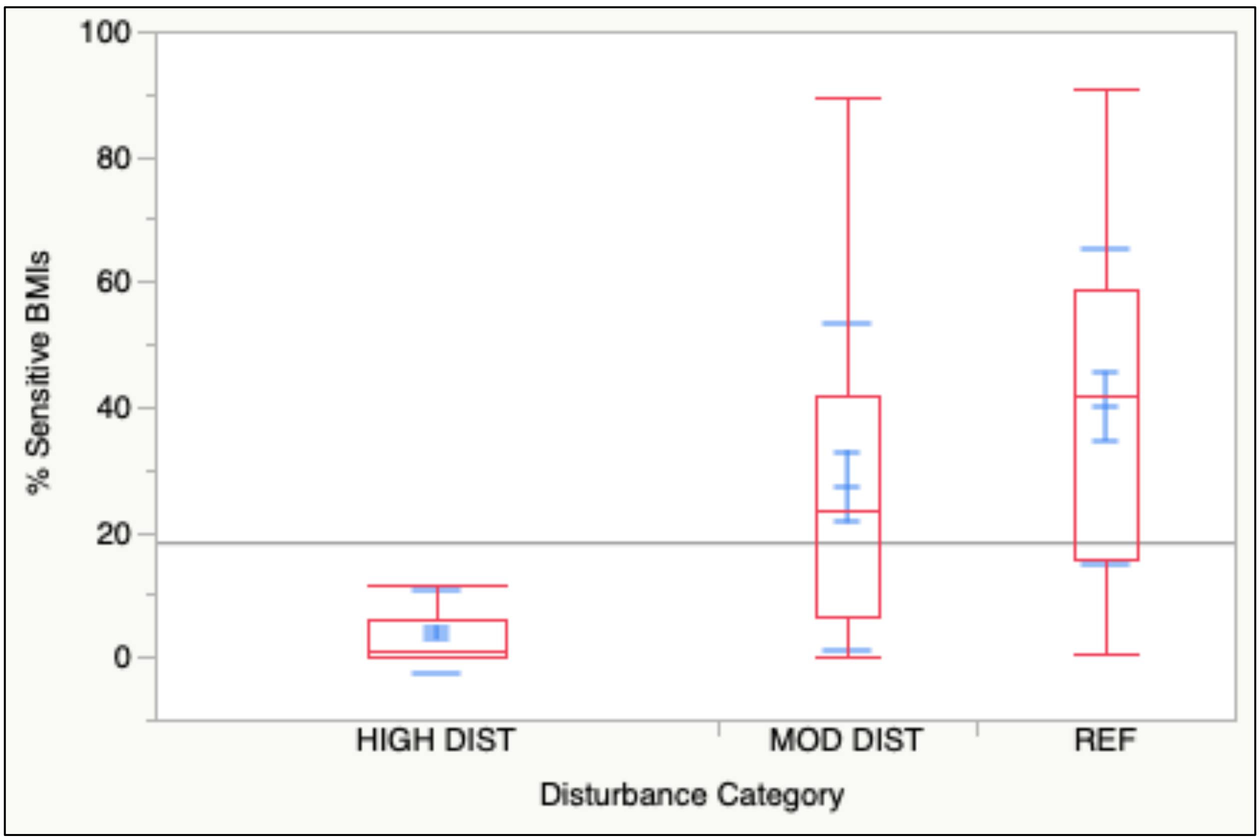
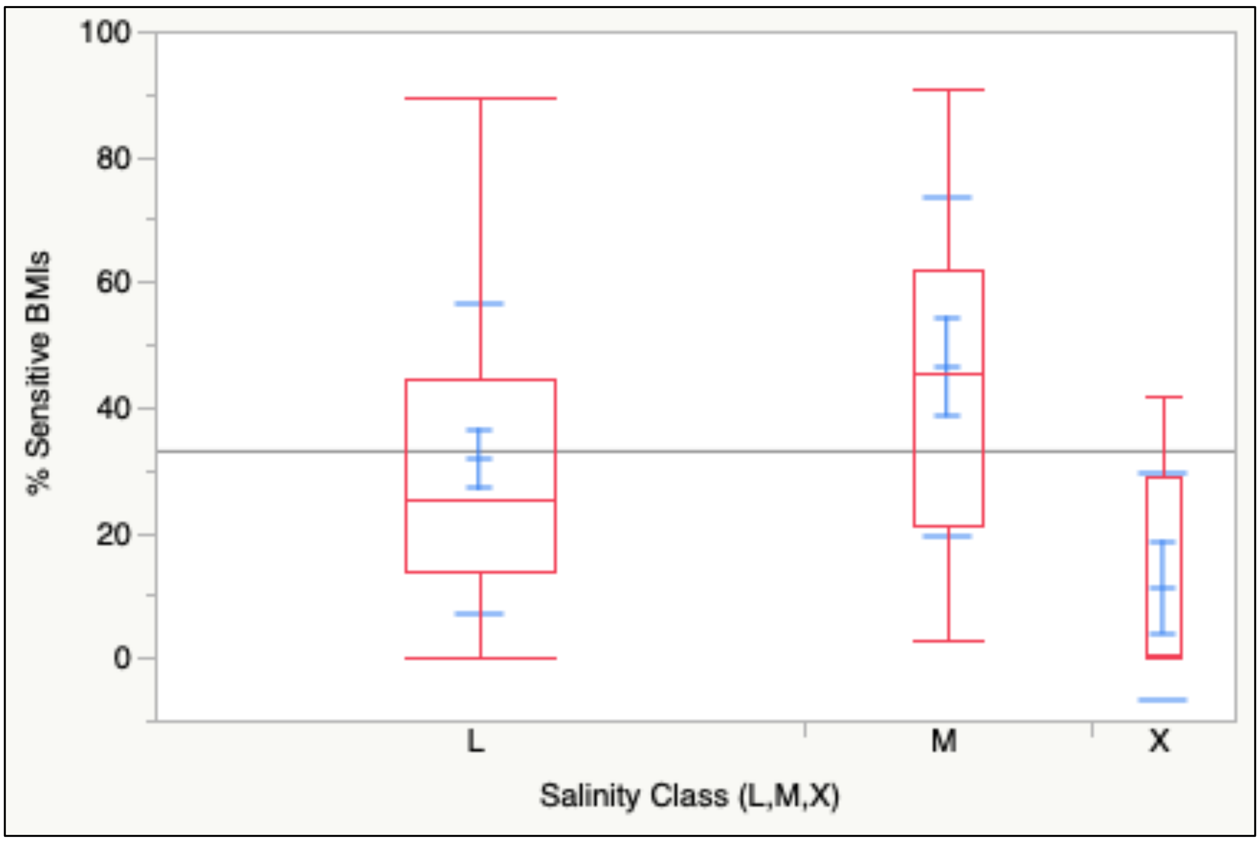


Figure 10: Oneway ANOVA of % Sensitive BMIs vs. Salinity Class, REF and MOD DIST Estuaries

Box plots are shown below of % sensitive BMIs by salinity class: Low (L), Moderate (M), and High (X). Only REF and MOD DIST estuaries (n=44) were included to minimize human disturbance confounds. Red boxes (i.e., box plots) represent 25th percentile (bottom), median (center line), and 75th percentile (top), with bottom and top bars representing 2.5th and 97.5th percentiles, respectively for each disturbance group. Blue bars represent means (centerline) standard error (inner bars), and standard deviation (outer bars). The ANOVA showed significant differences in mean % sensitive BMIs between salinity classes (r^2=0.16, p=0.02). The p value is for the ANOVA where % sensitive BMIs is the dependent variable and salinity class is the independent variable. The r^2 is the proportion of variation in % sensitive BMIs accounted for by salinity class.



There are patterns with respect to common, individual BMI taxa and salinity. Gastropoda, Acari, and Cladocera have been found only in low salinity (5 ppt or less). Baetidae, Dytisidae, Coenagrionidae, and Isopoda have been found in low to moderate salinity (up to 18 ppt). Chironimidae, Corixidae, Ostracoda, Oligochaeta, Copepoda, Isopoda, and Gammaridae have been found from low to high salinities. Polychaeta and Corophiidae have been found only in moderate to high salinities.

3. Scale of Biological Integrity

Table 8 below summarizes the accuracy of the Scale of Biological Integrity to date (n=92). Accurate classification of individual estuaries is based on the following criteria:

* REF sites scored as Good or Fair

* MOD DIST sites scored in the bottom half of Good range, Fair, or top half of Poor range

* HIGH DIST sites scored as Poor or Fair

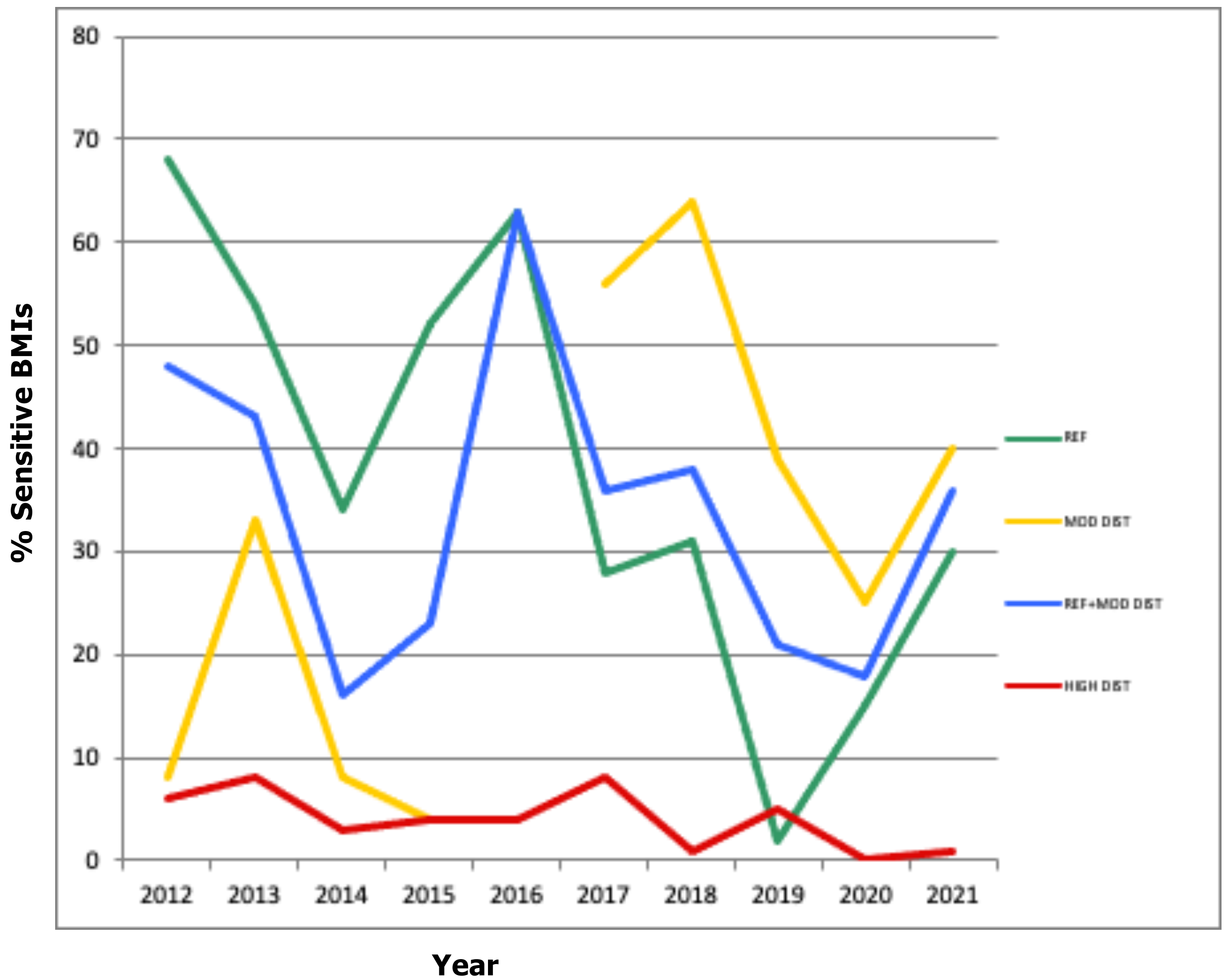
Table 7: Estuarine Scale of Biological Integrity, Classification Accuracy

Disturbance Group	Number of Sites	Good	Fair	Poor	Classification Accuracy
REF	22	17	3	2	20/22, 91%
MOD DIST	23	12	5	6	16/23, 70%
HIGH DIST	48	3	9	36	45/48, 94%
OVERALL	93	32	17	44	81/93, 87%

As shown in Table 7, 91% of REF and 94% HIGH DIST sites were classified accurately based on the criteria used. 70% of MOD DIST sites were accurately classified. MOD DIST site values for % sens BMIs have been widely spread (0-89%). Overall accuracy was 87% for all sites.

Figure 11 provides a graph of mean % sensitive BMIs by year and disturbance group. Overall there was distinct separation in mean % sensitive BMIs between REF and HIGH DIST sites from 2012 through 2018, with MOD DIST sites having mostly intermediate values skewed towards the lower end. HIGH DIST sites have remained low in mean % sensitive BMIs to present. Mean % sensitive BMIs at REF sites plummeted to 2 in 2019. Mean % sensitive BMIs at REF sites rebounded a bit to 15 in 2020, and 30 this past year. Meanwhile MOD DIST sites have averaged nearly 40% sensitive BMIs since 2017. When combined, REF and MOD DIST sites have averaged between 16-63% sensitive BMIs over the years, compared to 0-8 % sensitive BMIs for HIGH DIST sites.

Figure 11: Mean % Sensitive BMIs by Year in Estuaries by Disturbance Group



V. Closing

Over the past 22 years, the Program has provided a wealth of information regarding the physiochemical habitat conditions and biota, and in particular the BMI communities, present in local streams. The influences of natural physiochemical and climatic variability and human development on local stream communities have been extensively studied. The following statements can be made based on the research completed thus far:

- Negative impacts of human development on local stream communities (particularly BMIs) have been documented with highly significant statistical test results. Degradation of stream communities (e.g., lower IBI scores and loss of sensitive species) and physiochemical habitat conditions has increased linearly with increased watershed development. Urban development has been shown to have greater impacts on stream communities than has agricultural development.
- The IBI is highly effective as an indicator of biological integrity, as it has highly significant relationships with indicies of human disturbance. The IBI differentiates between REF, MOD DIST, and HIGH DIST groups with a high degree of accuracy and consistency.
- Major episodic disturbances including extreme stream flows, prolonged drought, and wildfires have been definitively shown, through rigorous statistical analyses, to negatively impact stream communities at REF and MOD DIST sites, as evidenced by lower IBI scores and loss or significant reduction of sensitive BMI taxa following such events. In past years, local stream BMI communities were resilient following floods and fires, typically showing dramatic recovery within 2 or 3 years. A string of events in recent years, starting with a prolonged drought (2013 to 2017), followed by the catastrophic Thomas Fire and extreme stream flows during a very wet winter (2018-2019), caused a prolonged trend of depressed overall IBI scores at REF and MOD DIST sites for several years. Rainfall and stream flows have been more consistent over the past few years, and IBI scores at non-fire affected REF and MOD DIST sites have generally improved. Continued monitoring will be needed to determine future trends in the BMI community of local streams.
- Due to a combination of wildfires, floods, and drought over an approximately 10 year period from 2008-2018, Rainbow trout appeared to be greatly reduced or eliminated in many study area streams including Mission Creek, Montecito Creek, Carpinteria Creek, and Rincon Creek and their tributaries. In today's world, it is inherently more difficult for southern steelhead trout to re-populate local streams impacted by these types of events. This is due in large part to their small numbers (i.e., Federally endangered), and also the presence of fish passage barriers and/or degraded habitat conditions in the lower reaches of all local streams. This year, trout were observed at M4 and MONT3 for the first time in several years. Their reappearance in these streams provides a ray of hope for the species locally, and perhaps suggests that numerous efforts to mitigate fish passage barriers and improve degraded stream habitat in the lower reaches of these streams are working to some degree. Another bright spot for the species locally is Arroyo Hondo (study reaches AH0 and AH1), where juvenile and adult steelhead/rainbow trout have been observed consistently over the past 20 years.
- Stream habitat restoration sites M2 and AB5 have shown improved habitat conditions, but significant improvements in the BMI community have not been observed 10 to 15 years post-restoration. Channel and riparian restoration at these sites did not address larger scale impairments in hydrology, geomorphology, water quality, and habitat continuity and

connectivity that have resulted from drastic human alteration of the respective watersheds. Although much of this watershed-scale impairment cannot be undone from a practical sense, there are opportunities to restore hydrology and water quality on a larger scale. Whether or not current and future restoration efforts at these and other stream habitat restoration sites (e.g., AB9, AB1, and AB2a) will improve the BMI community in local streams can only be evaluated via continued monitoring.

Based on the 10 years of data available for estuaries, the following can be stated:

- Determining the impacts of human land use and natural physiochemical variability to the BMI communities in local estuaries has proven to be more difficult compared with streams. One reason is there are fewer estuaries in the study area compared with streams, particularly in the REF category. Also, the wide salinity fluctuations that occur through time makes estuaries harsher environments where a relatively small number of BMI taxa can survive when compared with streams. However, we have identified several taxa that are more abundant in the REF estuaries, which are the basis of the metric % sensitive BMIs. This metric appears so far to be a fairly reliable indicator of estuary condition, and was used to produce the Scale of Biological Integrity for local estuaries. The Scale of Biological Integrity has been 87% accurate in classifying study estuaries based on their *a priori* disturbance category, including 91% for REF and 94% HIGH DIST sites.
- Salinity has been shown to influence the composition of the BMI community (i.e., the specific taxa present) in local estuaries. Mean % sensitive BMIs, the basis for the estuarine Scale of Biological Integrity, also appears to be influenced by salinity. This metric was intermediate in the Low salinity class (32%), highest in the Moderate salinity class (46%), and lowest in the High salinity class (11%) with significant statistical test results. It may prove advantageous to develop separate scales of biological integrity based on salinity class. More replication of sites, particularly in the High salinity class, will be needed to evaluate this possibility.

VI. Acknowledgements

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