



Fish-based Index of Biotic Integrity in the Caimito River Basin, Pacific drainage of Panama, Central America

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Abstract: The Caimito River Basin, adjacent to the Panama Canal watershed, is a freshwater system supplying drinking water to over 600,000 residents in West Panama. This study applies, for the first time in Panama's Pacific region, the Index of Biotic Integrity (IBI) using riverine fish assemblages to assess ecosystem condition. IBI results, evaluated alongside the Biological Monitoring Working Party (BMWP) metrics and physicochemical data, were examined in relation to an Environmental Quality Score (EQS) derived from integrated habitat and water-quality variables. Surveys at nine sites revealed an ecological gradient: about one-third of upper-basin sites scored "Good," with high native fish diversity and sensitive species; middle-basin scored "Fair," indicating moderate impacts yet retaining key attributes; lower-basin ranged from "Fair to Poor," due to pollution, exotic species, and habitat degradation. Despite localized stress, the basin remains recoverable. Strong IBI–BMWP–EQS correlations—EQS providing the best cross-system validation of IBI performance—support fish-based metrics as effective, replicable tools for integrated biomonitoring. Findings underscore urgent needs for riparian reforestation, pollution control, and governance reform to restore and protect the watershed. Climate change compounds risk, as declining rainfall and rising demand strain Panama's water resources, including the Canal. As a strategic ecosystem for regional resilience, the Caimito watershed offers a transferable framework for sustainable water and biodiversity management across Pacific Central American watersheds.

Keywords: Ichthyofauna, Bioindicators, Riparian management, Anthropogenic stressors, Water security.

Índice de integridad biótica basado en peces de la Cuenca del Río Caimito, vertiente Pacífica de Panamá, América Central. Resumen: La cuenca del río Caimito, adyacente a la cuenca del Canal de Panamá, es un sistema de agua dulce que abastece de agua potable a más de 600,000 habitantes en Panamá Oeste. Este estudio aplica, por primera vez en la región del Pacífico de Panamá, el Índice de Integridad Biótica (IBI) utilizando ensamblajes de peces de río para evaluar la condición del ecosistema. Los resultados del IBI, evaluados junto con métricas BMWP y datos fisicoquímicos, fueron analizados en relación con un Puntaje de Calidad Ambiental (EQS) derivado de la integración de variables de hábitat y calidad del agua. Los muestreos en nueve sitios revelaron un gradiente ecológico: aproximadamente un tercio de los sitios en la parte alta de la cuenca obtuvo clasificación “Buena”, con alta diversidad de peces nativos y presencia de especies sensibles; la zona media presentó condición “Regular”, indicando impactos moderados pero conservando atributos ecológicos clave; mientras que la parte baja osciló entre “Regular” y “Mala”, debido a contaminación, especies exóticas y degradación del hábitat. A pesar de presiones localizadas, la cuenca mantiene potencial de recuperación. Las fuertes correlaciones entre IBI, BMWP y EQS —siendo el EQS el que proporciona la mejor validación comparativa del desempeño del IBI— respaldan el uso de métricas basadas en peces como herramientas efectivas y replicables para el biomonitoreo integrado. Los resultados destacan la necesidad urgente de reforestación ribereña, control de la contaminación y reformas en la gobernanza para restaurar y proteger la cuenca. El cambio climático incrementa los riesgos, ya que la disminución de las lluvias y el aumento de la demanda presionan los recursos hídricos de Panamá, incluido el Canal. Como ecosistema estratégico para la resiliencia regional, la cuenca del Caimito ofrece un marco transferible para la gestión sostenible del agua y la biodiversidad en cuencas del Pacífico de Centroamérica.

Palabras clave: Ictiofauna, Bioindicadores, Manejo ribereño, Presiones antrópicas, Seguridad hídrica.

Introduction

The Southern region of Central America is characterized by high ecosystem representativity and diversity, and includes several rivers that drain towards the Tropical Eastern Pacific Ocean. Among these, the Isthmian Pacific Moist Forests stand out as a critical ecoregion, extending along the Pacific lowlands and foothills of southern Costa Rica and to central Panama (Palminteri *et al.* 1999, Powell *et al.* 2018). However, the region’s natural capital is facing multiple threats. The enhancement of infrastructure connectivity—particularly through the Pan-American Highway—has become a focal point of development efforts on both sides of the Panama-Costa Rica border. These initiatives aim to increase binational traffic via highway expansion and planned railway lines, including the proposed connection between Panama City and David (Forbes Centro América 2019, Newsroom Panama 2024, Álvarez *et al.* 2025). While these projects promise regional economic integration, they would also create a linear infrastructure barrier separating the coastal lowlands

from the uplands on the Pacific slope, fragmenting forests and watersheds and disrupting headwater-to-coast connectivity (wildlife movement, river flows, sediment and nutrient transport). These risks raise urgent concerns for sustainable development (ECLAC 2011, Gálvez 2018, Inter-American Development Bank-IDB 2018, LAC Geo 2022)

Rapid urban expansion and infrastructure growth along the Pacific drainage of southern Central America, particularly in central Panama around the Panama Canal, have intensified demographic and logistical pressures on adjacent watersheds. The Pacific Great Metropolitan Area continues to expand along major transport corridors, affecting basins located within narrow coastal-montane corridors that are inherently vulnerable to environmental degradation and salinization processes (Scodelaro *et al.* 2015, Comité de Alto Nivel de Seguridad Hídrica 2016). In the Caimito Basin, increasing agricultural activity and inadequate wastewater management have been associated with recent water-quality deterioration (Durán 2025). These pressures are further

compounded by climate-driven variability affecting water availability and regional water security, including operational constraints on the Panama Canal (Millard *et al.* 2024).

The increasing demand for water in nearby basins, driven by human and industrial use, places additional stress on aquatic ecosystems, which are further threatened by projects often promoted without considering long-term ecological consequences. This reflects the socio-environmental complexity of current development trajectories in Panama and the region (Di Baldassarre *et al.* 2018, Rodríguez 2019, Velásquez 2020, Hall *et al.* 2022). These pressures are compounded by the introduction of exotic fish species in the basin, such as tilapia, which may displace native fish, alter invertebrate communities, and contribute to eutrophication (Cassero *et al.* 2018). Given the multiple stressors affecting aquatic ecosystems in the region, it is crucial to implement robust biological monitoring tools to assess the current ecological status of rivers and support evidence-based decision-making. Establishing functional bioindicators is key for evaluating the ecological condition of freshwater systems, informing conservation strategies, and tracking future changes. These indicators should ideally reflect community composition, functional traits, and responses to anthropogenic pressures over time. Although methodologies such as the Index of Biotic Integrity (IBI) have been successfully applied in Mexico (Lyons *et al.* 1995, Ramírez-Herrejón *et al.* 2012), their application remains relatively absent in the Pacific lowlands of southern Costa Rica and Panama.

The Caimito River Basin is situated on the Pacific slope of the Central American Isthmus, within the freshwater biogeographic framework proposed by the Freshwater Ecoregions of the World (FEOW), this global regionalization delineates freshwater ecoregions primarily from patterns of freshwater fish composition and endemism, providing a standardized context for interpreting assemblage structure and ecological gradients among basins. In Pacific-draining systems of southern Central America, freshwater fish faunas reflect the history of Isthmian connectivity and isolation, with assemblages typically structured along marked longitudinal gradients from upland headwaters to lowland reaches. Framing the Caimito Basin within this freshwater-ecoregional context supports the interpretation of spatial patterns in fish assemblages and biological integrity using regionally comparable biotic expectations (Abell *et al.* 2008).

This study aims to address that gap by applying a fish-based IBI to the Caimito River Basin for the first time, establishing a baseline of biological integrity for this ecologically and strategically important watershed. In addition to evaluating the IBI and its component metrics across spatial scales, we examine the relationships between biological patterns and environmental variables (e.g., habitat, water quality) through correlation and multivariate analysis. We also use the BMWP index for cross-validation, enhancing the robustness of ecological interpretation. Ultimately, this study seeks to demonstrate the applicability of the IBI framework in a Central American tropical basin, generate recommendations for watershed management, and promote the use of standardized bioindicators for conservation and policy across the region.

Conceptual Framework and Regional Context for the Application of the IBI. A Fish-Based Index of Biological Integrity (IBI). Fish have been widely used as indicators of water quality and ecosystem condition, and the characterization of riverine fish assemblages has become a valuable tool for environmental assessment and decision-making (Duncan & Lockwood 2001, Boulton 1999). This has been achieved mainly through aquatic ecosystem quality indices (Karr *et al.* 1986, Soto-Galera *et al.* 1998, Kestemont *et al.* 2000, McDowall & Taylor 2000, Oberdorff *et al.* 2002), which can detect habitat degradation and help evaluate restoration success (Fausch *et al.* 1990, Scott & Hall 1997, Wichert & Rapport 1998, Paller *et al.* 2000). The Index of Biotic Integrity (IBI) was originally developed to evaluate fish assemblages in small streams of the north-central United States (Karr 1981, Karr *et al.* 1986, Lyons 1992, Lyons *et al.* 1996) and has since been adapted for temperate and tropical rivers. In the Neotropics and Mesoamerica, the IBI has been applied to evaluate fish assemblages and identify conservation priorities. For example, Araujo *et al.* (2003) applied the index in the Paraíba do Sul basin in Brazil, demonstrating its usefulness for assessing ecological integrity in large tropical river systems. In Mesoamerica, Lyons *et al.* (1995) provided one of the first regional applications and proposed a framework for its use in other countries such as Mexico. The fish-based Index of Biotic Integrity (IBI) used in this study follows the framework proposed by Karr and Dudley (1981), which defines biological integrity as the capacity of an aquatic ecosystem to sustain a balanced and adaptive biotic community comparable to minimally disturbed conditions. The IBI evaluates this integrity through the integration of

functional attributes of fish assemblages—such as species composition, trophic structure, habitat use, and tolerance to disturbance—rather than through direct responses to individual physicochemical variables such as conductivity or salinity (Hughes *et al.* 1998). Consequently, it functions as an integrative measure of cumulative ecological responses to natural and anthropogenic pressures over time rather than as a short-term chemical diagnostic tool (Karr & Dudley 1981, Hughes *et al.* 1998). Under this conceptual framework, natural longitudinal gradients occurring along river continua, including those typically observed in lower basin reaches, do not invalidate the index *per se*, provided that results are interpreted within their appropriate ecological and geomorphological context. The IBI has been applied to evaluate fish assemblages and identify conservation priorities. For example, Ramírez-Herrejón *et al.* (2012) used the index in the Angulo River sub-basin in central Mexico to assess ecological condition and habitat quality. In Central America, monitoring initiatives led by the ANAI Association have incorporated fish and macroinvertebrate indices in Caribbean-draining rivers such as the Sixaola and Estrella basins, later extending to the San San and Changuinola watersheds in the Greater Talamanca region (McLarney *et al.* 2008, ANAI Association 2015). Despite these advances, comparable long-term IBI monitoring programs remain largely absent from Pacific-draining basins of Panama and Costa Rica, highlighting the need for basin-scale applications supported by regional institutions and local stakeholders (Lyons 1992, Lyons *et al.* 1995, Ramírez-Herrejón *et al.* 2012). The IBI is closely linked to habitat condition and water quality, providing a range-based environmental quality indicator that can be compared with other ecological assessments (Lyons 1992, Lyons *et al.* 1995, Ramírez-Herrejón *et al.* 2012). This approach offers a functional perspective of basin condition and has proven useful as a benchmark for evaluating habitat and water quality patterns (Lyons 1992). In Panama, however, broader evaluations incorporating fish assemblage structure and conservation status are still limited. Applying the IBI therefore provides a dual opportunity: it supports biodiversity conservation through fish-community metrics while simultaneously integrating biological information into watershed monitoring and environmental assessment. The application of the IBI in tropical rivers has proven useful for conservation and management by distinguishing habitat disturbance from biodiversity patterns and providing a comparable monitoring framework

across basins. When combined with other biological and physicochemical indicators, it becomes a practical tool for institutional monitoring programs, particularly in regions where fish-based assessments remain limited. In lower Central America (Panama and Costa Rica), available baseline information on functional groups and ecological attributes supports its broader application (Bussing 1998, González-Gutiérrez 2021).

Fishes of the Pacific Slope of the lower Central American Isthmus. Panama harbors more than 200 freshwater fish species and is recognized as one of the most diverse countries in Central America (Smith & Bermingham 2005). Taxonomic and zoogeographic studies have established the basis for understanding species composition and distribution patterns in the country (Meek & Hildebrand 1916, Loftin 1965, Bussing 1998), yet ecological assessments relating fish assemblages to anthropogenic disturbance remain scarce. Within the biogeographic framework proposed by Smith & Bermingham (2005), which identifies five ichthyological provinces, Panama functions as a faunal bridge between Nearctic and Neotropical regions. The Caimito River Basin, located between the Tuira and Santa María provinces, has not previously been evaluated in terms of fish biodiversity or functional structure, making it a suitable system for examining ecological responses to disturbance gradients. Freshwater fishes are among the most threatened vertebrates globally due to habitat degradation, hydrological alteration, and climate change (Arthington *et al.* 2016), pressures intensified in Panama by poorly planned development and limited ecological research (Valdés-Pineda *et al.* 2014, ECLAC & OECD 2016, Contreras-MacBeath *et al.* 2022). Protected areas often do not overlap with critical freshwater fish habitats, leaving endemic or vulnerable species insufficiently protected (Tognelli *et al.* 2008). Although the Caimito Basin borders Campana National Park, aquatic ecological processes operate at the watershed scale, making the basin itself the appropriate unit for biodiversity conservation and management. Information on fish assemblages—particularly native and commercially relevant species—remains limited (MINSA-UCP 2016), and the impacts of infrastructure development on river systems further highlight the need for ecological studies in the region (Habit *et al.* 2006, Soto *et al.* 2006). This study addresses that gap by providing a taxonomically and ecologically grounded assessment of the fish community in this Pacific-slope basin near the Panama Canal.

Materials and Methods

Study Area. The study was conducted in the Río Caimito watershed (Basin 140 in Panama's national watershed classification), which covers approximately 453 km² and extends about 72 km across West Panama Province (IMHPA 2021). The river originates in the northeastern sector of Capira District, flows eastward through La Chorrera and Arraiján districts, and discharges into the Gulf of Panama in the Eastern Tropical Pacific. A major geomorphological feature in the upper-middle basin is El Chorro de La Chorrera, a waterfall of approximately 30 m that creates a marked vertical discontinuity along the river profile (Araúz *et al.* 2011). Hydrological dynamics are strongly seasonal, with most rainfall and streamflow occurring between May and December. The watershed supplies drinking water to Panamá Oeste Province (approximately 13.8% of the national population; INEC 2014) and contributes sediments and nutrients to adjacent coastal systems supporting estuarine fisheries (López *et al.* 2003). However, land-use change, urban expansion, and infrastructure development have degraded riparian zones and water quality in parts of the basin (Barranco-Pilides 2016, Comité de Alto Nivel de Seguridad Hídrica 2016, ATP 2020, Coronado 2023). Nine sampling sites were selected and grouped into upper, middle, and lower basin sectors to capture spatial variability in ecological and physicochemical conditions (Fig. 1). Most sampling was conducted directly in the river channel because recurring droughts have reduced water volume in Central American watersheds, including the Caimito Basin (Murphy *et al.* 2014, We Are Water Foundation 2024). In the lower basin, access to the main channel was restricted by infrastructure, so nearby tributaries were sampled to represent downstream conditions. Canopy cover and habitat structure were assessed following the method described by Zeni *et al.* (2019).

Upper Basin (Capira District). Altos del Cerro (Trinidad): Located at 476 m a.s.l., this site marks the river's origin at Cerro Trinidad within Campana National Park (Kiener-Ford 2020), also the source of rivers such as Chame, Perequeté, and Trinidad. River channel width ranged from 1.26 to 2.13 m, sampled depth range from 0.05 to 1.30 m, canopy cover reached average 60%, and structural shelters made up 65% of habitat features. Local nearby households engage in small-scale agriculture.

La Valdeza: At 213 m a.s.l., this site featured a wider channel from 5.59 to 7.90 m, sampled depth range from 0.29 to 1.02 m, with 67% canopy cover, and 77.5% structural shelters. Quarrying nearby

raises air and noise pollution concerns (Montenegro 2023).

Caimito: At 195 m a.s.l., this site had 5.53–6.83 m channel width, sampled depth range from 0.9 to 2.9 m and 30% canopy cover. Infrastructure and flow modifications are common in this area of 1,635 residents (INEC 2014).

Middle Basin (Chorrera District). Santa Rita: At 69 m a.s.l., with 3.14–8.93 m channel width, sampled depth ranged from 0.30 to 5.33 m and 62.5% canopy cover. Despite some urbanization (1,848 residents; INEC 2014), community vigilance helps maintain river conditions. Structural shelters reached 70%.

El Trapichito: At 41 m a.s.l., channel width 4.73–12.6 m, sampled depth range from 0.18 to 3.08 m, canopy cover 60.75% and structural shelter 68.75%. Adjacent to the Jaime Díaz Quintero Water Treatment Plant (IDAAN), serving La Chorrera, though threatened by land-use pressures (Montenegro 2019, Del Mar 2024).

Mastranto (*Mastranto Abajo*): Located in Barrio Colón at 32 m a.s.l., this site is hydrologically connected to the nearby El Chorro waterfall, a once iconic recreational area that has now become heavily polluted (Durán 2025). Surface waters flow between the two areas, highlighting the environmental interdependence. The channel at Mastranto ranges from 5.6 to 14.9 m in width, sampled depth ranges from 0.28 to 1.51 m with 40% canopy cover and 77% structural shelter. The site is significantly impacted by informal housing developments and the discharge of untreated sewage (Montenegro 2021) despite government efforts for sanitation in Panamá Oeste (Saneamiento Panamá 2018).

Lower Basin (Chorrera and Arraiján Districts). Maribel (*Puente Maribel, Chorrera*): The only accessible site along the main river in the lower basin. Located at 29 m a.s.l., the channel ranges from 8.9 to 16 m in width, sampled depth ranges from 0.12 to 3.38 m, canopy 50%, structural shelter 57.5%. Quarry activity nearby impact flow and cause flooding, along with sewage pollution (Moreno *et al.* 2021).

Martín Sánchez (*Arraiján*): Located at 20 m a.s.l., channel width ranged from 5.9 to 7.34 m, sampled depth range from 0.13 to 1.57 m, although canopy has relative higher percent of 60 %, it is adjacent to the Costa Verde development, therefore urban pressures are high including sewage pollution. The location is close to the coastal area of Puerto Caimito near Costa Verde; this stream remains freshwater, but it may have estuarine water intrusion during high tides (Velásquez 2020).

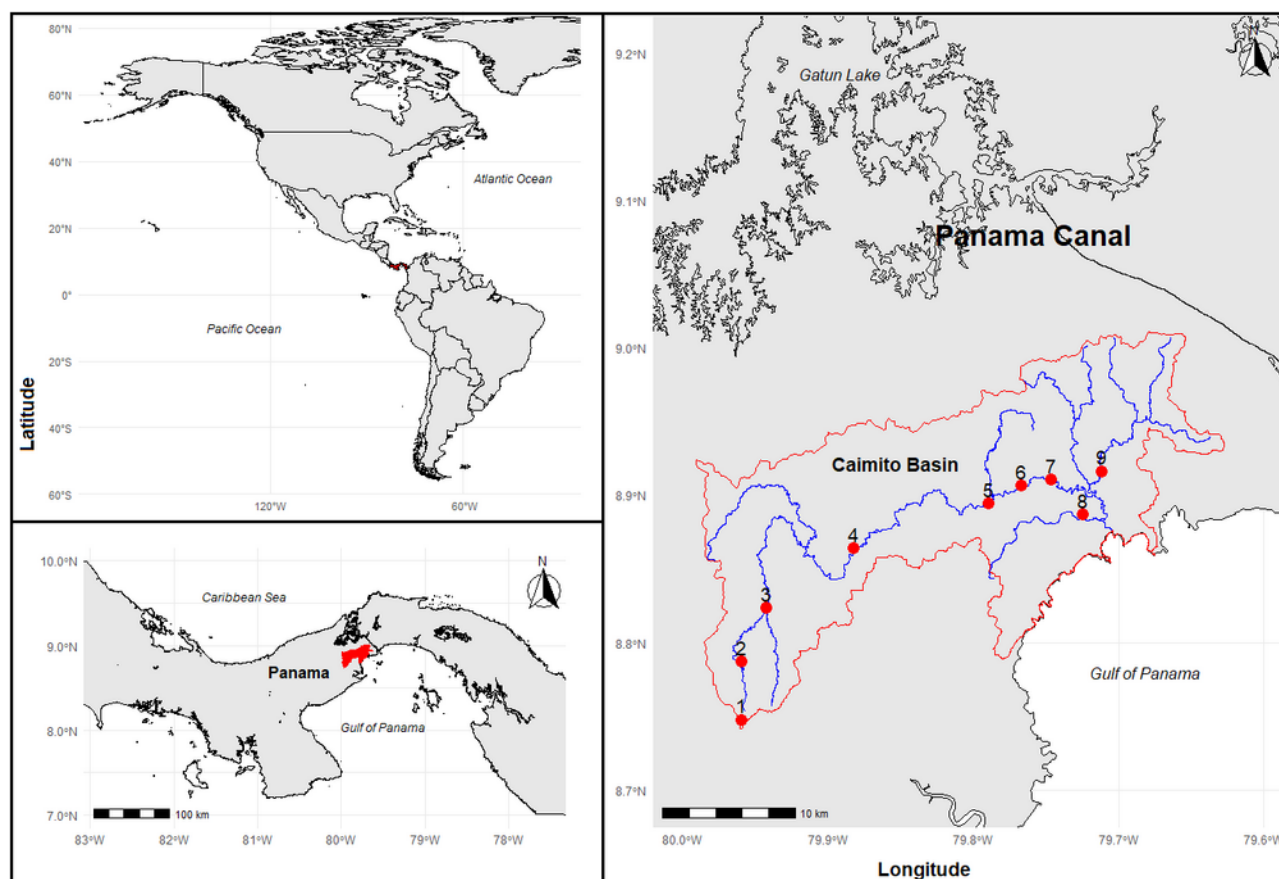


Figure 1. Location of the Río Caimito watershed (Basin 140) and distribution of the nine [study sites](#) across West Panama Province. Sites 1 (Altos del Cerro), and 2 La Valdeza) are located in Capira District; sites 3 (Caimito), 4 (Santa Rita), 5 (Trapichito), and 6 (Mastranto) in La Chorrera District; and sites 7 (Maribel), 8 (Martín Sánchez), and 9 (Aguacate) in Arraiján District.

Aguacate (Arraiján): A tributary sampled under the Inter-American Highway in Arraiján, near El Tecal and a city park (Parque Centenario). At 40 m a.s.l., the stream had a channel width ranging between 3.3–8.13 m; sampled depth ranged between 0.13 to 2.0 m; canopy cover was 27.5%, and structural shelter component was 73.75%. Urban and industrial activities have degraded conditions, contaminated wastewater ponds are located nearby (Velásquez 2020).

Fish Sampling. Three sampling campaigns were conducted at the nine study sites across the upper, middle, and lower sections of the Caimito River Basin: during the dry season (March 2022), the transition between dry and rainy seasons (May 2022), and the wet season (November 2022). At each site, a standardized 100-m transect was established within the active channel and the same sampling protocol was applied to ensure temporal and spatial consistency. Three complementary sampling methods were used following established stream assessment approaches (Angermeier &

Smogor 1995, Paller 1995). The combined use of gears increased detectability across habitats while maintaining standardized effort and a protocol replicable by local stakeholders with minimal technical requirements. Sampling techniques included electrofishing, cast netting, and seine netting, all applied within the same 100 m transect at each site and during each campaign. Electrofishing was conducted using a Smith-Root LR-20B unit. Voltage was adjusted according to measured conductivity, ranging from 250 V in high-conductivity waters ($>300 \mu\text{S}/\text{cm}$) to 750 V in sites with lower conductivity ($100\text{--}300 \mu\text{S}/\text{cm}$), values within the operational range recommended for backpack electrofishing in tropical freshwater systems. Sampling was conducted for 30 minutes per transect using a zigzag pattern against the current to ensure coverage of available microhabitats. Cast nets and seine nets complemented electrofishing (Newbury & Gaboury 1993, Pusey *et al.* 1998, Barbour *et al.* 1999) by improving detectability across a broader range of habitats and behavioral guilds that are not equally sampled by a single

method. For cast-netting (atarraya), a 1.83 m diameter (six-foot) net with 6.35 mm bar mesh ($\frac{1}{4}$ in) was used, and 20 standardized casts were performed along each 100 m transect. This method facilitated the capture of species occupying open-water sections and deeper runs, as well as individuals less susceptible to electrofishing under certain hydraulic conditions. Additionally, a 6 m-long seine net (chinchorro) with 6.35 mm bar mesh ($\frac{1}{4}$ in) was employed, and five standardized hauls of 10 m each were conducted within the same transect. Seine netting was effective in shallow margins, depositional zones, and structurally heterogeneous habitats. The three gears were therefore applied under fixed and equivalent effort at every site and in every seasonal campaign, ensuring consistent sampling of benthic, mid-water, and structurally associated species throughout the basin. Following capture, specimens were transferred to a 14-liter aerated tank for in situ identification, observation, and photography. Most individuals were released back into the river after processing. When necessary for verification and reference, a maximum of 30 individuals per species—depending on capture abundance—were preserved in 95% ethanol. Preserved specimens were deposited in the laboratory of the International Maritime University of Panama (UMIP), where identifications were corroborated to the lowest possible taxonomic level using regional and historical taxonomic keys and guides (Meek & Hildebrand 1916, 1923, 1928; Loftin 1965, Böhlke & Chaplin 1993, Bussing 1998, Valdés-Díaz 2020, González-Gutiérrez 2021). Some individuals were maintained alive in aquaria at the UMIP project facilities for identification, biological observations, and educational purposes promoting awareness of Pacific River fish diversity. Importantly, the same combination of gears and standardized effort (100 m transect, 30 minutes of electrofishing, 20 cast-net throws, and five seine hauls) were applied at all sites and during each sampling campaign, regardless of basin position. No gear was substituted or omitted in any section of the watershed. This multi-gear design was implemented to reduce potential detectability bias associated with channel morphology and to support comparisons of assemblage composition based on environmental variation rather than differences in sampling procedure. Fish capture, handling, transport, and temporary maintenance followed established ethical recommendations for research involving fishes (Jenkins *et al.* 2014). The principal investigator and some co-authors have participated in formal training programs on research ethics and animal care. These

good practices were communicated to the rest of the research team to ensure appropriate and consistent handling of fish during field and laboratory activities.

Macroinvertebrate Sampling. Macroinvertebrate sampling followed the Biomonitoring Protocol for Water Quality Monitoring (BMWP) in Surface Water Bodies of Panama (Cornejo *et al.* 2019), specifically Protocol 4 for multihabitat sampling. Sampling was conducted within the same 100-m reaches used for fish surveys. Three replicates were collected at the beginning (0 m), middle (50 m), and end (100 m) of each reach using a 500 μ m mesh D-net, performing 15 standardized drags per habitat type. Samples were preserved in 95% ethanol, transported to the UMIP laboratory, and identified to family level using a stereomicroscope and the taxonomic keys of Cornejo *et al.* (2019, 2023). The Biological Monitoring Working Party index adapted for Panama (BMWP-PAN) was calculated following Cornejo *et al.* (2019). For each site and sampling campaign, the three replicates were integrated to generate a consolidated list of macroinvertebrate families, and BMWP values were computed as the sum of the tolerance scores assigned to the families present according to the official scoring system (Table I). Campaign-specific BMWP values were used in all statistical analyses, including ordinations and correlations with fish-based metrics. According to the ecological quality classification established for the BMWP-PAN protocol (Cornejo *et al.* 2019), BMWP scores are interpreted as follows: ≥ 150 excellent ecological quality; 78–149 good quality; 59–77 regular quality; 39–58 contaminated conditions; 20–38 very contaminated conditions; and < 19 extremely contaminated conditions. Using the same sampling sites and timelines for fish and macroinvertebrates ensured direct comparability between the fish-based IBI and the BMWP index. Mean BMWP values from the three sampling campaigns were used for descriptive comparisons among sites.

Metrics used for IBI Calculation-scoring criteria. To apply the IBI in this new region, we replicated the metrics established by Lyons *et al.* 1995 using the criteria for basins larger than 400 km², since the Caimito Basin exceeds that area (453 km²), analyzing species composition, abundance, trophic levels, tolerance levels, water column position, fish health status, presence of native viviparous species, introduced species, and overall abundance (fishes collected in 30 minutes). For each site and sampling event, richness metrics were calculated as counts of distinct species per category, whereas percentage-based

Table I. BMWP tolerance scores assigned to macroinvertebrate families recorded in the Río Caimito Basin. Scores correspond to the official BMWP adaptation for Panama (Cornejo *et al.* 2019).

Family	BMWP
Atyidae	6
Baetidae	7
Belostomatidae	3
Caenidae	5
Calopterygidae	7
Ceratopogonidae	2
Chironomidae	2
Coenagrionidae	7
Collembola	6
Corbiculidae	7
Corydalidae	6
Cosmopterygidae	8
Crambidae	3
Culicidae	2
Ecnomidae	8
Elmidae	7
Glossosomatidae	6
Gomphidae	5
Hebridae	6
Helicopsychidae	4
Heptageniidae	6
Heteragrionidae	7
Hydrobiidae	5
Hydrobiosidae	7
Hydrophilidae	3
Hydropsychidae	7
Hydroptilidae	2
Hydroscaphidae	7
Lepidostomatidae	6
Leptoceridae	5
Leptohyphidae	7
Leptophlebiidae	5
Libellulidae	6
Limnychidae	5
Mesoveliidae	7
Naucoridae	3
Odontoceridae	4
Palaemonidae	4

Family	BMWP
Perlidae	6
Pseudothelphusidae	4
Philopotamidae	6
Physidae	3
Planariidae	5
Planorbiidae	3
Platystictidae	6
Polycentropodidae	7
Polythoridae	9
Psephenidae	4
Psychodidae	3
Ptilodactylidae	7
Scirtidae	5
Simuliidae	6
Sphaeriidae	7
Staphylinidae	8
Syrphidae	1
Tabanidae	7
Thiaridae	4
Tipulidae	2
Tubificidae	0
Veliidae	4
Xiphocentronidae	7

metrics were computed as $\%Group = 100 \times (nGroup/N)$, where $nGroup$ is the number of individuals in the functional group and N is the total number of individuals captured under standardized effort. Each metric was assigned an ordinal score $S_i \in \{0, 5, 10\}$ according to the threshold ranges presented in Table II (based on Lyons *et al.* 1995, where i identifies the metric ($i = 1-10$)). Metrics representing degradation (e.g., % tolerant, % exotic, % omnivores, % diseased/deformed) were inversely scored; thus, values of 0% received 10 points when within the “Good” threshold range. No additional normalization procedures were applied beyond percentage calculation. Absences were retained as zero values, and no minimum abundance threshold was applied to rare species. The IBI score was calculated as the unweighted sum of metric scores: $IBI = \sum_{i=1}^{10} S_i$, yielding a possible range of 0–100. Similar metrics have been used in other studies (Karr 1981, Karr *et al.* 1986, Newton *et al.* 1998, Mafla 2005, McLarney *et al.* 2008, Ramírez-Herrejón *et al.* 2012). As previously mentioned, fish-based IBI was combined

Table II. Scoring criteria for Metrics to calculate the Index of Biotic Integrity (IBI) based on fish in the Caimito River Basin (453 Km²), for a Basin Area of more than 400 Km² based on Lyons *et al.* (1995).

Metrics	Possible Scores		
	0 (Poor)	5 (Fair)	10 (Good)
Number of Native species	< 4	4 to 6	> 6
Number of Benthic species	< 5	5 to 25	> 25
Percent Native Live bearing species individuals	<25	25-75	>75
Number of Water column species	< 2	2 to 4	> 4
Number of Sensitive species	0	1 to 2	> 2
Percent of Tolerant Species	> 90%	90 to 10%	< 10%
Percent of Exotic species	> 25%	25 to 5%	< 5%
Percent of Omnivores	> 95%	95 to 85%	< 85%
# Fish/ 30 min	< 60	N/A	> 60
Percent of Diseased-Deformed individuals	> 5	5 to 1	< 1

with other indices, such as the Biological Monitoring Working Party (BMWP) score for macroinvertebrates, increasingly used for assessing water quality and ecosystem health. Studies in the Illinois River basin and the Ganjiang River system in China demonstrated that both fish and macroinvertebrates reflect different aspects of river health, supporting the value of multi-taxa approaches in ecosystem assessments (Holtz 2010, Xu *et al.* 2022). However, such combined studies are relatively rare in Central America, emphasizing the need for a holistic understanding of aquatic health alongside water security. The metrics range from very poor to good condition (Table II). We adopted an approach designed to minimize large sample requirements while facilitating collaborative and easy-to-implement monitoring methods for key stakeholders, including the Caimito River Basin Committee (Gaceta Oficial 2018) and associated government, civil society, and academic institutions. This strategy provides a practical and timely evaluation of environmental conditions in a watershed where management pressures continue to increase. Because detailed functional information for Panamanian freshwater fishes remains limited, species attributes were assigned using available regional literature and local ecological knowledge. Species were classified according to origin, trophic role, tolerance to degraded water quality, and position in the water column. Regional ichthyological references for Mesoamerica (Bussing 1998, Espinosa-Pérez *et al.* 2019, González-Gutiérrez 2021) were used to define functional categories and trophic groups, while the presence of native livebearers across multiple sections of the basin was considered following

Valdés-Díaz (2020). Field observations of diseased or deformed individuals were recorded during sampling and verified in the laboratory when necessary.

Sampling and analysis of physical, chemical, and biological water parameters were conducted at the nine specified sampling sites on the same dates as the fish and macroinvertebrate collections to develop a scale for a Water Quality Score (WQS), modified from Lyons (1992) and Lyons *et al.* (1995). The parameters analyzed included pH, temperature, dissolved oxygen, turbidity, total suspended solids, total solids, biochemical oxygen demand, chemical oxygen demand, nitrites, nitrates, and ammoniacal nitrogen. Most parameters were measured in the field using multiparameter devices and complemented by laboratory analyses. These variables were integrated into a traditional Water Quality Index (WQI; ICA), following the weighted additive approach described by Bhargava (1983), where each parameter is converted to a standardized sub-index (q_i , 0–100) and combined using assigned weights (w_i ; $\sum w_i = 1$)

$$WQI = \sum (w_i \cdot q_i)$$

To facilitate direct comparison with the Habitat Quality Score (HQS), the Index of Biotic Integrity (IBI; Lyons 1992, Lyons *et al.* 1995), and the BMWP-PAN index (Cornejo *et al.* 2019), the WQI (0–100) was linearly rescaled to a 0–10 Water Quality Score using:

$$WQS = WQI / 10$$

This transformation preserves the relative ranking among sampling sites and allows direct comparison across indices expressed on a common 1–10 scale.

The categorical ranges presented in Table III are interpretative and do not affect the linear transformation used to calculate WQS values. This scoring system also allowed us to standardize comparisons with HQS, which integrates qualitative and quantitative habitat information (Table IV).

Table III. Linear transformation of the Water Quality Index (WQI, 0–100) into the Water Quality Score (WQS, 0–10) used for comparison with IBI and HQS in the Caimito River Basin. WQS was calculated as $WQS = WQI / 10$. The transformation is strictly linear and preserves the relative ranking among sampling sites.

WQI (0–100)	WQS (0–10)	Water quality category
80–100	8.0–10.0	Excellent
51–79	5.1–7.9	Acceptable
26–50	2.6–5.0	Polluted
0–25	0.0–2.5	Heavily polluted

Habitat Quality Score (HQS). Habitat attributes were assessed along each 100-m transect by recording the percentage cover of substrate types (mud/clay, sand/gravel, pebbles/blocks), instream elements (leaf litter, logs/branches, cavities and structural shelters), canopy cover, pools, and bank condition (exposed or vegetated) using an adapted method from Zeni *et al.* (2019). Within the IBI framework of Lyons *et al.* (1995), habitat condition supports the interpretation of biological integrity, although no standalone numerical index was originally defined. Following that approach, habitat structure was operationalized using the percentage of structural shelters as an integrative proxy of habitat complexity, reflecting the availability of physical refuge and within-reach heterogeneity (Table IV). Structural shelters were estimated as the proportion of the reach containing refuge elements such as undercut banks, cavities, woody debris, and structural cover. This percentage was transformed to a 1–10 scale ($HQS = \text{structural shelters} [\%] / 10$). No categorization or threshold-based scoring was applied in the HQS calculation; the transformation is strictly continuous. The remaining habitat variables were used to characterize environmental context and support multivariate analyses.

Environmental Quality Score (EQS). The Environmental Quality Score (EQS) was calculated by integrating the Water Quality Score (WQS) and the Hab-

itat Quality Score (HQS) using a limiting-factor approach consistent with Lyons *et al.* (1995). Both WQS and HQS were first expressed on a standardized 1–10 scale. The EQS for each site and sampling period was then computed as the minimum of these two values, according to the rule: $EQS = \min(WQS, HQS)$. No additional weighting, averaging, or transformation was applied. This approach ensures that overall environmental quality reflects the most limiting condition between physicochemical water quality and structural habitat complexity. The resulting EQS values were subsequently used for comparison with biological indices (IBI and BMWP) as a conservative measure of environmental condition.

Data analysis. Based on Lyons *et al.* (1995), non-parametric Kruskal–Wallis tests were used to evaluate differences in individual IBI metric scores among sites and basin sections (upper, middle, and lower), allowing assessment of spatial variation in metric responses to environmental gradients. Given the seasonal sampling design, quantitative analyses were complemented with qualitative observations of water and habitat conditions. Variables used to calculate the Water Quality Score (WQS) and Habitat Quality Score (HQS) were analyzed using Principal Component Analysis (PCA) to explore relationships among physicochemical parameters, habitat attributes, and fish assemblage structure. Physicochemical and habitat variables were treated as active variables, whereas composite indices (IBI, WQS, HQS, EQS, BMWP) were projected as supplementary variables. For correlation and multivariate analyses, raw IBI and BMWP values calculated at the site $\times$ sampling event level were used, whereas site-level values correspond to arithmetic means across sampling events. Spearman rank correlations were conducted between IBI and WQS, HQS, EQS, and BMWP, and between BMWP and both HQS and EQS. All analyses were conducted in RStudio version 4.3.2 (R Core Team 2024).

Results and Discussion

In this study, we recorded 2,835 native individuals belonging to 34 species and 16 families (Table V). Freshwater resident taxa (primary and secondary freshwater species) represented more than 93% of total native individuals across the basin. Strictly marine–estuarine taxa accounted for less than 1% of individuals and were restricted to downstream sites, whereas diadromous species represented approximately 6% of total abundance, occurring mainly in the lower sector.

Table IV. Habitat Quality Scores (HQS) and attributes for the Caimito River Basin. The scores are derived from the percentage of structural shelters recorded in the field and transformed to a 1–10 scale ($HQS = \text{structural shelters } [\%] / 10$). The categorical descriptions below provide ecological interpretation of structural complexity associated with each score range. Percentage values mentioned in the descriptions reflect typical field conditions observed at those HQS levels and do not constitute independent components of the numerical HQS calculation.

Habitat Quality	Score	Habitat Quality Attributes
Good	8-10	High structural habitat complexity characterized by abundant physical refuge elements (e.g., undercut banks, cavities, woody debris, and structural cover). Typically associated with reaches where structural shelters exceed ~70% of the transect length, accompanied by heterogeneous substrates (pebbles, blocks, gravel), extensive canopy cover, and well-vegetated banks.
Fair	4-7	Moderate structural complexity with partial availability of refuge elements. Commonly associated with structural shelter coverage ranging approximately between 40–70%, variable substrate heterogeneity, and intermediate canopy cover and bank vegetation.
Poor	1-3	Low structural complexity with limited refuge elements. Often associated with structural shelter coverage below ~40% of the transect length, dominance of fine sediments (mud/clay), exposed or unstable banks,

Depth ranges overlapped among upper, middle, and lower basin sites, and the same multi-gear sampling protocol was applied consistently across locations and seasons. Because sampling effort and gear combinations were standardized, differences in assemblage structure are best explained by variation in habitat integrity, water quality, and anthropogenic pressure rather than by methodological bias. Although diadromous taxa and a few marine–estuarine species were recorded in downstream sites, their numerical contribution was low and restricted to the lower basin, indicating that marine influence did not dominate assemblage composition at the basin scale (Table V). The IBI metrics used here are based on functional composition (e.g., trophic structure, tolerance, habitat use, livebearers, exotics, and fish condition), so the presence of amphidromous or diadromous species is interpreted as a natural longitudinal feature of Pacific-draining tropical rivers. This interpretation is consistent with Lyons *et al.* (1995), who applied a fish-based IBI in Pacific rivers of Mexico where amphidromous taxa occur but biological integrity is evaluated through functional responses to disturbance. Following Lyons (1995), additional feeding categories such as herbivores and carnivores were recorded for ecological interpretation but excluded from the final IBI calculation.

Number of Native Species. Significant differences in native species diversity were observed across sites (p -value = 0.028). Altos del Cerro, for example, differed significantly from La Valdeza (2 vs 15 species), both at the high basin. Such results suggest that local environmental conditions, such as habitat structure and water quality, play a more significant

role in shaping the site's species richness. Moreover, Altos del Cerro is located at the river's head, showing a steeper topography as well as unique characteristics that require specific adaptations from certain species. Native species did not exhibit significant differences between basin portions (p -value = 0.595). This suggests that broader basin-wide factors, such as geographical scale and river characteristics, do not strongly influence their distribution. Instead, the lack of differences may reflect species exchanges across portions of the basin, facilitated by their ecological adaptations. This exchange of species between sites further underscores the role of site-specific habitat features in shaping the biodiversity patterns within the basin. Pusey *et al.* (2020) emphasizes the role of habitat complexity and flow regimes in structuring fish assemblages, showing that these factors influence the replacement of species across different parts of a river system.

Percent of Exotic Species. Significant differences in the percentage of exotics were detected across basins (p = 0.023) and across sites (p = 0.017), with the lower basin sites exhibiting a markedly higher percentage of exotic species compared to middle and upper basin areas (Fig. 2). The highest concentration of exotic fishes was in Aguacate, being dominated by *Oreochromis niloticus* (tilapia), followed by Martín Sánchez. This genus, used in aquaculture, is known globally for their capacity to outcompete and displace native fish communities, particularly in degraded environments with simplified habitats (Canonico *et al.* 2005). An isolated occurrence of one *Parachromis managuensis* (Guapote tigre) specimen was recorded at the high basin Caimito site, as well as an individual of *Hypostomus plecostomus* (Pez

Table V. Number of individuals and [species](#) captured at each study site in the Caimito River basin, West Panama Province. Numbers are total individuals per site. 'TOTAL species' counts the number of species present per site. Exotic species are indicated with an asterisk (*). Abbreviations: AC: Altos del Cerro, LV: La Valdeza, CA: Caimito, SR: Santa Rita, TR: Trapichito, MT: Mastranto, MB: Maribel, MS: Martín Sánchez, AG: Aguacate. Species names are hyperlinked to voucher photographs.

Family/species	AC	LV	CA	SR	TR	MT	MB	MS	AG	Total
Acestrorhamphidae										
<i>Astyanax panamensis</i>	0	55	123	107	108	44	13	19	100	569
Achiridae										
<i>Trinectes fluviatilis</i>	0	0	0	0	0	0	5	0	0	5
Bryconidae										
<i>Brycon argenteus</i>	0	53	16	45	1	0	0	0	0	115
<i>Brycon striatulus</i>	0	0	0	0	78	0	1	0	0	79
Cichlidae										
<i>Andinoacara coeruleopunctatus</i>	0	33	38	7	2	0	0	0	0	80
<i>Oreochromis niloticus</i> *	0	0	0	0	0	0	0	6	127	133
<i>Parachromis managuensis</i> *	0	0	1	0	0	0	0	0	0	1
Centropomidae										
<i>Centropomus unionensis</i>	0	0	0	0	0	1	0	0	1	2
Characidae										
<i>Compsura gorgonae</i>	0	21	6	0	9	0	0	0	0	36
<i>Roeboides occidentalis</i>	0	19	6	5	12	4	1	2	1	50
Curimatidae										
<i>Cyphocharax magdalenae</i>	0	0	0	0	0	0	0	0	14	14
Eleotridae										
<i>Dormitator latifrons</i>	0	0	0	0	0	0	0	25	58	83
<i>Eleotris picta</i>	0	0	0	0	0	21	1	0	9	31
<i>Gobiomorus maculatus</i>	0	0	0	0	0	6	9	9	9	33
<i>Leptophilypnus macrolepis</i>	0	0	0	0	0	0	1	0	0	1
Gerreidae										
<i>Eugerres brevimanus</i>	0	0	0	0	0	0	4	0	0	4
Haemulidae										
<i>Rhonciscus bayanus</i>	0	0	0	0	0	5	5	0	0	10
Heptapteridae										
<i>Pimelodella chagresi</i>	0	21	4	10	51	0	1	0	0	87
<i>Rhamdia guatemalensis</i>	0	0	0	0	1	0	0	0	0	1

Family/species	AC	LV	CA	SR	TR	MT	MB	MS	AG	Total
Hypopomidae										
<i>Brachyhypopomus occidentalis</i>	0	2	0	0	0	2	0	0	0	4
Loricariidae										
<i>Ancistrus chagresi</i>	0	15	0	1	7	0	0	0	0	23
<i>Leptoancistrus canensis</i>	0	10	0	0	8	0	1	0	0	19
<i>Hypostomus aspidolepis</i>	0	3	0	0	23	3	1	0	3	33
<i>Hypostomus plecostomus*</i>	0	0	0	1	0	0	0	0	0	1
<i>Rineloricaria uracantha</i>	0	3	1	0	0	0	0	0	0	4
<i>Sturisomatichthys panamensis</i>	0	0	0	101	458	6	38	0	0	603
Mugilidae										
<i>Dajaus monticola</i>	0	0	0	0	0	2	1	2	2	7
Oxudercidae										
<i>Awaous transandeanus</i>	0	0	0	1	0	0	0	1	2	4
<i>Sicydium salvini</i>	0	0	0	0	0	1	0	0	0	1
Poeciliidae										
<i>Brachyrhaphis episcopi</i>	254	89	1	14	1	1	0	0	0	360
<i>Neoheterandria tridentiger</i>	0	0	0	6	37	0	1	12	77	133
<i>Poecilia gillii</i>	0	15	48	2	9	6	1	11	15	107
<i>Poeciliopsis turrubarensis</i>	0	0	0	0	0	0	0	0	33	33
Rivulidae										
<i>Cynodonichthys brunneus</i>	14	0	0	0	0	0	0	0	0	14
Stevardiidae										
<i>Eretmobrycon emperador</i>	0	148	6	0	0	0	0	0	0	154
<i>Gephyrocharax atracaudatus</i>	0	61	4	28	26	4	0	0	9	132
Syngnathidae										
<i>Pseudophallus elcapitanensis</i>	0	0	0	0	0	0	1	2	1	4
TOTAL										
<i>Native individuals</i>	268	548	253	327	831	106	85	83	334	2835
TOTAL										
<i>Exotic individuals</i>	0	1	0	1	0	0	0	6	127	135
TOTAL										
<i>Native species</i>	2	14	12	12	16	14	17	9	15	34
TOTAL										
<i>Exotic species</i>	0	1	0	1	0	0	0	1	1	3



Figure 2. Distribution of exotic fish species across sampling sites in the Caimito River Basin, West Panama Province. Labels indicate the number of individuals and the percentage contribution of each exotic species to the local fish assemblage. Exotic species were concentrated primarily in lower basin sites, particularly Aguacate and Martín Sánchez, while only isolated individuals of *Parachromis managuensis* and *Hypostomus plecostomus* were recorded in upper and middle basin sites.

Diablo) at the middle site Santa Rita. Both species pose significant ecological risks (Barros *et al.* 2012, Scott *et al.* 2012). *Parachromis managuensis* is a large predatory cichlid capable of severely impacting native fish assemblages through direct predation and trophic disruption, with strong naturalization potential in tropical rivers (Holmes *et al.* 2020, González-Gutiérrez 2016). *Hypostomus plecostomus* affects the food web and destabilizes riverbanks through burrowing, exacerbating sedimentation, and competing with native benthic fishes (Pound *et al.* 2011). Beyond our study sites, *P. managuensis* is present in other Panamanian basins (e.g., Lakes Gatún and Alajuela, Contreras-MacBeath *et al.* 2022). Regionally it has been used in tilapia culture as a predator to limit recruitment (Dunseth & Bayne 1978). For *H. plecostomus*, we found no verified records from

other Panamanian basins; elsewhere its introductions are primarily linked to aquarium releases (Nico & Neilson 2025). Maintaining or restoring native biodiversity and habitat complexity can provide biotic resistance against invasive species (Moyle & Light 1996). Similarly, intact riparian zones and structurally complex stream habitats hinder the proliferation of aggressive species like tilapias by preserving ecological filters and stabilizing trophic networks (Canonico *et al.* 2005). Although direct competition was not evaluated in the Caimito Basin, the presence of resilient native communities and the maintenance of natural habitat features are likely key factors preventing a broader invasion at this stage. Restoration of riparian vegetation and reduction of anthropogenic disturbances should be prioritized to enhance

natural resistance to invasion and safeguard the biotic integrity of the Caimito River Basin.

Number of Benthic Species. Significant differences in the number of benthic species were observed across sites (p -value = 0.016), particularly highlighting the ecological distinctiveness of La Valdeza, which exhibits not only greater stream width (5.59–7.9 meters), compared to Altos del Cerro (1.26–2.13 meters), but also superior structural habitat quality (77.5% structural shelters) and a more heterogeneous substrate composition than most of the other sites where urbanization and anthropogenic pressures have reduced habitat quality and increased sedimentation. These conditions provide broader ecological niches for benthic fish species, supporting key ecological functions such as organic matter decomposition and nutrient cycling. The absence of significant differences at the basin level (p -value = 0.226) reflects internal ecological consistency within each basin grouping, where relatively homogeneous impacts or environmental conditions prevail. This finding highlights the capacity of benthic species metrics to sensitively reflect local habitat quality and degradation gradients when properly assessed (Lyons *et al.* 1995, Lyons *et al.* 2000).

Number of Water Column Species. Water column species are considered a positive metric within fish-based Index of Biotic Integrity (IBI) frameworks since they occupy the midwater portion of the water column and are highly dependent on variable flow regimes, high dissolved oxygen concentrations, and low sedimentation levels (Lyons *et al.* 1995, Barbour *et al.* 1999). Their life histories often include active swimming behaviors, visual foraging strategies, and reproduction strategies that require specific flow and oxygen conditions, making them highly sensitive to physical habitat degradation and water quality deterioration (Casatti *et al.* 2006, Casatti *et al.* 2009). In our study, significant differences in the number of water column species were detected across sites (p -value = 0.038), although no significant differences were observed between basins (p -value = 0.334). In well-preserved upper basin sites such as Altos del Cerro, the richness of water column species was naturally low, reflecting geomorphological constraints (steep gradients, narrow channels, and rapid flows) that inherently limit midwater habitat availability. Conversely, La Valdeza supported greater richness of mid-water species, associated with broader channels, moderate flows, and complex instream structures providing suitable niches. Moderate richness persisted in middle basin sites like Santa Rita and Trapichito, where habitat structure and riparian can-

opy are partially preserved. However, in lower basin sites such as Maribel, Martín Sánchez and Aguacate, significant degradation may result in a decline of mid-water species (Casatti *et al.* 2006). The lack of significant differences across basins, but clear site-level patterns, suggests that localized degradation processes—especially sedimentation, loss of cover, and altered flows—are the primary drivers affecting this metric (Barbour *et al.* 1999; Casatti *et al.* 2009). **Percent of Native Live Bearing Species.** Although the use of the percent of Native Live bearing Species metric has been discussed regarding its sensitivity limitations—due to the moderate tolerance of some livebearers (Lyons *et al.* 2000), our findings demonstrate, that the application of this metric is informative for assessing biotic integrity patterns in lower Central American basins. Significant differences in the percentage of native live bearing species were found across sites (p -value = 0.011) and basins (p -value = 0.010). In the well-preserved upper basin site Altos del Cerro, the fish assemblage was dominated by *Brachyrhaphis episcopi*, an endemic livebearer to Panama that we found in the less degraded habitats in this study, mainly in the high basin; this is consistent with the role of sensitive livebearers as indicators of ecological integrity (Lyons *et al.* 1995).

At La Valdeza, *Brachyrhaphis episcopi* exhibited a noticeable decrease in abundance, compared with Altos del Cerro, and was replaced by *Poecilia gillii* as we moved down the basin. *Poecilia gillii* is a native livebearer recognized for its moderate ecological tolerance and its ability to persist in moderately disturbed environments. We found it in greatest abundances at the last high basin site Caimito, and in the middle basin sites Santa Rita and Trapichito.

In highly impacted lower basin sites like Maribel, Aguacate and Martín Sánchez, a marked decline in native livebearers was observed, coinciding with intensified urbanization, increased sedimentation, and water quality degradation. Poecilids can inhabit a variety of freshwater conditions and even low-salinity estuarine habitats, some species are able to occupy transitional zones along the river continuum (Valdés-Díaz 2020). Even moderately resilient species such as *Poecilia gillii* declined in the most impacted sites, indicating that severe degradation surpasses their resilience threshold.

Number of Sensitive Species. The presence of sensitive fish species is one of the most critical metrics in fish-based Index of Biotic Integrity (IBI) frameworks, as these species tend to exhibit narrow environmental tolerances, requiring stable flow regimes, high dissolved oxygen concentrations, and structur-

ally diverse habitats to persist. Their absence or disappearance is a strong indicator of ecological degradation and stress, particularly from pollution, habitat fragmentation, or hydrological alteration (Lyons *et al.* 1995).

In our study significant differences in the number of sensitive species were observed between basins ($p = 0.025$), but not among sites ($p = 0.080$), suggesting that broader-scale environmental variation—particularly between the more conserved upper basin and the increasingly impacted lower basin—plays a stronger role in structuring the distribution of these environmentally demanding species. Sites in the upper basin, notably La Valdeza, exhibited higher richness of sensitive taxa, which include endemic or stenotopic species that are intolerant of siltation, chemical disturbance, or physical homogenization. The higher number of sensitive species in La Valdeza, despite its proximity to more impacted areas, may reflect remnant habitat quality and streamflow regulation conditions. Conversely, in sites located in the high basin (Caimito) and the middle and lower basin (Maribel, Martín Sánchez, and Aguacate), sensitive species were present in very low abundance or absent. In our study, the decline in sensitive species at impacted sites is consistent with previous findings that stressors such as turbidity, nutrient enrichment, and habitat simplification can eliminate these taxa even before noticeable shifts in overall richness occur (Barbour *et al.* 1999, Casatti *et al.* 2006).

Percent of Tolerant Species. Tolerant species are commonly used in IBI calculations to indicate, where they are dominant, sites affected by various forms of degradation, as their persistence often reflects the capacity to withstand low dissolved oxygen, turbidity, or habitat simplification (Lyons *et al.* 1995, Ramírez-Herrejón *et al.* 2012). In the Caimito River basin, the percentage of tolerant species varied significantly across sites ($p = 0.011$), indicating localized environmental degradation patterns. No significant differences were observed at the basin scale ($p = 0.167$), suggesting that broader geographic grouping does not fully capture the heterogeneity of anthropogenic pressures affecting fish assemblages at the site level.

Astyanax panamensis was classified as a tolerant species based on its high abundance across a gradient of anthropogenic pressures and the well-documented ecological plasticity within this genus (Schulz *et al.* 2001, Trujillo-Jiménez *et al.* 2011, Manna *et al.* 2012). This species, native to Costa Rica and Panama and relatively common in dis-

turbed river systems, may be presumed to exhibit resilience to eutrophication and altered flow regimes—patterns previously documented in other congeneric species inhabiting Neotropical rivers systems (Lima-Junior & Guarisso de Souza 2013). Our results indicate that *A. panamensis* dominates in mid- to low-basin sites where urban runoff, modified riparian vegetation, and sedimentation are more evident. This dominance is consistent with reported traits in *Astyanax* congeners, such as morphological plasticity, generalist feeding structures, and gill remodeling that enhance oxygen uptake under hypoxic conditions. Furthermore, although the genus *Poecilia* is considered tolerant (Gomes-Silva *et al.* 2020) and was present at the most degraded sites, its decreasing numbers relative to *A. panamensis* indicate that the latter is the primary contributor to this metric; *Poecilia* persists by performing aquatic-surface respiration and enduring warm, nutrient-rich, low-oxygen waters (Gomes-Silva *et al.* 2020). Likewise, some eleotrids—e.g., *Dormitator latifrons*—remain in impacted reaches owing to marked hypoxia tolerance supported by large gill surface area and cutaneous respiration, together with broad salinity tolerance (euryhalinity), traits that allow survival in low-dissolved-oxygen and variable-salinity waters (Vega-Villasante *et al.* 2021).

Omnivores. The proportion of omnivorous fish is widely used in IBI assessments as a negative indicator of habitat quality. According to Lyons *et al.* (2000), higher percentages of omnivores typically reflect environmental degradation because these generalist feeders can thrive in disturbed habitats where more specialized species may decline. In the Caimito River basin, we observed significant differences in the abundance of omnivorous species across sites (p -value = 0.012), particularly between Altos del Cerro and Caimito. At Altos del Cerro, which represents the headwater zone characterized by high elevation, steeper gradients, and more intact riparian cover, the abundance of omnivores was low. In contrast, the Caimito site, affected by urban runoff, low canopy cover (30%), and channel modifications, exhibited a much higher proportion of omnivores. This pattern aligns with ecological expectations that omnivores dominate in degraded systems due to their dietary plasticity and tolerance to habitat shifts (Karr *et al.* 1986, Ramírez-Herrejón *et al.* 2012). Although no significant differences were found across basins (p -value = 0.770), site-specific contrasts were evident. The lower basin sites (e.g., Martín Sánchez and Aguacate) also showed high omnivore percentages,

consistent with their altered physical structure and likely nutrient enrichment.

Fish per 30 Minutes. The abundance of native species, measured as the number of individuals collected during 30 minutes, is a key metric in the Index of Biotic Integrity (IBI). It serves as a positive indicator of ecological quality, since high native fish abundance reflects suitable habitat conditions and low environmental stress. Conversely, a decline in native fish abundance often signals habitat degradation or altered flow regimes (Lyons *et al.* 1995, Ramírez-Herrejón *et al.* 2012). In the Caimito River basin, native species abundance did not vary significantly across sites ($p = 0.091$), nor at the basin scale ($p = 0.053$), although the latter was close to significance. Despite these results, which suggest a tendency, impacted sites such as Aguacate, Martín Sánchez, and Caimito town exhibited lower fish abundance values, likely due to increased sedimentation, urban runoff, and altered substrate structure. This supports findings from other tropical systems, where local habitat integrity plays a greater role in sustaining native assemblages (Tedesco *et al.* 2005). Although the assemblage included a wide range of native freshwater taxa, the relatively low numbers of diadromous and estuarine-associated species compared with other Pacific-draining rivers in Eastern Panama (Pacora river) suggest that environmental degradation may be limiting their local abundance. These similar systems, with relatively lower levels of disturbance, have reported higher abundances of these taxa (Osorio 2024). Additionally, the presence of a major geomorphological barrier in the basin (El Chorro de La Chorrera, ~30 m waterfall) may also restrict longitudinal connectivity for some migratory fishes, while the high densities of exotic species such as *Oreochromis niloticus* in the lower basin may further contribute to reduced native fish abundance through competition and habitat alteration, as documented for invasive tilapias in tropical freshwater ecosystems (Canonico *et al.* 2005).

Percent of diseased or Deformed Individuals.

No significant differences were found in the percentage of diseased or deformed individuals across either sites ($p = 0.218$) or basins ($p = 0.747$), likely due to the overall low frequency of visibly affected fish in the samples, which limits the statistical power of this metric, but provides some qualitative ecological insight. A total of two sampling events (dry and intermediate season) recorded 3 native fish individuals in poor physical condition, and these were associated with sites of medium to high human disturbance. The affected species included relatively

tolerant species, one individual of *Poecilia gillii* (Caimito) with parasite spots, and two individuals of *Astyanax panamensis* (Trapichito and Martín Sánchez), with deformities in head and column. These findings are consistent with previous literature indicating that even small proportions of morphologically abnormal fish can signal chronic exposure to pollutants or environmental stressors (Adams *et al.* 1993, Teh *et al.* 1997). Conversely, no affected individuals were found in other study sites.

A summary of the Kruskal-Wallis tests for each IBI metric across sites and basins is provided in Table VI. These results support the site-level patterns discussed above and serve as the statistical foundation for the integrative index evaluation.

Physicochemical Variables. One of the primary aspects analyzed among physical variables was river width (stream size). However, this measurement did not show significant variation either by Basin (p -value = 0.064) or Site (p -value = 0.064), indicating that the river's dimensions remained relatively constant across the studied area. This lack of significant variation minimizes confounding effects when examining other ecological parameters, providing a stable baseline for comparison. This suggests that other factors, such as local habitat conditions, water quality, and food availability, are more likely to shape fish community structure among rivers if the width variation is not marked (Spurgeon *et al.* 2019). The analysis of individual IBI metrics provided a comprehensive understanding of how fish assemblages respond to localized environmental variation across the Caimito River Basin. Differences in native species' richness, benthic composition, trophic structure, and the occurrence of tolerant, sensitive, exotic, and diseased individuals clearly reflect spatial gradients in disturbance and habitat quality. However, these metrics also vary in sensitivity and spatial resolution. To integrate this ecological information into a cohesive site-level assessment of environmental condition, we subsequently evaluated composite indicators, including the Index of Biotic Integrity (IBI), the Water Quality Score (WQS), and the Habitat Quality Score (HQS). Physicochemical conditions varied across sites (Table VII), influencing fish assemblages and IBI metric performance. Upper basin sites such as Altos del Cerro and La Valdeza showed lower conductivity and higher dissolved oxygen. In contrast, lower basin sites like Aguacate and Maribel exhibited elevated conductivity, turbidity, and temperature—typical of urban disturbance. These variables are known to drive reductions in native fish diversity and favor tolerant and

Table VI. Summary of IBI Metric Variation by Site and Basin. The Kruskal–Wallis test was used to evaluate differences in IBI metrics across sites and basins ($\alpha = 0.05$).

Parameter	Factor	Kruskal-Wallis Chi-Squared	p-value	Significance
Number of Native species	Basin	1.0372	0.595	Not Significant
Number of Native species	Site	17.188	0.028	Significant
Number of Benthic species	Basin	2.969	0.226	Not Significant
Number of Benthic species	Site	18.734	0.016	Significant
Percent of Native Livebearing	Basin	9.1174	0.010	Significant
Percent of Native Livebearing	Site	19.814	0.011	Significant
Number of Water Column species	Basin	2.1887	0.334	Not Significant
Number of Water Column species	Site	16.278	0.038	Significant
Number of Sensitive species	Basin	7.3103	0.025	Significant
Number of Sensitive species	Site	14.03	0.080	Not Significant
Percent of Tolerant species	Basin	3.5711	0.167	Not Significant
Percent of Tolerant species	Site	19.704	0.011	Significant
Percent of Exotic species	Basin	7.488	0.023	Significant
Percent of Exotic species	Site	18.603	0.017	Significant
Percent of Omnivores	Basin	0.52268	0.770	Not Significant
Percent of Omnivores	Site	19.558	0.012	Significant
Fish/ 30 min	Basin	5.8687	0.053	Not Significant
Fish/ 30 min	Site	13.656	0.091	Not Significant
Percent of diseased or deformed	Basin	0.58147	0.747	Not Significant
Percent of diseased or deformed	Site	10.716	0.2183	Not Significant

exotic species (Casatti *et al.* 2009, Teresa & Casatti 2012). The environmental variation captured by these parameters contextualizes the biological patterns described earlier and serves as a foundation for the integrated index assessment.

Index based testing and validation. The Index of Biotic Integrity (IBI) scores revealed a consistent ecological gradient across the Caimito River basin, reflecting cumulative impacts on fish communities and habitat quality stated in the guideline for interpreting IBI scores (Table VIII).

La Valdeza (82) exhibited the highest score, consistent with the relatively well-preserved conditions of the upper basin, characterized by high canopy cover, heterogeneous substrates, elevated dissolved oxygen ($\sim 6 \text{ mg L}^{-1}$), and low turbidity ($\sim 10 \text{ NTU}$), among other favorable attributes. These findings agree with previous studies indicating that structurally complex habitats tend to support more intact biotic assemblages (Lyons *et al.* 1995, Casatti *et al.* 2009). In contrast, Altos del Cerro (65), also in

the upper basin, despite its generally favorable conditions, is surrounded by light small-scale agricultural activity. Together with Trapichito (68), Santa Rita (63), and Mastranto (56) in the mid-basin, these sites obtained moderate but progressively lower scores, reflecting partial habitat degradation yet retention of key functional groups, including sensitive taxa and intermediate trophic guilds. Caimito (55), also in the upper basin, while still within the “fair” category, showed a reduced score likely linked to urban runoff from the local town and riparian disturbance. Lower-basin sites—Maribel (55) and Aguacate (52)—also fell within the “fair” category, but their scores were close to the lower threshold, indicating higher environmental stress and reduced presence of sensitive taxa. These intermediate values suggest a transitional ecological status consistent with moderate environmental stress (Teresa & Casatti 2012). Martín Sánchez (38) was the only site classified as “poor,” with fish assemblages dominated by tolerant and exotic species and very low

Table VII. Average values (SD) of physicochemical parameters per site. pH = Potential of Hydrogen; Temp. = Temperature in degrees Celsius (°C); Turbidity = Measure of water clarity; TSS = Total Suspended Solids; TS = Total Solids; TDS = Total Dissolved Solids; BOD = Biochemical Oxygen Demand; COD = Chemical Oxygen Demand; Nitrite = NO₂⁻; Nitrate = NO₃⁻; Ammonia = NH₃ or NH₄⁺. All concentrations are in mg/L, except pH (unitless), temperature (°C), turbidity (NTU), and conductivity (µS/cm).

Site	pH	Temp (°C)	DO (mg/L)	Turbidity (NTU)	TSS (mg/L)	TS (mg/L)	TDS (mg/L)	BOD (mg/L)	COD (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Conductivity (µS/cm)
Altos del Cerro	7.37 (0.32)	24.90 (2.04)	6.05 (1.22)	10.35 (8.07)	13.83 (7.25)	95.67 (35.92)	65.83 (21.01)	4.17 (2.85)	14.00 (6.56)	0.06 (0.05)	2.17 (1.27)	0.04 (0.01)	100.80 (19.25)
Valdeza	7.88 (0.48)	26.47 (0.81)	5.97 (0.31)	11.11 (13.94)	8.46 (5.39)	107.00 (26.89)	87.90 (27.03)	4.97 (3.00)	19.67 (11.24)	0.94 (1.52)	1.33 (1.50)	0.04 (0.01)	132.16 (27.55)
Caimito	7.50 (0.64)	26.83 (0.31)	4.33 (1.53)	26.15 (32.19)	20.27 (14.40)	132.00 (17.44)	100.13 (8.89)	6.67 (5.03)	19.00 (3.61)	0.08 (0.04)	2.20 (1.31)	0.07 (0.03)	166.83 (48.45)
Santa Rita	7.70 (0.61)	27.07 (0.51)	6.23 (1.21)	48.37 (62.69)	59.99 (90.08)	147.74 (109.49)	83.67 (28.28)	5.67 (3.51)	27.33 (18.15)	0.07 (0.04)	2.33 (1.27)	0.04 (0.02)	115.56 (32.26)
Trapichito	7.72 (0.42)	27.30 (0.96)	5.77 (1.42)	43.97 (43.16)	14.25 (9.74)	99.00 (35.51)	79.03 (26.00)	6.53 (5.06)	23.33 (10.26)	0.10 (0.07)	2.67 (1.67)	0.29 (0.30)	120.86 (25.50)
Mastranto	7.83 (0.46)	27.83 (0.81)	6.85 (0.87)	46.57 (57.56)	15.31 (4.60)	104.00 (22.34)	81.35 (17.93)	9.73 (7.51)	25.00 (7.00)	0.12 (0.09)	2.97 (2.32)	0.38 (0.32)	131.53 (40.52)
Maribel	8.03 (0.32)	28.33 (0.81)	5.30 (0.86)	72.13 (58.66)	10.50 (2.78)	100.33 (22.01)	81.17 (10.77)	10.43 (7.47)	30.00 (9.17)	0.11 (0.09)	2.77 (2.06)	0.17 (0.21)	151.76 (23.84)
Martin	7.66 (0.43)	27.53 (0.42)	4.31 (1.79)	8.93 (0.59)	13.07 (6.81)	149.33 (26.10)	138.83 (25.96)	12.27 (9.21)	30.00 (8.89)	0.22 (0.17)	3.60 (1.01)	3.14 (3.19)	263.50 (72.35)
Sanchez Aguacate	7.80 (0.37)	27.73 (0.55)	4.22 (1.63)	47.29 (72.50)	15.02 (8.77)	157.33 (18.58)	140.27 (24.12)	12.03 (10.49)	26.67 (13.32)	0.15 (0.07)	3.20 (1.67)	1.02 (0.87)	250.26 (43.33)

Table VIII. Guidelines for Interpreting Scores in the Caimito River Basin adapted and adjusted from Lyons *et al.* (1995). BMWP ecological classes follow Cornejo *et al.* (2019) and are shown here only to facilitate qualitative comparison with IBI ratings.

Score	Rating	Fish Community Attributes
70-100	Good	High native species diversity (> 6 species), significant presence of benthic (> 25 species), Live Bearers (> 75%) and water column species (>4 species), high numbers of sensitive species (> 2 species), low numbers of tolerant species (< 10%), low numbers of exotic species (< 5%), low percentages of omnivores (< 85 %), high fish counts per half-hour sampling (> 30 individuals) and low percent of diseased individuals (< 1%). BMWP values within the “good” (78–149) or “excellent” (≥150) classes defined by Cornejo <i>et al.</i> (2019) generally correspond to sites rated as “Good” under the fish-based criteria.
45-69	Fair	Moderate native species diversity (4-6 species), presence of some benthic (5-25 species), Live Bearers (25-75%) and water column species (2-4 species), moderate numbers of sensitive species (1-2 species), higher numbers of tolerant species (90-10 %), higher numbers of exotic species (25-5%), moderate percentages of omnivores (95-85%), and moderate fish counts per half-hour sampling (N/A) and moderate percent of diseased individuals (5-1%). BMWP values within the “regular” class (59–77) or at the lower end of the “good” class (78–149) may correspond to sites rated as “Fair” under the fish-based framework (Cornejo <i>et al.</i> 2019).
0-44	Poor	Low native species diversity (< 4 species), few benthic (< 5 species) and water column species (< 2 species), low numbers of sensitive species (0 species), high numbers of tolerant species (> 90%), high numbers of exotic species (> 25%), high percentages of omnivores (> 95%), low fish counts per half-hour sampling (<30 individuals) and higher percent of diseased-deformed individuals (> 5%). BMWP values within the “contaminated” (39–58), “very contaminated” (20–38), or “extremely contaminated” (≤19) classes defined by Cornejo <i>et al.</i> (2019) correspond to sites rated as “Poor” under the fish-based criteria.

abundance of native taxa. Such low scores are characteristic of heavily impacted environments and underscore the IBI's sensitivity to cumulative degradation (Gonino *et al.* 2020). This site-specific variation supports the interpretation that local-scale stressors, including land-use change, substrate alteration, and water-quality deterioration, are stronger drivers of biotic integrity than basin-level position alone. Although most sites remain within the “fair” category, a good number are approaching the poor-condition threshold (Table VIII, Table IX and Fig. 3).

Environmental Correlations and Integrated Index Comparison. Ordination of environmental, habitat, and fish community variables using PCA: vectors contributing to ecological gradients in the Caimito River Basin. The two dimensions of the Principal Component Analysis (PCA) of environmental and biological variables explained 32.6% of the total variance across the first two axes (Dim 1 (PC1) = 16.9 %, Dim 2 (PC2) = 15.7%). Dimension 1 represented a gradient of ecological degradation, positively correlated with variables indicative of impacted con-

ditions such as conductivity, ammonia, and biological oxygen demand (BOD). Although increases in conductivity in the lower basin may partially reflect natural estuarine connectivity, elevated conductivity values were also recorded at upstream and where

Table IX. Final Index of Biotic Integrity (IBI) average scores and Condition Categories for the Caimito Basin, West Panama Province. Condition categories are based on threshold values adapted from Lyons *et al.* (1995).

Site	IBI Score	Condition Category
La Valdeza	82	Good
Trapichito	68	Fair
Altos del Cerro	65	Fair
Santa Rita	63	Fair
Mastranto	56	Fair
Caimito	55	Fair
Maribel	55	Fair
Aguacate	52	Fair
Martín Sánchez	38	Poor

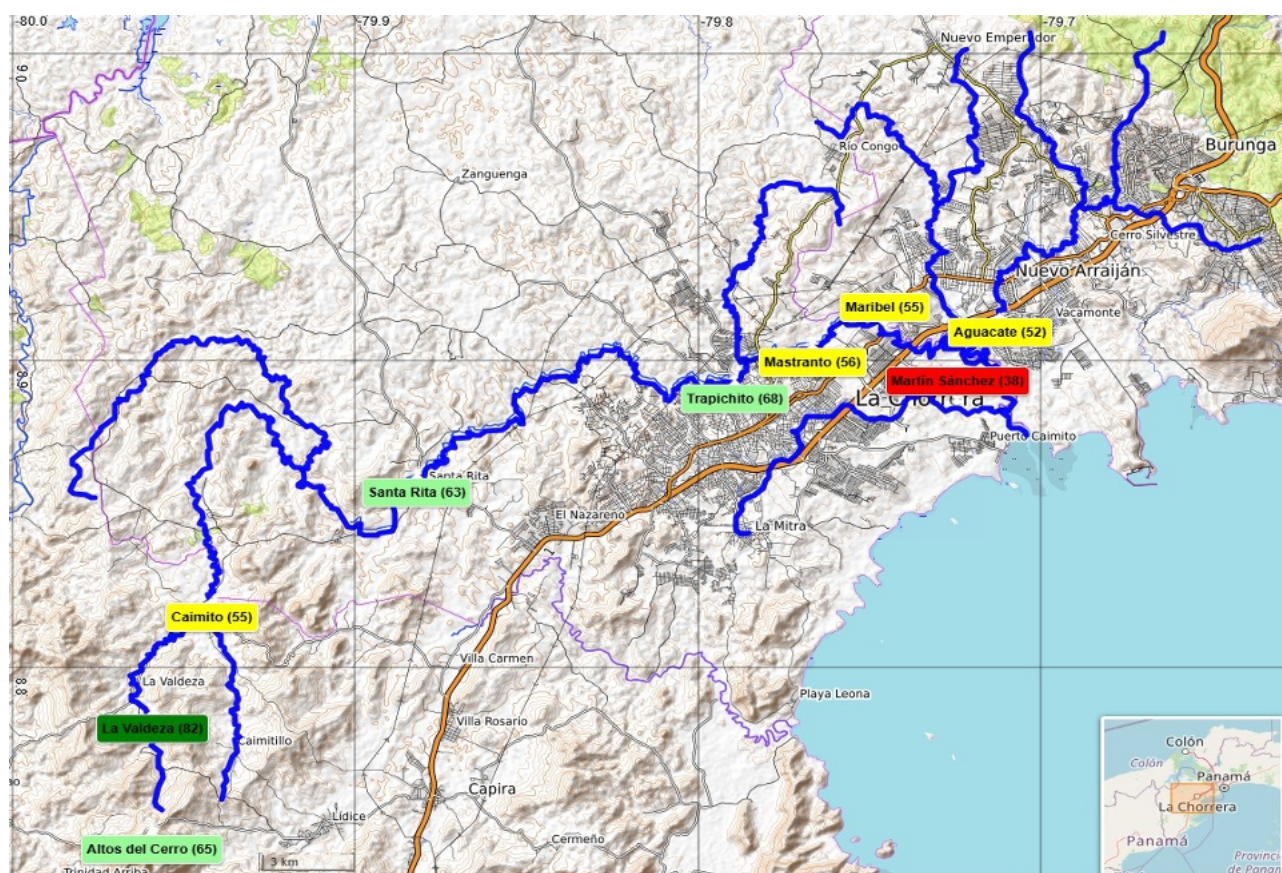


Figure 3. Caimito River Basin, West Panama Province: sampling sites and fish-based Index of Biotic Integrity (IBI) results. Labels indicate site name and IBI score. Color coding follows IBI categories with nuance within the “Fair” range: Good (70–100, dark green), Fair–High (65–69, light green), Fair–Low (45–64, yellow), and Poor (0–44, red). Blue = stream network; purple = basin boundary; orange = main roads; gray = terrain/urban base.

they co-occurred with higher ammonia and BOD concentrations (Table VII). This pattern indicates that conductivity is associated not only with downstream marine influence but also with watershed disturbance, supporting the interpretation of PC1 as an environmental impact gradient. In contrast, negative correlations along this axis included benthic species, native species richness, structural habitat features, and dissolved oxygen, suggesting better ecological integrity. Dimension 2 captured variation associated with river morphology and habitat complexity, such as pools, mud/clay substrate, canopy height, and live bearing species. While the vectors for composite scores like Environmental Quality Score (EQS), Habitat Quality Score (HQS), Water Quality Score (WQS), and Index of Biological Integrity (IBI) were oriented in the same direction as native richness and habitat structure, particularly structural shelters supporting their contribution to the integrity gradient, the BMWP was included in Dimension 2 along with the IBI score, reflecting a secondary gradient associated with habitat complexity and overall integrity (Fig. 4).

The PCA of study sites, grouped by basin, IBI quality, and site (Fig. 4), illustrates the spatial segregation of ecological conditions derived from the integrity gradient described in Figure 5. Sites such as Martín Sánchez and Aguacate are located at the positive end of Dim 1, overlapping with the “Poor” IBI quality class and the Low basin, indicating the most degraded conditions are driven by anthropogenic

pressure. Caimito, although situated in a highland basin, is also near the Poor cluster, suggesting that localized stressors are affecting its integrity despite the upstream setting.

In contrast, Valdeza aligns with the “Good” IBI quality class, reflecting comparatively better ecological conditions. Santa Rita and Trapichito are positioned near the “Fair” IBI group, representing intermediate conditions, while Maribel and Mastanto fall between “Fair” and “Poor”, suggesting transitional states. The clear separation of these groups underscores the gradient of ecological integrity from high-quality conditions (Good) to degraded ones (Poor), consistent with the patterns observed in environmental and biological variables (Fig. 5).

Relationships between Biotic Indices and Environmental Parameters. To validate the ecological representativeness of the IBI, we conducted a series of Spearman rank correlation analyses with independent environmental quality indicators. The correlation between the IBI and the Water Quality Score (WQS) was not statistically significant (Spearman's $\rho = 0.28$, $p = 0.15$), suggesting that physicochemical parameters alone may not fully capture the complexity of ecological degradation. However, the IBI was strongly and significantly correlated with the Habitat Quality Score (HQS) ($\rho = 0.55$, $p < 0.003$; Fig. 6a), underscoring the central role of habitat structure, substrate availability, and riparian condition in shaping fish community integrity (Altermatt *et al.* 2013).

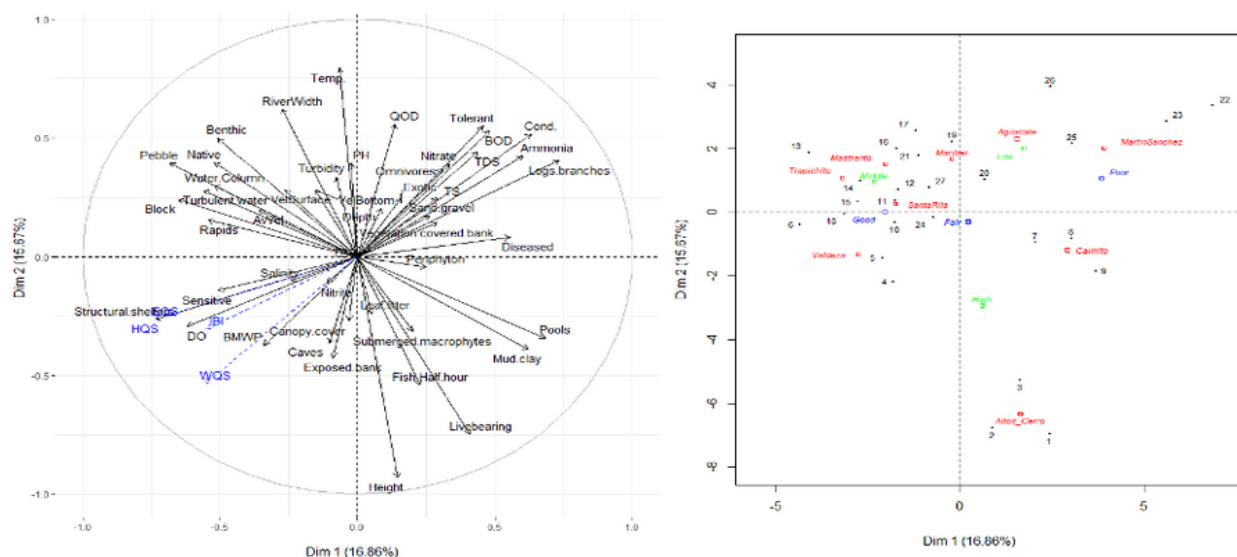


Figure 4. Principal Component Analysis (PCA) of environmental and biological variables. (A) Correlation of variables with the first two dimensions (Dim 1 = 16.86%, Dim 2 = 15.67%), showing a gradient from degraded conditions (e.g., high conductivity, BOD, ammonia) to better ecological integrity (e.g., native richness, habitat structure, dissolved oxygen). (B) Projection of sites and quality classes on the same ordination space, illustrating their distribution along the integrity gradient.

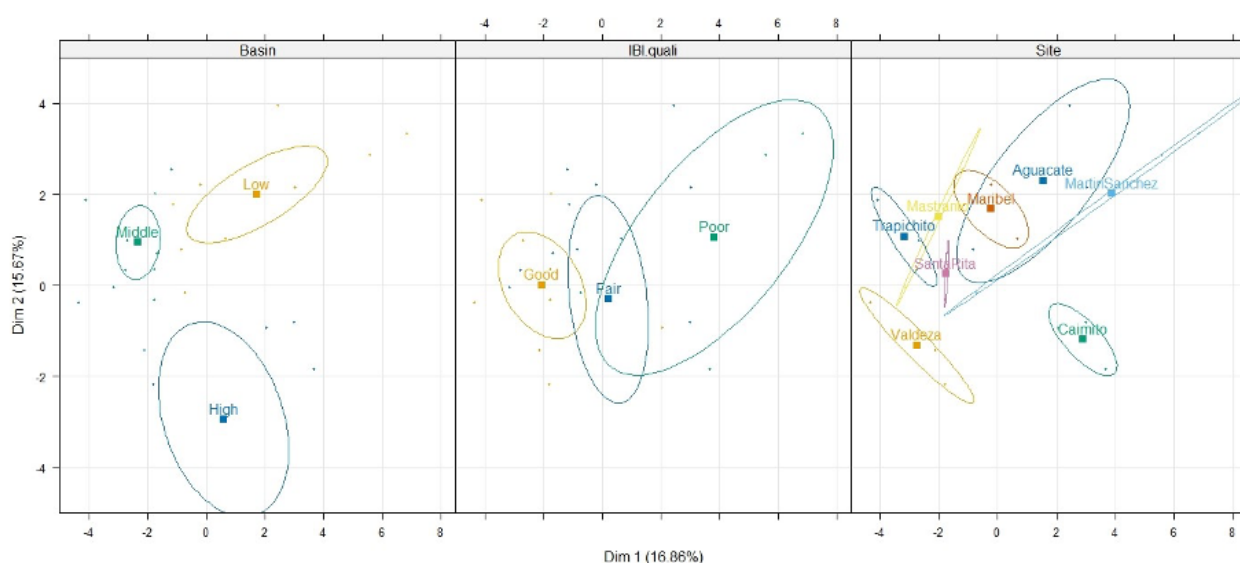


Figure 5. PCA of study sites grouped by basin, IBI quality, and site. The ordination highlights the integrity gradient described in Figure 2, with Aguacate and Martín Sánchez clustering with Poor, Caimito also near Poor, Valdeza with Good, and Santa Rita and Trapichito with Fair, while Maribel and Mastranto occupy an intermediate position between Fair and Poor.

When both WQS and HQS were integrated into the Environmental Quality Score (EQS), the correlation with the IBI remained significant ($p = 0.51$, $p = 0.007$; Fig. 6b), indicating that composite environmental metrics are more suitable for evaluating biological responses at the community level. These findings align with other studies that emphasize habitat variables as key drivers of ecological condition in tropical rivers. These results show similar tendencies to other studies, demonstrating that the use of EQS is the best approach to validate the effectiveness of the IBI in different geographical regions. (Lyons *et al.* 1995, Ramírez-Herrejón *et al.* 2012).

Validation with the BMWP. Benthic macroinvertebrate assemblages reflected a clear gradient of water quality across the Caimito River basin. BMWP scores varied among sites, with upper and middle basin locations generally exhibiting better ecological conditions than lower basin sites. Most upstream sites were classified within the good category, while intermediate locations showed Regular conditions. In contrast, the lowest basin sites exhibited markedly reduced BMWP values and were classified as Very contaminated according to the BMWP-PAN criteria (Cornejo *et al.* 2019). Mean BMWP scores for each sampling site across the three sampling campaigns are summarized in Table X.

The correlation between BMWP and HQS was positive and significant (Spearman's rank cor-

relation = 0.40, $p = 0.040$; Fig. 6c). Both biotic indices (BMWP and IBI) showed similar significance patterns with water-quality and environmental variables, as BMWP alone did not show a significant positive correlation with WQS (Spearman's rank correlation = 0.23, $p = 0.25$; Fig. 5d), while the correlation between BMWP and EQS was significant (Spearman's rank correlation = 0.40, $p = 0.050$; Fig. 6e), though weaker than the previous relationships. Validation using our IBI scores against BMWP at the same sites showed a positive, significant, and stronger association (Spearman's rank correlation = 0.72, $p < 0.001$; Fig. 6f).

Table X. Mean BMWP values per site in the Río Caimito Basin, West Panama Province, calculated from three sampling campaigns following the BMWP-PAN protocol (Cornejo *et al.* 2019). Condition categories correspond to the BMWP-PAN classification. Ordinations and correlations were conducted using campaign-specific BMWP values.

Site	BMWP	Category (Cornejo <i>et al.</i> 2019)
Altos del Co.	87	Good
La Valdeza	133	Good
Caimito	82	Good
Santa Rita	122	Good
Trapichito	64	Regular
Mastranto	71	Regular
Maribel	61	Regular
M. Sánchez	22	Very contaminated
Aguacate	33	Very contaminated

Recommendations for Conservation and Management. Our results support integrated conservation and restoration measures at the basin scale. Structural shelter was the variable with the strongest influence on Habitat Quality Score (HQS), followed by canopy cover (Lyons *et al.* 1995), while the Environmental Quality Score (EQS) and the BMWP macroinvertebrate index provided complementary highlight areas of ecological degradation and conservation opportunities within the Caimito River Basin. Lower IBI scores in urbanized, polluted and impacted sites, by several anthropogenic activities, including the presence of exotic species, and declining forest cover in certain areas, underscore the need for immediate and targeted management actions. The following recommendations aim to address site-specific pressures while also guiding regional policy alignment. These measures support the ecological resilience of the basin and provide a model for replication in other Pacific watersheds of Central America. These site-based actions must be embedded in a broader ecological and geopolitical framework. Although sampling was conducted at accessible locations, restoration efforts must be projected also into adjacent, unsampled areas to ensure comprehensive watershed recovery and water retention through forest recovery (Zhang *et al.* 2017, Zeni *et al.* 2019). The ongoing expansion of the Pacific Metropolitan Area into Panamá Oeste is accelerating land-use change, deforestation, and pressure on freshwater resources (Dirección de Planificación Urbana del Municipio de Panamá 2016).

As the Caimito River is the main freshwater source for this province, its conservation must be prioritized for the benefit of local residents, and planned diversions to megaprojects operations must be evaluated with respect to that and the Biological and Ecological Integrity of the Basin and the water resources (Zhu *et al.* 2023). In this context, technical training and biannual monitoring programs using fish-based indices (Index of Biotic Integrity [IBI]), macroinvertebrate-based indices (Biological Monitoring Working Party [BMWP]), and the Environmental Quality Score (EQS) should be institutionalized through collaboration with government agencies and local communities. To optimize the changes of success, these efforts should involve the interinstitutional Caimito River Basin Committee and key entities such as the 3 districts municipalities, the Ministry of Environment (MiAmbiente), Ministry of Health (Ministerio de Salud [MINSA]), Ministry of Housing and Land Management (MIVIOT), Ministry of Commerce and Industries (MICI), Ministry

of Public Works (MOP) the Water and Sewer Institute (Instituto de Acueductos y Alcantarillados Nacionales [IDAAN]), and the Aquatic Resources Authority of Panama (Autoridad de los Recursos Acuáticos de Panamá [ARAP]). The ARAP, along with MiAmbiente, in particular, are key for identifying sources of exotic species, regulating tilapia farming, and developing national policies to prevent their spread, as these species pose a significant threat to the ichthyological biodiversity of West Panama Province (Table XI).

At the regional level, infrastructure projects like the proposed Panama–David railway—with intended expansion toward the Costa Rican border (BNamericas 2024, Álvarez *et al.* 2025), underscore the urgency of binational collaboration. Transboundary environmental impacts demand harmonized monitoring strategies between Panama and Costa Rica. Applying methodologies such as the IBI and BMWP on both sides will allow for coordinated assessments of aquatic ecosystem health. Universities and academic institutions must take a leading role in applying these tools, promoting knowledge exchange with specialists who have already implemented them in the Caribbean basins of Costa Rica and Panama, and ensuring the involvement of community actors and government stakeholders through existing basin governance structures. Only through an integrated, multi-actor, and binational approach can the resilience of watersheds like Caimito be protected from climate pressures, urbanization, and extractive infrastructure development. Climate projections for tropical regions suggest that reductions in precipitation and prolonged drought periods may become more frequent under climate change scenarios. A hypothetical decrease of approximately 10–20% in precipitation could therefore alter hydrological conditions in rivers such as the Caimito basin through reduced discharge and longer low-flow periods. Because fish assemblages in tropical streams are strongly structured by flow regime and habitat heterogeneity, these changes are expected to influence several components of the Index of Biotic Integrity applied in this study. Alterations in natural flow regimes are known to modify fish diversity, abundance and functional composition (Poff *et al.* 1997), and drought events often favor tolerant or opportunistic species while reducing sensitive taxa (Lennox *et al.* 2019). Although the present study did not explicitly model hydrological scenarios, several of the IBI metrics used here are linked to ecological mechanisms affected by reduced precipitation. Table XII summarizes the expected directional response of

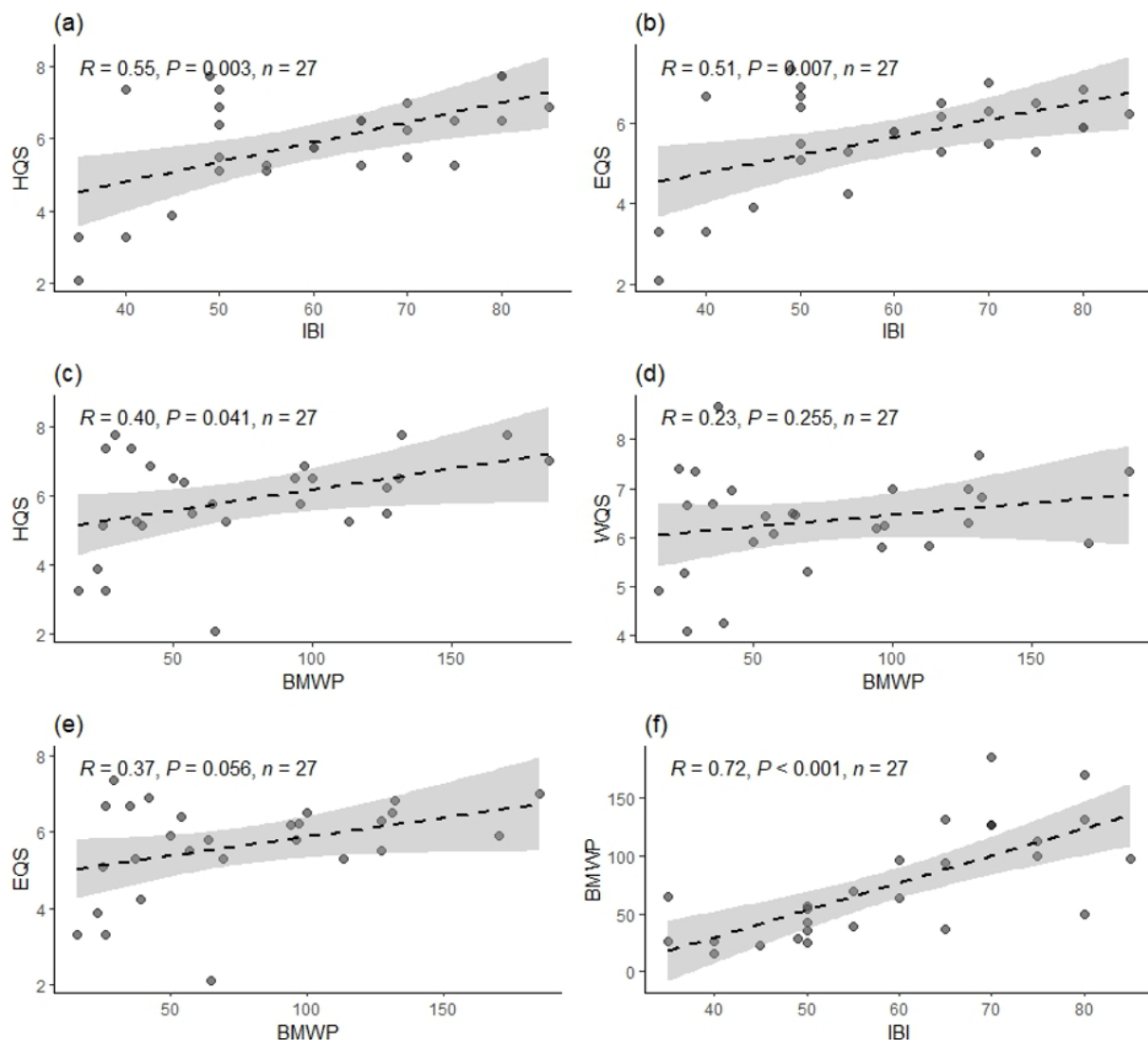


Figure 6. Spearman correlations: (a) Index of Biological Integrity (IBI) vs. Habitat Quality Score (HQS); (b) IBI vs. Environmental Quality Score (EQS); (c) HQS vs. Biological Monitoring Working Party (BMWP) index; (d) Water Quality Score (WQS) vs. BMWP; (e) EQS vs. BMWP; and (f) IBI vs. BMWP.

Table XI. Recommended site-specific interventions according to IBI scores and environmental pressures in the Caimito River basin, West Panama Province. IBI = Index of Biotic Integrity. Institutional acronyms are defined in the recommendations for Conservation and Management section.

Site (IBI, basin position)	Main Issues	Recommended Actions
Martín Sánchez (38, low)	Urban expansion; high domestic pollution; exotic species.	Monitor and contain invasive fish (ARAP, MiAmbiente). Regulate urban development. Implement sanitation infrastructure (MINSa, MiAmbiente). Control urban expansion (MIVIOT).
Aguate (52, low)	Industrial pressure; high domestic pollution; low forest cover; exotic species.	Identify pollution sources. Reforest corridors. Engage industrial actors. Implement sanitation infrastructure (MINSa, MiAmbiente). Control urban expansion (MIVIOT). Monitor and contain invasive fish (ARAP, MiAmbiente).

Site (IBI, basin position)	Main Issues	Recommended Actions
Maribel (55, low)	Flood risk; high domestic pollution; quarry impact.	Control runoff. Review quarry operations (MICI, MOP, MIVIOT). Improve drainage planning. Implement sanitation infrastructure (MINSA, MiAmbiente).
Caimito (55, upper)	Moderate canopy cover; urban encroachment; moderate pollution; quarry threats.	Restore riparian buffers. Improve wastewater management (MINSA, MiAmbiente). Remove cement structures blocking the river course. Control urban expansion (MOP, MIVIOT). Enhance natural areas for public use (Municipality, MiAmbiente). Monitor invasive species (ARAP, MiAmbiente).
Mastranto (56, middle)	High domestic pollution; informal housing.	Implement sanitation infrastructure (MINSA). Restore riparian zones (MiAmbiente). Restore 'El Chorro' recreational area. Enhance natural areas for public use (Municipality, MiAmbiente).
Santa Rita (63, middle)	Moderate urban pressure.	Prevent land-use change (MIVIOT). Support community water monitoring (IDAAN, MINSA). Enhance natural areas for public use (Municipality, MiAmbiente).
Altos del Cerro (65, upper)	High-quality forest habitat; nearby human activities.	Maintain forest cover. Promote conservation easements. Control agricultural and housing expansion (MiAmbiente, MIDA, MIVIOT).
Trapichito (68, middle)	Adjacent to the water treatment plant; moderate urban pressure.	Prevent land-use change (MIVIOT). Strengthen pollution control from upstream and adjacent infrastructure, including sewage outflows (MINSA, IDAAN). Enhance natural areas for public use (Municipality, MiAmbiente).
La Valdeza (82, upper)	High-quality riparian and river habitat; threatened by quarries and agricultural expansion.	Enforce restrictions on extractive activities. Monitor quarry impact. Regulate agricultural expansion (MICI, MiAmbiente, MIDA).

these metrics under a hypothetical 10–20% decrease in precipitation associated with climate change.

Conclusions

The application of the Index of Biotic Integrity (IBI) in the Caimito River Basin revealed a clear and ecologically meaningful gradient, underscoring the value of fish-based bioindicators for assessing watershed health in tropical systems. Approximately 77 % of the surveyed sites fell within the “fair” category and 11 % within the “good” range, suggesting that substantial portions of the basin still support structurally diverse habitats, high native species richness, and resilient functional fish communities. However, most sites in the “fair” category already exhibit signs of environmental degradation and could shift toward “poor” condition if effective management actions are not implemented. In contrast, 11 % of the sites were classified as “poor,” reflecting localized degradation associated with urbanization, pollution, and the presence of exotic species. Despite these pressures, the basin retains significant ecolog-

ical recovery potential, and targeted restoration measures—if implemented promptly—could halt or reverse ongoing degradation. The strong correlations observed between IBI, macroinvertebrate (BMWP) scores, and integrated habitat and water quality indicators (EQS), validate the IBI as a robust, replicable tool for guiding conservation actions in other Pacific Central American basins. These findings reinforce the critical need for immediate watershed-scale management interventions that prioritize riparian reforestation, water quality improvement, and native species protection. In the face of accelerating climate change and water scarcity risks—particularly for the densely populated West Panama Province—conserving and restoring the ecological integrity of the Caimito River Basin is not only feasible but imperative. Its rich ichthyofauna and remaining high-quality habitats represent both a conservation priority and a strategic natural asset essential for regional water security and long-term socio-ecological resilience in one of the most important basins on the west side of the Panama Canal.

Table XII. Expected response of IBI metrics under a hypothetical 10–20% reduction in precipitation associated with climate change in tropical river systems.

IBI metric	Expected response	Ecological mechanism
Number of native species	Decrease	Habitat contraction and reduced connectivity among river sections
Number of sensitive species	Decrease	Sensitive taxa affected by lower oxygen availability and thermal stress
Number of water column species	Decrease	Reduced flow velocity and simplified habitats
Number of benthic species	Decrease	Sediment accumulation and loss of benthic microhabitats
Percent tolerant individuals	Increase	Environmental stress favors tolerant species
Percent omnivorous individuals	Increase	Generalist feeding strategies become advantageous under disturbance
Percent exotic species	Potential increase	Disturbed habitats often facilitate invasive species
Fish abundance	Decrease	Reduced wetted habitat and physiological stress
Percent individuals with deformities or disease	Potential increase	Chronic environmental stress
Percent native livebearers	Variable	Species-specific responses depending on habitat conditions.

Ethical statement

Biological samples were collected following all applicable institutional and national ethical regulations regarding animal welfare and the collection of biological specimens. Field sampling was conducted under Scientific Collecting Permit No. ARB-021-2022 issued by the Ministry of Environment of Panama (MiAMBIENTE). Fish handling and processing were limited to the minimum necessary to accomplish the scientific objectives of the study.

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Supplementary material: Fish-based Index of Biotic Integrity in the Caimito River Basin, Pacific drainage of Panama, Central America

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Table S1: Complete species data matrix per site used to compute the IBI metrics is accessible through the following [hyperlink](#).

Table S2. Functional Trait Assignment SS: Sensitive Species TS: Tolerant WCS: Water Column BS: Benthic NS: Native EXS: Exotic (they don't apply to other metrics) OM: Omnivores NLB: Native livebearer ES: Endemic Species CI: Carnivores, Insectivores/Invertebrates CP: Carnivorous-Piscivores HB: Herbivores; Sources: González-Gutiérrez (2016), González-Gutiérrez (2021), Mc Larney *et al.* (2008), Valdés-Díaz (2020), and our own evaluations.

Family and Species	NL											
	SS	TS	WCS	BS	NS	ES	EXS	OM	CI	CP	HB	B
Acestrorhamphidae												
<i>Astyanax panamensis</i>		X	X		X			X				
Achiridae												
<i>Trinectes fluviatilis</i>				X	X							
Bryconidae												
<i>Brycon argenteus</i>	X		X		X			X				
<i>Brycon striatulus</i>			X		X			X				
Centropomidae												
<i>Centropomus unionensis</i>			X		X					X		

Characidae						
<i>Compsura gorgonae</i>	X		X	X		X
<i>Roeboides occidentalis</i>			X			X X
Cichlidae						
<i>Andinoacara coeruleo-</i>						
<i>punctatus</i>			X	X		
<i>Oreochromis niloticus</i>					X	
<i>Parachromis managuensis</i>					X	
Curimatidae						
<i>Cyphocharax magdalenae</i>		X		X		X
Eleotridae						
<i>Gobiomorus maculatus</i>	X		X	X		X
<i>Eleotris picta</i>		X	X	X		X
<i>Dormitator latifrons</i>		X	X	X	X	
<i>Leptophilypnus macrolepis</i>			X	X		
Gerreidae						
<i>Eugerres brevimanus</i>		X		X		X
Haemulidae						
<i>Rhonciscus bayanus</i>		X		X		
Hypopomidae						
<i>Brachyhypopomus occidentalis</i>			X	X		
Heptapteridae						
<i>Pimelodella chagresi</i>			X	X		X
<i>Rhamdia guatemalensis</i>			X	X		X
Loricariidae						
<i>Ancistrus chagresi</i>	X		X	X	X	
<i>Leptoancistrus canensis</i>	X		X	X		X
<i>Hypostomus aspidolepis</i>			X	X		X
<i>Hypostomus plecostomus</i>					X	
<i>Rineloricaria uracantha</i>			X	X		X
<i>Sturisomatichthys panamensis</i>			X	X		
Mugilidae						
<i>Dajaus monticola</i>	X	X		X		
Oxudercidae						
<i>Awaous transandeanus</i>			X	X		X
<i>Sicydium salvini</i>	X		X	X		X
Poeciliidae						
<i>Brachyrhaphis episcopi</i>	X			X	X	X
<i>Neoheterandria tridentiger</i>		X		X	X	X
<i>Poecilia gillii</i>		X		X	X	X
<i>Poeciliopsis turrubarensis</i>		X		X		X
Rivulidae						
<i>Cynodonichthys brunneus</i>	X					X
Stevardiidae						
<i>Eretmobrycon emperador</i>	X	X		X		X
<i>Gephyrocharax atracaudatus</i>	X	X		X	X	X
Syngnathidae						
<i>Pseudophallus elcapitanensis</i>						X