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Analysis of population size of *Pterygoplichthys multiradiatus* and its intake of microplastics in streams with different land uses

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Abstract

Most freshwater habitats have been substantially affected by anthropogenic factors such as fish introductions, plastic pollution, and river regulation. Urban rivers are highly vulnerable to impacts associated with land use changes resulting from increasing urbanization, including altering habitat and establishing aquatic biological communities in these areas. In turn, the introduction of exotic species into sensitive and threatened ecosystems such as tropical urban streams and their rapid establishment, such as *Pterygoplichthys multiradiatus*, was used as an ecological model to determine the relative population size of the species. Also, the species was used to evaluate the presence of microplastics (MPs) in the gastrointestinal tract (GIT) of fish in rivers with different land use history. Our results showed significant differences in pleco abundance between areas with high and low urban (LU) development in the watersheds. The study demonstrated that abiotic environmental factors directly influence the relative abundance of plecos at the range and watershed scales. In a total of 42 fish examined, only 85.7% showed MPs retained in the GIT, with fibers and fragments being the most common. A total of 22 pieces of microplastic were identified with Nile Red staining by slide analysis. A significant difference was found between the abundance of microplastic ingested per total fish length between streams with high and LU development reaches. Therefore, in relatively small amounts, microplastic ingestion appears to be common in *P. multiradiatus* species, regardless of the habitat in which they are found and the diet present.

1. Introduction

In recent years, urban development has had a negative impact, causing physical, chemical, and biological disturbances that significantly affect the composition and quantity of organisms inhabiting freshwater ecosystems [1]. For example, aquatic organisms inhabiting rivers in urban areas are exposed to a wide variety of environmental pollutants due to the high accumulation and toxicity of these wastes, which can have detrimental effects on free-living animals and cause population collapse [2]. Degradation due to changes in channel stability and geomorphology, alteration of water and sediment quality, and changes in the ecological status of receptors are described by the urban stream syndrome [3]. Furthermore, vulnerable impacts associated with changes in land uses contribute to the direct and indirect introduction of different forms of anthropogenic litter (e.g. plastic waste, used water discharges, pesticide or oil contamination, etc), which may or may not cause an imbalance in the ecosystem [4], being irreversible to its natural regeneration in many cases.

It is widely recognized that anthropogenic litter represents a severe threat to the environment on a global scale. Numerous studies have shown that plastics make up the majority of this concern, which is now known as plastic waste pollution [5]. Plastic use continues to increase worldwide, and some plastic waste is released into rivers unchecked. Although plastic pollution in rivers remains understudied [6]. Recent studies have shown excessive accumulation of different plastic fragments in freshwater systems [7–10], identifying sinks of plastic pollution in riverbanks, buried in riverbed sediments, and accumulation of plastics within freshwater

organisms [1, 11]. Consequently, this is a growing problem intimately linked to the current urbanization model.

Microplastics (MPs) (defined as plastics <5 mm), fall into two categories: (1) primary MPs, which are plastic particles originally manufactured in those sizes for commercial use (e.g. cosmetics, clothing, and other textiles); and (2) secondary MPs, particles resulting from the breakdown of larger plastic items (e.g. plastic bottles, cleaner and detergent containers, and many others). Ingestion of MPs by aquatic organisms (e.g. zooplankton, invertebrates, bivalves, and fish, among others) is the most studied interaction at present [12, 13] because of the potential for trophic transfer [14]. However, when MPs are ingested, they cause deleterious effects on the health of the organism (e.g. reduced survival, reproduction, feeding, immune function, growth rate, and lipid peroxidation) [14, 15]. They also serve as an entry point for the leaching of toxic chemicals and their introduction into the food chain [16, 17]. In this sense, several studies have used different organisms as bioindicators to characterize environmentally induced changes and the serious problem of plastic pollution for aquatic ecosystems. For example, in inland waters in Turkey, organisms such as the zebra mussel, *Dreissena polymorpha* (Pallas, 1771), have begun to be used to provide much more effective detection rates and characterization of MP [18]. There is increasing evidence that MPs can be transferred in the food chain. Also, studies have found up to 232 MPs in the gastrointestinal system in dominant fish species in freshwater ecosystems in Turkey [19], revealing the extent of MP contamination in aquatic ecosystems, especially in freshwater. For these reasons, there is increasing evidence that freshwater fish ingest MPs around the world [20, 21].

However, given the increased imperviousness and channelization projects caused by increased urbanization, urban rivers significantly impact how different fish species are distributed and how their communities are composed [22, 23]. For example, dam construction and some channel modifications act as barriers to native fish species in Puerto Rico, affecting their migratory patterns and assemblages. As a result, native species assemblages are overburdened due to altered or disrupted migratory patterns and life cycles, while the spread of exotic species continues to increase [24, 25]. In addition, it may be possible to study the population dynamics of exotic fish in urban streams in Puerto Rico, to serve as bioindicators of anthropogenic pollution (particularly plastic debris) and to better understand the connections between freshwater fish and their habitat and the impact of pollutants on altering aquatic ecosystems.

The freshwater fish fauna of Puerto Rico is dominated mainly by exotic fish species [22, 26], introduced from the southeastern United States, Africa, Asia, and South America [27, 28]. Exotic fishes were introduced to Puerto Rico through the aquaculture sector, pet shop trade, and game fishing [22, 26, 27, 29]. In fact, of the 45 primarily freshwater species on the island, 38 are introduced exotic species [22, 29, 30], with this proportion of exotic freshwater fishes being among the highest globally for island faunas [26, 28].

Recent introductions include the Orinoco sailfin catfish or pleco (*Pterygoplichthys multiradiatus*, Family: Loricariidae) (Hancock, 1828), an exotic species brought from Venezuela, probably introduced by aquarium hobbyists during the 1990s [27]. During the last few decades, several species of the genus *Pterygoplichthys* and putative hybrids have been widely introduced outside their native ranges [22, 29, 31, 32]. Currently, evidence has been obtained on the distribution of this exotic species in slow-flowing rivers and estuarine zones, as well as lakes and swamps [22, 29, 32], with a temperature range in tropical areas of 23 °C–27 °C and a pH of 6.5–7.8. *P. multiradiatus* has rapid growth during the first two years of life, living for more than ten years [33]. Their body sizes range from 30 to 50 cm, up to 70 cm in adults. They are bottom-dwelling fish, characterized by a depressed body covered by large bony plates forming a flexible armor and a flat bottom body shape [27]. The mouth is inferior (sucker), with surrounding lips forming a suction disk, and in combination with teeth, is an adaptation to attach to the substrate to consume algae, organic sediments, small invertebrates, and even eggs of other fish [34–36]. In addition, due to competition for food [36, 37], plecos show aggressive and territorial behavior in direct competition with native fish.

This species presents significant threats to native fish communities and aquatic habitats in the United States [36], affecting ecosystems at lower and upper trophic levels. In Puerto Rico, the Puerto Rico Department of Natural and Environmental Resources has cataloged *P. multiradiatus* as an invasive species, causing severe damage to sectors of the economy such as agriculture and fisheries and negative impacts on the island's diversity, constituting a public health risk for local communities. Among the adverse effects of this species are physical habitat degradation and ingesting native fish eggs [33, 36, 38]. Also, soil uptake and grazing affect primary productivity (e.g. by altering sediment size and algal growth cultures) and secondary productivity (e.g. by bypassing net food consumption).

The primary aim of this study was to determine the relative population size of *P. multiradiatus* and to compare abundance between watersheds that have different land uses and between urban and suburban areas within the watersheds. A secondary aim is to determine, quantify, and identify the types of MPs found within the gastrointestinal tract (GIT) of fish collected from different urban reaches in relation to different land use conditions between watersheds. We hypothesized that (1) the population of *P. multiradiatus* will be

higher in areas of LU density compared to areas of high urban (HU) density due to different habitat alterations, and (2) a high concentration of MPs could be found in the GIT of *P. multiradiatus* inhabiting highly developed urban areas because they are exposed to high levels of plastic pollution in these stream areas compared to areas of LU or rural development.

2. Materials and methods

2.1. Study sites

The Río Bayamón and Río Piedras watersheds were selected to describe and compare the population status of *P. multiradiatus*. These watersheds represent the highest levels of urbanization based on the percentage of urban coverage on the island. At each watershed, two sampling sites were selected, one located in the urban area and the other in the peri-urban area; each represents a different urban impact degree. Also, two pools were randomly selected at each sampling site, with approximately 100 m of separation between pools. Sampling sites within the watersheds were selected based on the percentage of urbanization in each watershed. For this, classifications provided by the US Census Bureau, identifying and defining the differences between individual urban areas and suburban areas, were used. HU reaches were defined as urbanized areas based on a minimum threshold of 2000 housing units or at least 2500–5000 people [39]. While LU reaches consisted of a population of fewer than 2500 people [39].

The Río Bayamón watershed (figure 1) is located in northern Puerto Rico and is one of the longest streams on the island. The stream has an elevation of up to 450 m above sea level. It originates in the mountainous areas south of Cidra, running approximately 40 km from the Cidra Reservoir to its mouth at the Atlantic Ocean, between Cataño and Levittown in northern Puerto Rico [40]. The Río Bayamón watershed has one of the highest urbanization rates on the island, with about 24% urban and 7% suburban developments [41]. Forests (41%) and pasture areas (26%) predominate in the upper part of the watershed. The water discharge of the Río Bayamón increases due to contributions from the Minillas and Guaynabo streams (downstream of the reservoir), along with another 22 primary streams and 36 secondary tributaries. Plecos were collected at the following stations: HU (18.3767° N, 66.1370° W) and LU (18.3470° N, 66.1359° W).

The Río Piedras watershed (figure 1) has the island's highest level of urban development. Its headwaters are located in the Caimito in the municipality of San Juan, and it flows northward for about 16 km at its mouth into San Juan Bay [42]. The Río Piedras is the only stream in San Juan that originates at about 150 m above sea level on the slopes of the mountains that separate the municipality of Trujillo Alto from San Juan, formed by the Los Guanos and Las Curias streams, the latter being the stream that feeds the Las Curias dam in the upper part of the watershed. Most of the watershed is urban, reaching about 60% urbanized area in the San Juan metropolitan area [41], except for the highlands south of the Experiment Station, with abundant vegetation. The watershed has forest cover (26.4%) and pasture areas (15.3%). There are no substantial agricultural activities in the watershed. Plecos were collected at the following stations: HU (18.4107° N, 66.0708° W) and LU (18.3912° N, 66.0578° W).

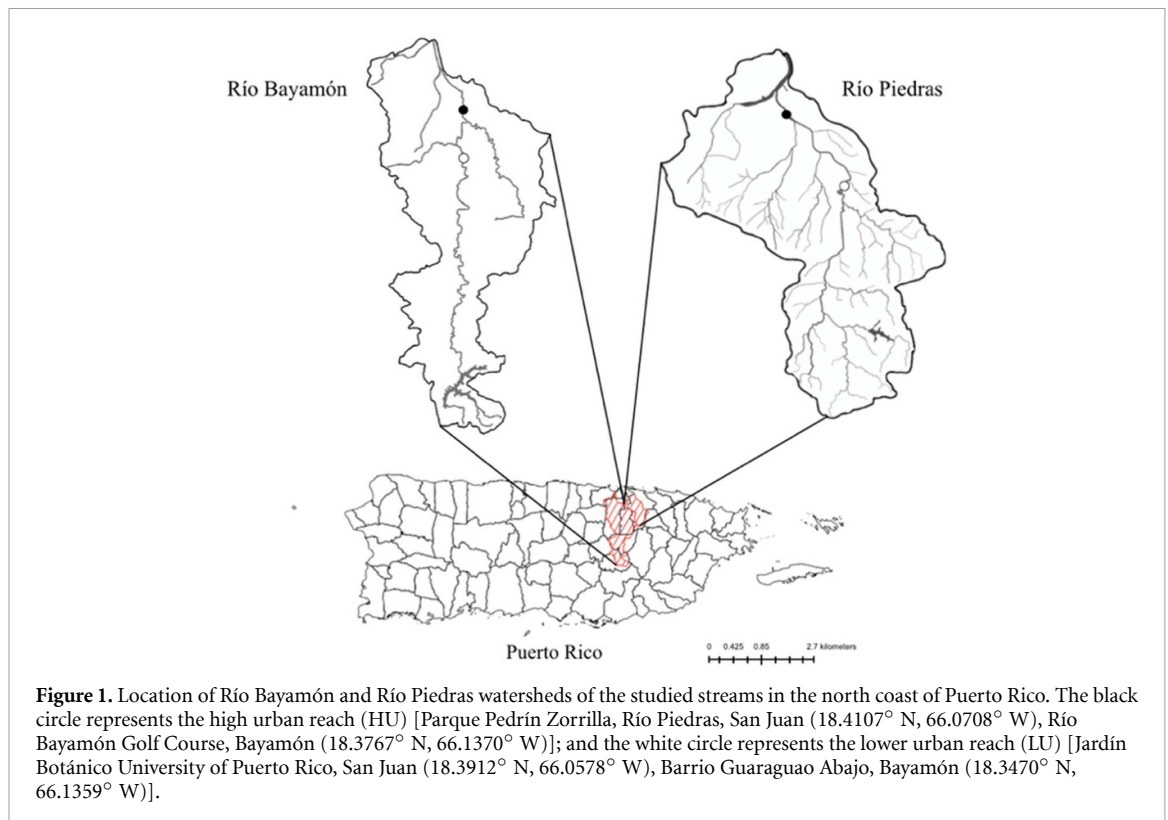
2.2. Physicochemical parameters

In each sampling site in each watershed, six chemical parameters were measured (temperature, pH, conductivity, total dissolved solids, dissolved oxygen, and salinity) using a multiparameter sonde. Also, stream habitat was quantified through direct measurements of the physical characteristics of the pools (length, width, depth, water flow, and substrate composition) at the four reaches (two per sampling site) with high and LU reaches.

The water discharge for each watershed was obtained from the United States Geological Service gauging station located nearest the stream sampling sites. The size composition of the substrate was estimated at each sampling reach where the fish were collected. A total of 500 rocks were randomly selected from each of the pools in the different sampling reaches using a gravelometer to determine and classify the size and structure of the substrate. The percentage categories were converted to a substrate index (SI) using the following formula: $SI = [(0.08)(\% \text{ bedrock}) + (0.07)(\% \text{ boulder}) + (0.06)(\% \text{ cobble}) + (0.05)(\% \text{ gravel}) + (0.04)(\% \text{ fine gravel}) + (0.03)(\% \text{ sand and fines})]$.

2.3. Fish sampling and morphological measurements

Plecos were collected in each pool per sampling site in the Río Bayamón and Río Piedras watersheds. Plecos were collected using a fishing net because the pools were shallow (<one meter deep). Collections consisted of capturing as many fish as possible during a time limit of 60 min per pass per pool, which was delimited by a fishing net to limit fish emigration or immigration during sampling. At the end of the capture time limit, each fish collected was counted and marked with plastic strips of a different color for each pool to avoid



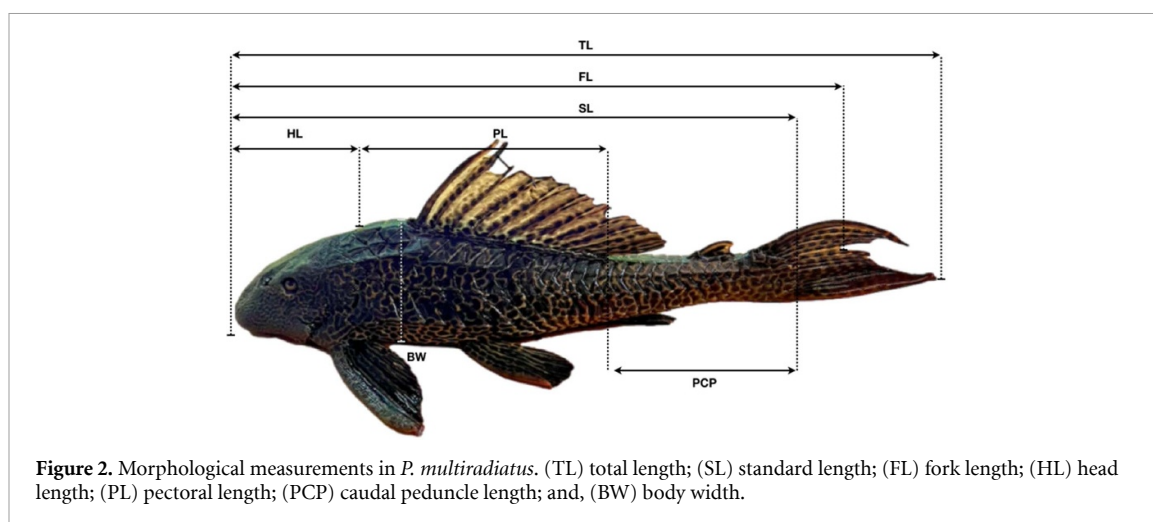
mixing fish between pools and to facilitate their identification for the recapture rounds of sampling. This procedure was carried out for four consecutive days during the first week and four consecutive days during the second week. Catch per unit effort (CPUE) was calculated for a total sampling of eight days. CPUE represented the number of plecos divided by the sampling effort (i.e. the number of catches among the eight days at each sampling site).

In order to determine the presence of microplastic particles in the GIT contents of fish, a total of 42 plecos were collected between urban reaches in both watersheds. In the Bayamon River watershed, 25 plecos were collected in the HU reach and five plecos in the LU reach. In the Río Piedras watershed, ten plecos were collected in the HU reach and two plecos in the LU reach. The captured fish were deposited in coolers with water and constant aeration until they were transported to the laboratory of the University of Puerto Rico Río Piedras campus, where they were identified (by location and date of capture), treated with an overdose of anesthetic (MS-222), kept on ice until prior use, and analyzed for GIT extraction.

At the laboratory, photographs of the lateral and ventral views and morphological measurements of the plecos were collected for visual comparison among samples. The following measurements were calculated: (i) total length (TL): defined as the length of a fish measured from the tip of the snout to the tip of the tail or caudal fin; (ii) standard length (SL): refers to the length of a fish measured from the tip of the snout to the posterior limit of the last vertebra or the mediolateral portion of the hypural lamina; (iii) fork length (FL): defined as the length of a fish measured from the tip of the snout to the end of the median rays of the caudal fin; (iv) head length (HL): defined as the length of the head from the tip of the snout to the most posterior portion of the operculum; (v) pectoral length (PL): defined as the length from the most posterior portion of the operculum to the end of the dorsal fin; (vi) length of the caudal peduncle (PCP): refers to the length from the end of the dorsal fin to the posterior limit of the last vertebra or the mediolateral portion of the hypural lamina; and, (vii) body width (BW): measured to determine the distance from the anterior part of the pectoral fin to the anterior part of the other pectoral fin, passing through the width of the animal (figure 2). It is important to mention that BW is not girth. The circumference is a contour measurement (it takes the shape of the fish).

2.4. Population estimation

The capture-mark-recapture method was used to determine the relative population size of *P. multiradiatus* at the different sampling sites in both watersheds. Peterson's Index was used to estimate population size, and a slightly more complex formula can be used to perform many additional capture-markings and markings in addition to the first one: $\hat{N} = M_2 \bullet C_2 + M_3 \bullet C_3 \dots / R_2 + R_3 \dots$ where N represents the population size, M



is the number of individuals marked in the population, C is the number of individuals captured in the sample, and R is the number of individuals recaptured from the sample.

2.5. MPs identification

In order to minimize the risk of contamination, the materials and tools used in our study were thoroughly cleaned with 70% ethyl alcohol before use and handling. Laboratory clothing made of non-plastic materials and gloves (nitrile) were used throughout the dissection and instruments were cleaned with pure filtered water after handling each specimen.

This study involved the use of two types of methods for the identification of MPs in the samples. One method involved the use of vacuum filtration, while the other involved the use of Nile Red staining. The organism was dissected using a method similar to previous studies [17]. The entire GIT from the 42 plecos was isolated, dissected into parts (esophagus, stomach, and intestine), and preserved in 250 ml glass vials with a 10% formaldehyde solution for two days at room temperature. The formaldehyde solution improved conservation time and did not destroy or alter tissue characterization. Each part of the GIT was washed with distilled water under controlled pressure to remove all contents adhering to the tissue. Prior to filtration of the contents, 1 ml of the stomach content sample was extracted to prepare slides with different dyes. Safranin was used to determine the presence of algae; floroglucinol for lignin detection; lactophenol cotton blue to recognize fungal chitin; alizarin red for animal chitin; and Nile red for microplastic detection. These dyes provided a balance in visibility and ease of internal stomach properties. Nile Red was adopted because it is the most effective in adsorption and fluorescence intensity for identifying plastic particles [43]. A total of five slides per staining per organ were performed in order to detect the highest composition of organic matter in fish. The stained residues on the prepared slides were examined with a stereo microscope, and microphotographs of the different types of organic matter and plastic particles were taken.

The solution with the remaining contents was vacuum filtered using mixed cellulose ester membrane filters (47 mm diameter, pore size of $1.0\ \mu\text{m}$), and examined for MPs under a stereomicroscope. Observations of MPs on filters and on slides prepared by staining with Nile red were classified according to their size and shape [13] and were categorized into (i) fragments: irregularly shaped particles, crystals, fluff, dust, films, flakes, granules; (ii) fibers: filaments, strands, threads; (iii) spheres: grains, microspheres; (iv) foam: polystyrene fragments, expanded polystyrene; and (v) pellets: resin or pre-production pellets. No plastic materials (beakers, Petri dishes, droppers, or micropipettes) were used in the MPs analysis.

2.6. Statistical analysis

The environmental and physicochemical variables were compared among study sites for both watersheds using one-way ANOVA analysis. Two-way ANOVA was used to compare the CPUE between streams and urban reaches (HU and LU); CPUE data were transformed to $\text{LN} + 1$. A cluster analysis was performed to determine the similarities characteristics between the urban reaches of both watersheds. Non-metric multidimensional scaling (NMDS) analysis was used to describe the relationship between the environmental variables (pool depth, pool length, pool width, water flow, % Substrate Index, temperature, pH, conductivity, total dissolved solids, dissolved oxygen, and salinity) and *P. multiradiatus* abundance. Measurements of stream habitat environmental variables in the different sampling reaches in Río Bayamón (RBHU—Río Bayamón HU and RBLU—Río Bayamón LU) and Río Piedras (RPHU—Río Piedras HU reach and RPLU—Río Piedras LU reach), were plotted as vectors. The Bray–Curtis dissimilarity index was used as a

Table 1. Mean ($\pm$ SE) water chemistry parameters in the high urban (HU) and low urban (LU) reaches in the Río Bayamón and Río Piedras watersheds. NS—not significant at $p = 0.05$; * $p < 0.05$.

Chemical parameters	Río Bayamón		Río Piedras		ANOVA
	HU	LU	HU	LU	
Temperature ($^{\circ}\text{C}$)	30.07 ± 0.08	31.27 ± 0.02	27.84 ± 0.07	27.08 ± 0.06	*
pH	7.41 ± 0.04	7.79 ± 0.07	7.69 ± 0.04	7.66 ± 0.04	*
Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$)	436 ± 5.03	425 ± 0.6	455 ± 4.6	473 ± 4.5	*
Total dissolved solids (ppm)	218 ± 2.4	212 ± 0.4	231 ± 2.0	236 ± 2.24	*
Dissolved oxygen ($\text{mg}\cdot\text{l}^{-1}$)	7.46 ± 0.1	5.20 ± 0.1	5.10 ± 0.2	5.10 ± 0.3	*
Salinity (ppt)	0.21 ± 0.002	0.20 ± 0.001	0.22 ± 0.002	0.22 ± 0.002	*

distance variable for NMDS ordination. All statistical analyses will be performed with PAST software version 4.04.

Descriptive analyses were performed to describe the amount and type of microplastic retained in the GIT of *P. multiradiatus*. A Chi-square test was performed to determine the sex of the plecos. To understand the variations in the abundance of MPs between the two different urban stream sites in each watershed, a one-way ANOVA was performed under a statistical significance level of $p < 0.05$. However, prior to ANOVA analysis, microplastic abundance data were tested using the Kolmogorov–Smirnov test to confirm normal distribution and Levene’s test ($p > 0.05$) to assess homogeneity of variances without any transformation. The total fish length in each urban reach of the watersheds was compared to the total amount of plastic particles found in the GIT using a Pearson correlation test.

3. Results

3.1. Physicochemical parameters

In the Río Bayamón watershed, the one-way ANOVA results for chemical parameters showed significant differences ($F_{(5,6)} = 4484$, $p < 0.05$) in temperature, pH, conductivity, total dissolved solids, dissolved oxygen, and salinity between the urban reaches (HU and LU) within the same watershed. The HU reach showed a mean water temperature of 30.07 ± 0.08 $^{\circ}\text{C}$ and a pH with a high mean value of 7.41 ± 0.04 . While in the LU reach the temperature and pH showed low mean values of 30.07 ± 0.08 $^{\circ}\text{C}$ and 7.41 ± 0.04 , respectively. The parameters of conductivity, total dissolved solids, dissolved oxygen concentration, and stream salinity in the HU reach were higher in comparison to the LU (table 1).

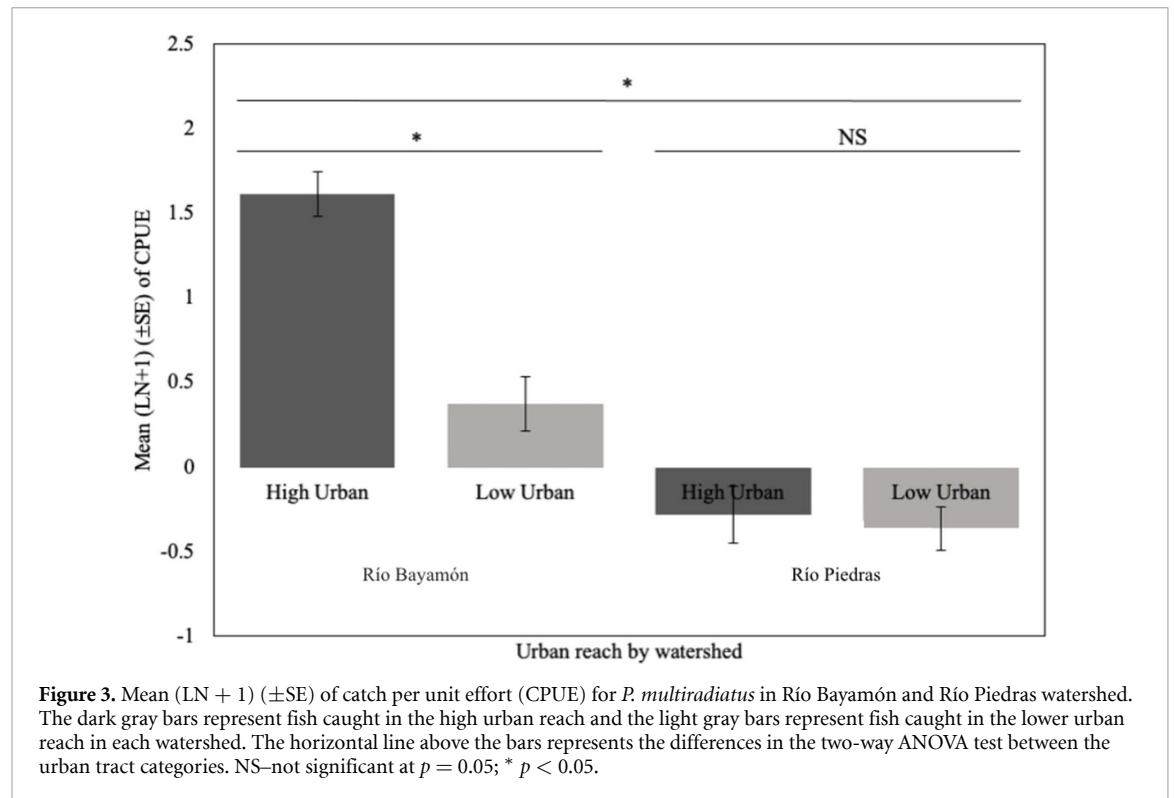
One-way ANOVA for chemical parameters in the Río Piedras watershed showed significant differences ($F_{(5,6)} = 616$, $p < 0.05$) in temperature, pH, conductivity, total dissolved solids, dissolved oxygen, and salinity between the urban reaches (HU and LU) within the same watershed. The high mean values of temperature and pH were recorded in the HU reach (27.84 ± 0.07 $^{\circ}\text{C}$ and 7.69 ± 0.04 , respectively), while in the LU, lower mean values were recorded (27.08 ± 0.06 $^{\circ}\text{C}$ and 7.66 ± 0.04 , respectively). The LU recorded high mean values for conductivity, total dissolved solids, and dissolved oxygen concentration, unlike the HU, which recorded low mean values. Salinity for both urban reaches was similar, with mean values of 0.22 ± 0.002 ppt (table 1).

Comparison of physicochemical parameters among urban reaches between the Río Bayamón and Río Piedras watersheds showed significant differences ($F_{(5,18)} = 2691$, $p < 0.05$) in temperature, pH, conductivity, total dissolved solids, dissolved oxygen, and salinity (table 1). Our results showed that the stream water temperature of the Río Bayamón watershed is significantly higher for all urban reaches compared to the Río Piedras watershed. The highest stream water temperature was observed in the LU reach of the Río Bayamón watershed (31.27 $^{\circ}\text{C} \pm 0.02$ $^{\circ}\text{C}$), while the temperature with low mean values was in the LU of the Río Piedras watershed (27.08 $^{\circ}\text{C} \pm 0.06$ $^{\circ}\text{C}$). Significant differences in conductivity, total dissolved solids, dissolved oxygen concentration, and salinity were observed between the watersheds (table 1). The urban reaches of the Río Piedras watershed had high mean values for Conductivity (HU: 455 ± 4.6 $\mu\text{S}\cdot\text{cm}^{-1}$; LU: 473 ± 4.5 $\mu\text{S}\cdot\text{cm}^{-1}$), total dissolved solids (HU: 231 ± 2.0 ppm; LU: 236 ± 2.24 ppm), and salinity concentrations (HU: 0.22 ± 0.002 ppt; LU: 0.22 ± 0.002 ppt), while in the Río Bayamón watershed, the urban reaches showed low mean values for these parameters. The mean values for dissolved oxygen concentration for the Río Bayamón watershed (HU: 7.46 ± 0.1 $\text{mg}\cdot\text{l}^{-1}$; LU: 5.20 ± 0.1 $\text{mg}\cdot\text{l}^{-1}$) were higher than those for the Río Piedras watershed (HU: 5.10 ± 0.2 $\text{mg}\cdot\text{l}^{-1}$; LU: 5.10 ± 0.3 $\text{mg}\cdot\text{l}^{-1}$) (table 1).

One-way ANOVA results comparing environmental variables (depth, water flow, and substrate index) of the urban reaches between the Río Bayamón and Río Piedras watersheds and between sampling sites showed statistically significant differences ($F_{(2,9)} = 15.3$, $p = 0.001$) (table 2). The urban reaches in the Río Bayamón

Table 2. Mean ($\pm$ SE) of physical measurements in the high urban (HU) and low urban (LU) reaches in the Río Bayamón and Río Piedras watersheds. NS—not significant at $p = 0.05$; * $p < 0.05$.

Physical measurements	Río Bayamón		Río Piedras		ANOVA
	HU	LU	HU	LU	
Depth (m)	37.5 $\pm$ 5.0	38.6 $\pm$ 4.5	51.2 $\pm$ 5.5	49.0 $\pm$ 5.4	*
Water flow	42.57 $\pm$ 9.93	12.10 $\pm$ 1.26	16.34 $\pm$ 1.37	29.5 $\pm$ 3.24	*
Substrate index	4.99 $\pm$ 1.37	4.98 $\pm$ 1.28	4.94 $\pm$ 1.09	4.94 $\pm$ 1.10	*



watershed showed no significant differences ($F_{(2,3)} = 2.76$, $p = 0.208$), while the urban reaches in the Río Piedras watershed did show significant differences ($F_{(2,3)} = 29.34$, $p = 0.01$) (table 2).

3.2. Fish sampling and population size

A total of 383 specimens of *P. multiradiatus* were collected in the Río Bayamón and Río Piedras watersheds. Higher numbers of *P. multiradiatus* were observed in the HU ($N = 264$), in contrast to the LU ($N = 80$) in the Río Bayamón watershed. For the Río Piedras watershed, the catch of organisms was lower than the Río Bayamón, with the HU ($N = 32$) having the highest number in the watershed and the LU ($N = 7$) having a lower abundance.

Significant differences were found in CPUE between Río Bayamón and Río Piedras watersheds (Two-way ANOVA, $F_{(3,56)} = 43.85$, $p < 0.05$) (figure 3). The urban reaches in the Río Bayamón watershed showed a statistically significant difference between the CPUE ($F_{(1,28)} = 45.57$, $p < 0.05$) (figure 3). While in the urban reaches of the Río Piedras watershed, there was no significant difference between CPUE ($F_{(1,28)} = 0.157$, $p = 0.694$) (figure 3). Our results showed that HU reaches in Río Bayamón (2.06 ± 0.21 CPUE) and Río Piedras (0.25 ± 0.56 CPUE) had significantly different mean CPUE. Similar results were found for LU reaches (figure 3).

The population estimate by the Petersen Index of *P. multiradiatus* in the urban reaches of HU and LU in Río Bayamón and Río Piedras showed variations between watersheds (table 3). The Río Bayamón watershed had the highest population estimate of *P. multiradiatus* in the HU reach, with a population size estimate of 408 individuals, while the LU showed a population size estimate of 19. On the other hand, the Río Piedras watershed obtained a much lower population estimate of *P. multiradiatus*, where the HU had 15 individuals and the LU had 7 (table 3).

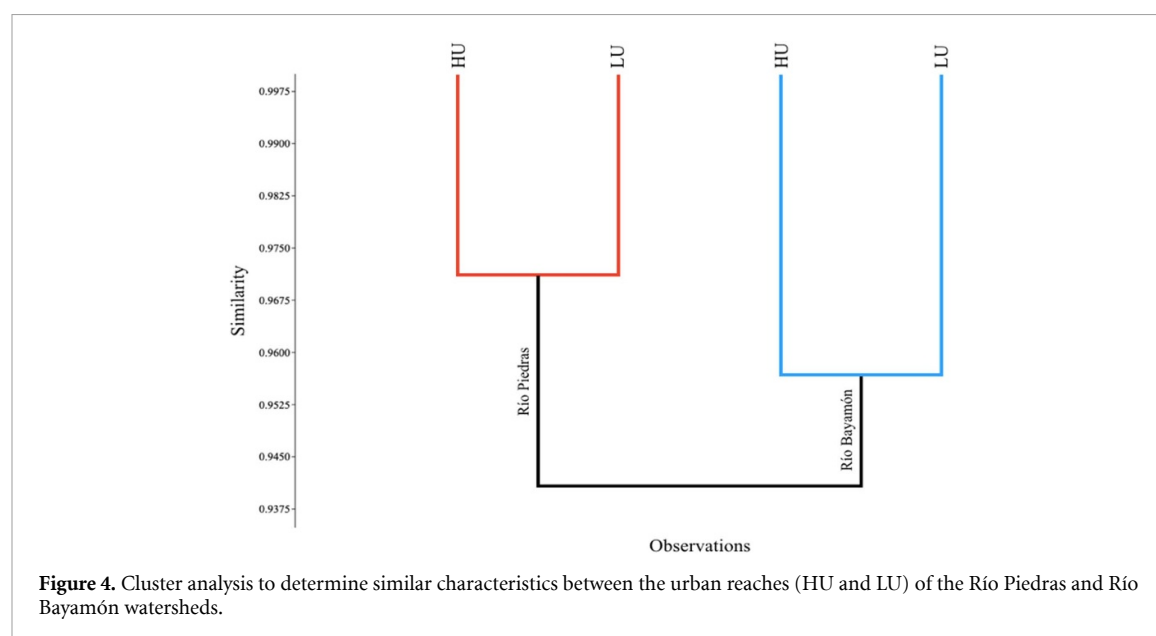
Plecos collected in the HU and LU reaches of the Río Piedras watershed had a greater TL than fish collected in the Río Bayamón watershed. The HU and LU reaches in the Río Piedras watershed showed a

Table 3. Population estimation by Petersen index (PI) of *P. multiradiatus* in the high urban (HU) and low urban (LU) reaches of the Río Bayamón and Río Piedras watersheds.

Watershed	Urban reach	Population size by PI
Río Bayamón	HU	408
	LU	19
Río Piedras	HU	15
	LU	7

Table 4. Mean ($\pm$ SE) morphological measurements of *P. multiradiatus* in the high urban (HU) and low urban (LU) reaches of the Río Bayamón and Río Piedras watersheds.

Watershed	Urban reach	Mean weight $\pm$ SE (g)	Mean total length $\pm$ SE (cm)	Mean fork length $\pm$ SE (cm)	Mean standard length $\pm$ SE (cm)	Mean head length $\pm$ SE (cm)	Mean pectoral length $\pm$ SE (cm)	Mean caudal length $\pm$ SE (cm)	Mean body width $\pm$ SE (cm)	Sex M:F (No. of fish)
Río Bayamón	High	483.62 $\pm$ 19.4	40.41 $\pm$ 0.6	36.41 $\pm$ 0.7	32.97 $\pm$ 0.6	7.53 $\pm$ 0.2	16.60 $\pm$ 0.3	10.57 $\pm$ 0.3	12.21 $\pm$ 0.3	18:7
	Low	345 $\pm$ 34.0	35.8 $\pm$ 0.9	31.34 $\pm$ 0.7	27.78 $\pm$ 0.7	6.52 $\pm$ 0.2	13.52 $\pm$ 0.5	9.42 $\pm$ 0.1	11.06 $\pm$ 0.3	2:3
Río Piedras	High	1068 $\pm$ 26.7	50.55 $\pm$ 1.3	46.29 $\pm$ 1.2	42.61 $\pm$ 1.1	9.68 $\pm$ 0.2	20.34 $\pm$ 0.6	14.82 $\pm$ 0.5	16.53 $\pm$ 0.2	8:2
	Low	495 $\pm$ 90	40.15 $\pm$ 2.7	35.3 $\pm$ 2.2	32.3 $\pm$ 2.3	8.65 $\pm$ 0.7	13.15 $\pm$ 0.7	11.35 $\pm$ 1.3	12.3 $\pm$ 1.0	1:1

**Figure 4.** Cluster analysis to determine similar characteristics between the urban reaches (HU and LU) of the Río Piedras and Río Bayamón watersheds.

total fish length with high mean values of 50.55 ± 1.3 and 40.15 ± 2.7 , respectively. Meanwhile, the HU and LU reaches in the Río Bayamón watershed showed low mean values of 40.41 ± 0.6 and 35.8 ± 0.9 , respectively (table 4). Similarly, plecos from the Río Piedras watershed presented high mean values in morphological measurements (FL, standard length, head length, pectoral length, caudal length, body width, and weight) in contrast to plecos from the Río Bayamón watershed (table 4). No significant difference was found between the sexes (Chi-square test, $X^2 = 2.977$, $df = 3$, $p = 0.3518$).

3.3. Multivariable analysis

Cluster analysis showed that the urban reaches (HU and LU) of the Río Bayamón watershed are similar to each other, as are the urban reaches of the Río Piedras watershed. However, the comparison at the watershed level showed that the Río Bayamón watershed is different from the Río Piedras watershed (figure 4).

NMDS ordination of *P. multiradiatus* abundance in the HU and LU reaches in the Río Bayamón and Río Piedras watersheds produced two main axes that explain most of the variation. Axes 1 and 2 were highly related to conductivity (0.7) and water flow (0.8), respectively (figure 5). Streams with positive values on NMDS axes 1 and 2 were located in the LU reach of Río Piedras watershed. Pool conductivity in the Río Piedras watershed represented the environmental variable that best explained variation in *P. multiradiatus* abundance on axis 1. The environmental variable that best explained variation on axis 2 was waterflow in the

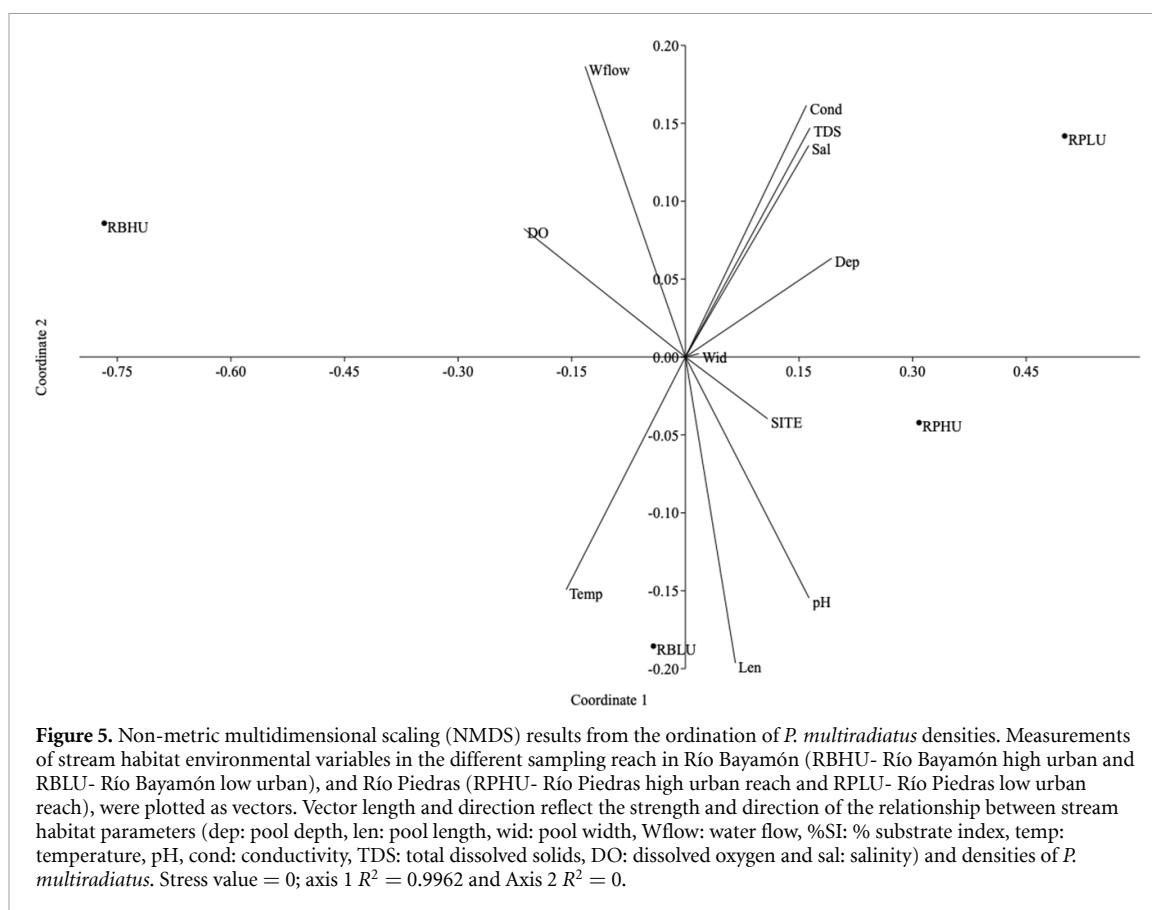


Table 5. Presence of microplastics (MP) recovered in the gastrointestinal tract (GIT) of *P. multiradiatus* in the high urban (HU) and low urban (LU) reaches of the Río Bayamón and Piedras watersheds.

Watershed	Urban reach	No. of fish GIT examined	No. of fish with MP presence	No. of fish with MP absence
Río Bayamón	HU	25	21	4
	LU	5	5	0
Río Piedras	HU	10	8	2
	LU	2	2	0

Río Bayamón watershed. Pool width, depth, total dissolved solids, salinity, and dissolved oxygen concentration were also important factors directly influencing *P. multiradiatus* densities (figure 5).

3.4. MPs identification

Among the samples corresponding to the sampling between the Río Bayamón and Río Piedras watersheds, MPs were identified in the GIT of 36 of the 42 specimens of pleco, representing 85.7% of the entire sample. In the Río Bayamón watershed in the HU reach, 25 individuals were collected, with 21 plecos having MPs present in the GIT, and in the LU reach ($n = 5$) all retained MP in their GIT (table 5). Meanwhile, in the Río Piedras watershed, plecos collected in the HU ($n = 10$) and LU ($n = 2$) reaches had MPs the GIT (8 and 2 fish, respectively) (table 5). The highest amount of plastic particles varied among the total fish lengths in the HU reach (mean 40.41 ± 0.6 cm Río Bayamón, mean 50.55 ± 1.3 cm Río Piedras) in both watersheds (table 6).

A total of 180 plastic particles were found in the GIT of the plecos, and there were 126 MPs (Río Bayamón—HU), 21 MPs (Río Bayamón—LU), 26 MPs (Río Piedras—HU) and 7 MPs (Río Piedras—LU) (table 6). Most of the MPs found in the GIT of the samples varied between fibers and fragments. The esophagus was the region of the GIT with the highest presence of MPs, followed by the intestine and stomach (table 7, figure 6). Most of the MPs retained in the sections of the GIT were fibers, and only a few individuals presented retention of fragments.

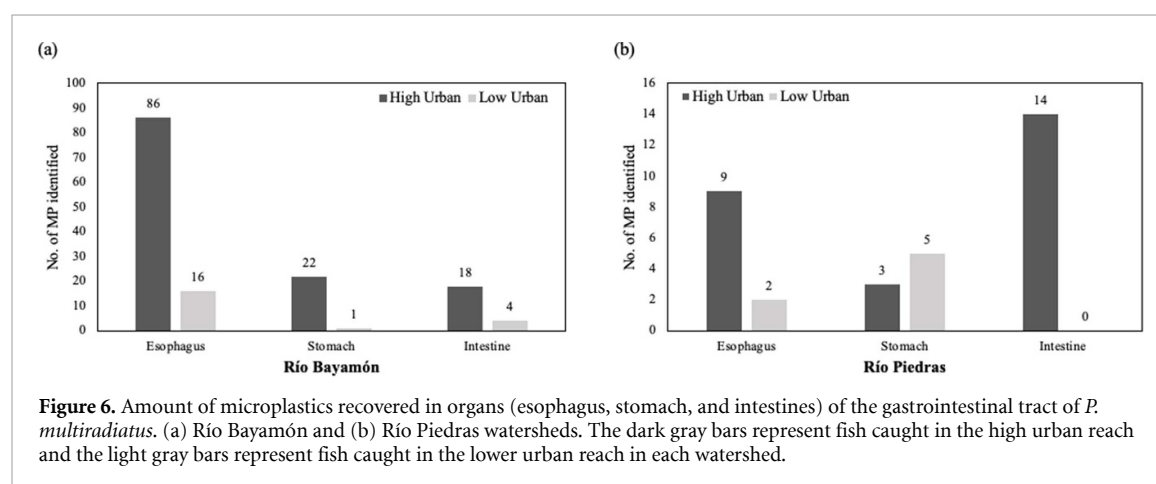
The one-way ANOVA to compare the abundance of MPs between the different categories of HU and LU showed no significant differences between the watersheds ($F_{(1,4)} = 2.42$, $p = 0.19$) (figure 7). The

Table 6. Mean ($\pm$ SE) length, width, weight, number, and type of microplastics (MP) recorded in the gastrointestinal tract (GIT) of *P. multiradiatus* in the high urban (HU) and low urban (LU) reaches of the Río Bayamón and Río Piedras watersheds.

Watershed	Urban reach	No. of fish GIT examined	Mean weight GIT $\pm$ SE (g)	Mean length fish $\pm$ SE (cm)	Mean width fish $\pm$ SE (cm)	Mean weight fish $\pm$ SE (g)	No. of MP identified (no. of fish)	Plastic type
Río Bayamón	HU	25	35.7 $\pm$ 1.9	40.41 $\pm$ 0.6	12.21 $\pm$ 0.3	483.62 $\pm$ 19.4	126 (21)	Fiber and fragment
	LU	5	28.2 $\pm$ 5.1	35.8 $\pm$ 0.9	11.06 $\pm$ 0.3	345 $\pm$ 34.0	21 (5)	Fiber
Río Piedras	HU	10	96.5 $\pm$ 6.0	50.55 $\pm$ 1.3	16.53 $\pm$ 0.2	1068 $\pm$ 26.7	26 (8)	Fiber and fragment
	LU	2	33.0 $\pm$ 1.0	40.15 $\pm$ 2.7	12.3 $\pm$ 1.0	495 $\pm$ 90	7 (2)	Fiber

Table 7. Total number of microplastics (MP) in the gastrointestinal tract (GIT) of *P. multiradiatus* by type in the high urban (HU) and low urban (LU) reaches of the Río Bayamón and Río Piedras watersheds.

Watershed	Urban reach	No. of fish GIT examined	Parts of the GIT (no. of MP identified)			Plastic type (no. of MP identified)
			Esophagus	Stomach	Intestine	
Río Bayamón	HU	25	(86)	(22)	(18)	Fiber (123) and fragment (3)
	LU	5	(16)	(1)	(4)	Fiber (21)
Río Piedras	HU	10	(9)	(3)	(14)	Fiber (14) and fragment (12)
	LU	2	(2)	(5)	(0)	Fiber (7)

**Figure 6.** Amount of microplastics recovered in organs (esophagus, stomach, and intestines) of the gastrointestinal tract of *P. multiradiatus*. (a) Río Bayamón and (b) Río Piedras watersheds. The dark gray bars represent fish caught in the high urban reach and the light gray bars represent fish caught in the lower urban reach in each watershed.

Kolmogorov–Smirnov test ($p > 0.150$), as well as the Levene test ($p > 0.506$), to confirm the normal distribution of the MPs abundance and evaluate the homogeneity of variances between watersheds, did not show a statistically significant difference between each of the variances.

The Pearson correlation test to compare the total fish length (cm) and the total amount of plastic particles found in the GIT showed positive and negative variations in the correlation coefficients. The concentrations of plastic particles identified in fish captured in the HU reach in the Río Bayamón watershed showed a positive degree of correlation in the total fish length ($r = 0.1192$, $p < 0.05$) (figure 8(a)). In comparison to the LU reach in the same watershed, which showed a negative correlation coefficient ($r = -0.4442$, $p < 0.05$) (figure 8(b)). However, fish caught in the HU and LU reaches in the Río Piedras watershed showed a negative ($r = -0.02120$, $p < 0.05$) and positive ($r = 1$, $p < 0.05$) correlation coefficient, respectively (figures 8(c) and (d)). There was no significant relationship between total fish length and the number of particles identified in the fish's GIT (figure 8).

No benthic filamentous algae, detritus, or small invertebrates could be found in the analysis of the filters. Therefore, multiple dyes (safranin, phloroglucinol, lactophenol cotton blue, alizarin red, and Nile red) were tested to identify some of the exposed properties of the stomach tissue of each GIT sampled (figure 9). No

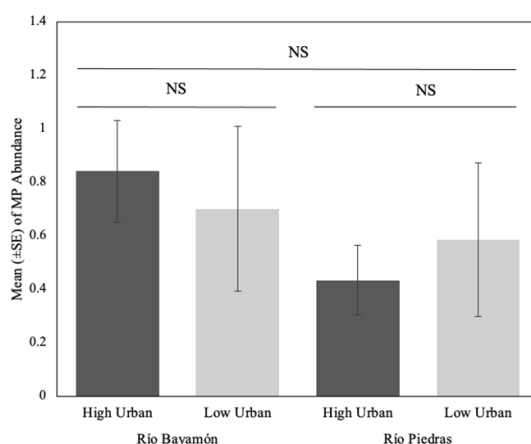


Figure 7. Mean ($\pm$ SE) of microplastics (MPs) abundance between the two different categories (high urban and low urban) between the Río Bayamón and Río Piedras watersheds. The dark gray bars represent fish caught in the high urban reach and the light gray bars represent fish caught in the lower urban reach in each watershed. The horizontal line above the bars represents the differences between the urban density categories in the one-way ANOVA test. NS—not significant at $p = 0.05$.

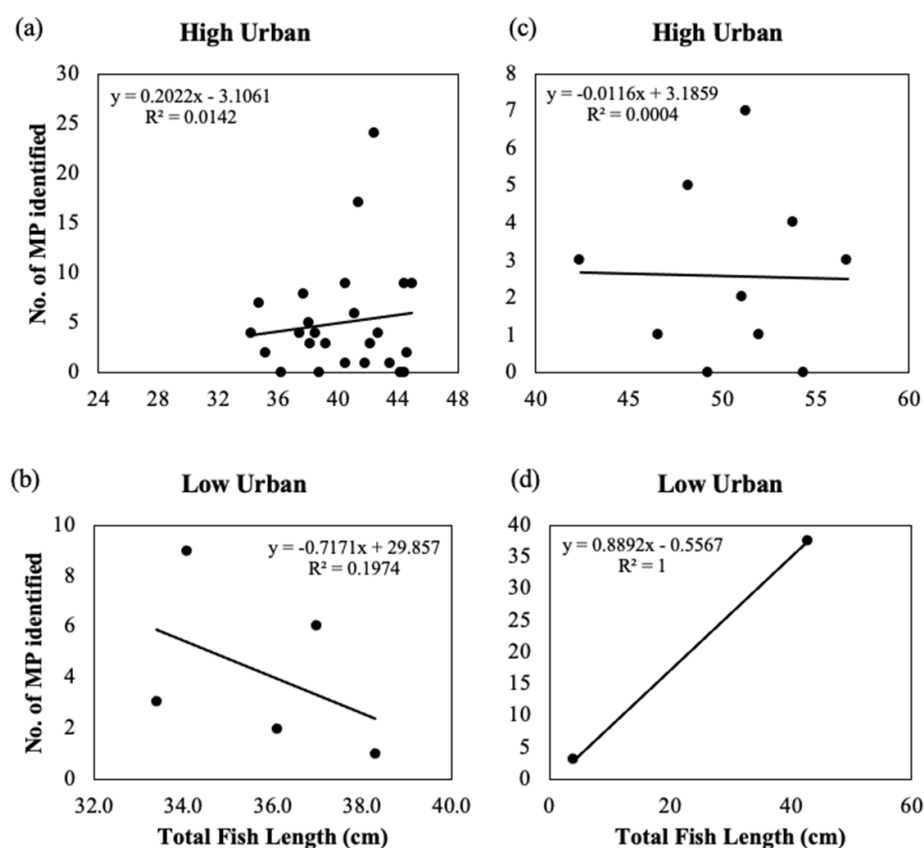


Figure 8. Correlation plots between microplastics (MP) identified and total fish length (cm) for *P. multiradiatus* captured in the HU and LU reaches in (a–b) Río Bayamón and (c–d) Río Piedras watersheds. $Y = 42.81 + 0.1460 \text{ MP} - 0.08897 \text{ total fish length}$; $R^2 = 0.012997$.

algae was observed in the specimens from both watersheds. The presence of lignin was only identified in individuals sampled in the Río Piedras watershed (HU, $n = 2$; LU, $n = 1$), while it was absent in the Río Bayamón watershed. For fish captured in both watersheds, in the HU and LU reaches in the Río Bayamón watershed, plecós showed the presence of fungal (HU, $n = 3$; LU, $n = 3$) and animal chitin (HU, $n = 17$; LU, $n = 3$). While in the Río Piedras watershed, only the plecós captured in the HU section showed the presence of fungal ($n = 2$) and animal chitin ($n = 8$). The presence of microplastic in individuals from both urban reaches in both watersheds was determined by staining plastic particles with Nile Red (figures 9(D)–(L)), with plastic-type fibers and fragments being the most common MPs identified. In the HU and LU reaches of

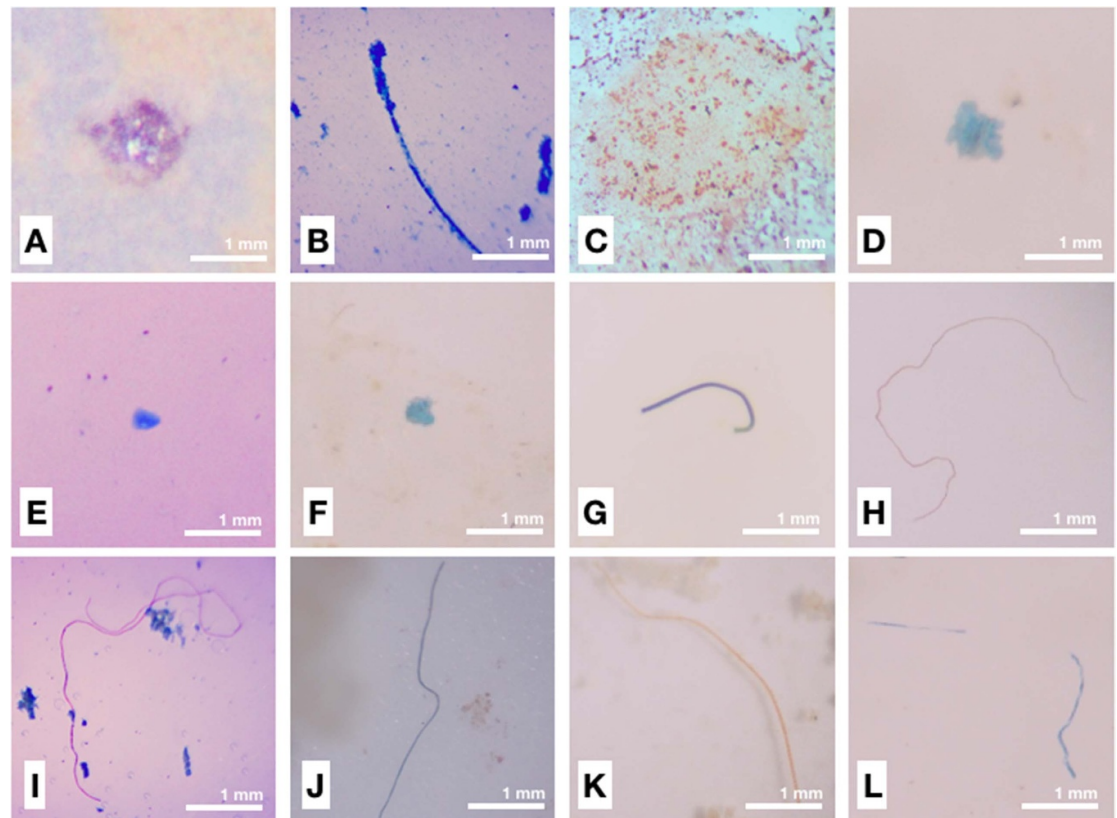


Figure 9. Images of the internal properties of *P. multiradiatus* stomach tissue with multiple dyes. (A) Lignin stained with Phloroglucinol; (B) fungi chitin stained with lactophenol cotton blue; (C) animal chitin stained with alizarin red; and, (D)–(F) fragment and (G)–(L) fiber stained with Nile red.

the Río Bayamón watershed, a total of 12 microplastic particles were identified, with fibers and fragments dominating. Meanwhile, in the Río Piedras watershed's urban reaches, 10 microplastic particles were identified.

4. Discussion

4.1. Population size of *P. multiradiatus*

Over the past decade, several publications have reported the presence of *Pterygoplichthys* outside its natural range, some suggesting new sites of introduction and others documenting expansion into existing populations [31, 34, 44, 45]. Related to the high environmental pressure caused by human activities, the introduction and expansion of *Pterygoplichthys* constitutes a severe threat to the reduction of native fish communities (e.g. *Gobiomorus dormitor*, *Dormitator maculatus*, *Eleotris pisonis*, *Sicydium plumieri*, *Awaous tajasica*, and *Awaous banana*) and the physical alteration of the aquatic ecosystems where they establish themselves (e.g. by altering sediment size and algal communities through competition for food) in the streams of Puerto Rico [33, 36].

This study showed no relationship in the abundance of *P. multiradiatus* between the HU and LU reaches in the Río Bayamón and Río Piedras watersheds. The HU reach in the Río Bayamón watershed had a higher mean CPUE and a higher estimated population of *P. multiradiatus* than the LU reach in the same watershed. The observed result confirms previous studies that found similar characteristics, such as shallow depth, sedimentary soils, rocky aquatic relief, aquatic plants, and a large amount of detritus [33, 36]. Although sections of the Río Bayamón are currently highly degraded by anthropogenic actions, it has a larger catchment area, a more or less constant depth, and the introduction of organic matter due to the canopy cover present. However, if the previously mentioned conditions are absent in a particular area, we would anticipate that the results would show a negligible or absent pleco population. This is why our results showed that the HU and LU reaches of the Río Piedras watershed had low mean CPUE values and a smaller estimated population of *P. multiradiatus* by the Petersen index than the urban reaches of the Río Bayamón watershed.

However, we cannot consider a direct relationship between pleco abundance and environmental parameters because it is contradictory to the low pleco catch in the urban reaches of the Río Piedras

watershed; there is an important contribution of organic matter present, the geological characteristics are favorable, and the relief is ideal for the establishment and development of pleco. However, the low abundance of this species in the Río Piedras watershed could be directly related to the construction of physical barriers such as dams (e.g. Las Curias dam), canalizations, and other human impacts such as degradation and fragmentation of the stream habitat. Similarly, poor water quality may influence the low presence of this species. The Puerto Rico Environmental Quality Board and the U.S. Environmental Protection Agency classify the waters of the Río Piedras as highly polluted [42]. As a consequence of these characteristics and according to our results, it is likely that plecos can migrate to adjacent areas, which explains their low abundance in the streams of the Río Piedras watershed.

The results of this study do not support the hypothesis. The abundance of plecos, which we found in both watersheds, indicates that reaches with HU development may be mostly influenced by abiotic factors such as temperature, pH, conductivity, total dissolved solids, dissolved oxygen concentration, and salinity; unlike reaches with LU development that may be less influenced by abiotic factors within the same watershed. Similar data are shown in previous studies, which have identified the areas with the highest number of species as well as the number of individuals, possibly due to the same abiotic factors [46–48]. In general, since it has been demonstrated that even a small population of the genus *Pterygoplichthys* can significantly reduce the abundance of aquatic vegetation and the physical habitat, the presence of these fish in any aquatic environment in Puerto Rico represents a significant threat to the biodiversity of native fish species in the region.

4.2. MPs analysis in the GIT of *P. multiradiatus*

MPs in freshwater fish have been documented since 2014 [49]. Since then, more studies have been conducted directly by MPs on these freshwater vertebrates and their relationship with plastic pollution. Due to their size, MPs can easily be mistakenly eaten as natural fish food, and their ingestion can cause injuries such as internal abrasions and obstructions [13, 14]. The present study documents the first record of MPs in the GIT of an invasive fish, *P. multiradiatus*, in the urban streams of Puerto Rico.

The analyses showed that the presence of MPs between these streams and urban areas was not different. Various factors can affect the distribution of MPs in surface waters, including source loading, plastic properties, and climatic and hydrodynamic conditions [50]. Similarly, the primary effluent entering the stream is likely due to the fragmentation of urban plastic products, and at the expense of this, these pollutants are likely to become more prevalent in aquatic organisms and environments, particularly those found in urban areas. Thus, the high concentration of MPs in the GIT of pleco, like other bottom-feeding species in urban aquatic environments, may serve as a good bioindicator for this type of pollution. However, the use of biomarkers is not limited to individual species that have limited environmental tolerance [18]. For example, findings from previous studies indicated the presence of MPs in the GIT (232 MP) in dominant fish species in freshwater ecosystems in Turkey [19]. Among the dominant species studied, chub (*Squalius cephalus*) was found to contain the most common type of plastic contaminants with fibers and fragments being the most common forms [19]. On the other hand, there are findings on the presence of MP in zebra mussels [18], despite the fact that this species can filter contaminants even when these contaminants are at low levels in the environment.

The correlation between the morphological measurements of *P. multiradiatus*, specifically the TL, and the abundance of MPs provides fundamental information on the availability of these plastic particles in freshwater bodies, influencing the direct intake of fish. However, no direct relationship between fish body size and the number of plastic particles ingested could be established. MPs were found in 36 of the 42 plecos analyzed, ranging from 7 to 126 plastic particles per fish caught in different urban reaches. A total of 180 MPs were found in the GIT of plecos in two categories: fibers and fragments, with the esophagus having the highest retention of MPs, followed by the intestine and stomach. Ingestion of this debris probably occurred accidentally during normal feeding activity. Likewise, plastic fibers and fragments are likely to be retained in the stomach along with other undigested waste due to MPs' unique shape and morphological characteristics, which cause longer retention [51]. Although plastic is normally expected to pass through the intestines along with any other non-digestible material,

In addition, the ingestion of MP by plecos could be more closely related to feeding habits than habitat occupancy; that is, it does not depend on different land uses due to urban development to determine the abundance of these small plastic particles in the GIT of fish. Adding to these disturbance conditions are the findings of this study, which demonstrate that the amount of MP recorded in the GIT in plecos is remarkably similar to that recorded for other species in aquatic environments near urban areas (e.g. [52–54]). This demonstrates the regular frequency of this type of plastic pollution input, which results from direct and indirect input of anthropogenic debris, together with the aforementioned topographic features and human influences.

Most of the MPs found and identified in fish were sources of secondary MPs, being fragments and fibers, one of the most common forms of plastic found in the environment [55], making them prone to being ingested by bottom-dwelling fish. Similar findings have been reported in numerous studies (e.g. [56–59]), in which the amount of MP found in freshwater aquatic environments is influenced by the degree of the surrounding environment. Analysis of stomach contents using a variety of dyes showed that selective ingestion with the detritivorous habits of these fish combined with the consumption of MP may be connected to the feeding habits of these organisms, but it is unclear whether the food availability influences the amount of particles ingested by these vertebrates in the environment in which they are found. Therefore, the presence and high concentration of MP in the GIT of the 42 specimens of *P. multiradiatus* studied indicate that the urban rivers of the Río Bayamón and Río Piedras watersheds have been heavily contaminated with plastic debris. However, more research is needed to determine if the diet of *P. multiradiatus* is related to the amount and type of plastic particles found in their digestive system.

5. Conclusions

The main objective of this study was to describe how human impact, as a function of urban density, influences microplastic pollution in urban streams of Puerto Rico and its effect on freshwater fish such as *P. multiradiatus*. To achieve this objective, a freshwater fish species, *P. multiradiatus* (Hancock, 1828), was used as a bioindicator, given its natural distribution range, in order to evaluate an experimental design to determine the effect of MPs on aquatic organisms. In addition, *P. multiradiatus*, being a freshwater fish, is reported as an invasive species in Puerto Rico, with increasing evidence of impacts on the ecosystem and biodiversity [22, 27, 28].

The accumulation of anthropogenic debris reaching rivers is known to increase the production of plastic debris of various sizes, to which a diversity of organisms living in freshwater environments are exposed [11]. Tropical rivers contain a variety of aquatic life, including fish, freshwater shrimp, and macroinvertebrates, that may be affected by anthropogenic activities related to urban development. In particular, freshwater fish are vulnerable to the physical, chemical, and biological degradation of river resources [23]. In addition to effects associated with land use change or increased imperviousness due to urbanization and increased channelization projects, urban streams have a major impact on the distribution of different fish species and community composition. As a result, native species assemblages are overburdened by disrupted migration patterns and life cycles, while the introduction and dispersal of exotic species continue to increase [24, 25]. However, it is not entirely clear which environmental and anthropogenic variables are most important in explaining the establishment of exotic fish populations in Puerto Rican streams.

This study found that urban areas have a high abundance of the freshwater fish *P. multiradiatus*. As a result, the difference in abundance of plecos found in both watersheds suggests that sites with HU development are mostly influenced by abiotic factors such as temperature, pH, conductivity, total dissolved solids, dissolved oxygen concentration and salinity; as opposed to sites with LU development, where the influence of abiotic factors is low.

Plastic debris contamination from anthropogenic activities in Caribbean aquatic environments is poorly documented, especially in Puerto Rico [15]. This study documented the first evidence of microplastic contamination in the GIT of *P. multiradiatus*, a freshwater fish species widely distributed in urban streams in Puerto Rico [29, 32]. We found that the distribution of MPs in different localities according to different land uses by urban development (HU and low urban) in streams is not statistically different. Furthermore, the high concentration of MPs in the GIT analysis of *P. multiradiatus* is due to anthropogenic activities, which are the main source of plastic pollution in aquatic ecosystems. This research also revealed that *P. multiradiatus* is a suitable test organism due to its high tolerance and dominance in disturbed environments [22], which can represent tropical freshwater environments altered by anthropogenic activities and disturbances related to unsustainable urban development.

In context, the presence of MPs in the GIT of the freshwater fish *P. multiradiatus* is alarming since it is part of an ecosystem where it interacts with a great diversity of aquatic species in the rivers of Puerto Rico. In addition, these wastes enter the food chain and are accidentally ingested, causing problems in the organism or causing bioaccumulation and retention problems. Therefore, future research should conduct additional studies to complement this work, either by extending the sampling period to have a higher fish capture rate or by generating a greater relationship between the different rivers and different land uses due to urban or rural development adopted on the island. In addition, create awareness programs on plastic pollution aimed at the population near the streams, especially the surrounding communities, merchants, and fishermen.

Data availability statement

The data cannot be made publicly available upon publication because they are not available in a format that is sufficiently accessible or reusable by other researchers. The data that support the findings of this study are available upon reasonable request from the authors.

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Ethical Statement

Ethical review and approval for this study was approved by the Institutional Animal Care and Use Committee (IACUC), protocol number 2021-02-15-3027, at the University of Puerto Rico, Río Piedras Campus.

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References

- [1] Roebroek C T J, Harrigan S, van Emmerik T H M, Baugh C, Eilander D, Prudhomme C and Pappenberger F 2021 Plastic in global rivers: are floods making it worse? *Environ. Res. Lett.* **16** 025003
- [2] Molbert N, Angelier F, Alliot F, Ribout C and Goutte A 2021 Fish from urban rivers and with high pollutant levels have shorter telomeres *Biol. Lett.* **17** 20200819
- [3] Walsh C J, Roy A H, Feminella J W, Cottingham P D, Groffman P M and Morgan R P 2005 The urban stream syndrome: current knowledge and the search for a cure *J. North Am. Benthol. Soc.* **24** 706–23
- [4] Hoellein T, Rojas M, Pink A, Gasior J and Kelly J 2014 Anthropogenic litter in urban freshwater ecosystems: distribution and microbial interactions *PLoS One* **9** e98485
- [5] Michielssen M R, Michielssen E R, Ni J and Duhaime M B 2016 Fate of microplastics and other small anthropogenic litter (SAL) in wastewater treatment plants depends on unit processes employed *Environ. Sci. Water Res. Technol.* **2** 1064–73
- [6] Schmidt C, Krauth T and Wagner S 2017 Export of plastic debris by rivers into the sea *Environ. Sci. Technol.* **51** 12246–53
- [7] Li J, Liu H and Chen J P 2018 Microplastics in freshwater systems: a review on occurrence, environmental effects, and methods for microplastics detection *Water Res.* **137** 362–74
- [8] Horton A A, Walton A, Spurgeon D J, Lahive E and Svendsen C 2017 Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities *Sci. Total Environ.* **586** 127–41
- [9] Eerkes-Medrano D, Thompson R C and Aldridge D C 2015 Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs *Water Res.* **75** 63–82
- [10] Wagner M *et al* 2014 Microplastics in freshwater ecosystems: what we know and what we need to know *Environ. Sci. Eur.* **26** 12
- [11] van Emmerik T and Schwarz A 2019 Plastic debris in rivers *Wiley Interdiscip. Rev. Water* **7** e1398
- [12] Lusher A 2015 Microplastics in the marine environment: distribution, interactions and effects *Marine Anthropogenic Litter* pp 245–307
- [13] Lusher A L, Welden N A, Sobral P and Cole M 2017 Sampling, isolating and identifying microplastics ingested by fish and invertebrates *Anal. Methods* **9** 1346–60
- [14] [FAO] Lusher A, Hollman P and Mendoza-Hill J 2017 Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety *Food and Agriculture Organization of the United Nations* (available at: www.fao.org/3/i7677e/i7677e.pdf)
- [15] Pérez-Alvelo K M, Llegus E M, Forestier-Babilonia J M, Elías-Arroyo C V, Pagán- Malavé K N, Bird-Rivera G J and Rodríguez-Sierra C J 2021 Microplastic pollution on sandy beaches of Puerto Rico *Mar. Pollut. Bull.* **164** 112010
- [16] Karthik R, Robin R, Purvaja R, Ganguly D, Anandavelu I, Raghuraman R, Hariharan G, Ramakrishna A and Ramesh R 2018 Microplastics along the beaches of southeast coast of India *Sci. Total Environ.* **645** 1388–99
- [17] Davison P and Asch R 2011 Plastic ingestion by mesopelagic fishes in the North Pacific Subtropical Gyre *Mar. Ecol. Prog. Ser.* **432** 173–80
- [18] Atamanalp M, Köktürk M, Gündüz F, Parlak V, Uçar A, Alwazeer D and Alak G 2023 The use of zebra mussel (*Dreissena polymorpha*) as a sentinel species for the microplastic pollution of freshwater: the case of Beyhan Dam Lake, Turkey *Sustainability* **15** 1422
- [19] Atamanalp M, Köktürk M, Parlak V, Uçar A, Arslan G and Alak G 2022 A new record for the presence of microplastics in dominant fish species of the Karasu River Erzurum Turkey *Environ. Sci. Pollut. Res.* **29** 1–11
- [20] Horton A A, Jürgens M D, Lahive E, van Bodegom P M and Vijver M G 2018 The influence of exposure and physiology on microplastic ingestion by the freshwater fish *Rutilus rutilus* (roach) in the River Thames, UK *Environ. Pollut.* **236** 188–94

- [21] Pazos R S, Maiztegui T, Colautti D C, Paracampo A H and Gómez N 2017 Microplastics in gut contents of coastal freshwater fish from Río de la Plata estuary *Mar. Pollut. Bull.* **122** 85–90
- [22] Rodríguez-Barreras R, Zapata-Arroyo C, Falcón L W and Olmeda M D L 2020 An island invaded by exotics: a review of freshwater fish in Puerto Rico *Neotrop. Biodivers.* **6** 42–59
- [23] Engman A C and Ramírez A 2012 Fish assemblage structure in urban streams of Puerto Rico: the importance of reach-and catchment-scale abiotic factors *Hydrobiologia* **693** 141–55
- [24] Greathouse E A, Pringle C M, McDowell W H and Holmquist J G 2006 Indirect upstream effects of dams: consequences of migratory consumer extirpation in Puerto Rico *Ecol. Appl.* **16** 339–52
- [25] Holmquist J G, Schmidt-Gengenbach J M and Yoshioka B B 1998 High dams and marine-freshwater linkages: effects on native and introduced fauna in the Caribbean *Conserv. Biol.* **12** 621–30
- [26] Kwak T J, Engman A C, Fischer J R and Lilyestrom C G 2016 Drivers of Caribbean freshwater ecosystems and fisheries *Freshwater, fish and the future: proc. global cross-sectoral conference Food and Agriculture Organization of the United Nations, Rome, Italy* pp 219–32 (available at: https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/8020676)
- [27] Bunkley-Williams L U C Y, Williams Jr E H, Lilyestrom C G, Corujo-Flores I R I S, Zerbi A J, Aliaume C and Churchill T N 1994 The South American sailfin armored catfish, *Liposarcus multiradiatus* (Hancock), a new exotic established in Puerto Rican fresh waters *Carib. J. Sci.* **30** 90–94
- [28] Erdman D S 1984 Exotic fishes in Puerto Rico *Distribution, Biology and Management of Exotic Fishes* ed WR Courtenay Jr and JR Stauffer Jr pp 162–76
- [29] Kwak T J, Cooney P B and Brown C H 2007 *Fishery Population and Habitat Assessment in Puerto Rico Streams: phase 1 Final Report: federal Aid in Sport Fish Restoration Project F- 50* (US Geological Survey, North Carolina Cooperative Fish and Wildlife Research Unit, Department of Zoology, North Carolina State University)
- [30] Neal J W, Lilyestrom C G and Kwak T J 2009 Factors influencing tropical island freshwater fishes: species, status, and management implications in Puerto Rico *Fisheries* **34** 546–54
- [31] Nico L, Butt P, Johnston G, Jelks H, Kail M and Walsh S 2012 Discovery of South American suckermouth armored catfishes (Loricariidae, *Pterygoplichthys* spp.) in the Santa Fe River drainage, Suwannee River basin, USA *Bioinvasions Rec.* **1** 179–200
- [32] Kwak T J, Smith W E, Buttermore E N, Cooney P B and Cope W G 2013 Fishery population and habitat assessment in Puerto Rico streams: phase 2 final report *US Geological Survey* (<https://doi.org/10.3133/70154939>)
- [33] Mendoza R E *et al* 2009 Armored Catfish (Loricariidae) trinational risk assessment *Trinational risk assessment guidelines for aquatic alien invasive species* (Commission for Environmental Cooperation)
- [34] Nico L, Loftus W and Reid J 2009 Interactions between non-native armored suckermouth catfish (Loricariidae: *pterygoplichthys*) and native Florida manatee (*Trichechus manatus latirostris*) in artesian springs *Aquat. Invasions* **4** 511–9
- [35] Covain R and Fisch-Muller S 2007 The genera of the Neotropical armored catfish subfamily Loricariinae (Siluriformes: loricariidae): a practical key and synopsis *Zootaxa* **1462** 1–40
- [36] Hoover J J, Killgore K J and Cofrancesco A F 2004 Suckermouth catfishes: threats to aquatic ecosystems of the united states? *Aquatic Nuisance Species Research Program Bulletin* pp 1–13
- [37] Mendoza R, Contreras S, Ramírez C, Koleff P, Álvarez P and Aguilar V 2007 Los peces diablo: especies invasoras de alto impacto *Biodiversitas* **70** 1–5
- [38] Williams Jr E H, Bunkley-Williams L and Lopez-Irizarry I 1992 Die-off of brown pelicans in Puerto Rico and the United States Virgin Islands *Am. Birds* **46** 106–1108
- [39] US Census Bureau 2023 Nation's urban and rural populations shift following 2020 census (*Census.Gov.*) (available at: www.census.gov/newsroom/press-releases/2022/urban-rural-populations.html)
- [40] Ewel J J and Whitmore J L 1973 The ecological life zones of Puerto Rico and the Virgin Islands *Institute of Tropical Forestry, San Juan, Puerto Rico. For Serv Res Pap ITF-18:1–72* (available at: www.srs.fs.usda.gov/pubs/rp/rp_itf018.pdf)
- [41] Pérez-Reyes O, Crowl T A and Covich A P 2015 Comparison of decapod communities across an urban-forest land use gradient in Puerto Rican streams *Urban Ecosyst.* **19** 181–203
- [42] Lugo A E, Ramos O and Rodríguez C 2011 The Río Piedras watershed and its surrounding environment. FS-980. US Department of Agriculture, Forest Service, International Institute of Tropical Forestry. 46 (available at: www.fs.usda.gov/treearch/pubs/39146)
- [43] Maes T, Jessop R, Wellner N, Haupt K and Mayes A R 2017 A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile red *Sci. Rep.* **7** 44501
- [44] Ng H H and Tan H H 2010 An annotated checklist of the non-native freshwater fish species in the reservoirs of Singapore *Cosmos* **6** 95–116
- [45] O'grady K and Cowx I 2000 Nonindigenous fishes introduced into Inland waters of the United States *Fish. Manage. Ecol.* **7** 470–1
- [46] Perdomo R and Zambrano G 2007 *Estructura y composición de la ictiofauna de la cuenca media del río Hacha en el piedemonte amazónico* (Trabajo de grado. Facultad de Ciencias Básicas. Universidad de la Amazonia. Florencia)
- [47] Mena Y, Murillo F, Rivas T and Rincón C 2003 Inventario Ictico de la Cuenca del Río Cabí, Chocó-Colombia (Revista Institucional, Universidad Tecnológica del Chocó) pp 39–44
- [48] Roman-Valencia C 1990 Lista y distribución de peces en la cuenca media del río Atrato, Chocó, Colombia *Caldasia* **16** 201–7
- [49] Sanchez W, Bender C and Porcher J M 2014 Wild gudgeons (Gobio gobio) from French rivers are contaminated by microplastics: preliminary study and first evidence *Environ. Res.* **128** 98–100
- [50] Zhao S, Zhu L and Li D 2015 Microplastic in three urban estuaries, China *Environ. Pollut.* **206** 597–604
- [51] Lenz R, Enders K, Beer S, Sørensen T K, Stedmon C A and Reeh L 2016 Analysis of microplastic in the stomachs of herring and cod from the North Sea and Baltic Sea *DTU Aqua National Institute of Aquatic Resources* vol 12 pp 1–21
- [52] Vidal C, Lozoya J P, Tesitore G, Goyenola G and De Mello F T 2021 Incidence of watershed land use on the consumption of meso and microplastics by fish communities in Uruguayan Lowland streams *Water* **13** 1575
- [53] McNeish R E, Kim L H, Barrett H, Mason S, Kelly J J and Hoellein T J 2018 Microplastic in riverine fish is connected to species traits *Sci. Rep.* **8** 11639
- [54] Silva-Cavalcanti J S, Silva J D B, de França E J, de Araújo M C B and Gusmao F 2017 Microplastics ingestion by a common tropical freshwater fishing resource *Environ. Pollut.* **221** 218–26
- [55] Anderson A, Andrady A, Hidalgo-Ruz V and Kershaw P J 2015 Sources, fate and effects of microplastics in the marine environment *Global Assessment; GESAMP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection*

- [56] Wang W, Yuan W, Chen Y and Wang J 2018 Microplastics in surface waters of Dongting Lake and Hong Lake, China *Sci. Total Environ.* **633** 539–45
- [57] Free C M, Jensen O P, Mason S A, Eriksen M, Williamson N J and Boldgiv B 2014 High-levels of microplastic pollution in a large, remote, mountain lake *Mar. Pollut. Bull.* **85** 156–63
- [58] Sadri S S and Thompson R C 2014 On the quantity and composition of floating plastic debris entering and leaving the Tamar Estuary, Southwest England *Mar. Pollut. Bull.* **81** 55–60
- [59] Eriksen M, Mason S, Wilson S, Box C, Zellers A, Edwards W, Farley H and Amato S 2013 Microplastic pollution in the surface waters of the Laurentian Great Lakes *Mar. Pollut. Bull.* **77** 177–82