SANTA BARBARA STREAMS AND ESTUARIES BIOASSESSMENT PROGRAM

2025 REPORT

Prepared for:

City of Santa Barbara,
Sustainability and Resilience

County of Santa Barbara,
Project Clean Water

Prepared By:

ECOLOGY
Consultants INC

Executive Summary

Introduction

This report summarizes the results of the 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 principal investigator for the Program, which was established 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 collected at study streams and estuaries using standardized methodology based on rapid bioassessment protocols originally established by the United States Environmental Protection Agency. For the purposes of this study, BMIs are aquatic invertebrates (insects, crustaceans, mollusks, worms, etc.) of a half-millimeter in size or greater that inhabit the bottom substrata of streams and estuaries for at least part of their life cycles. BMI samples are analyzed in the laboratory to determine BMI abundance and community composition in study streams and estuaries. Study sites have included the range available along a disturbance gradient, from "reference" sites that are fairly pristine in form with little human disturbance in their watersheds to "highly disturbed" sites that have been substantially altered in form and drain highly developed watersheds.

The BMI based Streams Index of Biological Integrity (IBI) developed by Ecology uses data from BMI samples to calculate IBI scores and classifications of biological integrity (i.e., Excellent, Good, Fair, Poor, or Very Poor) for each study stream reach. Similarly, the Estuary IBI developed by Ecology in 2022 is used to calculate IBI scores and classifications of biological integrity for study estuaries. The streams IBI was updated this year using data the full set of streams data collected from 2000 to 2025. Additionally, a separate field-based streams IBI was developed by Ecology this year using BMI taxa observations made during the field surveys from 2018 to 2025. The field-based streams IBI does not require laboratory analyses of BMI samples, allowing some streams sites to be surveyed for lower cost. The field-based streams IBI is not intended to replace the laboratory-based version. The intention is for both to be used in a complimentary fashion to expand the Program scope (i.e., number of study sites) and improve cost-effectiveness.

Study Area

The study area encompasses watersheds of the Santa Ynez mountains in coastal Santa Barbara County from Rincon Creek 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 (e.g., Alamo Pintado Creek) and Orcutt Creek near Santa Maria have also been studied. More than 70 different stream study reaches have been surveyed on one or more occasions during the 25 years of the Program, while 14 different estuaries have been studied once or more times in 14 years of study.

Results

Over the past 25 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 physiochemical habitat conditions and stream communities (e.g., lower IBI scores and loss of sensitive species) has increased linearly with increased watershed development. Urban development has been shown to have greater impacts on stream communities than has agricultural development.
- Natural variability in physiochemical conditions (i.e., elevation, gradient, watershed area, and water chemistry) has been shown to have mild effects on BMI communities, albeit not nearly as strong as the negative effects of intensive human disturbances.
- Variations in rainfall and resulting stream flows have been shown to impact BMI communities, particularly at REF and MOD DIST streams. Periods of high rainfall/flood flows and prolonged drought, as well as large wildfires (e.g., Jesusita, Thomas, Alisal, etc.) have caused downward trends in mean IBI scores at affected REF and MOD DIST streams the following spring, and in some cases for multiple years. This was particularly the case in 2005 (high rainfall/flood flows), from 2013 to 2018 (prolonged drought and Thomas Fire), and 2023 (high rainfall/flood flows). Periods of normal rainfall and stream flows have generally been marked by higher mean IBI scores in the REF and MOD DIST groups, including the upswing that has occurred the past two years.
- The diversity offered by the streams data set has allowed us to revise and update the streams IBI, making it more reflective of the overall range of BMI community conditions present in local streams over the past 25 years. This has improved the streams IBI's reliability as a scale or index by which to judge the biological integrity of each study stream site surveyed, and those to be studied in the future. Continued bioassessment monitoring through time will allow us to document the dynamic BMI communities occurring in our local streams as they respond to both human disturbances and natural physiochemical variability, including changing climate.
- The streams IBI's class criteria (i.e., Very Poor, Poor, Fair, Good, and Excellent) should be thought of as benchmarks by which to evaluate BMI community health, based on comparison to what has been present at a large, diverse collection of streams over many years. Departure from Fair/Excellent for a REF site, or below Poor/Good for a MOD DIST site, should serve as a "red flag", and make us ask, "why?" Is it explained by a naturally intermittent flow regime (i.e., frequent drying) of that site? Are recent extreme flood flows, wildfire, or prolonged drought the cause? If natural perturbations are the cause of low IBI scores, then IBI scores should recover within a couple of years provided that rainfall/stream flows, sediment loads, riparian canopy cover, stream temperature, etc. stabilize. If recovery of the BMI community does not occur, it is time to investigate other possibilities. Is there another natural cause, such as naturally hard groundwater, or are "natural" conditions (e.g., climate) changing with time and thereby affecting the BMI community? Is there a human cause such as water quality degradation from illicit wastewater flows, loss of stream flow due to upstream diversions, or some other human stressor?

- The new field-based streams IBI had a highly significant relationship with human disturbance, with similar p and r^2 as the laboratory-based IBI. Although it is not based on as large a data set nor as long a time period (i.e., only since 2018) as the laboratory-based version, the field-based streams IBI is a very useful indicator of biological integrity in local streams. The field-based streams IBI does not require laboratory analyses of BMI samples, allowing streams sites to be assessed at lower cost. The field-based streams IBI is not intended to replace the laboratory-based version. The intention is for both to be used in a complimentary fashion to expand the Program scope (i.e., number of study sites) and improve cost-effectiveness. Moving forward, it would advantageous to use the field method only at some study streams (perhaps half) each year, with the field method completed and BMI samples collected for laboratory analyses at others. The rotation of study sites between field-only vs. field + laboratory is to be determined.
- Stream habitat restoration sites including M2, AB1, AB2a, AB5, and AB9 have shown reach-level improvements in stream habitat complexity (i.e., depth, velocity, structure, cover, root mats, leaf litter, woody debris), streambed composition, and riparian vegetation. Thus far the improvements in reach-level habitat conditions at these sites have not been coupled with consistent, measureable improvements in the BMI community (i.e., IBI scores). Channel, floodplain and riparian restoration efforts at these sites do not address larger scale impairments in hydrology, geomorphology, water quality, and habitat continuity and connectivity at a watershed scale. Although much of this 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 will improve the BMI community in local streams can only be evaluated via continued bioassessment monitoring.
- Due to the combination of wildfires, floods, and drought over an approximately 10 year period from 2008-2018, Rainbow trout were greatly reduced or eliminated in many study streams including Mission Creek, Montecito Creek, Carpinteria Creek, and Rincon Creek and their tributaries. It is 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 nearly all local streams. In 2021 and 2022 trout were observed in small numbers at M4 and MONT3. Their reappearance in these streams provided a ray of hope for the species locally, and may indicate that 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 has been Arroyo Hondo (study reaches AH0 and AH1), where juvenile and adult steelhead/rainbow trout were observed consistently for 20 years during our field surveys. Trout were not observed in Arroyo Hondo from 2022 to 2024 presumably due to streambed scouring and sedimentation following the Alisal Fire in 2021. Trout were observed by others in Arroyo Hondo this past spring.

Based on the 14 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, more variable physiochemical conditions (e.g., input sources, salinity, temperature, etc.) make estuaries harsher, more dynamic environments where a relatively smaller number of BMI taxa can survive when compared with streams.

- Despite the harsher nature of estuaries, the estuarine IBI has proven to be effective as an indicator of biological integrity. The estuarine IBI has highly significant relationships with indicies of human disturbance, and effectively differentiates between REF, MOD DIST, and HIGH DIST groups.
- ANOVA results show that the IBI distinguished between disturbance groups appropriately with highly significant results in all three salinity classes (i.e., Low, Moderate, and High). However, based on the available data, there appears to be a skew towards higher scores in Low salinity, and towards lower scores in High salinity. If this trend continues, separate IBI classification scales should be created for each salinity class.

TABLE OF CONTENTS

PLATES

FIGURES

TABLES

I. Introduction

This report summarizes the results of the 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 principal investigator for the Program, which was established 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 collected at study streams and estuaries using standardized methodology based on rapid bioassessment protocols originally established by the United States Environmental Protection Agency. For the purposes of this study, BMIs are aquatic invertebrates (insects, crustaceans, mollusks, worms, etc.) of a half-millimeter in size or greater that inhabit the bottom substrata of streams and estuaries for at least part of their life cycles. BMI samples are analyzed in the laboratory to determine BMI abundance and community composition in study streams and estuaries. Study sites have included the range available along a disturbance gradient, from "reference" sites that are fairly pristine in form with little human disturbance in their watersheds to "highly disturbed" sites that have been substantially altered in form and drain highly developed watersheds.

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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 definitively 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 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 help guide the management 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, high 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.

Human development 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, roads, and rural residential uses west of Goleta to Point Conception.

Southerly view of Rattlesnake Canyon from East Camino Cielo

Generally, the magnitude of habitat degradation in 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.

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 pristine in form with little urbanization in their watersheds to highly disturbed sites that have been substantially altered in form and drain highly urbanized watersheds.

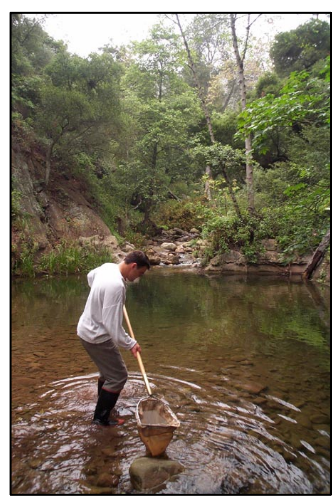

Jalama Creek estuary (low disturbance)

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, more dynamic environments where a relatively small number of BMI taxa can withstand the natural physiochemical conditions and survive when compared with streams.


Mission Creek estuary (high disturbance)

What is an 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 a waterbody. The term multimetric refers to an IBI being constructed by combining multiple 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.).


BMI sampling in Gobernador Creek

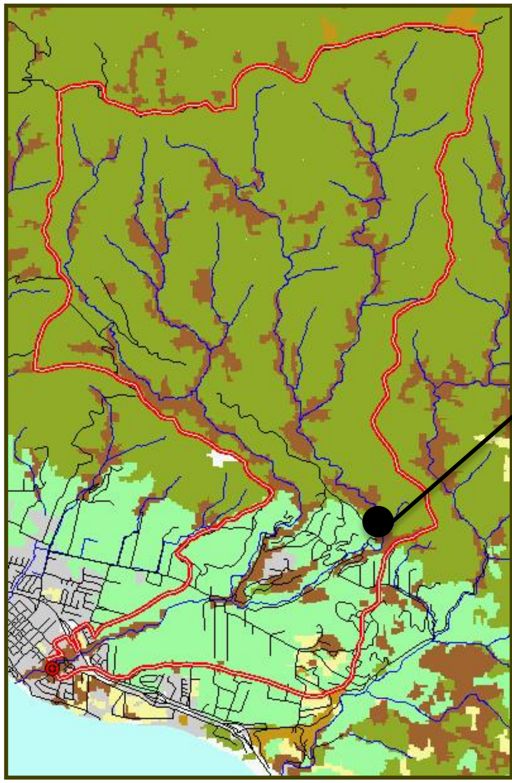
The laboratory-based streams IBI incorporates four “core metrics” calculated using BMI data from local stream reaches, while the new field-based streams IBI and estuaries IBI each incorporate two core metrics. Core metrics are highly sensitive to human disturbance as determined through rigorous statistical 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 site 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 IBIs classify the biological integrity of a given stream or estuary as Very Poor, Poor, Fair, Good, or Excellent based on the based on the overall score. By condensing complex biological data into an easily understood score and classification of biological integrity, the IBIs serve as effective tools for monitoring the condition of local streams and estuaries as they respond to natural and human perturbations, devising and prioritizing watershed management actions, and evaluating their benefits or consequences.

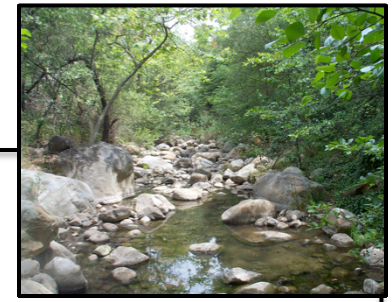
Why use BMIs?

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 can 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 cases of local streams and estuaries, BMIs also are far more abundant and diverse compared to aquatic vertebrates (e.g., fish and amphibians), and are relatively easy to observe and collect.

Stonefly (top) beetle (top right) and dragonfly (bottom), Birabent Creek

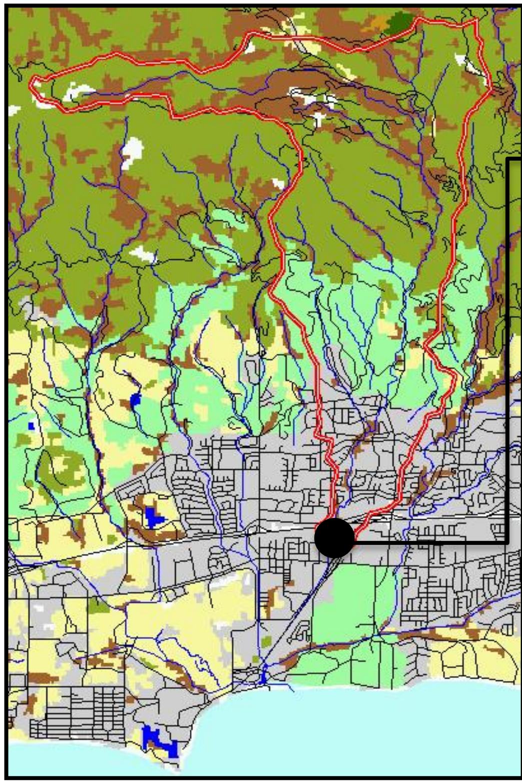
Plate 1: Reference Stream Reach Example





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 watersheds of the Santa Ynez mountains in coastal Santa Barbara County from Rincon Creek 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 (e.g., Alamo Pintado Creek) and Orcutt Creek near Santa Maria have also been studied. More than 70 different stream study reaches have been surveyed on one or more occasions during the 25 years of the Program, while 14 different estuaries have been studied once or more times in 14 years of study.

Figure 1 shows an overall map of the study area, and Figures 2 and 3 provide detailed maps for western and eastern portions of the study area, showing locations of all stream and estuary study reaches that have been surveyed over the years. The Orcutt Creek study reach is north of the other study sites, its approximate location shown in Figure 1. This year, 21 stream reaches and 7 estuaries were surveyed (see Table 1).

Table 1: 2025 Study Reaches

Stream Study Reaches	Location
AB1	Arroyo Burro at upstream end of Alan Rd.
AB2a	Arroyo Burro just upstream of Torino Rd.
AB3	San Roque Creek approx. 400m upstream of Foothill Rd.
AB5	Mesa Creek just upstream of entrance to Arroyo Burro estuary
AB10	San Roque Creek approx. 1.5 km upstream of Foothill Rd.
AH1	Arroyo Hondo, approx. 1.5 km upstream of U.S. 101.
AL1	Alamo Pintado Creek 400m downstream of Highway 246
AL2	Birabent Creek just upstream of Figueroa Mountain Rd. crossing
C3	Gobernador Creek, approx. 400m upstream of County detention basin
GAV1	Gaviota Creek at State Beach/Park just upstream of access road crossing
LH1	Lighthouse Creek just upstream of footbridge at Shoreline Park
M1	Mission Creek just downstream of De La Guerra St.
M2	Old Mission Creek at Bohnet Park
M3	Mission Creek at upstream end of Rocky Nook Park
M4	Rattlesnake Creek, approx. 1 km upstream of Las Canovas Rd. crossing
M6	Upper Mission Creek upstream of Tunnel Rd. trail
MONT3	Cold Springs Creek just upstream of Mountain Road.
OR1	Orcutt Creek just upstream of Blosser Ave.
SY2	Sycamore Creek 300m below Hwy. 192 crossing and Coyote/Sycamore confluence
SY4	East Fork Sycamore Creek at Mountain Rd.
SY7	West Fork Coyote Creek just above confluence with East Fork

Table 1: 2025 Study Reaches Continued	
Estuary Study Reaches	**Location**
ACe	Andria Clark Bird Refuge estuary
SYe	Sycamore Creek estuary
Me	Mission Creek estuary
ABe	Arroyo Burro estuary
Te	Tecolote Creek estuary
GAVe	Gaviota Creek estuary
Je	Jalama Creek estuary

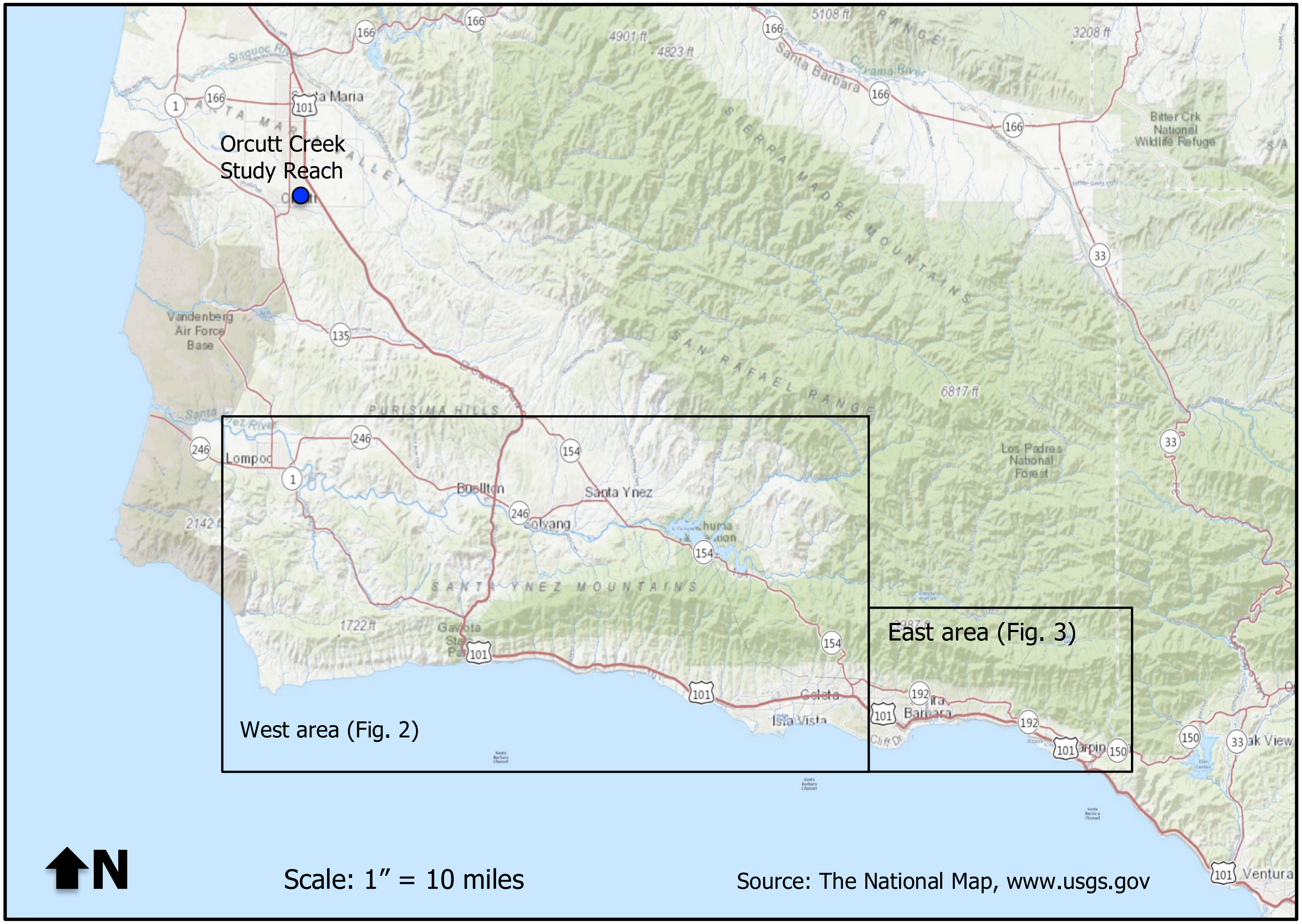


Figure 1: Study Area Map

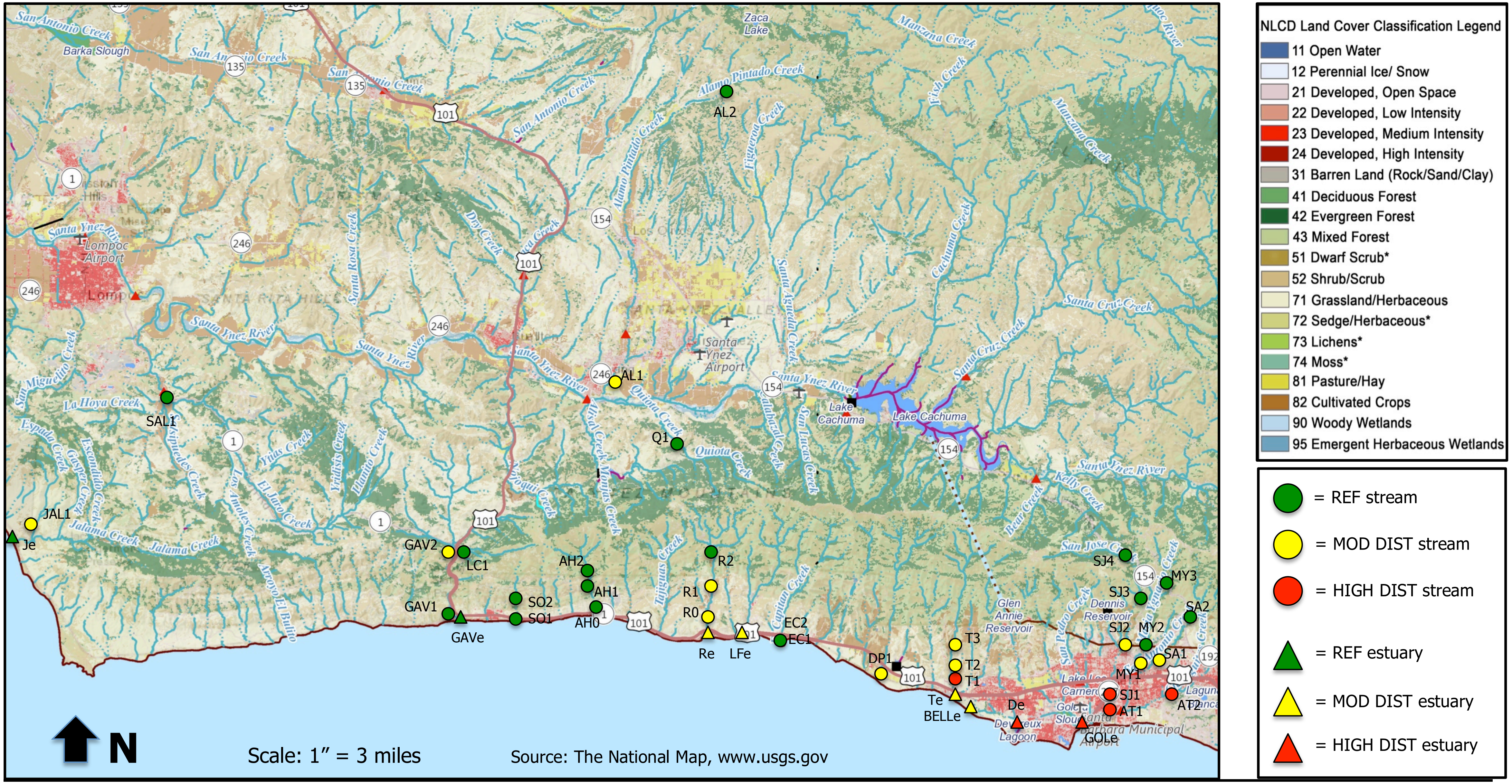


Figure 2: West Area Study Sites

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 using rapid bioassessment methodology. Our sampling methodology has been largely consistent since 2000. As in previous years, field surveys were conducted in the spring (late April or May) during base stream flow conditions. 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 identified and estimated as a percentage of the total reach length.
- Stream wetted width 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 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, placed in a clear plastic tub with water, and visually examined using a 2X magnification hand lens for 5 minutes. All types of BMIs identified were noted to the lowest discernable taxonomic level, as was an abundance factor

(i.e., 1= one individual, 2= 2 or 3, 3= more than 3) for each type of BMI observed. Following the visual assessment, the sample material 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 conducted 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 early fall (late September to 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 typically made at the downstream end of the estuary.
- BMI samples were collected at each monitoring station, one at the downstream end of the estuary, and in some cases another near the upstream end. Two separate samples were collected at each monitoring station; (1) an infaunal sample consisting of approximately the top 15 cm of sediments from two estuary bottom sites collected using a 10 cm diameter 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.
- 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 conducted 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., worms, crustaceans, mollusks, bivalves, 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. BMI identifications were completed 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 cover data 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

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 developed open space.

MOD DIST = Reaches that are moderately disturbed by human activities. Habitat assessment score is 50/100 or greater, and between 5 and 20 percent of the upstream watershed is developed through a combination of urban, agricultural and/or developed open space.

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 developed open space.

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 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 in some analyses.

F. Data Analyses for Streams

1. Calculation of BMI Tolerance Values for Study Area Streams

Tolerance values for all individual BMI taxa found in study area streams were assigned on a scale of 0 to 10 based on their perceived disturbance tolerance. A tolerance value of 0 indicates that a particular BMI is extremely intolerant of disturbance, with increasing scores indicating greater disturbance tolerance. To assess disturbance tolerance, all individual BMI taxa were evaluated for differences in their distributions between REF, MOD DIST, M/R F/P, and HIGH DIST study reach groups using one-way analysis of variance (ANOVA). An ANOVA 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 or less is generally accepted as indicating a statistically significant difference between group means.

Specific criteria for assigning tolerance values are provided in Figure 4. Basically, BMI taxa with low tolerance values (i.e., 0 to 4) have significantly greater abundances at REF sites, BMI taxa with moderate tolerance values (i.e., 5) have relatively even abundances across disturbance groups or significantly greater abundances at MOD DIST sites, while BMI taxa with high tolerance values (i.e., 6 to 10) have significantly greater abundances at HIGH DIST sites.

2. Calculation of Physiochemical and BMI Metrics

Numerous physiochemical parameters and BMI metrics were calculated for each stream study reach using the data collected. Table 2 lists each parameter calculated for the study reaches and the method of calculation (e.g., lab, field, etc.). BMI metrics calculated for each study reach reflect different aspects of community structure, including overall BMI density, richness, composition (i.e., taxa present), the relative and absolute abundances of component taxa or groups, trophic group representation, and sensitivity to human disturbance.

BMI density (number of individuals per 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. Functional feeding group parameters (e.g., % collector-gatherers, % scrapers, etc.) were determined using functional feeding group designations for individual taxa provided in Merritt and Cummins (2008).

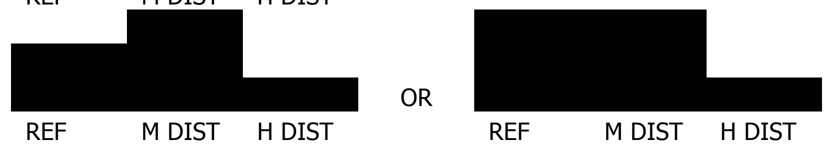
Figure 4: Tolerance Values Criteria

Sensitive (0-4):

0, 1: abundance significantly ($p<0.05$) highest in REF. MOD DIST and HIGH DIST not significantly different from one another. 0 for greater differences in mean values and lower p (generally <0.0001) between REF and MOD/HIGH DIST, 1 for less different means.

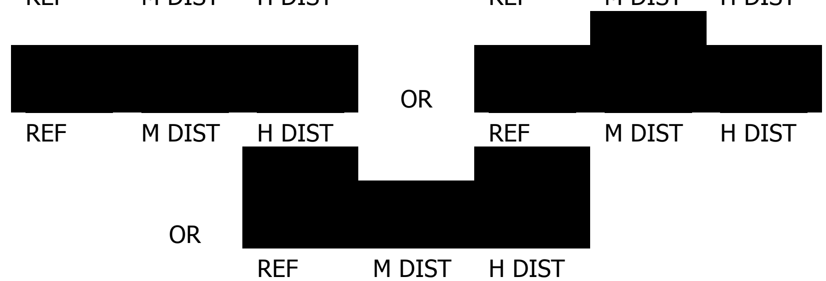
2,3: significant decrease in mean abundance from REF to MOD DIST to HIGH DIST, or from REF and MOD DIST to HIGH DIST. 2 for greater differences in mean values and p, 3 for lesser differences.

4: Mean abundance in REF significantly higher than in HIGH DIST, mean abundance in REF and MOD DIST not significantly different from each other, or significantly higher in MOD DIST.



Moderate

5: no significant difference in mean abundance between the three groups. Or mean abundance in MOD DIST sign. higher or lower, and REF and HIGH DIST means not sign. different from each other.



Tolerant (6-10):

6: Mean abundance in REF sign. lower than in HIGH DIST, mean abundance in REF and MOD DIST not significantly different from each other, or significantly higher in MOD DIST.

7, 8: significant decrease in mean abundance from HIGH DIST to MOD DIST to REF. 8 for greater differences in mean values and p, 7 for lesser differences.

9, 10: mean abundance significantly highest in HIGH DIST, REF and MOD DIST not significantly different from each other. 10 for greater differences in mean values and p, 9 for lesser differences.

TV AVG, or composite tolerance value average, was calculated by adding the tolerance values for each individual BMI in the sample, and dividing by the total number of individuals. % sens BMIs was calculated by adding the number of BMIs in the sample with a tolerance value of 4 or less, dividing by the total number of individuals in the sample, and multiplying by 100. % Vsens BMIs was calculated by adding the number of BMIs in the sample with a tolerance value of 3 or less, dividing by the total number of individuals in the sample, and multiplying by 100. % tol BMIs was calculated by adding the number of BMIs in the sample with a tolerance value of 6 or greater, dividing by the total number of individuals in the sample, and multiplying by 100.

Table 2: Physiochemical and BMI Metrics Calculated for Stream Study Reaches

Parameters	Abbreviation	Units	Method of Calculation
PHYSICAL PARAMETERS			
Elevation	None	Feet (ft.)	USGS Quad Maps
Stream gradient	None	None	USGS Quad Maps
Watershed area	None	Acres	GIS
% of watershed area disturbed	None	None	GIS
Wet stream width	None	Ft.	Field
Habitat assessment score	None	None	Field
% riparian canopy cover	None	None	Field
WATER CHEMISTRY PARAMETERS			
Stream temperature	None	°C	Field
pH	None	None	Field
Dissolved oxygen concentration	None	mg/l	Field
Specific conductance (corrected to 25° C)	None	µS/cm	Field
BMI PARAMETERS			
BMI density	None	#/m^2	Field/lab
# insect families	None	None	Field/lab
# Ephemeroptera/Plecoptera/Tricoptera families	# EPT Fams	None	Field/lab
% Ephemeroptera/Plecoptera/Tricoptera	% EPT	%	Field/lab
% EPT minus Baetidae	% EPT-Baetidae	%	Field/lab
% Plecoptera/Tricoptera	% PT	%	Field/lab
% Coleoptera	None	%	Field/lab
% Chironomidae	None	%	Field/lab
% Diptera	None	%	Field/lab
Composite tolerance value average	TV AVG	None	Field/lab
% sensitive BMIs	% Sens BMIs	%	Field/lab
% very sensitive BMIs	% Vsens BMIs	%	Field/lab
% tolerant BMIs	% Tol BMIs	%	Field/lab
% non-insect BMIs	None	%	Field/lab
% collector-gatherers	% cg	%	Field/lab
% scrapers	%sc	%	Field/lab
% shredders	%sh	%	Field/lab
% collector-filterers	%cf	%	Field/lab
% predators	%pred	%	Field/lab
% shredders + predators	%sh+pred	%	Field/lab
Composite tolerance value average (field)	TV AVG	None	Field
# EPT Taxa (field)	# EPT Taxa	None	Field
# sensitive BMI taxa (field)	# Sens BMI Taxa	None	Field
# insect taxa (field)	None	None	Field
# BMI taxa (field)	None	None	Field
# tolerant taxa (field)	# Tol Taxa	None	Field

3. Updating the Streams IBI

Updating the Streams IBI required the completion of several distinct steps, including: screening and selection of potential core metrics, defining scoring ranges for potential core metrics, defining IBI scoring categories and ranges, and evaluating the IBI's usefulness to assess the biological integrity of individual study reaches. These steps are discussed below.

a. Screening of BMI Metrics

Sensitivity to Human Disturbance

To evaluate their sensitivity to human disturbance, all of the BMI metrics calculated were evaluated for differences between the REF, MOD DIST, and HIGH DIST groups using ANOVA. The M/R I/F group reaches were included in the ANOVAs, but were not considered in the screening of metrics. BMI metrics that most significantly change (i.e., increase or decrease) with increasing levels of human disturbance (i.e., from the REF to MOD DIST to HIGH DIST groups) have the greatest potential to serve as measures of biological integrity and IBI core metrics.

Natural Relationships between Potential Core Metrics and Physiochemical Parameters

The BMI metrics most sensitive to human disturbance were screened for natural relationships with several physiochemical parameters, which is an important step in determining their suitability for inclusion in the IBI. In explanation, consider a situation where a BMI metric has a significant *negative* relationship with human disturbance, and also with a physiochemical variable, for example conductivity, in the absence of human disturbance (i.e., amongst REF study reaches). Since conductivity is *positively* influenced by increasing human disturbance (i.e., due to increased pollutants and mineral concentrations in urban runoff), it could be difficult to separate the negative effects of increasing human disturbance on the BMI metric versus those attributable to natural increases in conductivity. In such cases, comparing the strength of the relationship between the BMI metric versus human disturbance to that of the BMI metric versus the physiochemical parameter is key in determining whether the BMI metric can still be a reliable indicator of biological integrity.

To begin this part of the screening process, linear regression analyses were performed to evaluate relationships between two human disturbance indicies, % watershed disturbed (landscape level) and habitat assessment score (localized level), and several physiochemical parameters: elevation, gradient, watershed area, stream temperature, specific conductance, and dissolved oxygen concentration. A linear regression calculates a best-fit equation that best represents the relationship between a single independent variable (i.e., % watershed disturbed or habitat assessment score) and a single response variable (i.e., a physiochemical parameter). r^2, the correlation coefficient, is given as a value between 0 and 1, and indicates the proportion of the variation in the response variable accounted for by its relationship with the independent variable. The higher the r^2, the better the fit of the equation. P indicates the probability that the response variable and regressors are not related as predicted by the best fit equation, and is given as a value of between 0 and 1. A p of 0.05 or less is generally accepted as indicating a statistically significant relationship between the regressor and response variable.

For those physiochemical parameters having significant relationships with one or both of the human disturbance regressors, linear regressions were used to evaluate natural relationships between the physiochemical parameters and each of the potential core BMI metrics using data from only the REF study reaches. Using data solely from the REF study reaches provided an opportunity to evaluate these relationships as they occur naturally, or in the absence of human disturbance.

Potential core BMI metrics having no significant natural relationships with any of the physiochemical parameters passed this step of the screening process. Those potential core BMI metrics with significant natural relationships with one or more of the physiochemical parameters required further screening to determine if such natural relationships would confound the interpretation of the relationships between the potential core BMI metric and human disturbance. A confound would arise in one of two scenarios:

1. If the relationship between human disturbance and the physiochemical parameter is positive, and they both affect the BMI metric in the same way (i.e., both positively or both negatively) and with similar relationship strength (i.e., similar r^2 and p).
2. If the relationship between human disturbance and the physiochemical parameter is negative, and they affect the BMI metric in opposite ways (i.e., one positively and the other negatively), and with similar relationship strength (i.e., similar r^2 and p).

Any BMI metric having one or more such confounding relationship(s) was eliminated from consideration as a core metric in the IBI.

Correlations between Potential Core BMI Metrics

Remaining potential core BMI metrics were screened for inter-correlation. Where correlation coefficients were 0.75 or greater, only one BMI metric was retained as a core metric. In such a scenario, the BMI metric having the strongest relationship with human disturbance (per the ANOVA results) was retained as a core metric.

b. Selection of Core Metrics for Inclusion in the IBI

The BMI metrics that remained using the above screening processes were considered as potential core metrics for inclusion in the IBI. Selected core metrics have:

1. The most highly significant relationships with human disturbance, either increasing or decreasing between REF to MOD DIST to HIGH DIST groups.
2. No confounding significant natural relationships with physiochemical parameters that impair our ability to interpret the effects of human disturbance on the BMI metric.
3. Correlation coefficients of less than 0.75 with all other core metrics.

Collectively, the chosen core metrics should also represent different aspects of BMI community structure including richness, trophic structure, and disturbance sensitivity.

c. Core Metric Scoring Ranges

Scoring ranges were established for each core metric on a dimensionless scale of 0 to 10, 0 indicating the lowest level of biological integrity, and 10 indicating the highest level of biological integrity. For metrics that decrease with human disturbance (i.e., highest at REF sites), higher values corresponded with higher scores. For metrics that increase with human disturbance (i.e., highest at HIGH DIST sites), higher values corresponded with lower scores. The distributions of each metric in the REF, MOD DIST, and HIGH DIST groups were used to establish the scoring ranges. Scoring criteria are provided in Table 3.

Table 3: Streams IBI Core Metric Scoring Range Criteria	
Score	**Scoring Criteria**
10	The 75th percentile or greater of the REF group distribution for metrics that are highest in the REF group, or the 25th percentile or lower of the REF group for metrics that are lowest in the REF group
9	The median (50th percentile) to 75th percentile of the REF group for metrics that are highest in the REF group, or the 25th percentile to the median of REF group for metrics that are lowest in the REF group
8	The range between the REF group and MOD DIST group medians is divided and evenly partitioned to provide each scoring range for 6, 7, and 8
7	
6	
5	MOD DIST median is the top of the scoring range for 5
4	The range between the MOD DIST group and HIGH DIST group medians is divided and evenly partitioned to provide each scoring range for 5, 4, 3, and 2
3	
2	
1	The median to 25th percentile of the HIGH DIST group for metrics that are lowest in the HIGH DIST group, or the median to the 75th percentile of HIGH DIST group for metrics that are highest in the HIGH DIST group
0	The 25th percentile or less of the HIGH DIST group distribution for metrics that are lowest in the HIGH DIST group, or the 75th percentile or higher of the HIGH DIST group for metrics that are highest in the HIGH DIST group

d. Establishment of IBI Classifications of Biological Integrity

IBI scores for each study reach in the Test Group were calculated by totaling the scores for individual core metrics as determined using Table 3. Based on the distribution of IBI scores for REF and HIGH DIST groups, five categories of biological integrity were established: Excellent, Good, Fair, Poor, and Very Poor. Scoring criteria used to establish the categories is provided in Table 4.

Table 4: Streams IBI Classifications of Biological Integrity and Scoring Criteria	
Classification of Biological Integrity	**Scoring Range**
Excellent	Median of REF group or higher
Good	REF group 25th percentile to just below REF group median
Fair	From just above 75th percentile of HIGH DIST group to just below 25th percentile of REF group
Poor	From just above HIGH DIST group median to 75th percentile of HIGH DIST group
Very Poor	Median of HIGH DIST group or lower

4. Analyses of Streams IBI Scores using ANOVA

Oneway Analysis of Variance (ANOVA) statistical tests were used to evaluate mean values for IBI scores between the disturbance groups (i.e., REF, MOD DIST, M/R F/P, and HIGH DIST), both overall (i.e., all years of study combined), and for individual years. 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.

These ANOVAs were completed to examine:

(1) Overall relationships of BMI community health (i.e., IBI scores) vs. anthropogenic disturbance indices and natural physiochemical variables, and;

(2) Long-term trends in IBI scores through time in response to varying physiochemical conditions from natural and anthropogenic (i.e., human) causes.

Overall, there have been only minor changes in land use types, intensities, and footprints throughout the study area during the period of study (i.e., 2000 to present). However, it is widely accepted in the scientific community that anthropogenic increases in carbon-based air emissions are contributing to accelerated "global warming" or "climate change". While the effects of climate change are complex and uncertain, it is widely predicted that local effects may include more variable and overall warmer, drier weather (i.e., drought), less overall and more variable precipitation and stream flows, higher stream temperatures, and increased wildfire potential. Drought and wildfire related impacts have been shown to have strong downward effects on BMI community integrity (i.e., IBI scores), and populations of steelhead/rainbow trout and other sensitive aquatic vertebrates in local streams. These ANOVAs can used to explore long-term trends and help evaluate whether changing climate or other forces may cause significant changes in stream community health. Fire impacted and drought impacted (i.e., pools only) stream study reaches (i.e., M/R F/P sites) were left in their respective groups (i.e., MOD DIST or REF) for these analyses, as temperature, precipitation, stream flow and wildfire potential are greatly influenced by climate.

5. Analyses of BMI Field Metrics and Development of a Field-Based Streams IBI

Since 2018 we have collected field-based BMI data at streams study reaches (see Methods). Several metrics have been calculated based on field BMI observations, including # of BMI taxa, # of insect taxa, # of EPT taxa, # of sensitive taxa, # of tolerant taxa, and Tolerance Value Average (see Table 2). Richness metrics were calculated by summing the number of designated taxa observed. In many cases it is difficult to reliably distinguish between certain taxa in the field, so some taxa were combined into coarser taxonomic groupings compared to laboratory identifications. For example, Odonata were simplified into two groups, Anisoptera (dragon flies) and Zygoptera (damselflies). Other combined taxa included families of mayflies (e.g., Caenidae/ Leptohyphidae), caddisflies (e.g., Philoptomatidae/ Rhyacophilidae/ Psychomiidae), all families of Plecoptera (stoneflies), and the crustacean orders Ostracoda/Cladocera. Tolerance Value Average was calculated by multiplying the abundance factor (1=1 individual, 2=2 or 3, or 3=more than 3) and tolerance value (0-10) for each taxa observed, summing these values, and dividing by the sum of abundance factors.

The field BMI metrics were evaluated and a separate field-based Streams IBI was developed using the same process described above to update the laboratory-based Streams IBI.

G. Data Analyses for Estuaries

1. Calculation of IBI Scores for Estuaries

Core Metrics Calculations and Scoring Ranges

The estuarine Index of Biological Integrity (IBI) was developed using data from all estuaries surveyed from 2012 to 2022 (n=101), and uses two core metrics: % sensitive BMIs and # sensitive BMIs. The core metrics are sensitive to human disturbance and each represent different aspects of BMI community structure: richness and disturbance sensitivity. Core metrics are scored on a dimensionless scale of 0 to 10, 0 indicating the lowest level of biological integrity, and 10 indicating the highest level of biological integrity. Scoring criteria and ranges for the core metrics are provided in Table 5. See the 2022 Report for more information on how core metrics were selected and how scoring ranges were established.

Table 5
Estuary Core Metric Scoring Ranges

Score	% sens BMIs	# sens taxa
10	59+	4+
9	46-58	3
8	39-45	
7	33-38	
6	27-32	
5	21-26	2
4	16-20	
3	11-15	
2	6-10	
1	1-5	1
0	0	0

IBI Scores and Classifications of Biological Integrity

IBI scores for each study estuary site were calculated by totaling the scores for the two core metrics. Based on the distribution of IBI scores for REF and HIGH DIST groups, five categories of biological integrity were established: Excellent, Good, Fair, Poor, and Very Poor. Scoring criteria used to establish the categories are provided in Table 6. See the 2022 Report for discussion of how ranges were determined for the five classifications of biological integrity.

Table 6 Estuary Classifications of Biological Integrity	
Category	**Scoring Range**
Excellent	17 to 20
Good	14 to 16
Fair	8 to 13
Poor	3 to 7
Very Poor	0 to 2

2. Trends Analyses of IBI Scores using ANOVA

ANOVAs were used to evaluate mean values and distributions for IBI score between the disturbance groups (REF, MOD DIST, and HIGH DIST) using data from all years (2012-2025, n=121). These ANOVAs were completed to examine:

(1) Overall relationships between BMI community health (i.e., IBI scores) and human disturbance, and;

(2) Evaluate the overall reliability of the estuarine IBI as an indicator of BMI community integrity.

3. Salinity Effects on Estuarine IBI Scores

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.

Given these patterns, it is important to understand whether salinity shapes IBI scores and affects the IBI's ability to distinguish between estuaries of varying disturbance levels. To explore this, ANOVA was used to compare IBI scores by disturbance group (REF, MOD DIST, HIGH DIST) within three salinity classes: Low (<5 ppt), Moderate (5-18 ppt), and High (>18 ppt). This was done to evaluate how well the IBI distinguishes between disturbance groups in each the salinity class, and determine what if any limitations exist in utilizing the IBI in varying salinities.

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, and the number and percentage of native vs. introduced plant species observed. Table A-3 provides a list of vertebrate species observed at all study stream sites. 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, 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.

Report Cards for selected stream study reaches and estuaries (those surveyed five or more years) that were surveyed this year, and site photographs for this year's study reaches are provided in the Appendix. Report Cards provide a summary of IBI scores, BMI metrics, physiochemical variables, and vertebrate and plant species data for this year, and ranges of these parameters for all years of study.

B. Data Analyses for Streams

1. BMI Tolerance Values

Tolerance values (TVs) and sensitivity designations for individual BMI taxa were determined using ANOVAs as described in Methods. Table A-7 in the Appendix summarizes the results of the ANOVAs, and provides TVs for the individual taxa. Table 7 below also provides new TVs and sensitivity designations for streams BMI taxa, and old TVs (i.e., from 2019) for comparison. Overall, there are 37 sensitive, 25 moderate, and 13 tolerant taxa, respectively.

Table 7
Tolerance Values for Streams BMI Taxa

Sensitivity Designation	Taxa	Order/Class	New TV (2025)	Old TV (2019)
Sensitive	Heptagenidae	Ephemeroptera	0	1
Sensitive	Chloroperlidae	Plecoptera	0	0
Sensitive	Perlidae	Plecoptera	0	0
Sensitive	Perlodidae	Plecoptera	0	0
Sensitive	Helicopsychidae	Trichoptera	0	0
Sensitive	Lepidostomatide	Trichoptera	0	1
Sensitive	Gomphidae	Odonata	0	0
Sensitive	Ephemerellidae	Ephemeroptera	1	0
Sensitive	Limnophilidae	Trichoptera	1	0

Table 7 Tolerance Values for Streams BMI Taxa				
Sensitive	Dixidae	Diptera	1	1
Sensitive	Tipulidae	Diptera	1	0
Sensitive	Aeshnidae	Odonata	1	3
Sensitive	Cordulegastridae	Odonata	1	3
Sensitive	Corydalidae	Megaloptera	1	3
Sensitive	Caenidae	Ephemeroptera	2	1
Sensitive	Leptophlebiidae	Ephemeroptera	2	1
Sensitive	Nemouridae	Plecoptera	2	1
Sensitive	Rhyacophilidae	Trichoptera	2	3
Sensitive	Elmidae	Coleoptera	2	3
Sensitive	Psphenidae	Coleoptera	3	3
Sensitive	Sialidae	Megaloptera	3	3
Sensitive	Leptohyphidae	Ephemeroptera	4	3
Sensitive	Brachycentridae	Trichoptera	4	3
Sensitive	Glossostomatidae	Trichoptera	4	3
Sensitive	Hydropsychidae	Trichoptera	4	3
Sensitive	Polycentropodidae	Trichoptera	4	3
Sensitive	Psychomyiidae	Trichoptera	4	3
Sensitive	Sericostomatidae	Trichoptera	4	3
Sensitive	Dryopidae	Coleoptera	4	5
Sensitive	Dytisidae	Coleoptera	4	3
Sensitive	Blephariceridae	Diptera	4	5
Sensitive	Stratiomyidae	Diptera	4	3
Sensitive	Belostomatidae	Hemiptera	4	3
Sensitive	Gerridae	Hemiptera	4	5
Sensitive	Veliidae	Hemiptera	4	3
Sensitive	Libellulidae	Odonata	4	3
Sensitive	Acari	Acari	4	3
Moderate	Baetidae	Ephemeroptera	5	5
Moderate	Hydroptilidae	Trichoptera	5	5
Moderate	Leptoceridae	Trichoptera	5	5
Moderate	Philoptomatidae	Trichoptera	5	5
Moderate	Gyrinidae	Coleoptera	5	5
Moderate	Hydrophilidae	Coleoptera	5	10
Moderate	Ceratopogonidae	Diptera	5	5

Table 7 Tolerance Values for Streams BMI Taxa				
Moderate	Empididae	Diptera	5	5
Moderate	Muscidae	Diptera	5	5
Moderate	Psychodidae	Diptera	5	5
Moderate	Simulidae	Diptera	5	5
Moderate	Corixidae	Hemiptera	5	5
Moderate	Naucoridae	Hemiptera	5	5
Moderate	Notonectidae	Hemiptera	5	5
Moderate	Calypterygidae	Odonata	5	5
Moderate	Coenagrionidae	Odonata	5	5
Moderate	Lestidae	Odonata	5	5
Moderate	Collembola	Collembola	5	5
Moderate	Lepidoptera	Lepidoptera	5	5
Moderate	Hymenoptera	Hymenoptera	5	5
Moderate	Gastropoda	Gastropoda	5	5
Moderate	Nematomorpha	Nematomorpha	5	5
Moderate	Nemotoda	Nemotoda	5	5
Moderate	Maxillopoda	Maxillopoda	5	NA
Moderate	Bivalvia (clam)	Bivalvia	5	4
Tolerant	Halipidae	Coleoptera	6	6
Tolerant	Ephydridae	Diptera	6	7
Tolerant	Ostracoda	Ostracoda	6	5
Tolerant	Oligochaeta	Oligochaeta	6	7
Tolerant	Culicidae	Diptera	9	5
Tolerant	Decapoda	Decapoda	9	10
Tolerant	Amphipoda	Amphipoda	9	5
Tolerant	Chironomidae	Diptera	10	9
Tolerant	Copepoda	Copepoda	10	7
Tolerant	Cladocera	Cladocera	10	9
Tolerant	Isopoda	Isopoda	10	10
Tolerant	Hirudinea	Hirudinea	10	10
Tolerant	Turbellaria	Turbellaria	10	8

Sensitive taxa (TVs 0 to 4) included most of the EPT taxa (i.e., mayflies, stoneflies, and caddisflies), a few Coleoptera (Dryopidae, Dytisidae, Elmidae, and Psphenidae) and Diptera (Blephariceridae, Dixidae, Tipulidae and Stratiomyidae), Hemiptera (Belostomatidae, Gerridae and Veliidae), and Odonata (Aeshidae, Cordulegastridae, Gomphidae, and Libellulidae), both Megaloptera (Corydaliidae and Sialidae) and Acari (water mites).

A few EPT taxa including Baetidae (mayfly), Hydroptilidae, Leptoceridae, and Philoptomatidae (caddisflies) had moderate disturbance tolerance (TV 5), either showing no differences in mean abundance between disturbance groups, or significantly highest abundance in the MOD DIST group. Other moderately tolerant taxa include Coleoptera (Gyrinidae and Hydrophiliade), Diptera (Ceratopogonidae, Empididae, Muscidae, Psychodidae, and Simulidae), Hemiptera (Corixidae, Naucoridae, and Notonectidae), Odonata (Calypterygidae, Coenagrionidae and Lestidae), rare insect orders Lepidoptera, Hymenoptera, and Collembolla, and several non-insect taxa including Gastropoda, Nematomorpha, Nematoda, Maxillipoda and Bivalvia).

Tolerant taxa (TVs 6 to 10) included one Coleoptera (Halipidae), three Diptera (Chironomidae, Culicidae and Ephydridae), and several non-insect taxa including Ostracoda, Oligochaeta, Amphipoda, Copepoda, Cladocera, Decapoda, Isopoda, Hirudinea, and Turbellaria.

TVs for most BMI taxa were similar (i.e., unchanged or within the same sensitivity designation) compared with the 2019 results. Taxa with notable changes in TV were as follows:

Taxa	**NEW TV**	**OLD TV (2019)**
Dryopidae	4 (sensitive)	5 (moderate)
Blephariceridae	4 (sensitive)	5 (moderate)
Gerridae	4 (sensitive)	5 (moderate)
Hydrophillidae	5 (moderate)	10 (tolerant)
Culicidae	9 (tolerant)	5 (moderate)
Amphipoda	9 (tolerant)	5 (moderate)

2. Updating the Streams IBI

a. Screening of BMI Metrics

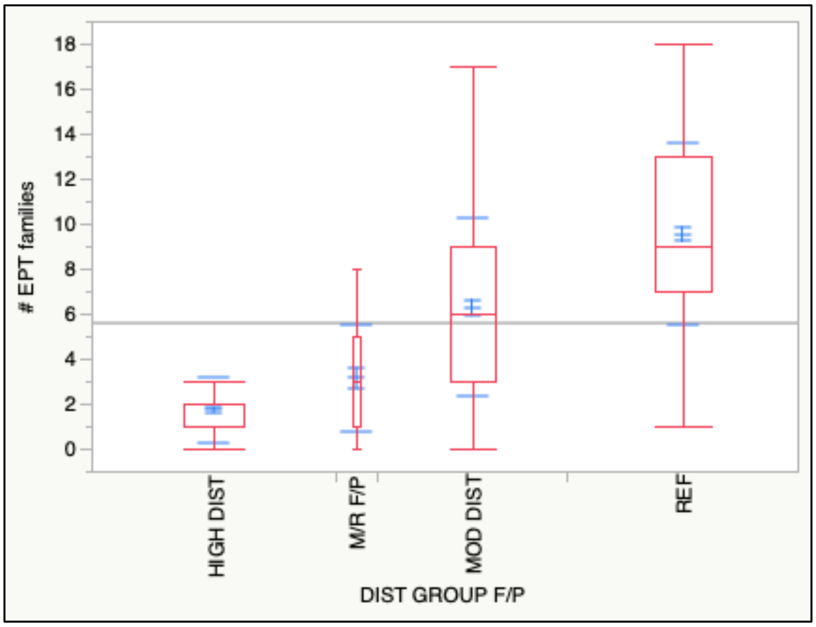
Sensitivity to Human Disturbance

Table A-8 summarizes the results of the ANOVAs conducted to evaluate the relationships of the BMI metrics with human disturbance (n=505). Overall, 16 of the 18 BMI metrics evaluated had significant differences among the REF, MOD DIST, and HIGH DIST groups, most with $p<0.0001$. The only metrics evaluated that did not show significant differences among the study reach groups were %SC and BMI density. %CF and %CG had relatively weak relationships with human disturbance.

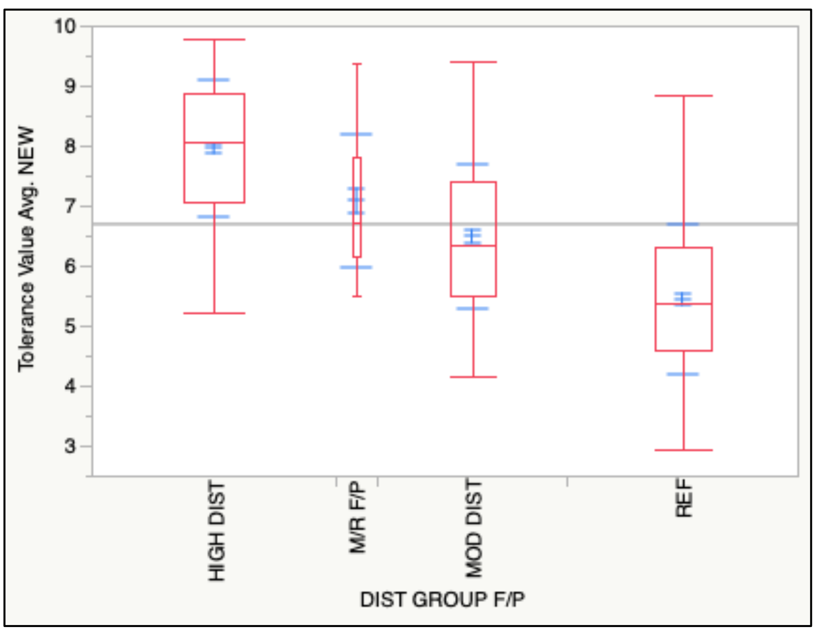
BMI richness metrics with the strongest responses (all negative) to human disturbance were # EPT Fams ($p<0.0001$, $r^2=0.5$), # Sens Taxa ($p<0.0001$, $r^2=0.47$), and # Insect Fams ($p<0.0001$, $r^2=0.43$). BMI composition metrics with the strongest negative responses to human disturbance were % Sens BMIs ($p<0.0001$, $r^2=0.46$), % EPT-Baetidae ($p<0.0001$, $r^2=0.45$), and % Vsens BMIs ($p<0.0001$, $r^2=0.44$). TV AVG had a strong positive response to human disturbance ($p<0.0001$, $r^2=0.43$). % sh+pred had the strongest response to human disturbance (negative, $p<0.0001$, $r^2=0.26$) among BMI metrics related to trophic structure. The 8 above-mentioned BMI metrics were all considered for further analyses as potential core metrics for the new IBI. Figure 5 illustrates the ANOVAS for # EPT Families and TV AVG.

Figure 5: Distributions of # EPT Families and TV AVG by Disturbance Group

Box plots of # EPT Families and TV AVG for the HIGH DIST, M/R I/F, MOD DIST, and REF groups (n=505) 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 p value is for the ANOVA where # EPT Families or TV AVG is the dependent variable and disturbance category is the independent variable. R^2 is the proportion of variation in the dependent variables accounted for by the disturbance categories.



N=505, $p<0.0001$, $r^2 = 0.5$



N=505, $p<0.0001$, $r^2 = 0.43$

b. Natural Relationships between Potential Core Metrics and Physiochemical Parameters

Table A-9 summarizes the results of the linear regression analyses conducted to evaluate relationships between the disturbance regressors (% watershed disturbed and habitat assessment score) and the physiochemical parameters elevation, gradient, watershed area, stream temperature, specific conductance, and dissolved oxygen. All of the physiochemical parameters were significantly related to one or both of the disturbance regressors, and all at $p<0.0001$ except dissolved oxygen ($p=0.001$). All 6 physiochemical parameters were used in the ensuing linear regressions.

Table A-10 summarizes the results of the linear regressions conducted to evaluate natural relationships between each of the potential core metrics and physiochemical regressors amongst the REF study reaches (n=165). Table A-10 provides a breakdown of each potential relationship between the potential core metrics, physiochemical parameters, and human disturbance, and the strength and direction of the relationships (i.e., positive or negative). Based on the strength and direction of the relationships and the logic described in the Methods, conclusions about confounds introduced by relationships among biotic, physicochemical, and human disturbance metrics were made.

Most of the potential core metrics had significant natural relationships with one or more physiochemical parameters across the REF sites. However, in most cases these relationships were either (1) out of alignment with physiochemical vs. human disturbance relationships, and/or (2) weak compared to relationships between the potential core metrics and human disturbance metrics (i.e., significantly lower r^2 and higher p). One of the potential core metrics, %Vsens BMIs, had moderately significant natural relationships with elevation ($p<0.0001$, $r^2=0.11$) and conductivity ($p<0.0001$, $r^2=0.11$) that could confound the interpretation of relationships between the potential core metrics and human disturbance. % Vsens BMIs was eliminated from further consideration.

c. Correlations between Potential Core BMI Metrics

Table A-11 presents the results of the correlation analyses between the potential core metrics. # sens taxa and # Insect Fams were both eliminated as potential core metrics due to high correlations (0.9 and 0.86, respectively) with # EPT Fams, which had a stronger relationship with human disturbance as demonstrated by higher r^2 in the ANOVAs. %EPT-Baetidae was eliminated due to a correlation of 0.90 with % sens BMIs, which has a stronger relationship with human disturbance and thus was retained as a core metric.

d. Core Metric Selection

Based on the results presented above, four core metrics were retained for inclusion in the IBI.

- % sens BMIs
- # EPT families
- TV AVG
- % sh+pred

The core metrics selected were the most sensitive to human disturbance while also having no or weaker confounding natural relationships with physiochemical parameters. In addition, the core metrics are diversified. Collectively, they represent different aspects of community structure including taxa richness, composition, disturbance sensitivity, and trophic structure.

e. Scoring Categories and Ranges for Core Metrics

Scoring ranges were developed for the core metrics using the criteria presented in Methods. The scoring ranges are provided below in Table 8.

Table 8
Streams IBI Core Metric Scoring Ranges

Score	# EPT Fams	% sens BMIs	TV AVG	% Sh+Pred
10	13+	49+	4.58 or lower	17+
9	10 to 12	34 to 48	4.59 to 5.01	11 to 16
8	9	27 to 33	5.02 to 5.46	10
7	8	20 to 26	5.47 to 5.91	9
6	7	13 to 19	5.92 to 6.35	8
5	6	11, 12	6.36 to 6.80	7
4	5	8 to 10	6.81 to 7.15	6
3	4	5 to 7	7.16 to 7.60	5
2	3	2 to 4	7.61 to 8.05	4
1	2	1	8.06 to 8.88	2, 3
0	0 to 1	0	8.89+	0, 1

f. IBI Classifications of Biological Integrity

IBI scoring ranges for five categories of biological integrity were determined as described in Methods, and are provided in Table 9.

Table 9
Streams IBI Classifications of Biological Integrity

Category	Scoring Range
Excellent	32 to 40
Good	24 to 31
Fair	11 to 23
Poor	7 to 10
Very Poor	0 to 6

g. Data Analyses Using the Updated Streams IBI

IBI Scores by Disturbance Group

ANOVA was used to evaluate differences in mean IBI score between the study reach groups using the entire data set of streams study reaches as replicates (n=505). This ANOVA is illustrated in Figure 6. The updated IBI has a very strong negative response to increasing human disturbance, with highly significant declines in mean IBI score from REF (30) to MOD DIST (21) to M/R I/F (12) to HIGH DIST (7) groups ($p<0.0001$, $r^2=0.57$).

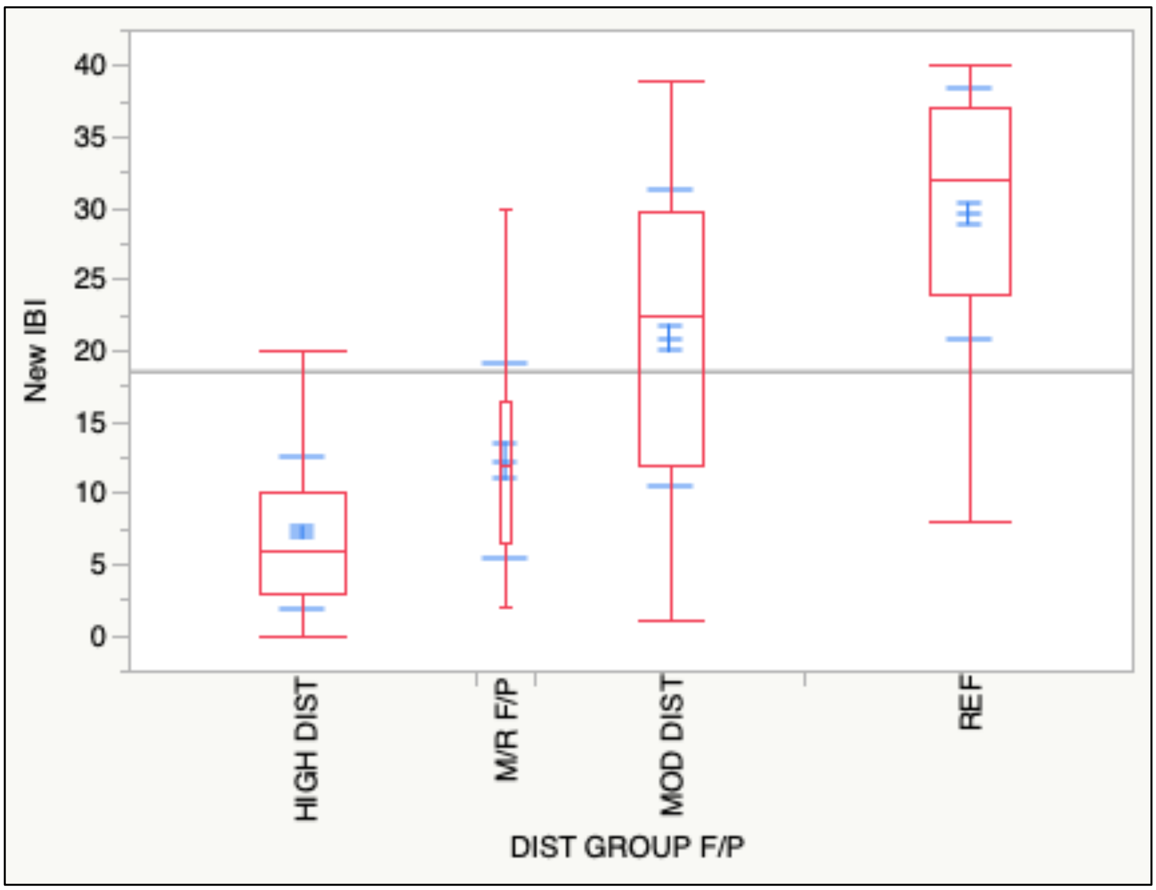
IBI Scores for the HIGH DIST group ranged from 0-24, with the overall mean (6) and median (7) at the Very Poor/Poor margin. By design, 75% of HIGH DIST sites score in the Very Poor to Poor range. Nearly all of the remaining HIGH DIST streams have scored in the Fair range, with one study site just reaching into the Good range. This remarkable consistency in IBI scores within the HIGH DIST group shows that degradation of the BMI community at high human disturbance levels has been very predictable and consistent.

IBI Scores for the REF group ranged from 3-40, with the overall mean (29) and median (31) in the Good range. By design, 75% of REF sites score in the Good to Excellent range of the IBI. Of the remaining REF 25% of REF sites, most, about 15%, scored in the Fair range. Less than 10% of REF sites scored in the Very Poor to Poor range, nearly all which were affected by major natural disturbances the previous year, again those being very high flood flows, prolong drought, or highly destructive wildfires with near complete burns of the upstream watershed. Excluding those recently affected by such episodic disturbances, less than 3% of REF sites scored below Fair. This consistency within the REF range shows that BMI communities in streams with low levels of human disturbance have been predictably diverse and healthy unless recently impacted by major episodic disturbances. BMI communities are also resilient, typically showing significant recovery from major episodic disturbances within a couple of years.

IBI Scores for the MOD DIST group ranged from 1-39, with the overall mean and median (both 20) in the Fair range. Approximately 75% of the MOD DIST sites scored between Poor and Good, which was expected due to the intermediate and somewhat variable magnitude of human disturbances present at these sites. In general, MOD DIST sites that were close to but fell just short of REF criteria such as AB3, M3, and SY4 tended to score on the higher end of the IBI (Fair to Excellent), while MOD DIST sites that were closer to HIGH DIST criteria such as AB2a, AB9, SY2 tend to have lower IBI scores (Poor to Fair). Similar to REF reaches, many MOD DIST sites that scored Very Poor were directly affected by episodic disturbances. Of the MOD DIST sites not directly impacted by episodic disturbances, only about 10% scored Very Poor.

Figure 6: Distribution of Streams IBI Scores by Disturbance Group

Box plots of IBI score for the HIGH DIST, M/R I/F, MOD DIST, and REF groups (n=505) 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=0.57, p<0.0001). The p value is for the ANOVA where IBI score is the dependent variable and disturbance category is the independent variable. R^2 is the proportion of variation in the dependent variables accounted for by the disturbance categories.



The streams IBI is based on the BMI data collected from over 500 study stream reaches surveyed in the study area over the past 25 years. Collectively, this data set represents the BMI communities from a tremendous range of stream conditions with respect to physiochemical variability and human disturbance. Study area streams have been surveyed through many years having highly variable rainfall and stream flows, including some of the wettest years on record, prolonged droughts, and years of more average rainfall. Many stream sites have been studied as they have responded through time to several highly destructive wildfires. The diversity offered by the streams data set has allowed us to revise and update the streams IBI, making it more reflective of the overall range of BMI community conditions present in local streams over the past 25 years. This has improved the streams IBI's reliability as a scale or index by which to judge the biological integrity of each study stream site surveyed, and those to be studied in the future.

Year by Year Trends in IBI Scores

Figure 7 illustrates mean IBI score by year (i.e., from 2000 to 2025) for the HIGH DIST, MOD DIST, and REF groups. ANOVA results are summarized in Table A-12.

Mean IBI score for the HIGH DIST group has ranged from 3 to 12 amongst the 25 years studied, with no significant differences in mean IBI scores between years ($p=0.08$, $r^2 = 0.19$, $n=175$).

The MOD DIST group had wider fluctuations in mean yearly IBI score (6 to 30). Years in which mean IBI score was significantly lower compared to most in the MOD DIST group include 2001 (11), 2018 (10) and 2023 (6) ($p<0.0001$, $r^2 = 0.39$, $n=154$).

The REF group is less confounded by variable human disturbance impacts compared to the MOD DIST and HIGH DIST groups, and yields the most meaningful information regarding long-term trends in IBI scores. Yearly mean IBI score for the REF group has ranged from 14 (2023) to 39 (2002) in 25 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, 2012, and 2025) and several lower scoring years including 2005, 2014, 2015, 2016, 2017, 2018, and 2023 ($p<0.0001$, $r^2 = 0.54$, $n=176$).

Figure 7: Mean IBI Score for Stream 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 = 0.54, n=176)

MOD DIST group (YELLOW): ($p<0.0001$, r^2 = 0.39, n=154)

HIGH DIST group (RED): ($p=0.08$, r^2 = 0.19, n=175).

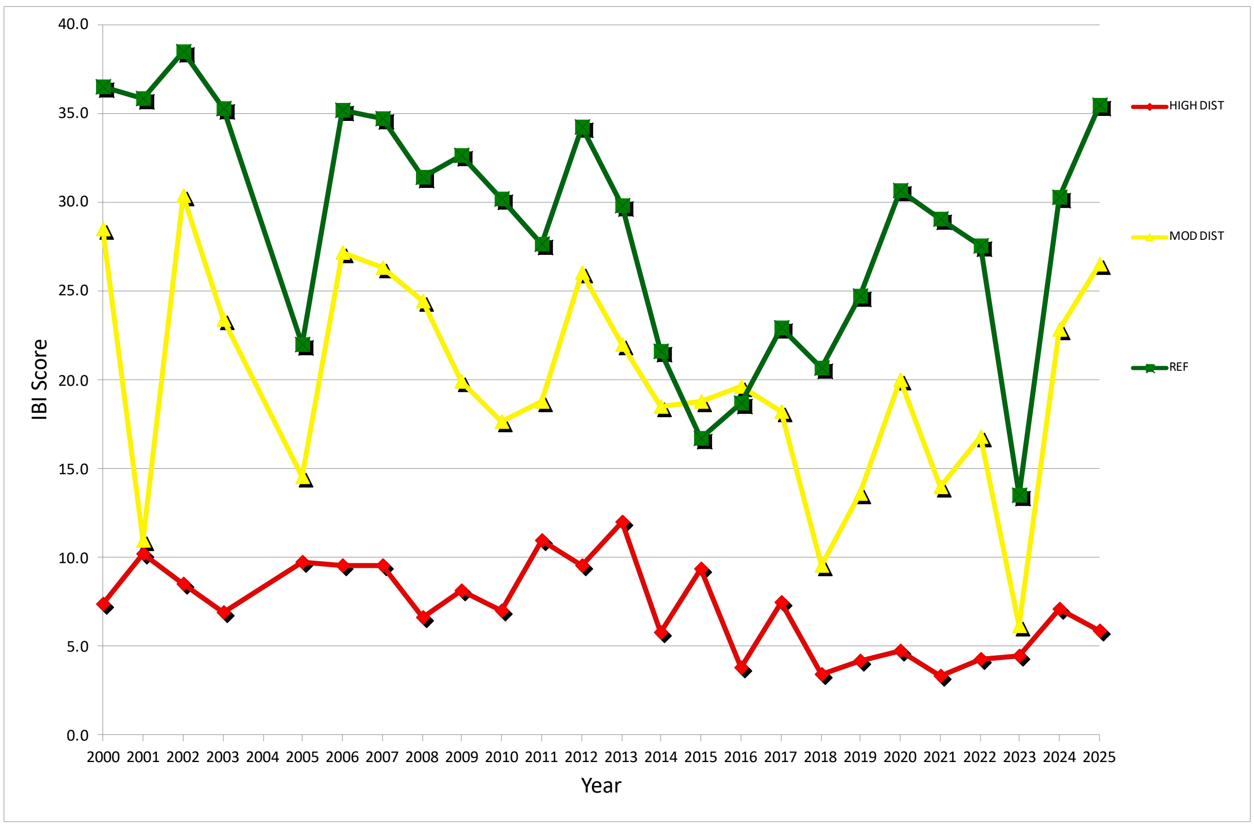


Figure 7 illustrates the general pattern that has occurred at the REF sites:

- In general, mean IBI score was relatively high at the REF sites from 2000 to 2013, a period of above average rainfall at the downtown Santa Barbara and San Marcos Pass rain gauging stations (County of Santa Barbara, 2025). Mean IBI score for the REF group was low (22) in 2005, which followed one of the wettest winters on record when extremely high stream flows largely scoured out stream sediments and inhabitants.
- A five-year drought occurred from 2012 to 2017, with well below average rainfall at the downtown Santa Barbara and San Marcos Pass stations each year (County of Santa Barbara, 2024). As streams began to dry from the drought, the effects on local stream communities became evident in 2014, with relatively low mean IBI scores in the REF group in 2014, 2015, 2016, 2017, and 2018 (22, 27, 19, 23, and 21, respectively). The Thomas Fire in late 2017, followed by a very wet winter with extreme scouring stream flows, prolonged the depression of IBI scores into 2018.
- IBI scores began to recover somewhat in 2019 (25) and continued upward in 2020 (31), likely in response to more stable stream flows caused by the very wet winter in 2017-18 and above average rainfall in winter 2019-20. 2021 and 2022 saw below average rainfall and decreasing stream flows, with mean REF IBI scores of 29 in 2021 and 28 in 2022.
- Mean IBI score (14) for REF sites was lowest in 2023 since the inception of the Program. Similar to 2005, rainfall and peak stream flows were extreme the previous winter, and heavy streambed scouring occurred repeatedly.
- The 2024 surveys followed a winter of above average rainfall, though not extreme as in the previous one. Mean IBI scores at the REF reaches recovered to 30, which is the overall mean through the years for REF reaches. This year's surveys (2025) followed a winter of near average rainfall, and mean IBI score (35) at the REF reaches was the highest in 20 years. It appears that generally higher rainfall and more consistent stream flows the past several years have allowed the BMI communities to recover from the negative effects of the prolonged drought and highly destructive wildfires that occurred from 2012-2017.

2025 Results

The following provides a synopsis of IBI scores and aquatic vertebrate observations in 2025 by disturbance group. Data for individual streams study reaches in 2025 (and previous years) is provided in Appendix Tables A-1, A-2, and A-3. Individual Report Cards for selected study reaches (those surveyed 5 or more years) and site photographs are also provided in the Appendix. Table 10 provides a list of stream study reaches surveyed this year and their individual IBI scores. Figure 8 illustrates ANOVA results of IBI scores by study reach group for this year (2025). The IBI appropriately separated between disturbance groups with a high level of significance (p<0.0001, r^2 = 0.82, n=21), with mean IBI score increasing from HIGH DIST (6) to MOD DIST (27) to REF (35) groups.

Figure 8: Oneway ANOVA of Stream IBI Score by Disturbance Group, 2025

Box plots of IBI score for the HIGH DIST, MOD DIST and REF groups (no M/R F/P reaches this year) 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=0.82, p<0.0001, n=21). 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.

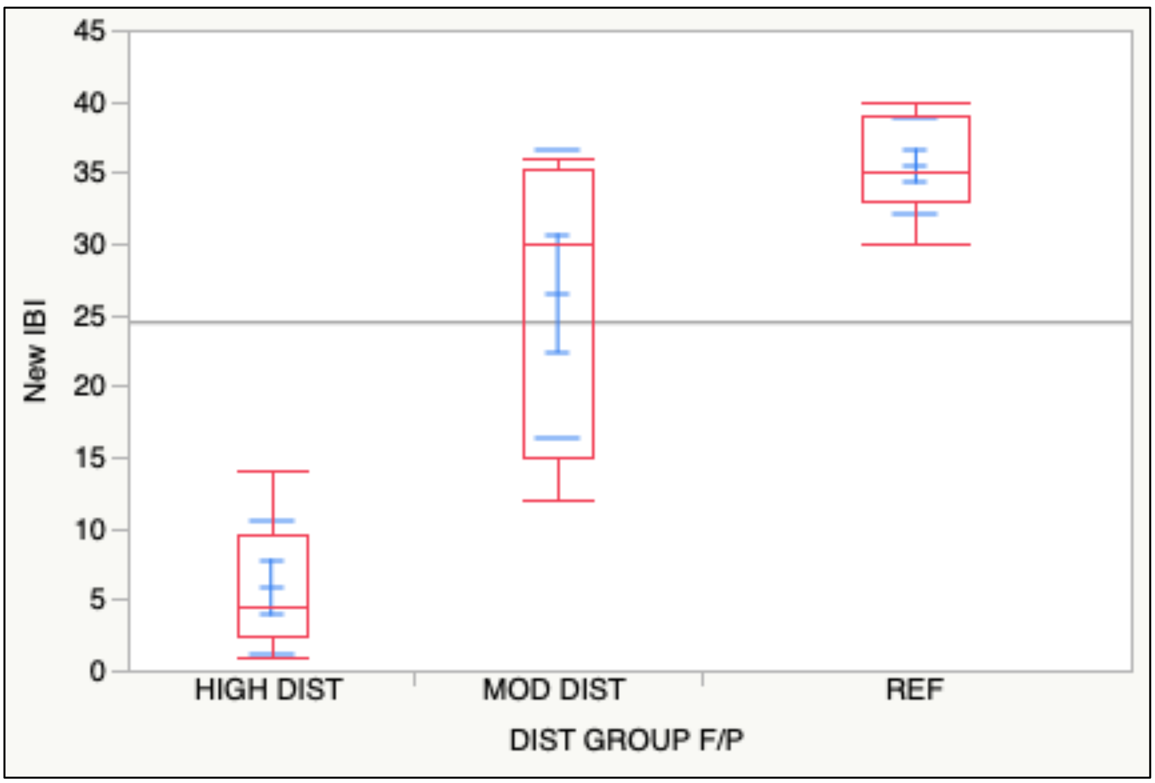


Table 10 IBI Scores for 2025 Stream Study Reaches			
Study Reach	**Disturbance Group**	**IBI Score**	**IBI Classification**
M1	HIGH DIST	1	Very Poor
AB5	HIGH DIST	3	Very Poor
OR1	HIGH DIST	3	Very Poor
M2	HIGH DIST	6	Very Poor
AB1	HIGH DIST	8	Poor
LH1	HIGH DIST	14	Fair
AB2a	MOD DIST	12	Fair
AL1	MOD DIST	16	Fair
SY2	MOD DIST	29	Good
SY7	MOD DIST	31	Good
M3	MOD DIST	35	Excellent
SY4	MOD DIST	36	Excellent
MONT3	REF	30	Good
GAV1	REF	32	Excellent
AL2	REF	34	Excellent
C3	REF	34	Excellent
AH1	REF	35	Excellent
M6	REF	36	Excellent
AB10	REF	38	Excellent
AB3	REF	40	Excellent
M4	REF	40	Excellent

REF Group

The REF group included 9 study reaches this year: AB3 (San Roque Creek) AB10 (upper San Roque Creek), AH1 (Arroyo Hondo), AL2 (Birabent Creek), C3 (Gobernador Creek), GAV1 (Gaviota Creek), M4 (Rattlesnake Creek), M6 (upper Mission Creek), and MONT3 (Cold Springs Creek). IBI score at REF sites ranged from 30 at MONT3 (Good) to 40 (Excellent) at AB3 and M4, with a mean score of 35.

MOD DIST Group

The MOD DIST group included 6 study reaches this year: AB2a (Arroyo Burro), AL1 (Alamo Pintado Creek), M3 (Mission Creek), SY2 (Sycamore Creek), SY4 (East Fork Sycamore Creek), and SY7 (West Fork Coyote Creek). IBI score at MOD DIST sites ranged from 12 at AB2a (Fair) to 36 (Excellent) at SY4, with a mean score of 27.

HIGH DIST Group

The HIGH DIST group included 6 study reaches this year: AB1 (Arroyo Burro), AB5 (Mesa Creek), M1 (lower Mission Creek), M2 (Old Mission Creek), OR1 (Orcutt Creek), and LH1 (Lighthouse Creek). IBI scores were Very Poor to Fair at these sites, ranging from 1 (M1) to 14 (LH1), with a mean score of 7.

4. Developing the Field-based Streams IBI and Data Analyses

a. Screening of BMI Metrics

Sensitivity to Human Disturbance

Table A-13 summarizes the results of the ANOVAs conducted to evaluate the relationships of the field-based BMI metrics with human disturbance (n=174). All of the field-based metrics showed highly significant responses along a human disturbance gradient (i.e., from REF to MOD DIST to M/R F/P to HIGH DIST groups). Statistical test results were strongest for TV AVG, which increased along the disturbance gradient ($p<0.0001$, $r^2=0.45$), and # EPT Taxa ($p<0.0001$, $r^2=0.44$), which decreased with increasing disturbance. # Sensitive Taxa ($p<0.0001$, $r^2=0.43$), # Insect Taxa ($p<0.0001$, $r^2=0.39$), and # BMI Taxa ($p<0.0001$, $r^2=0.32$) decreased along the disturbance gradient, while # Tolerant Taxa ($p<0.0001$, $r^2=0.25$) increased.

TV AVG, # EPT Taxa, # Sensitive Taxa, and # Insect Taxa had the strongest statistical results and were all considered for further analyses as potential core metrics for the Field-based Streams IBI.

b. Natural Relationships between Potential Core Metrics and Physiochemical Parameters

Table A-14 summarizes the results of the linear regressions conducted to evaluate natural relationships between each of the potential core metrics and physiochemical regressors across the REF study reaches (n=59). Three of the potential core metrics (TV AVG, # Sensitive Taxa and # Insect Taxa) had significant natural relationships with one or more physiochemical parameters across the REF sites. However, these relationships were either (1) out of alignment with physiochemical vs. human disturbance relationships, and/or (2) weak compared to relationships between the potential core metrics and human disturbance metrics (i.e., significantly lower r^2 and higher p). Therefore all four potential core metrics were retained for further consideration.

c. Correlations between Potential Core BMI Metrics

Table A-15 presents the results of the correlation analyses between the potential core metrics.

Sensitive Taxa had high correlations (>0.75) with two metrics, # EPT Taxa (0.81) and # Insect Taxa (0.83), and was therefore eliminated from further consideration as a core metric. # Insect Taxa and # EPT Taxa were also highly correlated (0.76), and therefore both cannot be included in the IBI. # EPT taxa had a stronger relationship with human disturbance and was therefore retained as a core metric, while # Insect Taxa was eliminated.

d. Core Metric Selection

Based on the results presented above, four core metrics were retained for inclusion in the field-based streams IBI.

- # EPT Taxa
- TV AVG

The core metrics selected were the most sensitive to human disturbance while also having no to minor confounding natural relationships with physiochemical parameters. In addition, the core metrics represent different aspects of community structure including taxa richness and disturbance sensitivity.

e. Scoring Categories and Ranges for Core Metrics

Scoring ranges were developed for the core metrics using the criteria presented in Methods. The scoring ranges are provided below in Table 11.

Table 11
Field-Based Streams IBI Core Metric Scoring Ranges

Score	# EPT Taxa	TV AVG
10	5+	2.8 or lower
9	4	2.9 to 3.4
8	3	3.5 to 3.8
7		3.9 to 4.2
6		4.3 to 4.6
5	2	4.7 to 4.9
4		5.0 to 5.1
3	1	5.2 to 5.3
2hre		5.4 to 5.5
1		5.6 to 6.4
0	0	6.5+

f. Field-Based Streams IBI Classifications of Biological Integrity

IBI scoring ranges for five categories of biological integrity were determined as described in Methods, and are provided in Table 12.

Table 12
Field-Based Streams IBI Classifications of Biological Integrity

Category	Scoring Range
Excellent	16 to 20
Good	13 to 15
Fair	9 to 12
Poor	5 to 8
Very Poor	0 to 4

g. Data Analyses using the Field-Based Streams IBI

ANOVA was used to evaluate differences in mean field-based streams IBI score between the study reach groups (n=174). This ANOVA is illustrated in Figure 9. The field-base streams IBI has a very strong negative response to increasing human disturbance, with highly significant declines in mean IBI score from REF (16) to MOD DIST (12) to M/R F/P (8) to HIGH DIST (4) groups ($p<0.0001$, $r^2=0.56$). Based on these results, the field-based streams IBI is a highly reliable indicator of biological integrity in local streams, with nearly identical p and r^2 as the laboratory-based streams IBI. It is remarkable that field-based assessments of the BMI community have thus far resulted in the creation of this tool (i.e., field-based streams IBI) having similar reliability in indicating BMI community integrity, as measured by its relationship with human disturbance, as does the tool based on laboratory analyses of BMI samples (i.e., streams IBI).

5. Aquatic Vertebrates in Local Streams

Rainbow trout (*Oncorhynchus mykiss*) have not been observed for the past three years during our spring stream surveys. Trout were also seen in small numbers in 2021 and 2022 at MONT3 and M4. Rainbow trout were routinely observed in several REF and MOD DIST study streams in the 2000s and early 2010s, most notably Arroyo Hondo (AH1), Gobernador Creek (C3), Rattlesnake Creek (M4), and Cold Springs Creek (MONT3). Overall, trout sightings have been greatly reduced the past decade as local trout populations have been decimated by a combination of events including the long drought from 2012-2017 and several major wild fires, most notably the devastating Thomas Fire in 2017, and the Alisal Fire in 2021, with a burn area that included much of Arroyo Hondo's watershed. While trout were not observed during our survey this year, Arroyo Hondo's Preserve Manager informed us there were several trout sightings in the creek during the spring of 2025 (John Warner, personal communication).

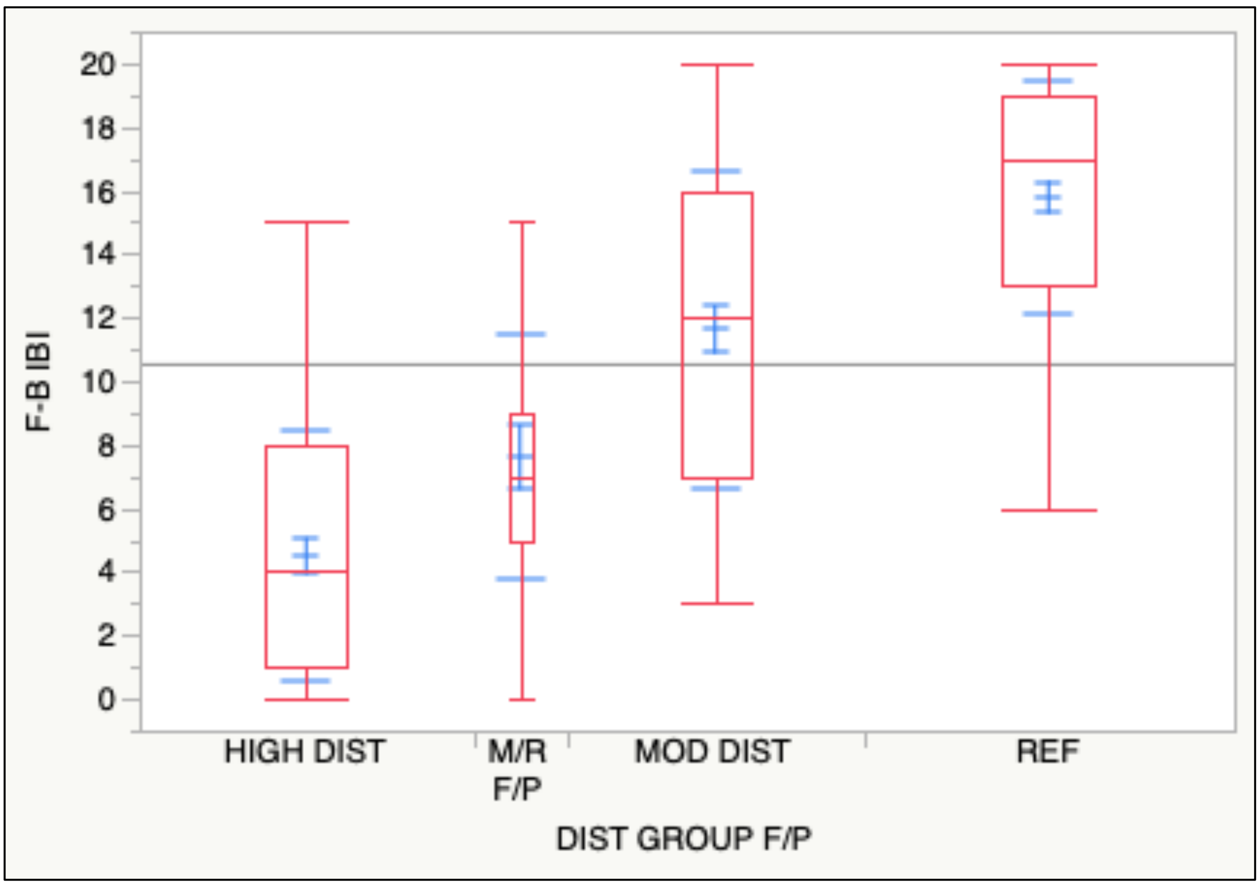
California newts (*Taricha torosa*) have been observed, sometimes in large numbers, at higher gradient, higher elevation REF and MOD DIST study streams, most notably C3, M4, M6, AH1, and MONT3, as have California tree frogs (*Pseudacris cadaverina*) and two-striped garter snakes (*Thamnophis hammondii*).

California red-legged frog (*Rana draytonii*) and southwestern pond turtle (*Clemmys marmorata*) have been observed mostly in lower gradient, lower elevation REF streams including Gaviota Creek (GAV1), Salipuedes Creek (SAL1), Jalama Creek (JAL1) and Arroyo Hondo (AH0 and AH1).

Pacific tree frog (*Pseudacris regilla*) and Western toad (*Bufo boreas*) are fairly ubiquitous, and have been found most commonly in lower elevation, low gradient streams of varying disturbance levels, as have three-spine stickleback (*Gasterosteus acleatus*).

Figure 9: Distribution of Field Based Streams IBI Scores by Disturbance Group

Box plots of field-based streams IBI score for the HIGH DIST, M/R I/F, MOD DIST, and REF groups (n=174) 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=0.56, p<0.0001). The p value is for the ANOVA where IBI score is the dependent variable and disturbance category is the independent variable. R^2 is the proportion of variation in the dependent variables accounted for by the disturbance categories.



C. Data Analyses for Estuaries

1. Trend Analyses of IBI Scores by Disturbance Group

ANOVA results of estuarine IBI scores by disturbance group for all years studied (2012-2025, n=131) are illustrated in Figure 10. As shown, IBI score significantly decreased in mean value from REF (15) to MOD DIST (10) to HIGH DIST (4) (p<0.0001, r^2=0.45). Figure 11 provides a graph of mean IBI score by year and disturbance group. Overall, mean IBI score has been considerably higher at REF sites each year compared with HIGH DIST sites, with MOD DIST sites generally having intermediate values. The exception to this was 2019, when mean IBI score was 4 at both REF and HIGH DIST sites, and 15 at MOD DIST sites. When IBI scores are averaged for REF and MOD DIST sites, they have been higher every year compared to HIGH DIST sites. This year mean IBI score was 4 at HIGH DIST sites (n=5), 15 at the lone MOD DIST site, and 16 at REF sites (n=4).

2. Salinity Effects on Estuary IBI Scores

Appendix Table A-16 summarizes the results of the ANOVAs of mean IBI Score by Disturbance Group in Low (<5 ppt), Moderate (5-18 ppt), and High (>18 ppt) salinity classes. Figure 12 provides a graphical representation. The ANOVA results show that the IBI distinguished between disturbance groups appropriately (i.e., increasing mean IBI scores from HIGH DIST to MOD DIST to REF) with highly significant results in all three salinity classes.

The IBI best distinguished between disturbance groups at Moderate salinity, with mean IBI score rising from HIGH DIST (5) to MOD DIST (9) to REF (16) groups (p<0.0001, r^2=0.55). Replication is limited for the MOD DIST (n=4) at moderate salinity. A similar pattern occurred at Low salinity, albeit with less robust statistical results (p=0.0005, r^2=0.28). HIGH DIST IBI scores were more variable and overall higher (mean of 10) compared to other salinity classes.

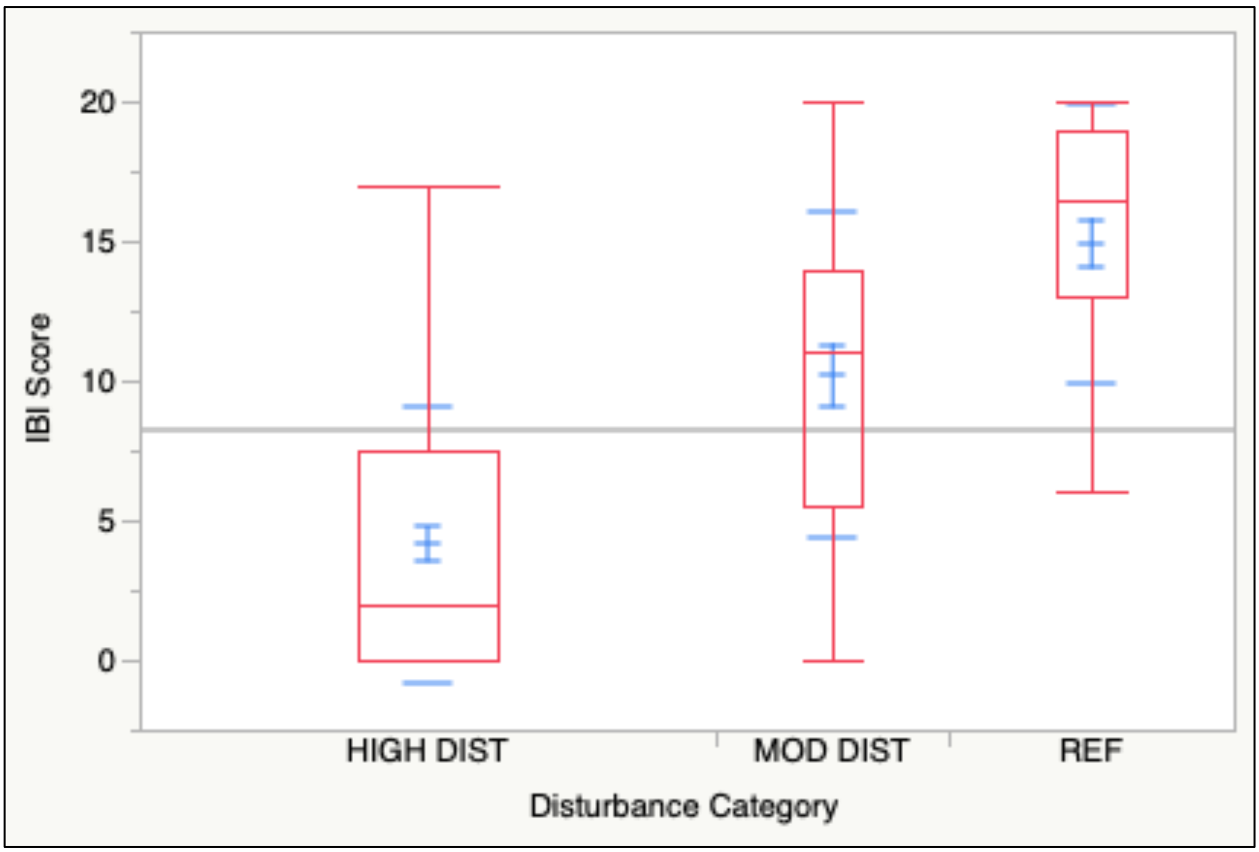
The appropriate pattern existed at High salinity also, with mean IBI score increasing from HIGH DIST (1) to MOD DIST (4) to REF (9) groups (p=0.0005, r^2=0.47). There is low replication in MOD DIST (n=4) and REF (n=7) groups compared to HIGH DIST (n=30) at High salinity.

Based on the data available thus far, there appears to be a skew towards higher IBI scores in Low salinity, and towards lower IBI scores in High salinity. If this trend continues, separate IBI classification scales should be created for each salinity class.

The connection between high salinity and reduced IBI scores is supported by data from 2024, when mean IBI scores were depressed at MOD DIST sites (4) and REF sites (11). High bottom salinity conditions existed during field surveys at Tecolote (29.4 ppt), Gaviota (28.3 ppt and 27.6 ppt), and Jalama (23.1 ppt) estuary sites, and nearly so at Carpinteria (17.8 ppt). The high salinity was caused by significant ocean inputs from king high tides, which were observed during some field surveys. Bottom dissolved oxygen was very low or very high at these sites, and bottom water temperature was elevated to high. Many aquatic species likely moved out of these areas due to the extreme water chemistry changes created by the sudden influx of large amounts of ocean water, thereby resulting in depressed IBI scores. The upstream site from Jalama estuary had an Excellent IBI score (19) in 2024. This site had moderate bottom salinity (6.8 ppt), buffered from the king tides by its distance from the estuary/ocean mouth.

Figure 10: Oneway ANOVA of IBI Score in Estuaries by Disturbance Group

Box plots of mean IBI score 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 IBI score between disturbance groups (r^2=0.45, p<0.0001, n=131). 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 the disturbance categories.



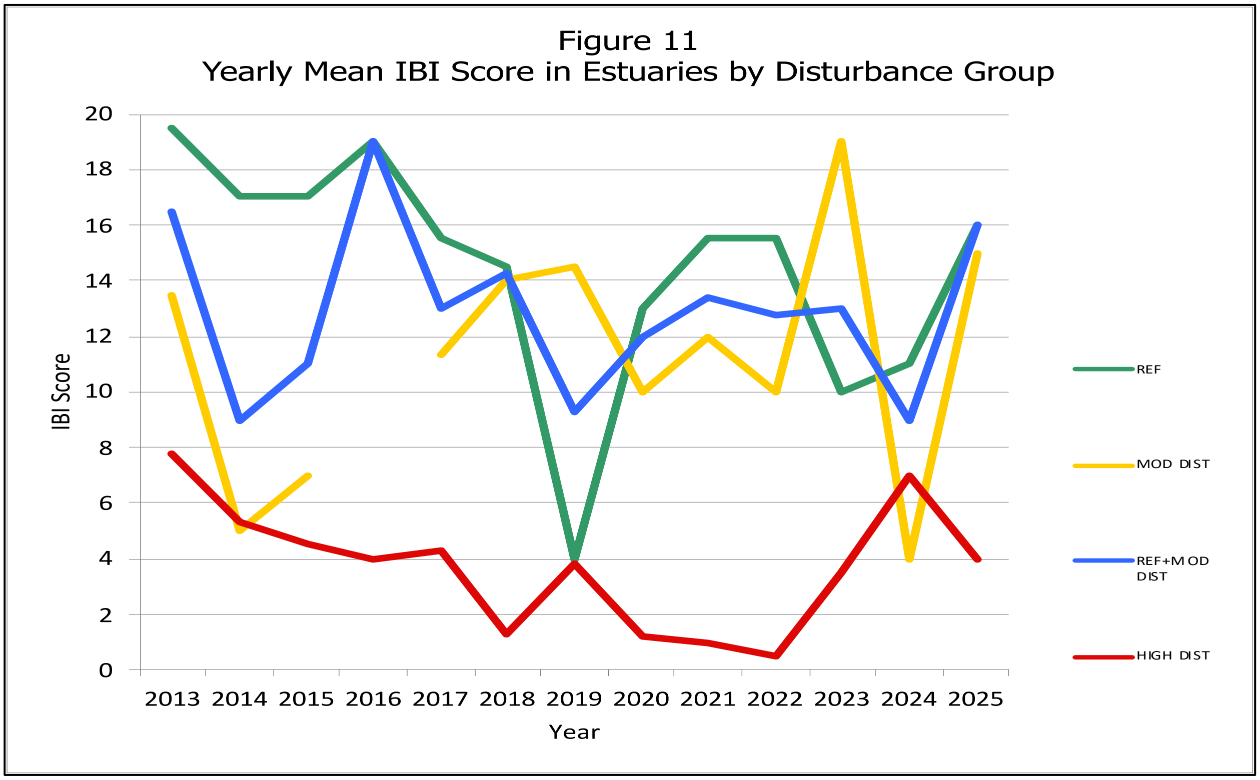


Figure 11
Yearly Mean IBI Score in Estuaries by Disturbance Group
IBI Score
20
18
16
14
12
10
8
6
4
2
0
2013 2014 2015 2016 2017 2018 2019 2020 2021 2022 2023 2024 2025
Year
REF
MOD DIST
REF+MOD DIST
HIGH DIST

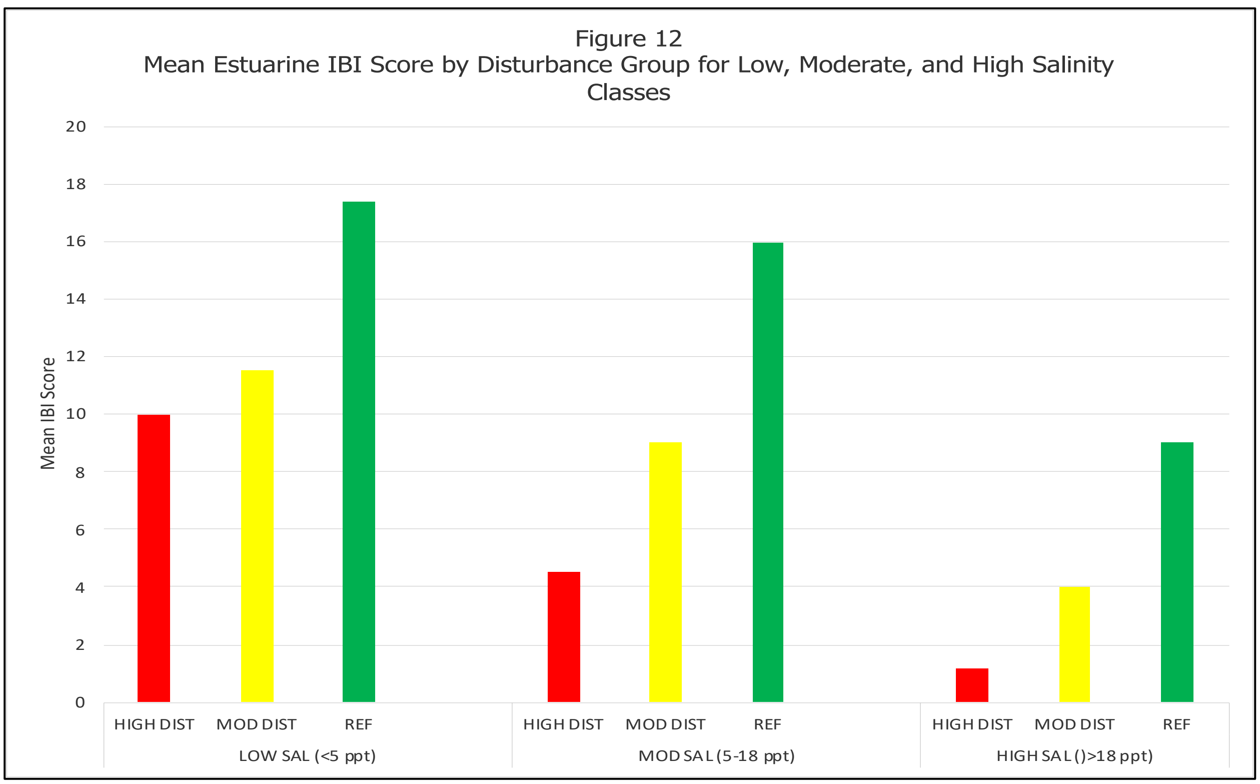


Figure 12
Mean Estuarine IBI Score by Disturbance Group for Low, Moderate, and High Salinity Classes
Mean IBI Score
20
18
16
14
12
10
8
6
4
2
0
HIGH DIST
MOD DIST
REF
LOW SAL (<5 ppt)
HIGH DIST
MOD DIST
REF
MOD SAL (5-18 ppt)
HIGH DIST
MOD DIST
REF
HIGH SAL ()>18 ppt)

V. Conclusions

A. Streams

Over the past 25 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 physiochemical habitat conditions and stream communities (e.g., lower IBI scores and loss of sensitive species) has increased linearly with increased watershed development. Urban development has been shown to have greater impacts on stream communities than has agricultural development.
- Natural variability in physiochemical conditions (i.e., elevation, gradient, watershed area, and water chemistry) has been shown to have mild effects on BMI communities, albeit not nearly as strong as the negative effects of intensive human disturbances.
- Variations in rainfall and resulting stream flows have been shown to impact BMI communities, particularly at REF and MOD DIST streams. Periods of high rainfall/flood flows and prolonged drought, as well as large wildfires (e.g., Jesusita, Thomas, Alisal, etc.) have caused downward trends in mean IBI scores at affected REF and MOD DIST streams the following spring, and in some cases for multiple years. This was particularly the case in 2005 (high rainfall/flood flows), from 2013 to 2018 (prolonged drought and Thomas Fire), and 2023 (high rainfall/flood flows). Periods of normal rainfall and stream flows have generally been marked by higher mean IBI scores in the REF and MOD DIST groups, including the upswing that has occurred the past two years.
- The diversity offered by the streams data set has allowed us to revise and update the streams IBI, making it more reflective of the overall range of BMI community conditions present in local streams over the past 25 years. This has improved the streams IBI's reliability as a scale or index by which to judge the biological integrity of each study stream site surveyed, and those to be studied in the future. Continued bioassessment monitoring through time will allow us to document the dynamic BMI communities occurring in our local streams as they respond to both human disturbances and natural physiochemical variability, including changing climate.
- The streams IBI's class criteria (i.e., Very Poor, Poor, Fair, Good, and Excellent) should be thought of as benchmarks by which to evaluate BMI community health, based on comparison to what has been present at a large, diverse collection of streams over many years. Departure from Fair/Excellent for a REF site, or below Poor/Good for a MOD DIST site, should serve as a "red flag", and make us ask, "why?" Is it explained by a naturally intermittent flow regime (i.e., frequent drying) of that site? Are recent extreme flood flows, wildfire, or prolonged drought the cause? If natural perturbations are the cause of low IBI scores, then IBI scores should recover within a couple of years provided that rainfall/stream flows, sediment loads, riparian canopy cover, stream temperature, etc. stabilize. If recovery of the BMI community does not occur, it is time to investigate other possibilities. Is there another natural cause, such as naturally hard groundwater, or are "natural" conditions (e.g.,

climate) changing with time and thereby affecting the BMI community? Is there a human cause such as water quality degradation from illicit wastewater flows, loss of stream flow due to upstream diversions, or some other human stressor?

- The new field-based streams IBI had a highly significant relationship with human disturbance, with similar p and r^2 as the laboratory-based IBI. Although it is not based on as large a data set nor as long a time period (i.e., only since 2018) as the laboratory-based version, the field-based streams IBI is a very useful indicator of biological integrity in local streams. The field-based streams IBI does not require laboratory analyses of BMI samples, allowing streams sites to be assessed at lower cost. The field-based streams IBI is not intended to replace the laboratory-based version. The intention is for both to be used in a complimentary fashion to expand the Program scope (i.e., number of study sites) and improve cost-effectiveness. Moving forward, it would advantageous to use the field method only at some study streams (perhaps half) each year, with the field method completed and BMI samples collected for laboratory analyses at others. The rotation of study sites between field-only vs. field + laboratory is to be determined.
- Stream habitat restoration sites including M2, AB1, AB2a, AB5, and AB9 have shown reach-level improvements in stream habitat complexity (i.e., depth, velocity, structure, cover, root mats, leaf litter, woody debris), streambed composition, and riparian vegetation. Thus far the improvements in reach-level habitat conditions at these sites have not been coupled with consistent, measureable improvements in the BMI community (i.e., IBI scores). Channel, floodplain and riparian restoration efforts at these sites do not address larger scale impairments in hydrology, geomorphology, water quality, and habitat continuity and connectivity at a watershed scale. Although much of this 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 will improve the BMI community in local streams can only be evaluated via continued bioassessment monitoring.
- Due to the combination of wildfires, floods, and drought over an approximately 10 year period from 2008-2018, Rainbow trout were greatly reduced or eliminated in many study streams including Mission Creek, Montecito Creek, Carpinteria Creek, and Rincon Creek and their tributaries. It is 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 nearly all local streams. In 2021 and 2022 trout were observed in small numbers at M4 and MONT3. Their reappearance in these streams provided a ray of hope for the species locally, and may indicate that 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 has been Arroyo Hondo (study reaches AH0 and AH1), where juvenile and adult steelhead/rainbow trout were observed consistently for 20 years during our field surveys. Trout were not observed in Arroyo Hondo from 2022 to 2024 presumably due to streambed scouring and sedimentation following the Alisal Fire in 2021. Trout were observed by others in Arroyo Hondo this past spring.

B. Estuaries

Based on the 14 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, more variable physiochemical conditions (e.g., input sources, salinity, temperature, etc.) make estuaries harsher, more dynamic environments where a relatively smaller number of BMI taxa can survive when compared with streams.
- Despite the harsher nature of estuaries, the estuarine IBI has proven to be effective as an indicator of biological integrity. The estuarine IBI has highly significant relationships with indicies of human disturbance, and effectively differentiates between REF, MOD DIST, and HIGH DIST groups.
- ANOVA results show that the IBI distinguished between disturbance groups appropriately with highly significant results in all three salinity classes (i.e., Low, Moderate, and High). However, based on the available data, there appears to be a skew towards higher scores in Low salinity, and towards lower scores in High salinity. If this trend continues, separate IBI classification scales should be created for each salinity class.

VI. Acknowledgements

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