

## ABSTRACT

MYERS, BONNIE JEAN EVALINE. Effects of Extreme Flows and Non-Native Species on Freshwater Fish Assemblages in the Caribbean with Potential Implications for River Ecosystem Services (Under the direction of Dr. Augustin C. Engman)

Tropical island freshwater ecosystems are regarded as highly vulnerable to climate change and invasion by non-native species—two of the main threats to global biodiversity. However, tropical islands are some of the least researched. Extreme events, such as droughts and floods, can alter fish assemblage structure by causing mortality, reduced recruitment, and facilitating invasion and establishment of non-native species. Non-native fish negatively affect native assemblages through increased competition, predation, and reduced growth and fitness.

Projections suggest Puerto Rico, a tropical island in the Caribbean, will experience increased drying, more intense drought, and more intense hurricanes and floods. Increases in extreme events coupled with the expansion of non-native fish on the island may have detrimental impacts on recreationally and culturally important native freshwater fish. An increased understanding of how extreme flows and expansion of non-native fish impact the tropical native fish in the Caribbean is needed for effective conservation and management.

We sought to investigate the effects of extreme events and non-native taxa on fish assemblages in Puerto Rico at multiple scales using in situ data and experiments. We also assessed the importance of ecosystem services and river uses of 14 rivers to communities. We found extreme events and non-native fishes have complex effects on native species and assemblage structure using an extensive dataset coupled with flow across rivers in Puerto Rico and 15 years of data in one specific river. At the islandwide scale, we built generalized linear models to identify the most critical flow variables driving variations in native and non-native fish fishes. Annual average high flows had a positive effect on native biomass; the most extreme high

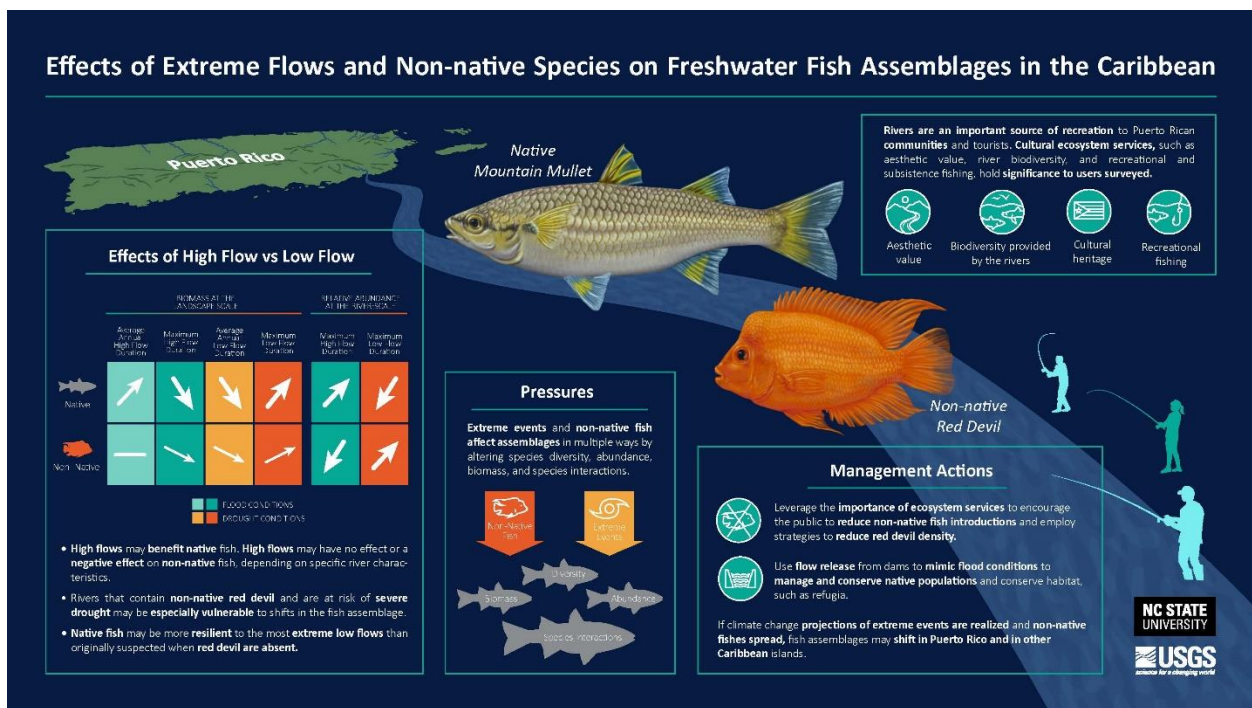
flows had a negative effect on native and non-native biomass. Annual average low flows had a negative effect on both native and non-native fish biomass, but the effect on native biomass was almost one and half times larger. At the river-scale, we found native fish abundance decreased with longer extreme low flows, and non-native fish abundance increased with longer extreme low flows. We found two assemblage changepoints during 2008-2023 between 2014-2015 ( $p=0.005$ ) and 2017-2018 ( $p=0.753$ ). The year 2015 was characterized by an increase in non-native relative abundance; this coincided with an extreme drought in 2015. The year 2017 was characterized by an increase and decrease in native and non-native relative abundance, respectively; this shift occurred a year after Hurricane María. The main fishes driving these shifts were native mountain mullet *Dajaus monticola* and non-native red devil *Amphilophus* spp.

Experimentally, we found non-native red devil *Amphilophus* spp. altered mountain mullet behavior and habitat use in high and low flow artificial stream treatments. When red devil were not present in simulated drought, mountain mullet were actively searching for refuge from low flows; however, when red devil were present mountain mullet shifted their behavior and competed for habitat. Mountain mullet may need to exert more energy to occupy space and engage in aggressive interactions due to red devil.

Our findings show that extreme events and non-native fish affect assemblages in multiple ways, including altering biomass, abundance, competition, and behavior. We also found native species may be more resilient to extreme low flows than expected; however, this resilience may be affected by the presence of non-native fishes. Ecosystem functioning and services may be altered if non-native fishes continue to expand on the island. This could affect human uses and value of the rivers. We showed through 177 surveys that users valued cultural services, such as biodiversity provided by the river, recreation, and aesthetic value. Recreational fishing was

important to a subset of users. Differences in users existed between visits to estuarine and inland river locations and between fishers and non-fishers. Economic benefit to users was greater for visits to estuarine sites (\$22.22 per trip) and for fishers (\$21.28 per trip) compared to inland river sites and non-fishers.

Our results provide a more comprehensive understanding of how extreme flows may alter assemblages when non-native fishes are present. Specifically, rivers that contain non-native red devil and are at risk of severe drought may be especially vulnerable to novel shifts in the fish assemblage. This information could guide prioritization, management, and conservation of native fishes and rivers in Puerto Rico, the Caribbean, and other tropical islands globally.



**Figure 1:** Dissertation graphical abstract illustrating the main findings of the effects of extreme flows and non-native species on freshwater fish assemblages in the Caribbean with potential implications for river ecosystem services.

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Effects of Extreme Flows and Non-Native Species on Freshwater Fish Assemblages in the  
Caribbean with Potential Implications for River Ecosystem Services

by  
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## **DEDICATION**

I dedicate my dissertation to my wonderful, strong, and caring mom, Marie, who always gave me the courage and confidence to take on new challenges, to my dad, Bill, who instilled in me a passion for the outdoors and natural world, and last but not least, to my amazing advisor, mentor, and friend, Dr. Thomas J. Kwak, who supported me and provided sound guidance and unwavering support throughout my Ph.D. journey.

## **BIOGRAPHY**

I come from a small town in Northwest Arkansas. I grew up backpacking and canoeing with my family, which instilled in me a strong connection to nature. I received my Bachelor of Science from the University of Wyoming in Wildlife and Fisheries Biology and Management and my Master of Science from Virginia Tech in Biological Conservation. I have been lucky to have had so many great opportunities to work with the National Oceanic and Atmospheric Administration, the U.S. Geological Survey National Climate Adaptation Science Center, and the United Nations at the Intergovernmental Platform on Biodiversity and Ecosystem Services.

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Finally, I could not have finished without the unwavering support from my family and friends. Specifically, my brother, Will, for always listening to my field work woes and challenges throughout finishing my dissertation. Finally, I must thank the best dog in the world,

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## CHAPTER 1

### The effects of flow extremes on native and non-native stream fishes in Puerto Rico

#### ABSTRACT

Freshwater fishes are among the most vulnerable taxa to climate change globally but are generally understudied in tropical island ecosystems. Climate change is predicted to alter the intensity and frequency of extreme flow events on the Caribbean island of Puerto Rico. These changes may impact native and non-native stream ecosystems and biota in complex ways. We compiled an extensive dataset of fish assemblages collected at 119 sites across Puerto Rico from 2005 to 2015. We coupled these data with stream flow indices and dam height to understand how flow drives fish assemblage structure. Sixteen percent of sites contained exclusively non-native species, 34% contained exclusively native species, and 50% contained a mix of native and non-native species. We built generalized linear models to identify the most critical flow variables for explaining variation in native and non-native species richness and biomass. Mean daily flow was the most important flow variable in driving native species richness ( $\beta=-0.22$ ;  $p<0.05$ ), while flashiness was the most important for non-native species richness ( $\beta=1.47$ ;  $p<0.05$ ). Extreme flows were important in explaining variations in native and non-native biomass. Native biomass increased by 5.10 kg/ha with longer average high flows and decreased with longer maximum high flows. Model averages showed native and non-native biomass decreased by -8.84 kg/ha and -5.92 kg/ha, respectively, with longer annual average low flows and increased by 3.37 kg/ha and 2.19 kg/ha, respectively, with longer annual maximum low flows. Annual average low flows had a negative effect on both native and non-native assemblages, but the effect on native biomass was almost one and half times larger. Native species may be more resilient to one-off extreme low flow events, and non-native species may be more tolerant to the natural flashy flows in the Caribbean than expected. Our results are informative for island ecosystems globally and can

guide management and conservation of native fish, particularly in the context of climate change and non-native species. Managers may prioritize maintaining connectivity, refugia, and habitat complexity in rivers while preventing non-native introductions and establishment to conserve native diversity and biomass in Caribbean rivers.

## **INTRODUCTION**

Flow regime structures the physical habitat of rivers and influences river ecosystems and biota (Humphries et al., 2014; Poff et al., 1997). Changes to flow regimes create atypical conditions that can adversely impact fish assemblages. These conditions can increase the pressure on fishes to adapt to novel conditions, including more extreme flows. Extreme drought and floods impact water flow, dissolved oxygen content, sediment load, and other habitat characteristics (Carpenter et al., 1992; Grill et al., 2019). These flow-induced changes can affect fish assemblage composition, diversity, and biotic interactions via changes in species presence, density, biomass, invasive species establishment, and disruption of food webs (Hershkovitz & Gasith, 2013; Kwak et al., 2016; Martins et al., 2022). Invasive species, dams, disease, and urbanization may interact with changes to the flow regime to further affect fish assemblages (Datry et al., 2018; Engman & Ramírez, 2012; Greathouse, Pringle, & Holmquist, 2006). For example, infectious disease, particularly in tropical ecosystems, has caused extinctions through reduced survival of freshwater species globally (Reid et al., 2019).

Native species may adapt behaviorally, phenologically, physiologically, and morphologically in response to extreme flows up to a certain threshold (Hershkovitz & Gasith, 2013). These adaptations can enhance assemblage resilience (the capacity of an assemblage to return to its previous state following a disturbance) and resistance (the capacity to resist change during a disturbance) to extreme events—a common theme in disturbance ecology documented in

tropical ecosystems, including marine, mangrove forest, wetland, estuary, and freshwater ecosystems (Lake & Barmuta, 1986; Patrick et al., 2022; Wijaya et al., 2023). Life history strategies (e.g., opportunistic, periodic, and equilibrium strategies) associated with spawning, recruitment, maturation, and parental care also contribute to assemblage responses to environmental disturbance (McManamay & Frimpong, 2015; Winemiller, 2005; Winemiller & Rose, 1992). For example, species with periodic life history strategies (large, long-lived species with late maturation) may respond differently to extreme flows compared to species with opportunistic life history strategies (small, short-lived species with early maturation) (e.g., Ferguson et al., 2013). Increases in extreme flows beyond species' adaptive capacity may affect their resistance and resilience and affect assemblage structure.

Greater intensity and higher frequency of extreme high and low flows may have inverse effects on native and non-native fish. High flow events that are part of the natural flow regime may benefit native species compared to their non-native counterparts (Diez et al., 2012; Yoon et al., 2011). Native fish occupying habitats with naturally occurring high flows possess unique adaptations, such as the ability to spawn after floods, move to shelter habitats, and ovarian development cued by floods (Lytle & Poff, 2004). The lack of these high flow adaptations in non-native species could put them at a competitive disadvantage (Minckley & Meffe, 1987; Rahel & Olden, 2008). Alternatively, low flows may benefit non-native species. Low flows can cause fish assemblage shifts from native to non-native dominant, which has been observed in freshwater ecosystems (Lennox et al., 2019; Ramírez et al., 2018; Rogosch et al., 2019). These trends have not been observed consistently across all ecosystems and are dependent on the intensity and type of disturbance (Brokaw et al., 2012).

Tropical island streams, such as those in Puerto Rico, are characterized by frequent, natural flow disturbances (Church et al., 2006; Smith et al., 2003), including flash floods and drought (Taniwaki et al., 2017). These extreme events are increasing in intensity and frequency due to climate change (Cashman et al., 2010; Thomas & Benjamin, 2018). The Caribbean region, for example, has experienced shifts in temperature and precipitation extremes resulting in more intense rainfall events and longer periods of drought (Karmalkar et al., 2013). Climate projections suggest continued drier dry periods and wetter wet periods across the region (Bowden et al., 2021). The Intergovernmental Panel on Climate Change highlighted the unique effects of climate change on small islands, concluding that they have already experienced disproportionate impacts that will continue to intensify (Nurse et al., 2014). Our current knowledge about the effects of climate-driven changes in precipitation and flow on tropical ecosystems and biota is limited. However, even what little is known helps assist island ecosystem management and conservation (Frauendorf et al., 2019).

Recent syntheses on the status of freshwater fish assemblages in Puerto Rico suggested the island provides an ideal landscape to determine the resistance and resilience of native and non-native fish to extreme events (Kwak et al., 2016, 2019). Puerto Rico streams have naturally low fish assemblage diversity, supporting up to nine native species (Cooney & Kwak, 2013; Engman et al., 2019; Neal et al., 2009). The native fish provide important ecological services that contribute to ecosystem functioning, such as transportation of nutrients from marine to freshwater environments, and cultural services that support subsistence and recreational fisheries (Engman et al., 2017; Kwak et al., 2016). With the introduction of non-native fish, understanding what drives native and non-native assemblage structure is critical for tropical island stream conservation and management. We anticipate that native species may exhibit greater resistance

and resilience to the impacts of extreme high when compared to non-native species. Here, we address the need to inform stream conservation planning and practice in the region and island ecosystems with the following research objectives: (1) investigate trends in native and non-native fish assemblages using a spatially representative dataset of Puerto Rico streams, (2) quantitatively evaluate the relationship among environmental flow and biotic parameters across sites, and (3) compare relationships between native and non-native biotic and flow variables that capture mean flows, variation in flow, and extreme flows.

## **METHODS**

### *Study Area*

Puerto Rico is the smallest of the Greater Antilles islands in the Caribbean, measuring approximately 175 kilometers in length and 62 kilometers in width. The Cordillera Central mountain range runs east-west through the island's interior (Neal et al., 2009). Small streams that originate in the mountains form 50 river basins draining into the Atlantic Ocean to the north and the Caribbean Sea to the south. Most of the rivers are impounded by dams to supply water and hydroelectric power, creating 13 large (>100 hectares) reservoirs, which, along with small dams and other barriers, impact the migratory native fish fauna (Cooney & Kwak, 2013; Greathouse, Pringle, & Holmquist, 2006; Neal et al., 2008).

Puerto Rico's freshwater systems contain nine native fish species and approximately 37 non-native species (mostly aquarium species introduced intentionally or unintentionally), including catfish, cichlids, and poecilids (Engman et al., 2019; Neal et al., 2009; Rodríguez-Barreras et al., 2020). The nine native fish include mountain mullet *Dajaus monticola* (Mugilidae), bigmouth sleeper *Gobiomorus dormitor* (Eleotridae), smallscaled spinycheek sleeper *Eleotris perniger* (Eleotridae), fat sleeper *Dormitator maculatus* (Eleotridae), river goby

*Awaous banana* (Gobiidae), sirajo goby *Sicydium* spp. (Gobiidae), and American eel *Anguilla rostrata* (Anguillidae). All but one of Puerto Rico's native fishes are amphidromous (Engman et al., 2017). River goby, sirajo goby, smallscaled spinycheek sleeper are small-bodied, benthic fish; bigmouth sleeper is a larger-bodied, benthic species. Mountain mullet is larger-bodied and the only native species that occupies the water column.

### *Data Synthesis*

We compiled a spatially representative dataset of fish assemblage metrics across Puerto Rico using an existing quantitative dataset (Kwak et al., 2007, 2013). The dataset included 119 sampling sites in 49 river basins, spanning the years 2005 to 2015 (Kwak et al., 2007; Neal et al., 2009; Figure 1). Sampling followed a standardized protocol described in detail in Kwak et al. (2007, 2013). Fish assemblages were sampled in 100-150 meter stream reaches using a standardized three-pass removal method with pulsed DC backpack or barge electrofishing. Sites were sampled once except for one site which was repeated. All 119 sites included data on species richness. One hundred and eleven sites included data on fish density (number/ha) and biomass (kg/ha).

We identified the United States Geological Survey (USGS) stream gage for the 119 sites in the corresponding river to quantify flow (U.S. Geological Survey, 2022). If a river did not contain a stream gage, we identified the stream gage in the closest river in the same watershed as a proxy. We used the gage data to generate a suite of flow metrics for the year preceding the sampling date. We selected 1-year prior to the sampling date to capture the wet (~April to November) and dry (~December to March) seasons. The importance of flows during the year preceding sampling is supported by previous research showing that this period explains the most variation in species richness compared to flows two and three years prior (Knouft & Chu, 2015).

In total, we calculated 11 flow metrics that represent baseflows, temporal variations in flow, high and low flows, and the Richard-Baker Flashiness Index (Baker et al., 2004). For a list of the flow variables and definitions, see table S1 in the supplementary material. We calculated flow metrics using the “Hydrostats” package in RStudio (Version 2022.02.3 Build 492) (Bond & Bond, 2022).

To account for the influence of dams and migration barriers in our models (described below), we used a continuous variable of dam height (m) of the highest dam downstream of the sampling point. Downstream dams can restrict native amphidromous species movement to upstream sites following their marine life history stage and can account for decreased abundance of native species (Cooney & Kwak, 2013). Downstream dams can also impact the presence of both native and non-native species (Holmquist et al., 1998).

### *Quantitative Analysis*

We constructed generalized linear mixed effects models for native and non-native species richness and biomass. We included eight flow variables, a dam height variable, and river sampled as independent variables in the models. We selected the independent flow variables using a Variance Inflation Factor (VIF) stepwise approach to reduce collinearity. We excluded variables with a VIF greater than 10 (Naimi et al., 2014). We removed the median flow variable from the analysis because of its strong correlation ( $R^2 \sim 1$ ) with mean daily flow (MDF). We removed the minimum low and high flow duration variables due to lack of variation. We first built the generalized linear mixed effects models, including the nine independent variables and one random effects variable. The random effect included a spatial variable (river) to account for spatial autocorrelation. We then built a second set of generalized linear models with no random effects. We compared the  $\Delta$ Akaike information criterion (AIC) between models including and

excluding the random effects for native and non-native species richness and biomass to determine if the random effects provided a better fit. We selected the models based on the lowest AIC for further analyses. We built the generalized mixed effects models and the generalized linear models using the “lme4” package (Bates et al., 2009). All analyses were conducted using RStudio (Version 2023.03.0 Build 386).

Using an information theoretic approach (Anderson & Burnham, 2004), we determined the most important flow variables explaining variation in species richness and biomass. We conducted model selection and model averaging using the "MuMIN" package (Barton, 2010). We determined the relative importance of flow variables for each set of models (Grieshaber et al., 2018). Relative variable importance was determined by summing the model weights of the top models that included the flow variable. We report the variable importance in models with a  $\Delta AICc < 2$  relative to the best model, the coefficients and significance value for the best model, and the coefficients based on model averaging of all models with a  $\Delta AICc < 2$ . The model results include native and non-native species richness (N=119) and native and non-native biomass (N=111). We assumed Gaussian (i.e., normal) distributions for all continuous dependent variables; and species richness was assumed to follow a Poisson distribution.

## **RESULTS**

### *Islandwide native and non-native assemblage structure*

Native fishes dominated the species richness of Puerto Rico's lotic mixed fish assemblages (Figure 1-2). All native fishes were present in the dataset with the exception of fat sleeper, which largely occurs in lowland rivers and coastal lagoons that are not efficiently sampled with backpack or barge electrofishing. Mountain mullet, bigmouth sleeper, river goby, sirajo goby, and American eel were encountered at the most sites (Table 1). Fifteen non-native

fishes were present in the dataset (Table 1). Of those 15 non-native species, Mexican molly *Poecilia sphenops* (Poeciliidae), guppy *Poecilia reticulata* (Poeciliidae), green swordtail *Xiphophorus hellerii* (Poeciliidae), tilapia species (Cichlidae) were present at the most sites. Sixteen percent of the sites contained only non-native species; 50% of sites contained a mix of native and non-native species (i.e., at least 1 of either type); and 34% of sites contained only native species (Figure 1). Seventy-nine percent of sites were dominated (i.e., >50% of species present) by native species, and 21% of sites were dominated by non-native species when both assemblages were present (Figure 1). In 65% of the mixed-assemblage streams, native biomass was greater than that of non-native biomass (Figure 1). Non-native species tended to be more dominant ( $\geq 50\%$  proportional biomass, density, and species richness) in the central areas of Puerto Rico (Figure 1), often upstream of dams that limit the migration of native species.

**Table 1:** Native and non-native fish assemblage information for 119 sampling sites in Puerto Rico sampled 2005-2015. Missing data were omitted from calculations.

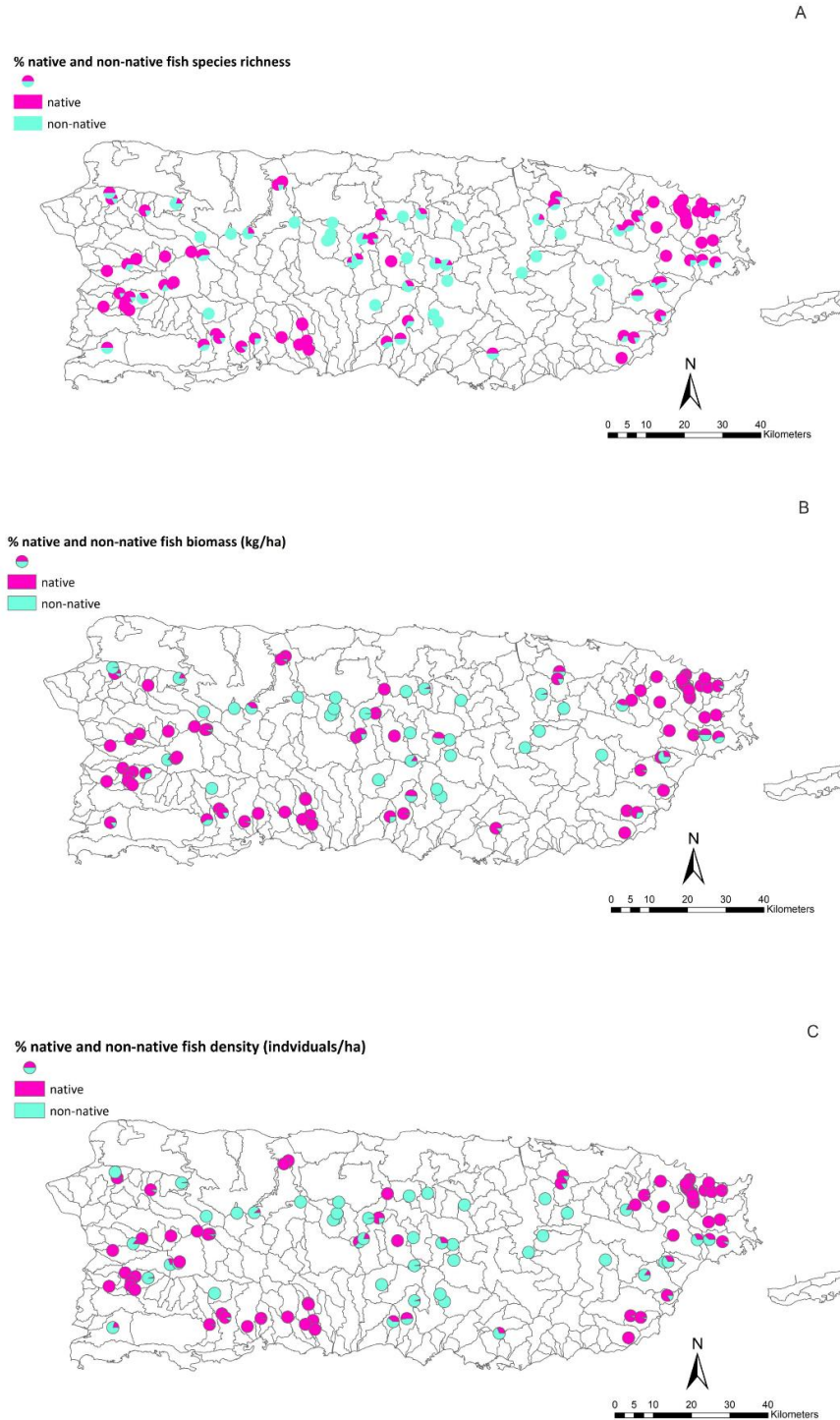
Fish species and family			
Scientific name	Common name	Family	Native or non-native
<i>Anguilla rostrata</i>	American eel	Anguillidae	native
<i>Awaous banana</i>	River goby	Gobiidae	native
<i>Dajaus monticola</i>	Mountain mullet	Mugilidae	native
<i>Eleotris perniger</i>	Smallscaled spinycheek sleeper	Eleotridae	native
<i>Gobiomorus dormitor</i>	Bigmouth sleeper	Eleotridae	native
<i>Lutjanus griseus</i>	Gray snapper	Lutjanidae	native
<i>Mugil curema</i>	White mullet	Mugilidae	native
<i>Rhanciscus croco</i>	Burro grunt	Haemulidae	native
<i>Sicydium spp.</i>	Sirajo goby	Gobiidae	native
<i>Amatitlania nigrofasciata</i>	Convict cichlid	Cichlidae	non-native
<i>Coptodon rendalli</i>	Redbreast tilapia	Cichlidae	non-native
<i>Gyrinocheilus aymonieri</i>	Siamese algae eater	Gyrinocheilidae	non-native
<i>Ictalurus punctatus</i>	Channel catfish	Ictalurida	non-native
<i>Lepomis auritus</i>	Redbreast sunfish	Centrarchidae	non-native
<i>Lepomis macrochirus</i>	Bluegill	Centrarchidae	non-native
<i>Micropterus nigricans</i>	Largemouth bass	Centrarchidae	non-native
<i>Oreochromis mossambicus</i>	Mozambique tilapia	Cichlidae	non-native
<i>Oreochromis niloticus</i>	Nile tilapia	Cichlidae	non-native
<i>Pethia conchonius</i>	Rosy barb	Cyprinidae	non-native
<i>Poecilia latipinna</i>	Sailfin molly	Poeciliidae	non-native
<i>Poecilia mexicana</i>	Mexican molly	Poeciliidae	non-native
<i>Poecilia reticulata</i>	Guppy	Poeciliidae	non-native
<i>Pterygoplichthys pardalis</i>	Amazon sailfin catfish	Loricariida	non-native
<i>Xiphophorus helleri</i>	Green swordtail	Poeciliidae	non-native

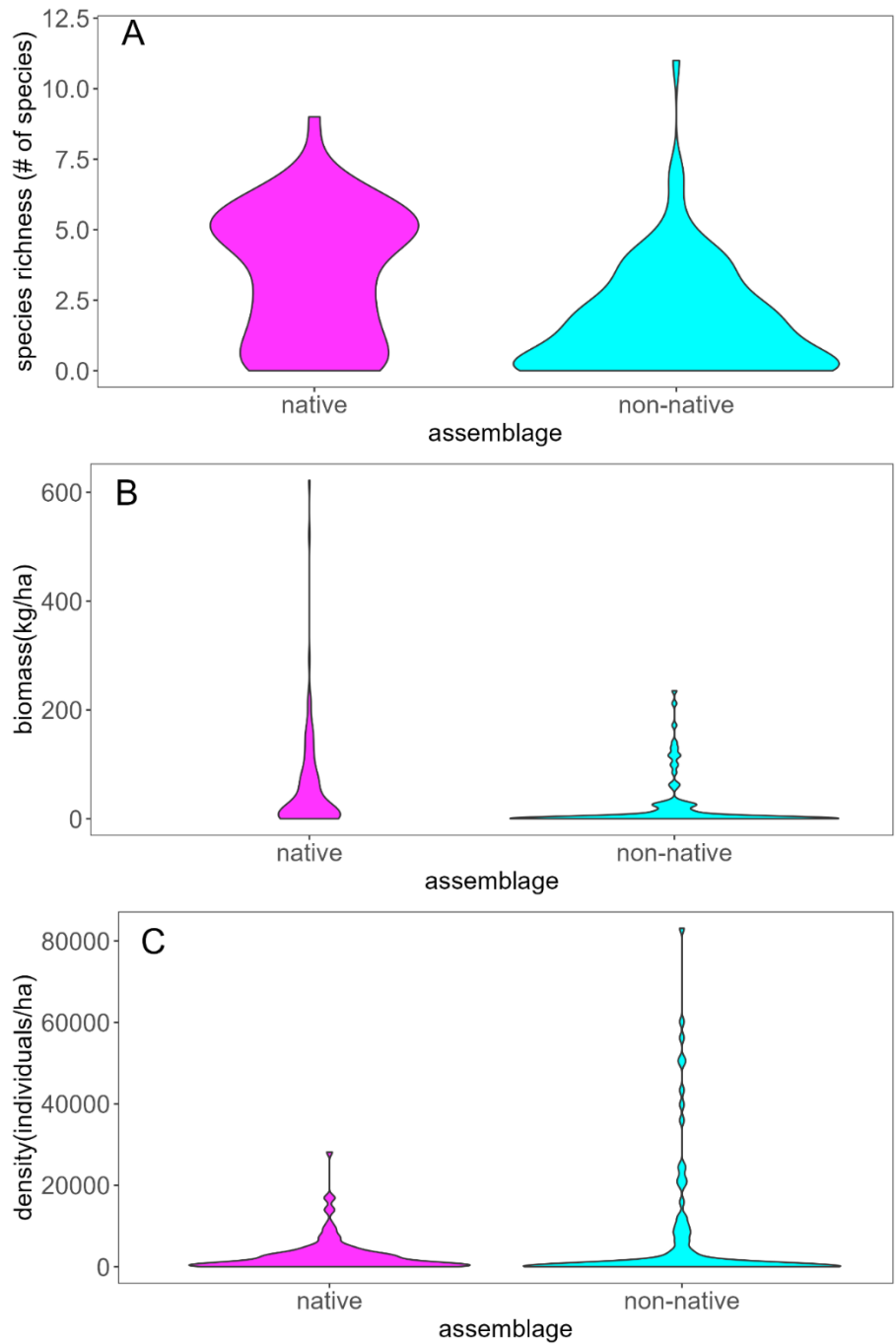
Fish assemblage metrics						
	Native			Non-native		
	Species richness	Biomass (kg/ha)	Density (number/ha)	Species richness	Biomass (kg/ha)	Density (number/ha)
<b>Mean</b>	3.42	56.32	2710.90	1.61	20.96	6192.90
<b>Standard deviation</b>	2.32	92.76	4194.93	1.85	45.09	14490.58
<b>Minimum</b>	0.00	0.00	0.00	0.00	0.00	0.00
<b>Maximum</b>	9.00	621.90	28139.70	11.00	235.10	83100.70

**Table 2:** Hydrology summary statistics for 119 sampling sites in Puerto Rico, sampled from 2005-2015.

	Stream flow metrics							
	Mean daily flow (cms)	Mean Richard Baker-Flashiness index	Annual coefficient of variation (%)	Monthly coefficient of variation (%)	Annual average high flow duration (days)	Annual average low flow duration (days)	Mean maximum high flow duration (days)	Mean maximum low flow duration
<b>Mean</b>	3.66	0.47	37.34	66.73	5.11	5.45	16.88	14.47
<b>Standard deviation</b>	4.56	0.16	25.60	26.18	2.82	2.89	6.86	7.11
<b>Minimum</b>	0.02	0.19	10.40	35.26	2.18	1.37	6.00	2.00
<b>Maximum</b>	18.85	1.08	241.34	162.23	26.00	14.00	50.00	33.00



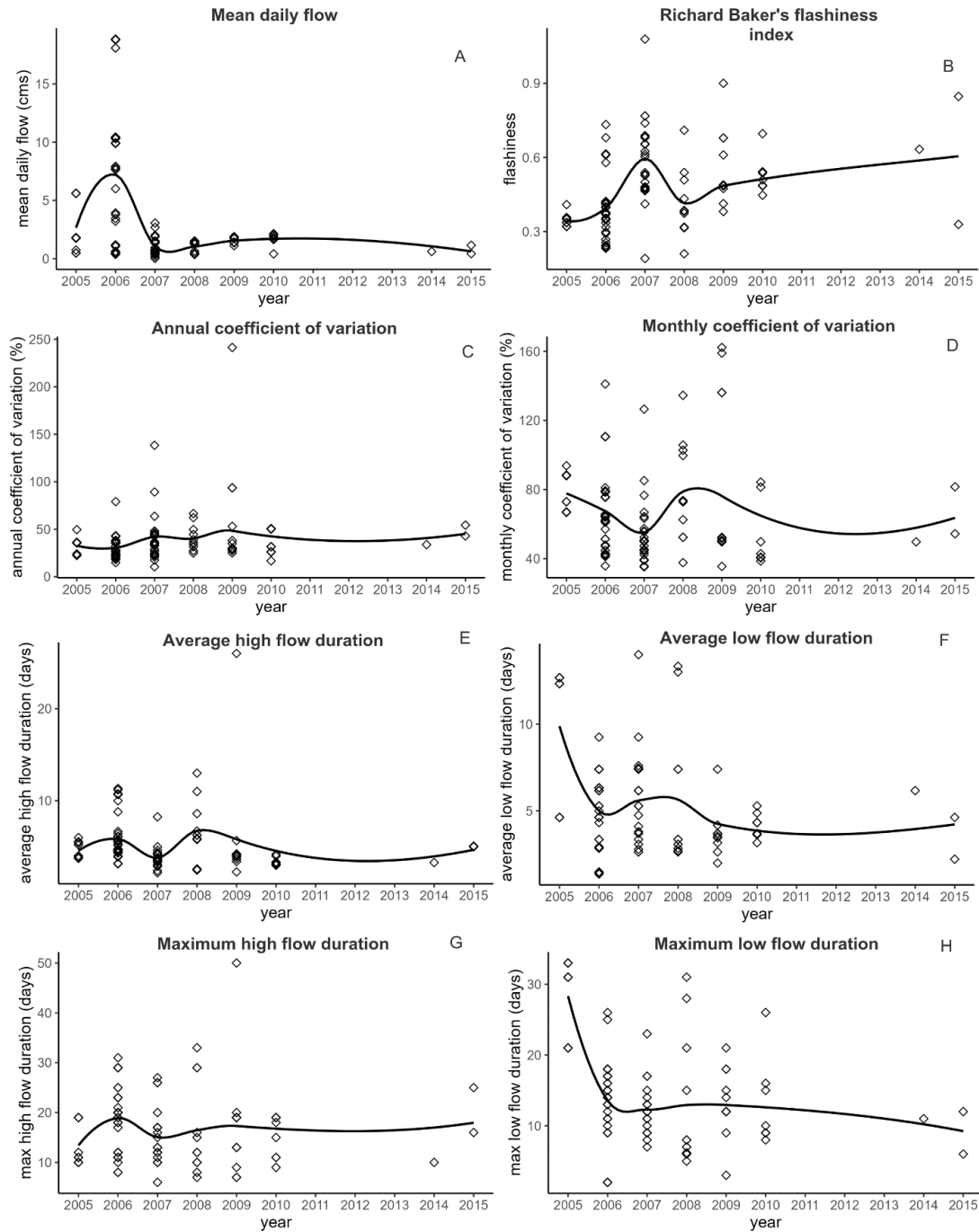
**Figure 1:** Map of study sites across Puerto Rico illustrating the proportions of native (magenta) and non-native (turquoise) fish species richness from 119 sites (A), native species biomass versus non-native species biomass from 111 sites (B), and the proportion of native species density versus non-native species density from 111 sites (C) sampled from 2005-2015.



**Figure 2:** Islandwide fish assemblage metric violin plots representing distribution of native (magenta) and non-native (turquoise) fish species richness (number of species) (N=119) (A), biomass (kg/ha) (N=111) (B), and density (number of individuals/ha) (N=111) (C) sampled across Puerto Rico from 2005-2015.

*Islandwide flow characteristics*

Ninety-one rivers or tributaries were sampled in the islandwide dataset, broadly representing Puerto Rico's stream flow. Flow statistics varied across sites annually with mean daily flow ranging from 0.02 to 18.85 cms and annual coefficient of variation ranging from 10.40 to 241.34 (Table 2; Figure 3). High flow durations and mean daily flows suggested the years 2006-2009 tended to be wetter years; however, some sites during this time period experienced longer low flow durations. We considered 2005 as a dry year based on mean daily flows and low flow duration values.



**Figure 3:** Distribution of mean annual flow metrics from 2005-2015 (N=119 sites) sampled across Puerto Rico, including mean daily flow (cms) (A), Richard Baker's Flashiness index (B), annual coefficient of variation (%) (C), monthly coefficient of variation (%) (D), average annual high flow duration (days) (E), average annual low flow duration (days) (F), maximum annual high flow duration (days) (G), and maximum annual low flow duration (days) (H).

### *Native and non-native fish species richness and biomass model results*

Random effects did not improve the fit of the global model for native species richness, but they did for the non-native species richness and the biomass global models. We did not include the random effects for the non-native species richness global model as including the random effects resulted in very large eigenvalues and overfitting. Although fit improved for native and non-native biomass models, the random effects caused overfitting. To avoid issues with overfitting, we did not include random effects for the native and non-native biomass models in favor of the more parsimonious, fixed effects-only models. However, we included river as a fixed effect in the models to control for the location of sampling and spatial autocorrelation.

The top models revealed similarities and differences in the flow variables that best explained variations in native and non-native species richness. We reported the coefficients for the lowest AICc model and the model average coefficients for the flow variables included in models with a  $\Delta\text{AICc} < 2$ . Models with a  $\Delta\text{AICc} < 2$  suggested any of these models could best explain the variation in the fish variables (Anderson & Burnham, 2004). We presented the importance of each variable present in the top models of native and non-native species richness and biomass in figure 4. Native species richness was best explained by dam height, mean daily flow, high flow durations, monthly coefficient of variation, and flashiness (Table 3; Figure 4). Model averages revealed average high flow duration and flashiness were positively associated with native and non-native species richness. The top model predicts that native species richness decreases by 0.06 species with each cubic meter per second increase in mean daily flow and decreases by 0.22 species with each meter increase in dam height, while holding all other variables constant ( $p < 0.05$ ). Non-native species richness was best explained by six variables, including high flow durations, low flow durations, mean daily flow, flashiness and dam height

(Table 3; Figure 4). The top model predicts non-native species richness increases by 1.47 species with one-unit increase in flashiness and increases by 0.02 species with each meter increase in dam height, while holding all other variables constant ( $p < 0.05$ ). Model averages also illustrated a positive association between non-native species richness and high and low flow durations and mean daily flow (Table 3). Low flow variables were present in the top models for non-native species richness but were absent in the native species richness top models (Figure 4).

All the extreme flow variables were important predictors of native and non-native biomass. Maximum high flow duration was a significant variable in the native biomass top model. Average and maximum low flow durations were significant variables in the non-native biomass top model. Based on model averages, average low flow duration had a one and a half times larger negative effect on native biomass than non-native biomass (Table 4). Conversely, average high flow duration had a positive effect on native biomass and no effect on non-native biomass. The top model predicts native biomass decreases by 4.14 kg/ha with longer maximum high flow durations and increases by 0.96 with one percent increase in monthly coefficient of variation. Model averages showed a positive association of native fish biomass with average high flow duration and maximum low flow duration. Dam height and mean daily flow were also important variables in the top models with a variable importance of 0.40 or higher and exhibited a negative association with native biomass (Table 4; Figure 4). The top model predicts non-native biomass decreases by 6.21 kg/ha with longer average low flow durations, increases by 1.22 kg/ha with each meter increase dam height, and increases by 2.39 kg/ha with longer maximum low flow durations (Table 3). Model averages revealed a positive association between non-native biomass and mean daily flow, and flashiness (Table 3). Model averages showed maximum high flow duration was negatively associated with non-native biomass (Table 3). Low

flow durations, dam height, and flashiness were the most important variables in the top models of non-native biomass (Figure 4).

**Table 3:** Native and non-native species richness model selection results illustrating the top model based on Akaike's Information Criteria (AICc) and model averages for all models with a  $\Delta AICc < 2$ . \*represents significant independent variables ( $p < 0.05$ ) of the best model.

Model selection										
Global model: native species richness ~ mean daily flow + annual_cv + monthly_cv + max high flow duration + average high flow duration + max low flow duration + average low flow duration + flashiness + dam_height + river										
Native species richness	intercept	average high flow duration	dam height	max high flow duration	mean daily flow	monthly coefficient of variation	flashiness	degrees of freedom	AICc	weight
	1.69*		-0.22*		-0.06*			3	411.00	0.36
<b>Model averages</b>	<b>1.63</b>	<b>0.01</b>	<b>-0.22</b>	<b>0.00</b>	<b>-0.07</b>	<b>0.00</b>	<b>0.15</b>			

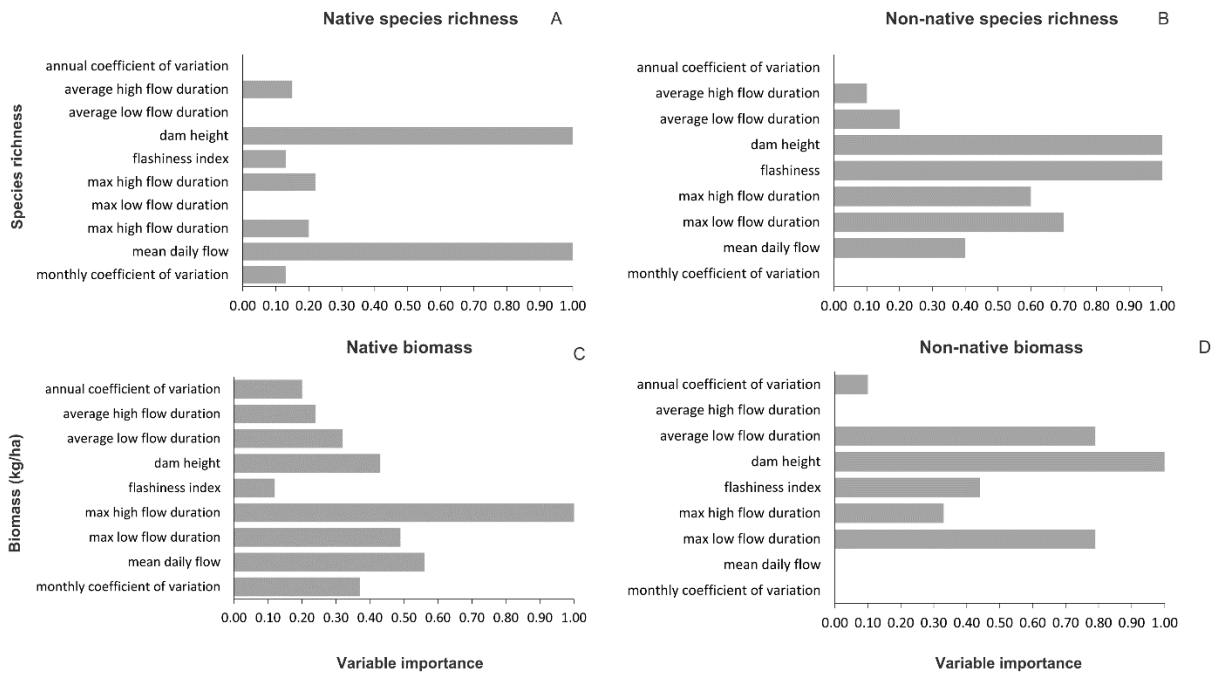
Model selection											
Global model call: non-native species richness ~ mean daily flow + annual_cv + monthly_cv + max high flow duration + average high flow duration + max low flow duration + average low flow duration + flashiness + dam_height + river											
Non-native species richness	intercept	average high flow duration	average low flow duration	dam height	max high flow duration	max low flow duration	mean daily flow	flashiness	degrees of freedom	AICc	weight
	-1.02*			0.02*	0.02	0.02		1.47*	5	412.80	0.20
<b>Model averages</b>	<b>-0.92</b>	<b>0.04</b>	<b>0.04</b>	<b>0.02</b>	<b>0.02</b>	<b>0.01</b>	<b>0.03</b>	<b>1.49</b>			

**Table 4:** Native and non-native species biomass model selection results illustrating the top model based on Akaike's Information Criteria (AICc) and model averages for all models with a  $\Delta AICc < 2$ . \*represents significant independent variables ( $p < 0.05$ ) of the best model.

Model selection													
Global model: native biomass ~ mean daily flow + annual_cv + monthly_cv + max high flow duration + average high flow duration + max low flow duration + average low flow duration + flashiness + dam_height													
Native species biomass	intercept	annual coefficient of variation	average high flow duration	average low flow duration	dam height	flashiness	max high flow duration	max low flow duration	mean daily flow	monthly coefficient of variation	degrees of freedom	AICc	weight
	107.31*	-0.80					-4.14*	1.53	-3.75	0.96*	11	1315.00	0.05
<b>Model averages</b>	<b>102.00</b>	<b>-0.74</b>	<b>5.10</b>	<b>-8.84</b>	<b>-0.98</b>	<b>-76.31</b>	<b>-3.64</b>	<b>3.37</b>	<b>-3.43</b>	<b>0.76</b>			

Model selection												
Global model: non-native biomass ~ mean daily flow + annual_cv + monthly_cv + high flow duration + average high flow duration + max low flow duration + average low flow duration + flashiness + dam_height												
Non-native species biomass	intercept	annual coefficient of variation	average high flow duration	average low flow duration	dam height	flashiness	max high flow duration	max low flow duration	mean daily flow	degrees of freedom	AICc	weight
	-5.96			-6.21*	1.22*	39.64		2.39*		9	1144.00	0.24
<b>Model averages</b>	<b>8.95</b>	<b>-0.15</b>		<b>-5.92</b>	<b>1.27</b>	<b>42.60</b>	<b>-0.66</b>	<b>2.19</b>	<b>0.16</b>			



**Figure 4:** Variable importance graphs across the top models for native species richness (A) and non-native species richness (B) and native biomass (C) and non-native biomass (kg/ha) (D) from the general linear model results from 119 and 111 sites, respectively, in Puerto Rico sampled from 2005-2015.

## DISCUSSION

### *Resistance and resilience of native and non-native species to high flows*

Average high flow duration is an important factor in structuring fish assemblages in Puerto Rico. We found average high flow duration benefit native fishes (Figure 5; Table 4). High flows may increase habitat complexity by providing more pool habitat and promoting riparian vegetation growth providing more space and cover for fish. Increased habitat complexity may also reduce the effects of competition and predation (Alexander et al., 2015). Existing habitat structures can also dampen the impact of floods by reducing water velocity and providing micro-habitat as refuge (Pearsons et al., 1992). Engman & Ramírez (2012) found habitat complexity

was positively correlated with species richness and biomass in streams in Puerto Rico. Substrate diversity, variation in depth, channel morphology, and percent cover contribute to habitat complexity and were positively associated with fish assemblage structure. Species may withstand the effects of increasing average high flows by moving to pool habitats or taking advantage of available habitat structures, such as coarse woody debris or large substrate. Habitat complexity could explain the positive effect of average high flow durations on native biomass across the island.

However, the effect of average high flows only benefits native compared to non-native fishes. We found that average high flow duration had a positive effect on native biomass and did not positively affect non-native species (Figure 5; Table 4). This finding suggests native species are resistant and resilient to average high flow durations and can thrive under longer average high flow conditions. Native fish in Puerto Rico exhibit a mix of opportunistic and periodic life history strategies that are ideal for streams that experience a gradient of disturbance and high seasonal variability (Smith, 2012; Tedesco et al., 2008; Winemiller, 2005). Species with opportunistic and periodic life history strategies inhabit environments prone to frequent and intense disturbance (Winemiller, 2005; Winemiller & Rose, 1992). Flood-resilient life history characteristics, such as early maturation to reproductive age or producing many offspring to increase survival potential, increase native species resilience to frequent and heavy flooding (Smith, 2012; Xiang et al., 2021). For example, Engman et al. (2017) documented a positive relationship between native goby (Gobiidae) recruitment and stream discharge, and peak recruitment during the rainy season. Non-native fish like guppies that exhibit similar life history strategies, such as employing continuous reproductive efforts early maturation, to native fish can also withstand increasing environmental disturbance (Blondel, 2021; Zengeya et al., 2013). This

may explain the lack of an effect of average duration high flows on non-native biomass. Finally, native fish may be more historically adapted to periodic increases in average high flows, which would explain the positive effect of average high flows compared to non-native fish. For example, mountain mullet and bigmouth sleeper exhibit rapid recovery through increased recruitment and movement upstream to replace displaced adults following flooding (Smith, 2012). Other research has shown these opportunistic and periodic life history strategies dominated assemblages following flooding events in the Tonle Sap floodplain in Cambodia (Chea et al., 2020). Thus, life histories may be key for resilience of native tropical fish assemblages to high flow events.

The most extreme high flow durations are detrimental to native and non-native fish assemblages. We found one-off extended maximum high flow durations may negatively affect native assemblages more than non-native (Figure 5; Table 4). Several mechanisms may be driving this finding, including effects on recruitment of native species. Frederico et al. (2016) found tropical native species were highly sensitive to the duration of floods. The authors attributed this sensitivity to a potential mismatch in seasonal recruitment cues for the native species with periodic life histories. Similarly, recruitment of native species in Puerto Rico is cued by high flows; thus, timing of these maximum high flow events may be detrimental to native recruitment (Engman et al., 2017). Alternatively, non-native species from the family Poeciliidae, which we found dominated the non-native assemblages, have year-round recruitment that is not cued by high flow events (Echelle et al., 2020). Poeciliidae's recruitment strategy may make them less susceptible and more resilient to increases in the duration of one-off maximum high flows. This finding suggests that in the future, the most extreme high flows could pose a greater threat to native assemblages.

Reduced water quality and mortality of larger-bodied fish may also explain the larger negative effect of maximum duration high flows on native assemblages. Floods can reduce dissolved oxygen concentration in tropical rivers, increase dissolved organic carbon, and increase sedimentation, which can cause fish mortality (Mishra et al., 2021; Puig et al., 2016; Whitworth et al., 2012). Poeciliidae can withstand low oxygen levels; thus, may resist the lower dissolved oxygen levels during the most extreme floods (Echelle et al., 2020). Engman & Ramírez (2012) found dissolved oxygen was an important factor structuring fish assemblages in Puerto Rico. The authors found streams with wide shallow channels, characterized by lower dissolved oxygen, favored non-native over native species. The negative effect of maximum high flows on biomass may also be due to reduced body size distribution through mortality of larger individuals. Saniei & Crispo (2022) found mean length of mountain mullet and green swordtail (*Xiphophorus hellerii*) decreased significantly following Hurricane Irma and Hurricane Maria; while, guppy experienced the smallest decrease in mean length. These changes in fish size may be contributing to the negative effects of one-off maximum high flow events we documented. Further research on the timing of the most extreme high flow events and species-specific research on the physiological mechanisms decreasing individual population biomass can provide important management information at the population-level.

#### *Resistance and resilience of native and non-native species to low flows*

The effect of average low flow durations exhibits a greater adverse influence on native fish assemblages compared to non-native counterparts. We found a larger decrease in native biomass than non-native biomass with longer periods of low flows (Figure 5). Species-specific behavioral strategies may contribute to these observed trends. Behavioral strategies, including increased mobility and changes in foraging behavior, and physiological adaptations, such as

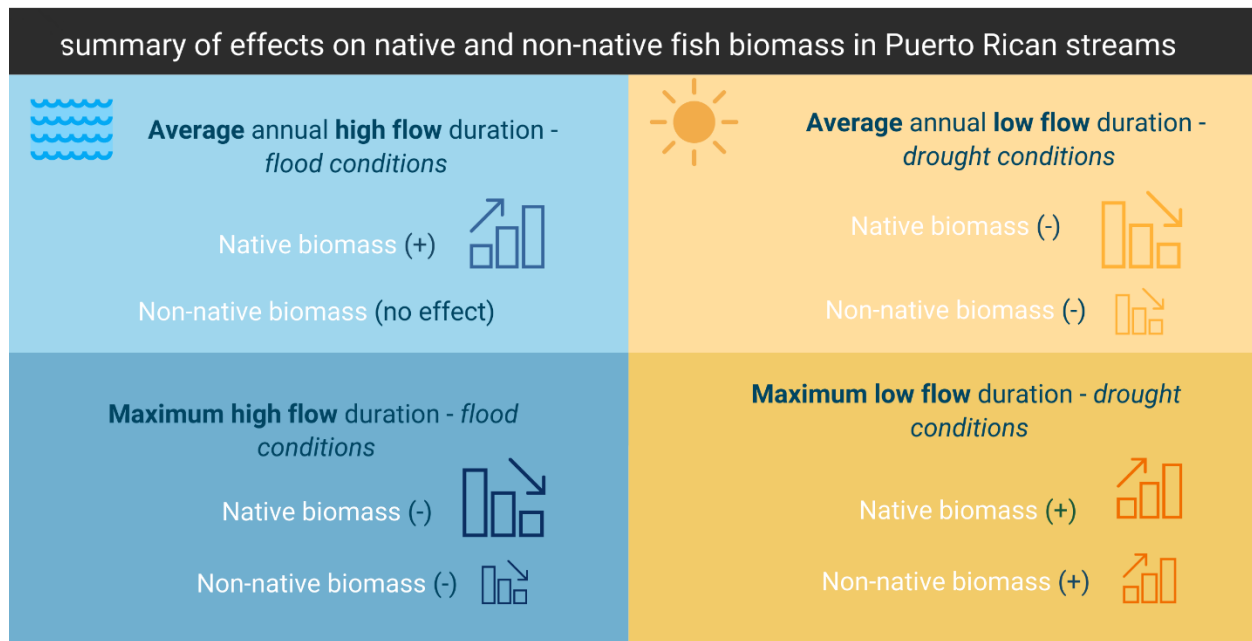
body armor and air-breathing, may allow non-native species to be more resistant to long-duration drought events (Magoulick & Kobza, 2003). For example, guppy and other species from Poeciliidae exhibit shoaling behavior, tending to swim and forage in groups and exploring new environments during disturbances (Camacho-Cervantes, 2015; Echelle et al., 2020). This mobile behavior may promote their ability to withstand drought better by moving to available habitats during periods of limited habitat availability or increased predation during drought (Magoulick & Kobza, 2003; Townsend & Hildrew, 1994). Competition for habitat could also explain the difference in responses. For example, non-native species may be outcompeting native species for limited space and shelter during low flows (Ribeiro et al., 2009). Ramírez et al. (2018) also observed a shift in fish assemblage composition dominated by native to non-native species during drought.

In contrast, one-off maximum low flow events do not have the same detrimental effect on native and non-native biomass (Figure 5). Our findings align with previous research showing non-native fish biomass increased with longer low flow durations (Table 4; Arthington & Balcombe, 2011; Sánchez-Pérez et al., 2020). Wilkinson et al. (2019) also found a dominant freshwater fish in tropical Southeast Asia to be more resilient to an extreme drought in 2016 than expected. Changes in refugia from varying lengths of drought may explain why native and non-native biomass decreased with longer average duration of low flows and increased with longer maximum low flow events (Magoulick & Kobza, 2003). Availability and type of refugia vary with the extent and magnitude of drought (Magoulick & Kobza, 2003). The ability of fish to migrate downstream or to suitable refugia may also contribute to their resistance to low flows (Magoulick & Kobza, 2003). Native fish in Puerto Rico live and spawn in freshwater; larvae develop into post-larvae in estuarine and marine environments before migrating back to

freshwater (Engman et al., 2017; Smith & Kwak, 2014). Previous research has shown native fish may move to more suitable environments during extreme events in Puerto Rico (Smith & Kwak, 2015). We concluded that native species might be less affected by longer, single low flow events than expected at the islandwide scale (Figure 5).

Multiple factors may explain the positive effect of the most extreme low flow events on native and non-native fishes. We suggest a combination of life history strategies (e.g., periodic and opportunistic) (Winemiller, 2005), the behavior of amphidromous native species (Engman, 2016), and the availability of refugia could facilitate the resistance and resilience of native and non-native fish the one-off extreme drought events. However, to support these conclusions, future research could focus on investigating individual river conditions, the interacting effects of high and low flow events, refugia's role, and refugia availability at finer scales following low flow events (Thomas & Benjamin, 2018). Our results suggest the negative effects of average duration low flow events may be more critical for managers to consider than extended, one-off maximum duration low flow events (Figure 5).

# High flow vs. Low flow



**Figure 5:** Graphical abstract of the effects of high and low flow durations on native and non-native fish biomass from 111 sites in Puerto Rico from 2005-2015 based on generalized linear model averages. Larger graph icons denote a larger magnitude effect of the flow variable on native or non-native fish biomass.

## *Native and non-native assemblages and variation in flow*

Stream flashiness and short-term variations in flow constitute an important component of the flow regime, exerting an influence on fish assemblage structure. Our model average coefficients showed native and non-native species richness increased with flashier streams (Table 3). Clilverd et al. (2019) also found stream flashiness is critical to fish assemblage composition and ecosystem functioning in tropical island streams in Hawaii. Fish life history traits are strongly related to their responses to stream flashiness (Gido et al., 2019). Behavioral

adaptations, such as the ability to disperse or move to suitable refuge habitat (e.g., the native sirajo goby that can climb up rocks or other structures), as well as life history and reproductive strategies discussed contribute to species' ability to persist in flashy and variable systems (Gido et al., 2019; Winemiller, 2005). Studies in non-tropical regions, such as headwater streams in the Ozark Plateau in the United States, have not demonstrated increased species richness with flashiness (Magoulick et al., 2021). Smith and Kwak (2015) also found no change in species richness following flash floods in one river in Puerto Rico. However, Olden et al. (2006) found native species with periodic and opportunistic life histories, like those life history strategies exhibited by native and non-native species in Puerto Rico, dominated fish assemblages when flows were more variable and unpredictable (Smith, 2012). However, Smith (2012) found flashiness had a more substantial positive effect on non-native than native species biomass. We found increased short-term variability in flow benefited native biomass, but increased flashiness was detrimental to native biomass. This finding aligns with research showing native fish abundance decreases with flashiness (Ramírez et al., 2018). Historically, non-native species cannot withstand flashy flow regimes in tropical streams (Brasher, 2003). In Puerto Rico, the non-native species are native to tropical environments characterized by intense rainfalls and predictable seasonal flooding (Boulton et al., 2008), which could explain this positive effect (Table 3 and 4).

#### *Native and non-native assemblage dynamics and implications of dams*

Including a dam height variable is crucial when investigating fish assemblage structure in flashy, tropical island streams. We showed dam height was among the most important variables in explaining variation in native and non-native species richness and biomass (Figure 4). Dams globally hinder upstream and downstream migration of biota and are a major cause of loss of

connectivity in rivers (Grill et al., 2019; Nilsson et al., 2005). Modeling the effects of dam height is essential considering its significant impact on native and non-native fishes and is a critical correlate to consider in studies of flow and assemblage structure in heavily modified systems (Cooney & Kwak, 2013; Ramírez et al., 2009). The presence and size of dams and the flow regime are inextricably linked in streams (Grill et al., 2019). The higher the dam, particularly dams above 1.9 meters, the fewer native species are present (Cooney & Kwak, 2013). Native amphidromous fish species in Puerto Rico can only migrate above structures they can jump and climb, so as dam height increases, native species richness and density decrease (Cooney & Kwak, 2013; Holmquist et al., 1998; Greathouse et al., 2006). Non-native fish do not require the same longitudinal connectivity with the ocean as native fish (Holmquist et al., 1998). Our results support the significance and effect of dam height on the structure of native and non-native assemblages.

Overall, our results on the effects of average high flows do not fully support that extreme high flows may act as a filter for the establishment of non-native species, potentially due to dam effects (Table 3 and 4). The presence of dams may dampen any adverse impacts of long-duration high flows on non-native species assemblages. Given the significant effect of dams on non-native species, it is reasonable that average high flow durations did not negatively impact non-native species biomass, as anticipated (Greathouse, Pringle, McDowell, et al., 2006). Leprieur et al. (2008) found anthropogenic activities outweighed natural processes, such as biotic resistance to invasions, to non-native species invasions in over 1,000 river basins globally. The presence of dams increases non-native species richness and biomass in Puerto Rico, similar to findings from dam impacts in New Zealand rivers (Jellyman & Harding, 2012).

*Management implications and future research needs*

Our results increase the knowledge base regarding fish assemblages and changes in flow regimes in tropical ecosystems (e.g., Havel et al., 2015; Welcomme & Halls, 2004). Notably, we showed native fishes may be more resilient to prolonged drought than expected and to longer duration average high flow events than non-native fishes (Figure 4; Table 4). Although, non-native fishes may be adapted to the natural flashy flows, they may not benefit from increases in average high flow durations (Table 3). Average annual high flows most likely aid in downstream dispersal of native fish larvae to ocean and estuarine habitats, while also allowing for connectivity of instream habitats for upstream migration of post larvae. Our results suggest managers could consider flow regulations that mimic natural levels of periodic high flow events as well as mitigate the negative effects of average duration droughts to conserve native fishes in the assemblage. Managers may also consider maintaining habitat complexity or restoring river connectivity to sustain the resistance and resilience of native species to high and low flow events (e.g., Grill et al., 2019).

Shifts in the length and intensity of high and low flow events undoubtedly will impact freshwater fish assemblages in Puerto Rico. Our results suggest the effects on native and non-native fishes will be complex as high and low flows affected native and non-native fishes in similar directions but to varying degrees (Figure 5). Downscaled projections of Coupled Model Intercomparison Project Phase 5 global climate models show increased drying for Puerto Rico from 2041-2060, which may drive native and non-native assemblage structure in the coming decades (Bowden et al., 2021). The Caribbean region has experienced more intense rainfall events but also more prolonged periods of drought, which are projected to continue in the future (Cashman et al., 2010; Karmalkar et al., 2013; Thomas & Benjamin, 2018). If the intensity and duration of single tropical storms and hurricanes continues to increase, native fishes could

decline. However, due to the patchiness of heavy rainfall during tropical storms, only a proportion of rivers on the island will face an extreme event during any given storm. Therefore, streams that only face a higher average annual flow duration may be less affected.

The effects on fish assemblages will depend on the flow disturbance type, frequency, magnitude, and other anthropogenic stressors. Anthropogenic activities, such as land-use change, also affect tropical fish assemblages (Leitão et al., 2018). We did not consider land-use change in this study, but further research incorporating agricultural and urbanization land-use factors coupled with flow data could provide further information on fish assemblage responses to changing flows. Fish assemblages fluctuate yearly, therefore temporal changes in native and non-native assemblages are also crucial to individual river ecosystem functioning. Further, the timing of high and low hydrologic flow events may also play a critical role on native species. Research using long-term data and repeated sampling that captures seasonal and repeated fluctuations in fish assemblages and flow could improve understanding of how finer scale changes affect assemblages and help prioritize rivers with the highest need for management interventions.

Many of the streams in Puerto Rico remained dominated by native fishes; managers may be able to conserve these native-dominated assemblages in the coming decades. Native fishes exhibited positive relationships with at least one of the high and low flow variables in our study (Figure 5), suggesting if proper management of non-native species occurs, native fishes may be able to withstand negative effects of climate-induced increases in high and low flow events. Thus, managers could develop strategies to remove new non-native species introductions and further educate the public to prevent future introductions.

We showed increases in average annual high flow durations do not negatively affect the fish assemblages and may provide positive benefits, especially to native fishes, that promote

increased resistance and resilience to floods (Table 4; Figure 5). However, we also showed average duration low flows did have a smaller negative effect on non-native fishes, which puts native species at a disadvantage if climate change increases the average duration of extreme drought (Figure 5). There is a clear need for additional research on the impacts of non-native species on native species coupled with changes to extreme events, the role of refugia during low and high flows, and empirical studies to document climate change effects on Caribbean fish assemblages. Increased long-term monitoring could provide vital information on seasonal shifts in assemblages to high and low flow events. Our results could inform conservation and stream flow management on other islands characterized by mixed fish assemblages that face similar stressors globally and add to needed stream research on tropical islands.

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## CHAPTER 2

### Flow and non-native species shift Caribbean fish assemblage structure in an urban river

#### ABSTRACT

Tropical island freshwater ecosystems are some of the least researched systems but are regarded as highly vulnerable to climate change and invasion by non-native species. Climate change and non-native species are two main drivers of ecosystem transition and transformation globally. The Río Piedras in Puerto Rico supports all nine native fish species of Puerto Rico. The native assemblage has shifted in recent years due to extreme drought and floods and the establishment of non-native species. Understanding the responses of native assemblages to changes in extreme flows and the presence of non-native fish species is essential for protecting native biodiversity and ensuring the conservation of freshwater ecosystems on the island. Gaps remain in understanding how fish assemblages will shift with changes to extreme low and high flow events. To investigate these gaps, we (1) evaluated the effects of flow, specifically flow extremes, on the Río Piedras fish assemblage structure over a 15-year period; (2) quantitatively identified changepoints in native and non-native relative abundance; (3) estimated impacts of flow on native and non-native relative abundance and body size; and (4) identified potential future shifts in fish assemblages in a tropical river ecosystem. Fish assemblage composition in the Río Piedras fluctuated from native to non-native dominated, following one extreme low flow and one extreme high flow event. Assemblage evenness varied from 2008-2023, with mountain mullet (*Dajaus monticola*) and non-native red devil cichlids (*Amphilophus* spp.) driving changes in assemblage structure and relationships with flow. We identified a changepoint in native and non-native abundance in 2014 ( $p=0.005$ ) and 2017 ( $p=0.753$ ). The year 2015 was characterized by the start of an extreme drought. Between 2014 and 2015 non-native relative abundance increased by 44%. Hurricane Maria occurred in 2017; native relative abundance increased and

non-native relative abundance decreased by 61% between 2017 and 2018. Log-transformed native abundance decreased by 0.267 with one cms increase in mean daily flow ( $p=0.022$ ) and decreased by 0.013 with one day increase in maximum low flow duration ( $p=0.019$ ). Log-transformed non-native abundance increased by 0.212 with one cms increase in mean daily flow ( $p=0.037$ ) and increased by 0.011 with one day increase in maximum low flow duration ( $p=0.021$ ). Our results reveal the complex interactions between changes in flow and non-native species and their potential to trigger changepoints in fish assemblages and highlight the need for continued monitoring and effective, integrated conservation and management of tropical freshwater fish.

## **INTRODUCTION**

In freshwater ecosystems, climate change and non-native species are crucial drivers of ecosystem transition and transformation (Dunham et al., 2022; Rahel, 2022). Transitions occur when an ecosystem shifts from one stable state to an alternative stable state following an environmental change (Clements & Ozgul, 2018). The movement into alternate stable states is often characterized by changes in assemblage parameters that affect population densities and are large enough to move an ecosystem from one domain to another stable equilibrium domain (Beisner et al., 2003). Ecosystem transformation reorganizes prior ecosystem states prompting shifts in species composition, structure, and ecosystem functioning and are thought to be irreversible (Crausbay et al., 2022; Williams et al., 2021). Both ecosystem transitions and transformations can be prompted by changes to temperature, flow regimes, and extreme events. These changes affect fish physiology (Whitney et al., 2016), demographic processes, evolutionary processes (Lynch et al., 2016; Myers et al., 2017), distribution (Comte et al., 2013), species interactions (Gilman et al., 2010), and behavior (Bartolini et al., 2015), which can lead to the emergence of novel fish assemblages (Havel et al., 2015; Scheffer et al., 2001). Additionally,

non-native species introductions contribute to ecosystem transitions by altering assemblage and functional diversity and composition (Toussaint et al., 2016), food web dynamics (Bruestle et al., 2019), and physical habitat (Emery-Butcher et al., 2020). These changes are geographically dependent but impact fishes and ecosystems globally, from the Arctic to temperate and tropical ecosystems (Barbarossa et al., 2021). Investigating regionally specific ecosystem transitions and transformations in freshwater ecosystems is crucial for designing tailored conservation and management strategies that improve outcomes under future climate change (Lynch et al., 2021; Paukert et al., 2016; Richard & Rypel, 2013).

Extensive research has shown invasion of non-native species is a major driver of biodiversity loss (e.g., Didham et al., 2005; Ricciardi & MacIsaac, 2011; Thomaz et al., 2015). Biodiversity scenarios for 2100 project that ecosystem change resulting from non-native species is the fourth largest driver of biodiversity shifts and is expected to occur more in freshwater compared to terrestrial ecosystems (Sala et al., 2000). Freshwater ecosystems are especially diverse given the area they represent globally. Forty percent of global fish diversity is contained in freshwaters (Balian et al., 2008). Insularity and high endemism make freshwater ecosystems particularly susceptible to the effects of non-native species (Moorhouse & Macdonald, 2015). Once established in a new habitat, most non-native species are nearly impossible to eradicate (Britton et al., 2023). Non-native species effects on fish assemblage structure impact ecosystem services, including subsistence and recreational fishing, tourism, and nutrient availability (Kiruba-Sankar et al., 2018; Rahel & Olden, 2008); however, these changes will manifest differently and depend on local conditions. For example, tropical species are expected to have the highest species extinction and losses due in part to the presence of non-native species (Brook et al., 2008).

Invasive and non-native species alter ecological interactions generally resulting in a decrease of native abundance and diversity (Gallardo et al., 2016; Kernan, 2015). Shifts in species interactions may be an important component in understanding changes in assemblage structure (Jackson et al., 2001; Lavergne et al., 2010). Non-native species may increase competition, niche overlap, and predation (Angert et al., 2013; Rahel & Olden, 2008). Competition and niche overlap, specifically, may decrease species ability to fill their niche space (Klanderud & Totland, 2007). For example, Haubrock et al. (2021) found competitive interactions between native and non-native fish shifted native species niche space resulting in displacement. Similarly, Córdova-Tapia et al. (2015) found little niche overlap among native species but a large overlap between native species and two non-native species (i.e., common carp *Cyprinus carpio* and tilapia *Oreochromis spp*), resulting in declines in native populations. In addition to increased competition for resources and predation, larger-bodied non-native species globally have shifted the mean body size in freshwater assemblages (Blanchet et al., 2010); larger mean body size can affect assemblage structure, food web dynamics, and overall ecosystem functioning, prompting assemblage shifts from previous states (e.g., Toussaint et al., 2016; Townsend, 2003). The interplay of competition, predation, and body size variation between native and non-native fishes can reveal mechanisms influencing shifts in assemblage structure.

Early warning signals could be used to identify future tipping points in ecosystems (Wang et al., 2021). Tipping points in physical and biological systems can be defined as the point at which a small change or disturbance can prompt a large change in a system (Gladwell, 2006; Lenton et al., 2008). Tipping points can occur from multiple processes, such as species interactions and abiotic processes (i.e., climate change; Lenton, 2013). Tipping points and related

concepts, such as changepoints, have been investigated in experimental studies in terrestrial, marine, and freshwater ecosystems globally (Carrier-Belleau et al., 2022). Abrupt transitions in ecosystems can be determined by identifying changepoints, defined as the point where a statistical change can be identified in time series data (Cael et al., 2022). Ecosystem states refer to a numerical representation of an ecosystem, including one or more ecological responses (Capon et al., 2015). In this study, we use relative abundance as the ecological response used to describe different ecosystem states. Understanding early warning signals of tipping points and transitions in ecosystem states is essential to managers to develop preemptive strategies (Clements & Ozgul, 2016; Gsell et al., 2016).

Tropical freshwater ecosystems are some of the least researched but also some of the most threatened by climate change and non-native species (Barbarossa et al., 2021; Miqueleiz et al., 2020). The tropics are expected to show the earliest changes in climate from small, abrupt shifts in temperature and precipitation (Mora et al., 2013). These small, abrupt changes may have a strong negative effect on species with narrower physiological tolerances. For example, tropical species may be more sensitive to small increases in temperature because they are typically adapted to less temperature variation and have narrower temperature ranges (Campos et al., 2021; Tewksbury et al., 2008). Fish, shrimps, and crabs tend to have a lower acclimation capacity and warming tolerance than temperate species (Vinagre et al., 2016). Although changes in temperature are important, changes to the flow regime may have stronger effects on tropical aquatic ecosystems and biota (Winemiller, 2004). Native fish species are adapted to the natural flow regime in rivers (Lytle & Poff, 2004), including frequent, periodic flow disturbances that are characteristic of tropical rivers (Ngor et al., 2018; Ward & Stanford, 1995; Winemiller et al., 2008). Projections suggest tropical ecosystems will experience changes in the timing and

intensity of extreme rainfall events (Pachauri et al., 2014), which will have implications on the flow regime and fish assemblage structure (Strauch et al., 2015; Taniwaki et al., 2017). For example, regime shifts in fish assemblage functional groups in the Amazon were documented following an extreme drought (Röpke et al., 2017). Extreme weather events can alter fish populations within an assemblage by causing mortality, displacement, alterations in recruitment (Smith, 2012), and body size (Ramírez et al., 2018), which can lead to ecosystem transitions (Scheffer et al., 2001; Smith, 2012).

The Caribbean island of Puerto Rico, is home to nine native fish species, including amphidromous species (i.e., fish characterized by a life history strategy of spawning in freshwater followed by larvae that drift to the ocean to develop before migrating back to freshwater streams) (Engman, 2016; Kwak et al., 2007). These species provide essential services to ecosystems and society, including the transfer of nutrients and biomass from marine to freshwater environments, provisioning of recreational and artisanal fisheries, and are a component of the freshwater food web (Engman, 2016; Engman et al., 2017; Hein & Crowl, 2010). In recent years, researchers and managers have become concerned with how changes in flow, specifically extreme events, impact the tropical native fish fauna (Díaz et al., 2022; Ramírez et al., 2018; Smith & Kwak, 2015). In addition to increased drought, Puerto Rico has also experienced increases in extreme precipitation events. An inventory of extreme weather events in Puerto Rico documented seven extreme weather events from 2000 to 2017, including the destructive Hurricane María, which occurred in September 2017 (Clarke et al., 2021). The U.S. Drought Monitor also reported Puerto Rico experiencing moderate drought in 2021 (droughtmonitor.unl.edu).

The Río Piedras is an urbanized river that supports all nine native freshwater fishes in Puerto Rico (Engman & Ramírez, 2012). These include the mountain mullet *Dajaus monticola*, three sleeper Eleotridae species, four goby Gobiidae species, and American eel *Anguilla rostrata*. The Río Piedras has suffered from severe pollution, urbanization pressures, and the presence of non-native species, degrading the river's water quality and suitable habitat (Ramírez et al., 2018). Given the many stressors, managing and conserving the valuable ecosystem services it provides remains challenging. Despite the growing body of research on freshwater fish in Puerto Rico (Cooney & Kwak, 2013; Engman et al., 2017, 2017; Kwak et al., 2007, 2016; Neal et al., 2009; Ramírez et al., 2018; Smith & Kwak, 2015), there are significant knowledge gaps regarding fish assemblage structure shifts, particularly with the establishment of red devil species *Amphilophus* spp. since 2012 in the river system. Filling these knowledge gaps will allow researchers and managers to develop strategies to mitigate the impacts and ensure the long-term health and sustainability of Puerto Rico's freshwater ecosystems (Carrubba et al., 2022). To address these gaps, the objectives of our study were to: (1) evaluate the effects of flow, specifically flow extremes, on the Río Piedras fish assemblage structure over a 15 year period; (2) quantitatively identify changepoints in native to non-native relative abundance; (3) estimate early warning signals in flow on native and non-native relative abundance and body size; and (4) identify potential future shifts in fish assemblages in a tropical river ecosystem.

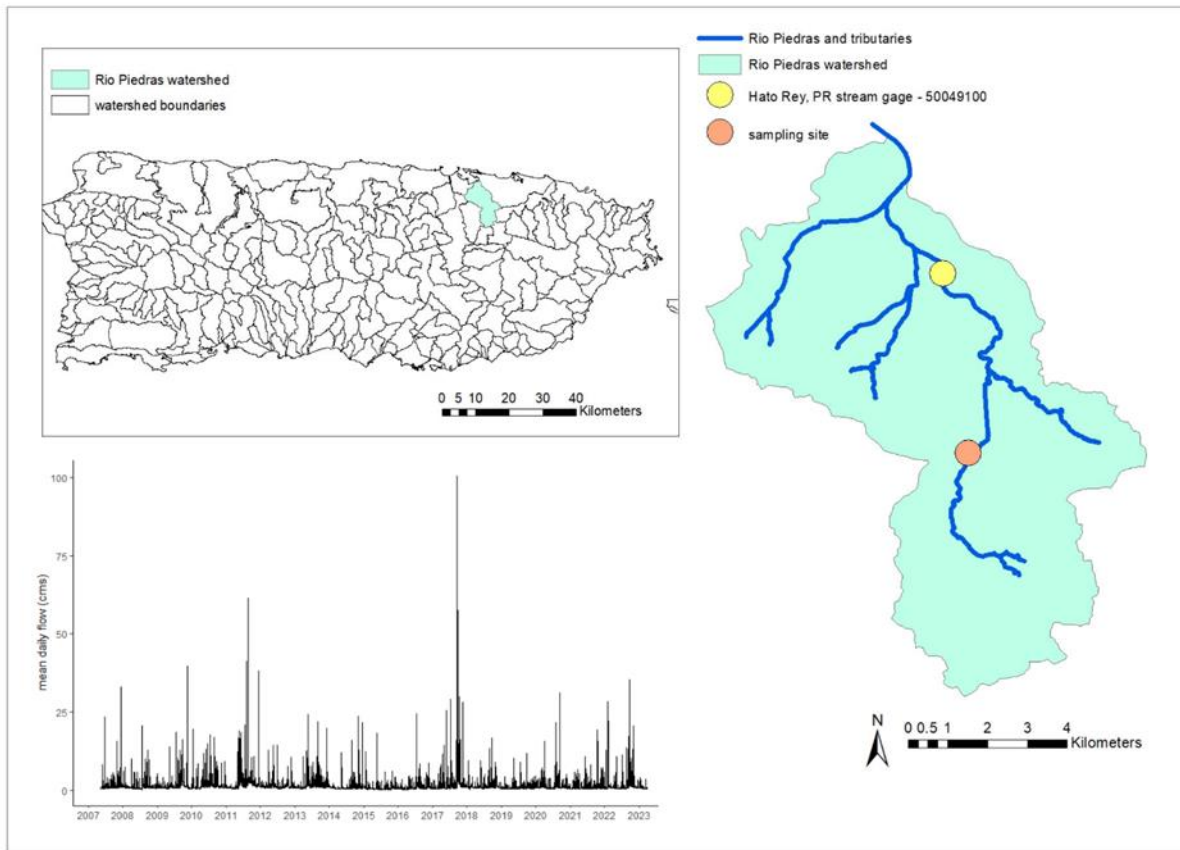
## **METHODS**

### *Study area*

The Río Piedras is located in the metropolitan area of San Juan, Puerto Rico, in the Northeast part of the island (de Jesús-Crespo & Ramírez, 2011; Figure 1). This is the most highly developed watershed in Puerto Rico, with approximately 2 to 2.5 million inhabitants (Torres-

Perez & Perez-Reyes, 2023). The Río Piedras runs 16 km North from its headwaters through Río Piedras, Hato Rey, and Puerto Nuevo (Lugo et al., 2011). Our study site in the Río Piedras is a long-term sampling location, sampled annually since 2008 (Ramírez et al., 2018). Ramírez et al. (2018) documented thirteen species, including six native species and seven non-native species, at the study site from 2008-2016. The study site is on the main stem of the river, approximately 10.8 kilometers from San Juan Bay and the Atlantic Ocean (Figure 1). A U.S. Geological Survey (USGS) stream gage (Hato Rey; identifier 50049100) is downstream of the study site and collects daily flow measurements and water quality parameters, such as calcium, sodium, magnesium, and potassium concentrations (Figure 1; U.S. Geological Survey, 2023). We conducted one 3-pass depletion of an approximately 100-meter reach during each sampling event using Smith-Root pulsed DC backpack electrofishing units and block nets placed up and downstream of the sampling reach. We collected all fish encountered during each pass, identified each individual to genus or species, and recorded the length of each fish.

Sampling occurred once a year from 2008-2023 from May-July, except for the years 2015, 2027, 2020, 2021, and 2022. We conducted three, 3-pass depletion samplings in June, September, and December of 2015. We conducted two, 3-pass depletion samplings in 2017 (August and October), 2021 (January and June), and 2022 (April and June). We did not conduct sampling in 2020 because of the COVID-19 pandemic. Some sampling error may have been introduced to the dataset as result of different teams conducting the sampling; however, sampling methods were kept consistent across years.



**Figure 1:** Map of Puerto Rico highlighting the Río Piedras watershed, main stem, its tributaries, the locations of the Río Piedras Hato Rey, PR stream gage, and the long-term sampling site and hydrograph of mean daily flow (cubic meters per second) for the study years 2008-2023 (bottom left corner).

### *Quantifying assemblage structure*

We used a long-term dataset of fish sampled from 2008 to 2023 to calculate annual fish species richness and relative abundance for native and non-native species or taxon. We calculated relative abundance using the following equation:

(1) *Relative abundance*

$$= \frac{\sum \# \text{ of individuals recorded per species or genus}}{\sum \# \text{ of individuals recorded across all species and genus}}$$

Relative abundance is unitless and measures the proportion of species or genus present relative to other species in the assemblage. It provides information on how rare or common a species is. We developed rank abundance curves for each sampling event to compare fish assemblage structure from the 20 unique sampling events. Rank abundance curves are an essential tool in community ecology analysis and provide a helpful visual for comparing assemblage richness and evenness changes over time (Avolio et al., 2019; Whittaker, 1965). To develop the rank abundance curves, we calculated the relative abundance of each species or taxon for each sampling event and ranked species from highest to lowest abundance. We then calculated the mean rank shifts between consecutive years; mean rank shifts reveal the relative change species rank abundance between two consecutive time periods (codyn R package; Hallett et al., 2020). For the years 2015, 2018, 2021, and 2022, which included multiple sampling events, we used the last sampling event conducted to calculate mean rank shifts. Mean rank shifts reveal the relative change in species abundances over time and is calculated using the following equation (Collins et al., 2008):

$$(2) \text{ Mean rank shift} = \sum_{s=1}^n (|relative\ rank_{s,t+1} - relative\ rank_{s,t}| \div n)$$

where  $n$  is the number of species common between two consecutive years,  $t$  is the year, and  $s$  is the relative rank of species  $s$  in year  $t$ .

### *Analysis of environmental flows and assemblage structure*

We calculated a suite of flow variables from the daily mean flow estimates from the USGS Hato Rey stream gauge using the *waterData* R package (Ryberg & Vecchia, 2012). These included mean daily flow (cubic meters per second; cms), annual and monthly coefficient of variation of mean daily flow (%), annual number of high (>90th percentile) and low (<10th percentile) flow events, the annual average duration of high and low flow events (days), the maximum duration of high and low flow events (days), and Richard-Baker's Flashiness index (package *Hydrostats*; Bond & Bond, 2022). Flow variables were calculated for the year prior to the sampling event.

We conducted a hierarchical cluster analysis to categorize years based on flow variables. We calculated spearman correlations on the suite of ten flow variables to identify significant correlations between variables (package *stats*; R Core Team et al., 2018). We did not use variables that were significantly correlated with one or more variables in our hierarchical cluster analysis to reduce collinearity in the analysis. The following six flow variables were removed from the analysis: flashiness, the monthly coefficient of variation, the annual mean duration of high and low flows, and the number of low and high flow events. The remaining four flow variables, which included mean daily flow, annual coefficient of variation, and the maximum annual duration of high flow and low flow events, were used in the analysis. We normalized the flow variables in the cluster analysis then calculated a dissimilarity matrix using Euclidean distance among the four flow variables. We identified four clusters based on the cluster tree and the distinction among the mean statistics of the four flow variables used.

We conducted a constrained redundancy analysis to analyze associations between the species that drove assemblage variation and the flow variables characterizing the four clusters

(Legendre & Gallagher, 2001). We transformed relative abundances using Hellinger distance to linearize the data and reduce rare species' impact (Legendre & Gallagher, 2001). We then used the “envfit” function to fit four environmental flow factors on the ordination and displayed species that accounted for  $\geq 0.30$  of the variation across assemblages (vegan package; Oksanen et al., 2022). Assemblages of years closer together in the ordination space were considered more similar than assemblages farther apart. Finally, we applied fitted environmental contours of the flow variables to the ordination space (Wood, 2003). This allowed us to visualize associations among the top ranked species and mean daily flows, annual variation in flow, and extreme high and low flow variables.

#### *Changepoint analyses for native and non-native assemblage structure*

We qualitatively and quantitatively investigated changepoints in native and non-native relative abundance (McClelland et al., 2012; Vanacker et al., 2016). Qualitatively, we identified potential changepoints in native and non-native abundance as points where the assemblage shifted from native dominant to non-native dominant and vice versa. Dominance was considered to be  $>50\%$  of the assemblage abundance. Next, we used Pettitt's test to quantitatively identify changepoints in the relative abundance of native and non-native fishes (Pettitt, 1979). Pettitt's test identifies a changepoint where the maximum shift occurs in the central tendency of time series data. Pettitt's test identifies one changepoint in the data revealing the maximum change. To account for this aspect, we conducted two tests using all sampling events (2008-2023; N=20) and using years after the establishment of red devils (2015a-2023; N=13). We tested the null hypothesis that there was no significant change exists across fish assemblages against the alternative hypotheses that a significant change exists. We conducted the changepoint analyses using the *trend* package in RStudio (Pohlert et al., 2016).

To investigate potential values of flow associated with changepoints in native and non-native species relative abundance, we conducted segmented regression analyses on the flow variables. We used the Shapiro-Wilks test to test the assumption of normality (Guy & Brown, 2007). Native and non-native abundance was log-transformed abundance to adhere to the assumption of normality. We included the same four flow variables in the native and non-native segmented regression models as the hierarchical cluster analysis. We calculated the variance inflation factor (VIF) for the covariates included in the native and non-native species models, and no variables had a VIF greater than ten (Guy & Brown, 2007). We statistically tested the significance of the flow value where a changepoint was estimated using Davies Test, which tests for a non-constant regression parameter in a linear predictor (Muggeo & Muggeo, 2017; Vanacker et al., 2016). One limitation of the segmented regression model is this method only identifies one changepoint in the data.

We then summarized length frequencies of the six species or taxon that accounted for the most variation in the assemblages as identified through the redundancy analysis. The length frequency analysis included the following fish: mountain mullet *Dajaus monticola*, bigmouth sleeper, smallscaled spinycheek sleeper, river goby *Eleotris perniger*, red devil *Amphilophus* spp., and armored catfish *Loricariidae* sp. We also conducted segmented regression analysis for each species lengths across years and flow variables using the *segmented* R package and Davies test (Muggeo & Muggeo, 2017; Vanacker et al., 2016). In addition to the four flow variables, we added the relative abundance for native in the non-native species models and non-native relative abundance in the native species models. Segmented regression identifies points where there is a shift in the linear regression, which is considered the changepoint in the dependent variable (Carrier-Belleau et al., 2022). We considered these values to be associated with a significant

change point at the 0.05 significance level. All statistical analyses were conducted in RStudio (Version: 2022.12.0+353.pro3).

## **RESULTS**

### *Annual time-series assemblage characteristics*

Fish assemblage structure fluctuated through the long-term monitoring. Overall species richness ranged from four to nine species (mean = 7) (Table 1). Native species richness ranged from four to six; non-native species richness ranged from zero to four. Non-native species richness increased on average starting in 2015 through 2023. Total relative abundance ranged from 25 to 321 individuals. Native mountain mullet and non-native cichlids (red devil) had the highest mean abundance across all years (47.52 and 45.00 individuals, respectively) followed by native river goby (mean abundance = 13.47 individuals) and native bigmouth sleeper (mean abundance = 12.06 individuals) (Table 1). Red devil were first detected in 2012, with abundance increasing starting in 2015.

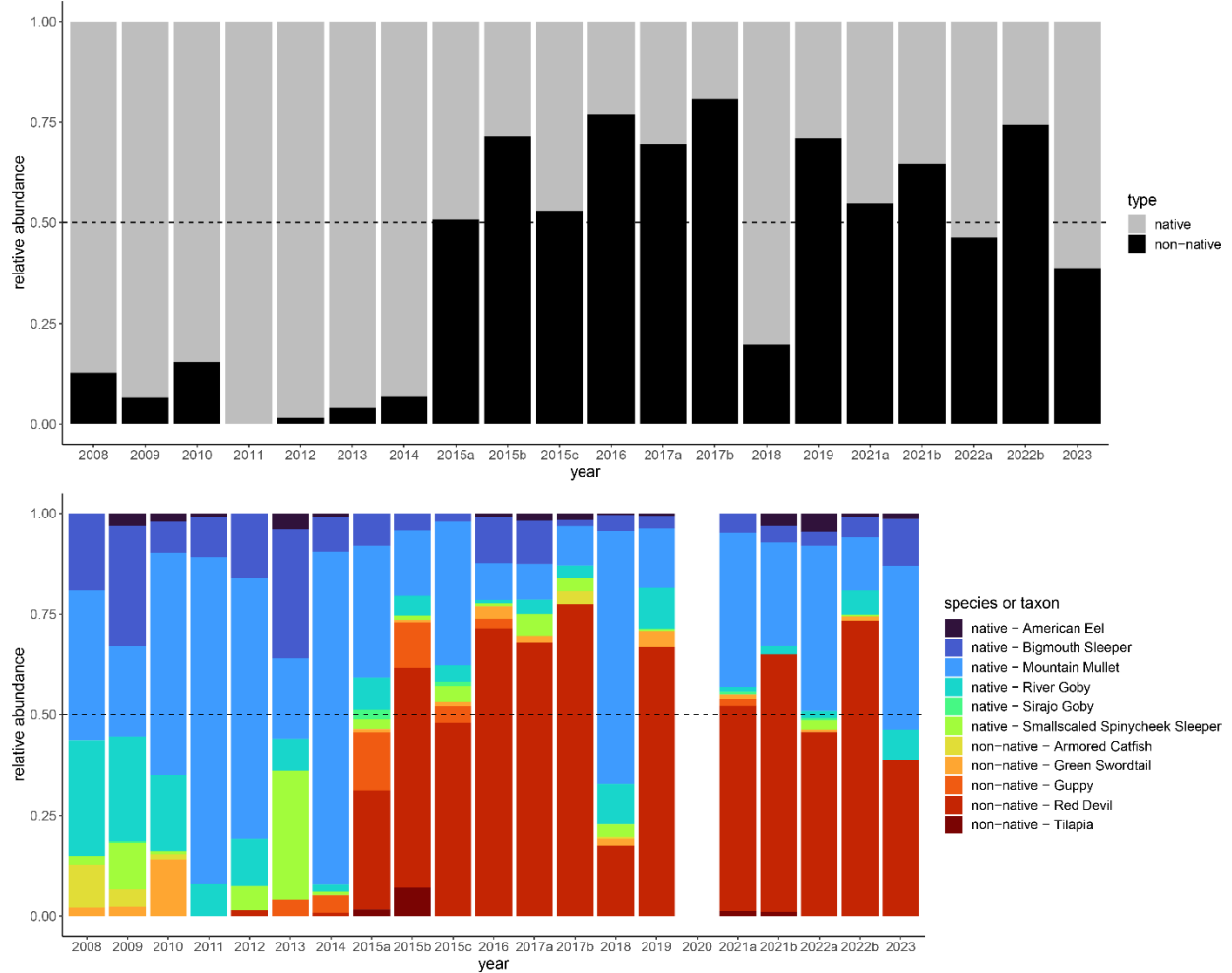
The relative abundances of native and non-native fish showed a clear shift in 2015 from 93% to 49% native species, as previously documented by Ramírez et al. (2018), and in 2018 from 19% to 80% native species (not previously documented) (Figure 2). The assemblage shifted at three distinct points. Native fish dominated assemblage abundance from the start of the long-term monitoring in 2008 through 2014. The first shift occurred in 2015, which was marked by a 44% increase in non-native abundance. Non-native abundance continued to dominate the assemblage until the second shift in 2018 when there was a 61% decrease in non-native abundance. The third shift occurred in 2019 when non-native species rebounded and made up 75% of the assemblage. Non-native fish continued to dominate the assemblage in 2021 (55-65% of the assemblage abundance) and in the second sampling event in 2022. An increase in native

abundance was observed between the first and second sampling events in 2021 and 2022 (Figure 2). Native fish dominated again in 2023. Mountain mullet comprised the highest relative abundance in 2008, 2010, 2012, 2014, and 2018. Mountain mullet, bigmouth sleeper, and river goby had the highest relative abundance in 2009. Red devil had the highest relative abundance in 2015b-2017b and from 2021a, 2022b, and 2022b. Red devil contributed the most to non-native species abundance across all years after its establishment in 2015 (2015a-2023).

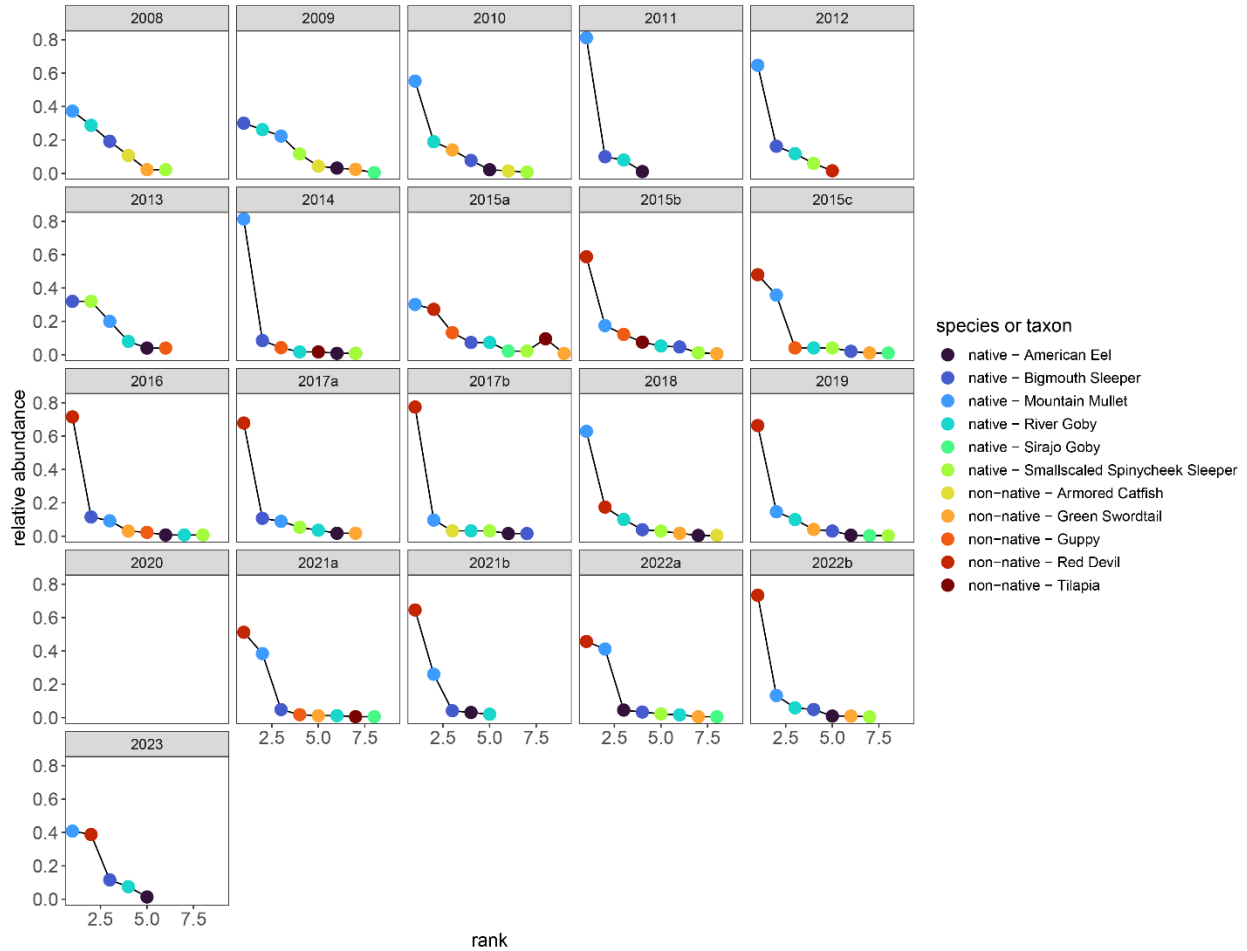
Annual rank abundance curves revealed variation in evenness across years (Figure 3). Steeper slopes in the rank abundance curves correspond to less evenness, while shallower slopes correspond to more evenness in the assemblage (Avolio et al., 2019; Guy & Brown, 2007). The years 2008, 2009, 2013, 2015a, and 2015b had more even assemblages compared to the other years (Figure 3). Mountain mullet and red devil underwent a transposition, with each fish occupying the top ranked position depending on the year (Figure 3). Mountain mullet had the highest rank abundance for 7 of the 20 sampling events; Red devil had the highest rank abundance for 10 of the 20 sampling events. In 2023, mountain mullet and red devil were the same rank. Bigmouth sleeper dominated the remaining two years (Figure 3). The average pairwise differences between each successive year varied from slight differences (i.e., 0.08) to more considerable differences (i.e., >0.30). The mean rank shifts revealed four main shifts. These occurred between 2009 and 2010, 2014 and 2015, 2015 and 2016, and 2016 and 2017. These years exhibited the highest difference in species composition. Comparisons between the years 2010-2011, 2011-2012, 2021-2022, and 2022-2023 showed the smallest change in mean rank differences.

**Table 1:** Summary of native and non-native fish raw abundance (number of individuals) estimates for the sampling events spanning 2008 to 2023 (excluding 2020) at the Río Piedras long-term sampling site in Puerto Rico.

Native/Non-native	native						non-native				
	Common name	American Eel	Bigmouth Sleeper	Mountain Mullet	River Goby	Sirajo Goby	Smallscaled Spinycheek Sleeper	Armored Catfish	Green Swordtail	Guppy	Red Devil
Scientific name	<i>Anguilla rostrata</i>	<i>Gobiomorus dormitor</i>	<i>Dajaus monticola</i>	<i>Awaous banana</i>	<i>Sicydium</i> spp.	<i>Eleotris perniger</i>	<i>Pterygoplichthys</i> sp.	<i>Xiphophorus hellerii</i>	<i>Poecilia reticulata</i>	<i>Amphilophus</i> spp.	<i>Oreochromis</i> spp.
2008	0	18	35	27	0	2	10	2	0	0	0
2009	8	78	58	68	1	30	11	6	0	0	0
2010	3	11	79	27	0	1	2	20	0	0	0
2011	1	10	82	8	0	0	0	0	0	0	0
2012	0	11	44	8	0	4	0	0	0	1	0
2013	1	8	5	2	0	8	0	0	1	0	0
2014	1	10	96	2	0	1	0	0	5	1	2
2015a	0	10	41	10	3	3	0	1	18	37	13
2015b	0	8	30	9	0	2	0	1	21	101	0
2015c	0	2	35	4	1	4	0	1	4	47	0
2016	1	15	12	1	0	1	0	4	3	93	0
2017a	1	6	5	2	0	3	0	1	0	38	0
2017b	1	1	6	2	0	2	2	0	0	48	0
2018	1	9	144	23	0	7	1	4	0	40	0
2019	2	10	47	32	1	1	0	13	0	213	2
2021a	0	8	63	2	1	0	0	2	3	84	1
2021b	3	4	25	2	0	0	0	0	0	62	0
2022a	8	6	72	3	1	4	0	1	0	80	0
2022b	2	10	27	12	0	1	0	2	0	149	0
2023	2	17	60	11	0	0	0	0	0	57	0



**Figure 2:** Relative abundance of native and non-native fish (top graph) and individual species or taxon (bottom graph) from 2008-2023 (excluding 2020) at the Río Piedras long-term sampling site in Puerto Rico. The dotted line illustrates the 50% dominance threshold between native and non-native species.



**Figure 3:** Rank abundance curves showing species richness and rank relative abundance for individual species or taxon from 2008-2023 (excluding 2020) at the Río Piedras long-term sampling site in Puerto Rico.

*Annual time-series flow characteristics and hierarchical cluster analysis*

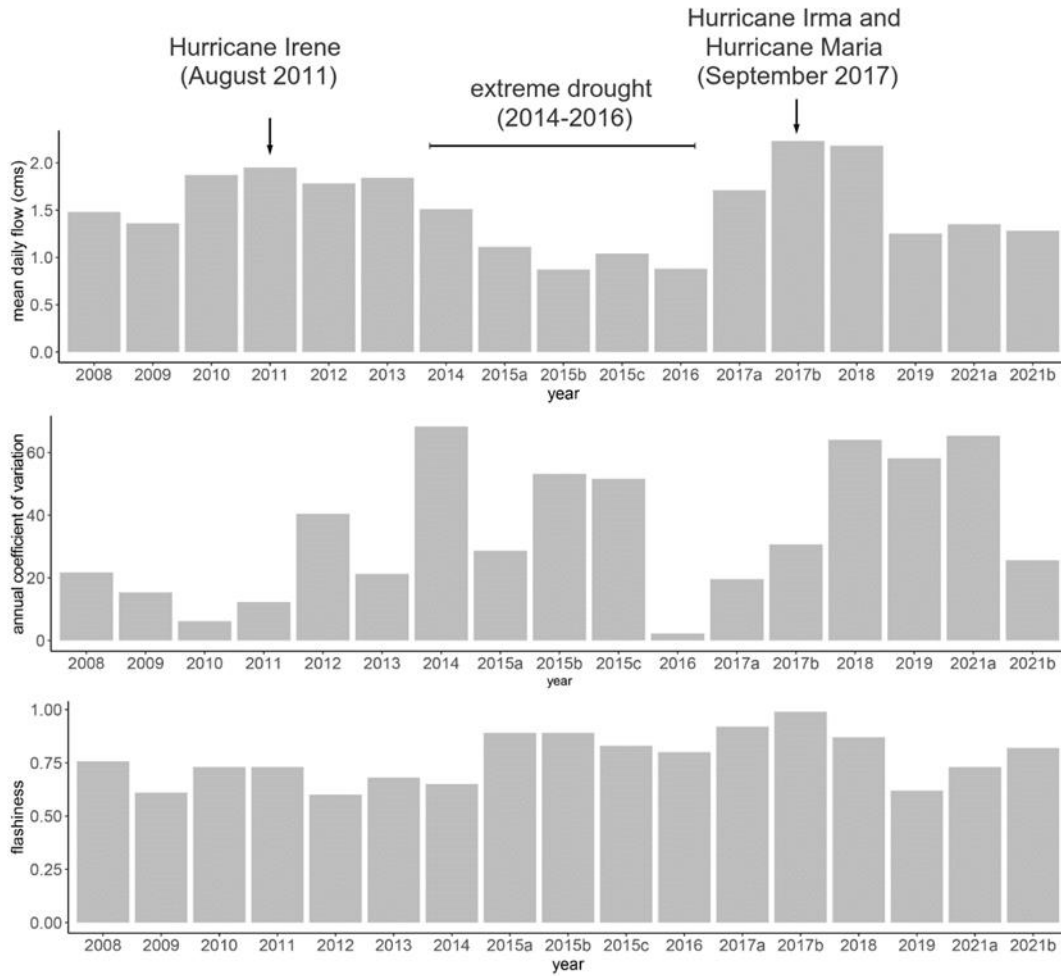
Mean daily flows, annual coefficients of variation, and flashiness were variable across years. The Río Piedras flow regime varied from 0.87 to 2.23 cms ( $\mu=1.49$  cms) in mean daily flows and 2.24-68.30% ( $\mu=33.12\%$ ) in annual coefficients of variation (Table 2; Figure 4). daily flows decreased from 2014-2016, characterized as drought years by the National Integrated Drought Information Systems (NDIS, 2021). The sampling events 2014, 2015, 2018, 2019, 2021a, and 2022b had the highest annual flow variation. Annual flow variation was lowest

during the start of the monitoring (2008~2013) and the years 2015a, 2016, 2017, 2021b, 2022a, and 2023 (Figure 4). Richard-Baker's Flashiness Index ranged from 0.60-0.99 ( $\mu=0.78$ ).

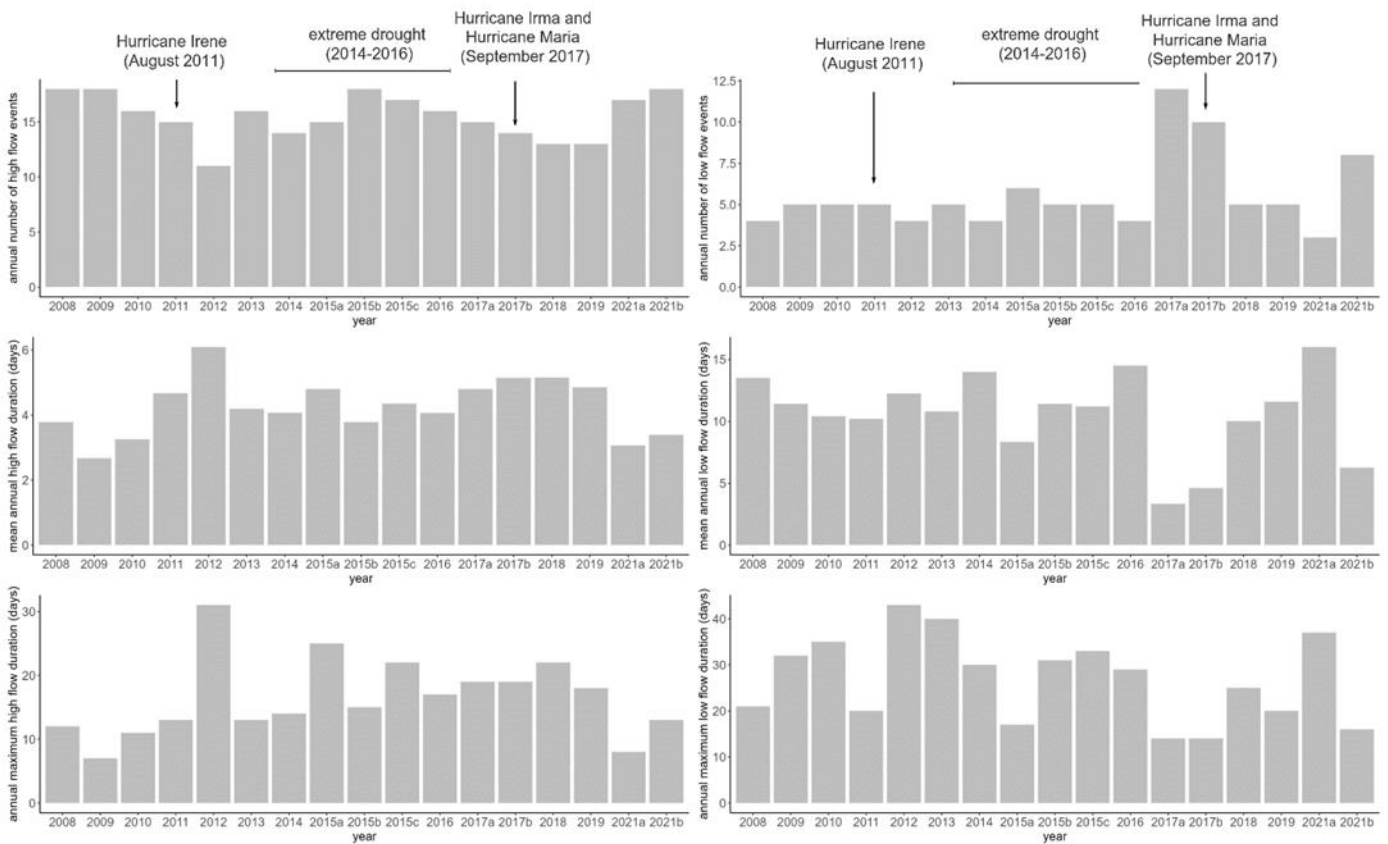
The study period included several extreme flow events and variation in high and low flows. Two extreme drought events occurred during the study period in 2014-2015 and 2021; two major hurricane events also occurred in 2011 (Hurricane Irene) and 2017 (Hurricane María) (Figure 4; Figure 5). Extreme high and low flows varied from year to year in the number of high flow events ( $\mu=15.3$ ; range=11.00-18.00) and low flow events ( $\mu=5.45$ ; range=3.00-12.00) and the annual mean duration of the high flow events ( $\mu=4.27$  days; range = 2.67-6.09 days) and low flow events ( $\mu=10.57$  days; range=3.33-21.20 days) (Table 2). The number of high flow events tended to be higher than the number of low flow events across all years (Figure 5). The annual mean duration of extreme high flows only varied by +/- 3 days, while we found more variation in the annual mean duration of low flows by +/- 18 days. The annual maximum duration of both high flow events ( $\mu=16.41$  days; range=7.00-31.00 days) and low flows events ( $\mu=26.88$  days; range=14.00-43.00 days) showed maximum duration of low flow events tended to be higher than high flow events. Years 2009-2010, 2012-2013, 2015b and 2015c, and 2021a were characterized by the longest maximum duration low flows. The years 2009, 2015a, 2019, 2021a, 2022a, and 2023 had extended annual mean and maximum duration high flows. The year 2017b, which includes Hurricane María was characterized by having a low number of extreme low flow events, approximately average low flow duration, less than average maximum low flow duration, and one of the longest mean annual high flow durations (>19 days).

The hierarchical flow cluster analysis binned the years into four clusters based on the summary statistics of each cluster: (1) non-drought years (2) low variability drought years, (3) high variability drought years, and (4) outlier year (Table 2; Figure 6). Cluster 1, the non-drought

years cluster, contained six sampling events and showed that mean daily flow values were greater than the overall mean, mean annual coefficients of variation were less than the overall mean, and were near the overall mean maximum high and low flow durations. Non-drought years represented non-extreme years. Cluster 2, low variability drought years, contained six of the sampling events and demonstrated median mean daily flows that were slightly less than the overall mean, mean annual coefficients of variation were less than the overall mean, low maximum high flow duration, and the highest maximum low flow duration ( $\mu=33$  days). Cluster 3, high variability drought years, contained seven sampling events and showed slightly lower mean daily flows compared to the overall mean, the highest annual coefficient of variation ( $\mu = 59.16$ ), approximately mean maximum high flow duration, and high maximum low flow duration ( $\mu=29.14$  days). High variability drought years represented extreme low flow conditions coupled with longer maximum duration high flow and higher annual variations in flow compared to the low variability drought years (Figure 6). The outlier year cluster contained one sampling event, which had the longest maximum high and low flow duration.



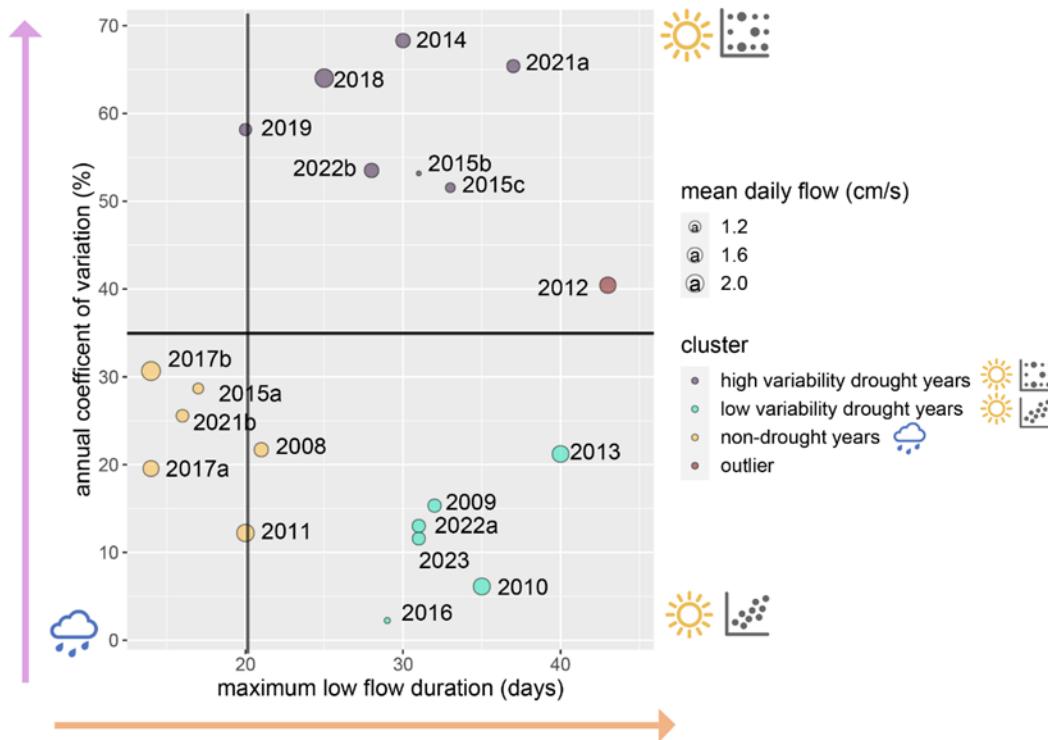
**Figure 4:** Mean annual mean daily flow (cms) (top graph), annual coefficient of variation (middle graph), and Richard-Baker Flashiness Index (bottom graph) from 2008-2023 (excluding 2020) based on daily flow measures taken from the Hato Rey USGS stream gage.



**Figure 5:** Mean extreme annual flow variables including annual number of high (top left graph) and low flow events (top right graph) (number of events), mean annual high (middle left graph) and low flow duration (middle right graph) (days), and annual maximum high (bottom left graph), and annual maximum low flow duration (days) (bottom right graph) from 2008-2023 (excluding 2020) based on daily flow measures taken from the Hato Rey USGS stream gage.

**Table 2:** Annual estimates and summary statistics of the four flow variables (mean daily flow, annual coefficient of variation, maximum high flow duration, and maximum low flow duration) included in the hierarchical cluster analysis and qualitative descriptions of the four clusters of the Río Piedras long-term sampling site in Puerto Rico for years 2008-2023 (excluding 2020).

cluster category	year	cluster description	mean daily flow (cms)	annual coefficient of variation	maximum annual high flow duration (days)	maximum annual low flow duration (days)
non-drought years	2008	> mean MDF; < mean annual variation in flows; ~ mean max high flow duration; ~ minimum low flow duration	1.48	21.70	12	21
	2011	> mean MDF; < mean annual variation in flows; ~ mean max high flow duration; ~ minimum low flow duration	1.95	12.21	13	20
	2015a	> mean MDF; < mean annual variation in flows; ~ mean max high flow duration; ~ minimum low flow duration	1.11	28.67	25	17
	2017a	> mean MDF; < mean annual variation in flows; ~ mean max high flow duration; ~ minimum low flow duration	1.71	19.55	19	14
	2017b	> mean MDF; < mean annual variation in flows; ~ mean max high flow duration; ~ minimum low flow duration	2.23	30.66	19	14
	2021b	> mean MDF; < mean annual variation in flows; ~ mean max high flow duration; ~ minimum low flow duration	1.28	25.56	13	16
	summary statistics for cluster	mean	<b>1.66</b>	<b>23.33</b>	<b>17.80</b>	<b>16.20</b>
	minimum	<b>1.28</b>	<b>19.55</b>	<b>13.00</b>	<b>14.00</b>	
	maximum	<b>2.23</b>	<b>30.66</b>	<b>19.00</b>	<b>16.20</b>	
low variability drought years	2009	median MDF; < 1st quantile annual variation in flows; ~ mean max high flow duration; > 3rd quantile low flow duration	1.36	15.33	7	32
	2010	median MDF; < 1st quantile annual variation in flows; ~ mean max high flow duration; > 3rd quantile low flow duration	1.87	6.12	11	35
	2013	median MDF; < 1st quantile annual variation in flows; ~ mean max high flow duration; > 3rd quantile low flow duration	1.84	21.21	13	40
	2016	median MDF; < 1st quantile annual variation in flows; ~ mean max high flow duration; > 3rd quantile low flow duration	0.88	2.24	17	29
	2022a	median MDF; < 1st quantile annual variation in flows; ~ mean max high flow duration; > 3rd quantile low flow duration	1.36	13.01	25	31
	2023	median MDF; < 1st quantile annual variation in flows; ~ mean max high flow duration; > 3rd quantile low flow duration	11.58	54.48	25	31
	summary statistics for cluster	mean	<b>3.15</b>	<b>18.73</b>	<b>16.33</b>	<b>33.00</b>
	minimum	<b>0.88</b>	<b>2.24</b>	<b>7.00</b>	<b>29.00</b>	
	maximum	<b>11.58</b>	<b>54.48</b>	<b>25.00</b>	<b>40.00</b>	
high variability drought years	2014	< mean MDF; > 3rd quantile annual variation in flow; ~ mean max high flow duration; ~ median max low flow duration	1.51	68.30	14	30
	2015b	~ mean max high flow duration; ~ median max low flow duration	0.87	53.17	15	31
	2015c	< mean MDF; > 3rd quantile annual variation in flow; ~ mean max high flow duration; ~ median max low flow duration	1.04	51.55	22	33
	2018	< mean MDF; > 3rd quantile annual variation in flow; ~ mean max high flow duration; ~ median max low flow duration	2.18	64.03	22	25
	2019	< mean MDF; > 3rd quantile annual variation in flow; ~ mean max high flow duration; ~ median max low flow duration	1.25	58.16	18	20
	2021a	< mean MDF; > 3rd quantile annual variation in flow; ~ mean max high flow duration; ~ median max low flow duration	1.35	65.39	8	37
	2022b	< mean MDF; > 3rd quantile annual variation in flow; ~ mean max high flow duration; ~ median max low flow duration	53.51	86.26	18	28
summary statistics for cluster	mean	<b>8.82</b>	<b>63.84</b>	<b>16.71</b>	<b>29.14</b>	
	minimum	<b>0.87</b>	<b>51.55</b>	<b>8.00</b>	<b>20.00</b>	
	maximum	<b>53.51</b>	<b>86.26</b>	<b>22.00</b>	<b>37.00</b>	
outlier	2012	longest max high and low flow durations	1.78	40.44	31	43
Summary statistics for all years	mean	6.07	37.54	16.96	26.95	
	median	1.42	27.11	17.50	29.50	
	minimum	0.87	2.24	7.00	14.00	
	maximum	2.23	68.30	31.00	43.00	



**Figure 6:** Infographic of the four sampling event clusters based on three annual flow variables with the most variation across years, including annual coefficient of variation (y-axis), maximum low flow duration (x-axis), and mean daily flow (size of points) from 2008-2023 in the Río Piedras in Puerto Rico.

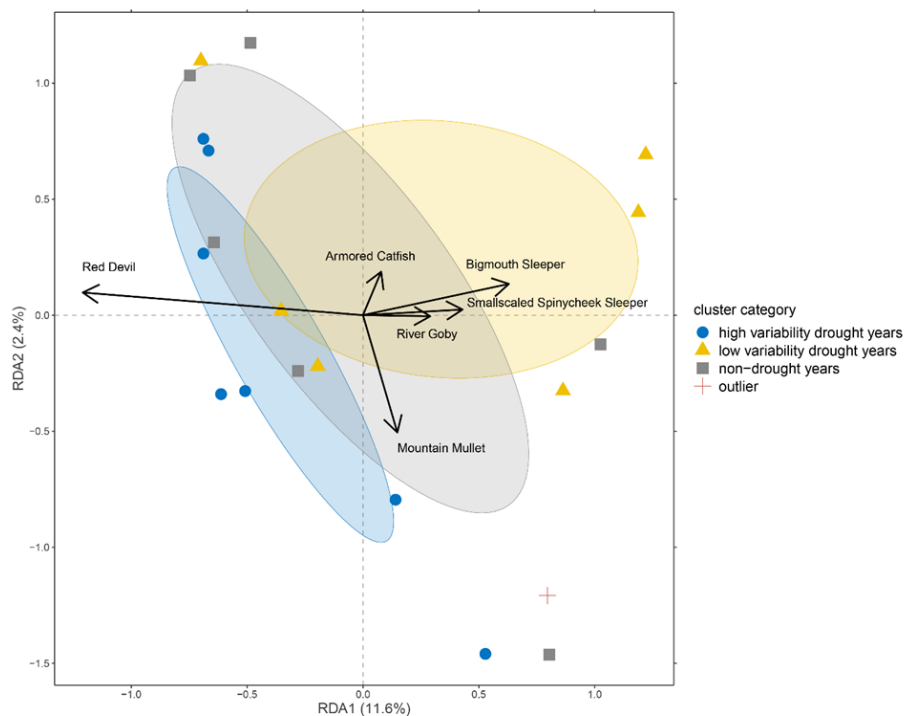
*Redundancy analysis of environmental flows and assemblage structure*

The redundancy analysis illustrated variation within the clusters across fish assemblages. The main species driving variation in the fish assemblages, in order of importance, were non-native red devil (98.60%), native mountain mullet (95.07%), native bigmouth sleeper (75.10%), native river goby (55.44%), native small-scaled spinycheek sleeper (47.05%), and non-native armored catfish species (33.74%) (Figure 7). The overlap between ellipses suggested fish assemblages had some similarities among the year clusters (Figure 7). The low variability drought years and high variability drought years had the smallest overlap suggesting these fish

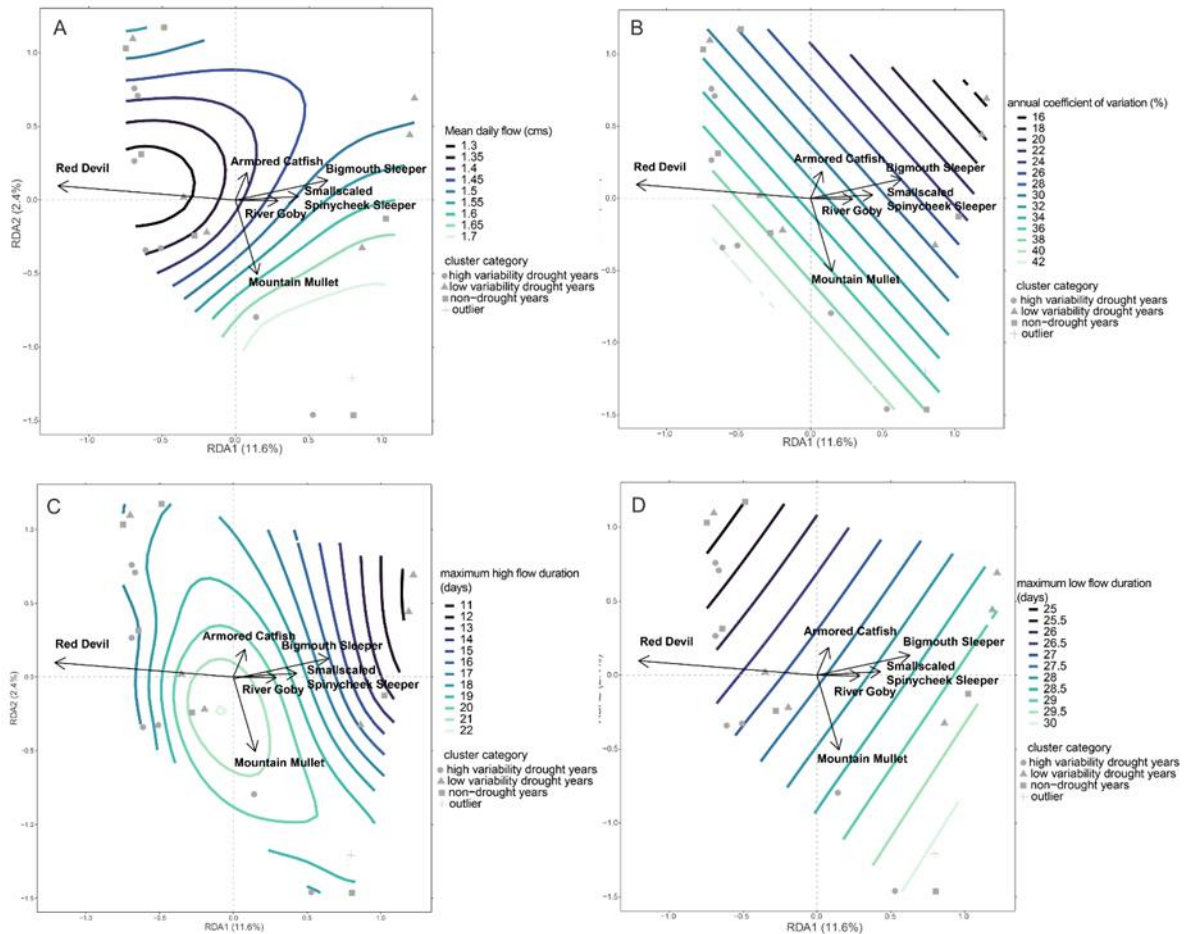
assemblages exhibited distinct patterns across those years, while non-drought years overlapped with the other two clusters (Figure 7). The high variability drought years tended to be characterized by fish assemblages with higher relative abundances of red devil (Table 2; Figure 7). Mountain mullet followed by bigmouth sleeper and river goby had the highest relative abundance compared to the other species in certain years within the non-drought years cluster (2008-2011) and low variability and drought years cluster (2009-2011). The non-drought years, 2017a and 2017b, were dominated by red devil (Table 2; Figure 7). Year clusters were variable with some fish assemblages not clustering tightly within the flow year clusters.

The environmental flow contour plots revealed the gradient of flow variables included in the redundancy analysis and how the flow variables contribute to the species and clusters in the ordination space. These plots revealed optimal environmental conditions for the native and non-native species analyzed where colors were concentrated, or the contour lines were close together. Our flow contour plots revealed red devil and armored catfish abundance were associated with lower mean daily flows and mountain mullet associated with higher mean daily flows (Figure 8; panel A). Similarly, river goby, bigmouth sleeper, and smallscaled spinycheek sleeper were associated with higher mean daily flows (Figure 8; panel A). Both red devil and mountain mullet abundance increased with annual variation in flows; however, the plots reveal their abundance tends to increase in different directions (Figure 8; panel B). Armored catfish and the three other native species abundances increased with lower annual coefficient of variation. Armored catfish, bigmouth sleeper, river goby, and small-scaled spinycheek sleeper abundance decreased with maximum high flow durations (Figure 8; panel C). Red devil and mountain mullet abundance increased with longer high flow durations with mountain mullet trending towards the highest values (Figure 8; panel C). Interestingly, we found native species abundance tended to be

associated with longer maximum low flow durations than red devil and armored catfish (Figure 8; panel D). However, maximum low flow durations did not appear to have a strong association as contour lines were shallow and did not exhibit any distinct shifts in their shape. Based on the shape of the contour plots, mean daily flow and maximum high flow durations had stronger associations driving variation in the fish assemblages than annual coefficient of variation and maximum low flow durations.



**Figure 7:** Constrained redundancy analysis ordination plot of fish abundance data across the 20 sampling events in the Río Piedras illustrating the year cluster classification fit to the ordination of assemblage data. Arrows represent trends in abundance of the main species driving variation in the fish assemblages, including native mountain mullet *Dajaus monticola*, bigmouth sleeper *Gobiomorus dormitor*, river goby *Awaous banana*, and smallscaled spinycheek sleeper *Eleotris perniger*, and non-native red devil *Amphilophus spp.* and armored catfish *Loricariidae sp.*



**Figure 8:** Constrained redundancy analysis contour plots for each of the flow variables included in the cluster analysis related to the fish assemblage (circles and triangles represent the two different clusters). (A) Mean daily flow (cms), (B) annual coefficient of variation, (C) annual maximum high flow duration (days), and (D) maximum low flow duration (days). Arrows represent trends in abundance of the species or taxa driving variation in the fish assemblages, including native mountain mullet *Dajaus monticola*, bigmouth sleeper *Gobiomorus dormitor*, river goby *Awaous banana*, and smallscaled spinycheek sleeper *Eleotris perniger*, and non-native red devil *Amphilophus spp.* and armored catfish *Loricariidae sp.*

### *Changepoints in native and non-native relative abundance*

We qualitatively identified four possible changepoints in 2014, 2017b, 2018, 2021b, 2022a, and 2022b based on the 50% threshold (Figure 2). Quantitatively, we found two changepoints in 2014 ( $p=0.005$ ) and 2017b ( $p=0.753$ ). The two most distinct qualitative changepoints (2014 and 2017b) aligned with the two quantitative changepoints we identified. The 2017b changepoint was not significant; however, this could be a function of the lower sample size used to estimate the changepoint following the establishment of red devil. These quantitative changepoints reveal statistical shifts in relative abundance of native and non-native fish in the assemblage over time.

The overall model for native abundance was significant ( $p=0.040$ ) and explained 32.66% of the variation in native abundance. The overall model revealed log-transformed native abundance decreased by 0.267 with one cms increase in mean daily flow ( $p=0.022$ ) and decreased by 0.013 with one day increase in maximum low flow duration ( $p=0.019$ ). Although not significant, native abundance has a positive association with maximum high flow duration. None of the flow changepoints identified by the segmented regression model were significant (Table 3). However, the model revealed that log-transformed native abundance decreased following changepoints in maximum low flow duration (slope=-0.004), maximum high flow duration (slope = -0.030), and the annual coefficient of variation (slope=-0.068), while controlling for other flow variables in the model (Table 3). Native abundance increased once mean daily flow reached 1.02 cms (slope = 7.49). When maximum low flow duration reached 37 days or more, native abundance starts to decrease; when maximum high flow duration reached ~14 days, native abundance also starts to decrease.

The overall model for non-native abundance was almost significant ( $p=0.058$ ) and explained 28.62% of the variation in non-native abundance (Table 3). Log-transformed non-native abundance increased by 0.212 with one cms increase in mean daily flow ( $p=0.037$ ) and increased by 0.011 with one day increase in maximum low flow duration ( $p=0.021$ ). Although not significant, non-native abundance was negatively associated with maximum high flow duration. None of the flow changepoints identified by the segmented regression model were significant at the 0.05 or 0.10 significance level. The model revealed that non-native relative abundance increased once maximum low flow duration reached 40 days and above (slope = 0.0003), once maximum high flow duration reached 19 days (slope = 0.029), and once the annual coefficient of variation reached 65.39% (slope = 0.029). Non-native abundance decreased after mean daily flow reached 1.02 cms (Table 3). We found native and non-native relative abundance had opposite relationships with the extreme high and low flow variables.

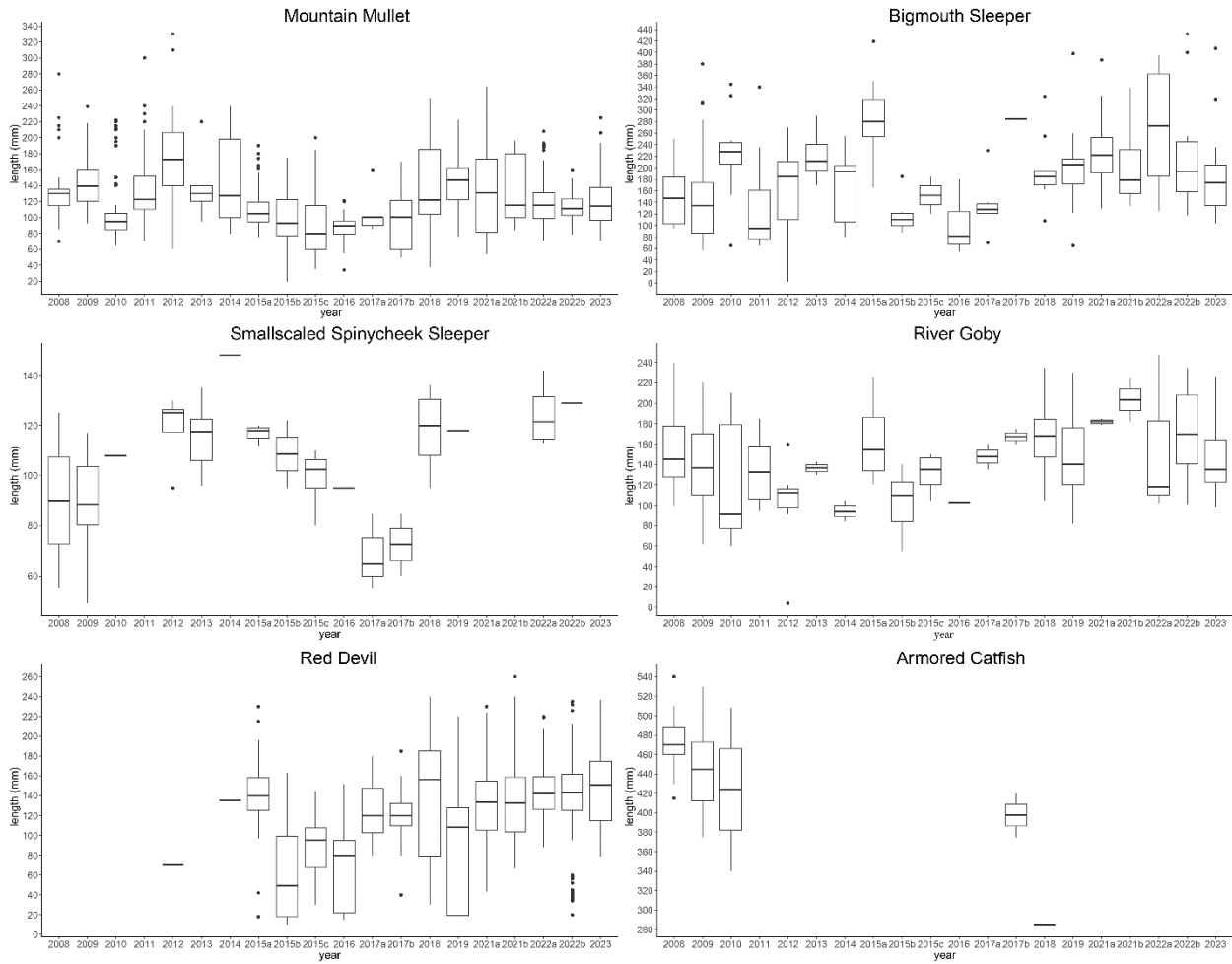
**Table 3:** Changepoints in flow identified by the segmented regression analyses for log-transformed native and non-native relative abundance in the Río Piedras in Puerto Rico from 2008-2023.

Model	Flow variable	Estimated changepoint	Slope after changepoint	P-value
Non-native	mean daily flow (cms)	1.02	-7.63	0.814
	maximum low flow duration (days)	40.00	0.003	0.690
	maximum high flow duration (days)	19.33	0.029	0.198
	annual coefficient of variation (%)	65.39	0.029	0.345
Native	mean daily flow (cms)	1.02	7.49	1
	maximum low flow duration (days)	37.11	-0.004	0.404
	maximum high flow duration (days)	13.68	-0.030	0.248
	annual coefficient of variation (%)	12.7	-0.068	0.529

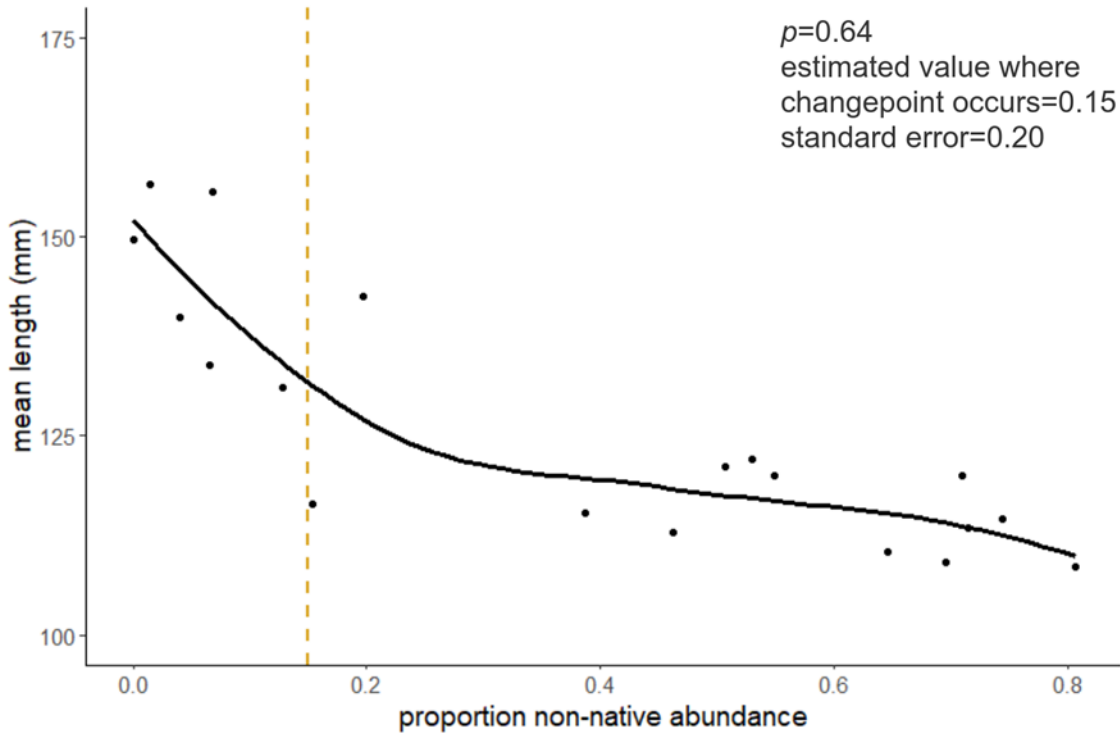
### *Changepoints in native and non-native body size*

The length distributions of the six most-abundant fishes varied through the years of this study (Figure 9). Mountain mullet were generally larger from 2011-2015 and from 2018-2023. Bigmouth sleeper tended to have smaller mean lengths and smaller range of length distribution from 2015-2017a. Smallscaled spinycheek sleeper had the largest mean lengths from 2010-2015a and 2018-2022. The range of river goby length tended to be higher from 2008-2011, 2015, 2018-2019, and 2022-2023. Red devil mean lengths and the range of length distribution varied after their establishment from 2015 to 2019 then red devil mean length appeared to stabilize from 2019-2023. Finally, armored catfish mean length tended to decrease over the years the species was present, from 2008-2010 and 2017-2018.

The overall model for mountain mullet mean length as a function of flow and proportion of non-native species abundance was the only significant model for mean species lengths ( $p=0.03$ ; Adjusted  $R^2=0.41$ ). Mountain mullet mean length decreased by -56.57 mm with a one-unit increase in non-native species relative abundance, while controlling for the four flow variables ( $\beta=-56.57$ ;  $p=0.006$ ). Mountain mullet mean length increased by 0.35 mm with a 1% increase in the annual coefficient of variation, while controlling for the other variables ( $\beta=0.35$ ;  $p=0.07$ ). The segmented regression revealed a possible changepoint in mountain mullet mean length at 0.15 non-native relative abundance (not significant) (Figure 10). Mountain mullet mean length decreased with increasing non-native relative abundance; however, the change in slope shows the rate of decrease slowed as non-native species increased in abundance beyond 0.15 (Figure 10).



**Figure 9:** Box plots of species length distributions for the six species driving at least 30% or greater of the variation across the assemblages for years 2008-2023 (excluding 2020) at the Río Piedras long-term sampling site in Puerto Rico.



**Figure 10:** Segmented regression analysis results for predicted mean length of mountain mullet as a function of non-native relative abundance, while holding the flow variables constant, from the Río Piedras in Puerto Rico from 2008-2023. The chart contains the p-values, estimated values of the independent variable where a changepoint was identified, and standard error of the values. The orange dashed line shows the value of the estimated changepoint.

## DISCUSSION

### *Temporal variations in assemblage abundance driven by a native and non-native fishes*

The fish assemblage in the Río Piedras shifted from an assemblage composed predominantly of native fishes to one dominated by non-native fishes and vice-versa at multiple points from 2008-2023 (see Figure 2). Our results provide evidence that builds on Ramírez et al. (2018) showing the introduction and establishment of non-native red devil in the Río Piedras has altered assemblage structure since 2015. We found native fishes abundance increased in 2018 following Hurricane María. The assemblage shifted again to non-native dominant in 2019, two

years after Hurricane María. This shift lasted three years until native species became dominant again in the last year of sampling. The replacement of native mountain mullet by red devil was the main factor driving these shifts and highlighted the importance of the red devil in the river. We showed other non-native fish (i.e., armored catfish and guppy *Poecilia reticulata*) did not have a similar impact in shifting assemblage structure (Figure 2). Bernardo et al. (2003) observed the same trend in a study in a Mediterranean river. The authors found non-native species increased over a 15-year period dominating the assemblage. Similar to our study, the Mediterranean assemblage shifted to native dominant in response to environmental conditions that were more favorable to native species. Leal-Flórez et al. (2008) also found non-native *Oreochromis niloticus* abundance replaced native *Cathorops mapale* abundance in a Caribbean estuary. Similar to our results, species composition fluctuated over time with shifts in native and non-native species abundance primarily driven by two species.

Our results show red devil success as a non-native fish in the Río Piedras. Chabrerie et al. (2019) highlighted key concepts in the invasion process that we believe are particularly relevant to the success of red devil. These include preadaptation (Mack, 2003), phenotypic plasticity (Richardson & Pyšek, 2006), species invasiveness (Van Kleunen et al., 2010), and environmental disturbance (Meyer et al., 2021). We assert that red devil is preadapted to succeed in tropical rivers based on their evolution from tropical regions. Non-native species tend to have greater phenotypic plasticity in their invaded range (Chabrerie et al., 2019; Richardson & Pyšek, 2006). Cichlids from the genus *Amphilophus* have exhibited phenotypic plasticity displaying trophic polymorphism, which decreases intraspecific competition (Burress, 2015). Red devil also exhibit plasticity in their diet and maturation that shifts with environmental conditions, and can evolve quickly (Abate & Noakes, 2021; Campbell et al., 2011; McConnell & Lowe-McConnell, 1987;

Villéger et al., 2010). Red devil may also have become abundant in our study river due to their high capacity for invasion (Van Kleunen et al., 2010). Cichlid success as invaders has been documented in North and Central America. For example, cichlids Puerto Rico was the most invaded region in Central America with 15 out of the 23 non-native species reviewed present in Puerto Rico (Agostinho et al., 2021).

Red devil may exhibit multiple competitive advantages that also contributed to their success. For example, red devil are aggressive and territorial, which increases their competitive advantage for food and space (Abate & Noakes, 2021). Red devil have been shown to successfully colonize new habitats and are adapted to use a range of trophic niches (Ohee et al., 2018). Bigmouth sleeper and river goby abundance also tended to decrease with the presence of red devil. However, we observed the biggest decrease in mountain mullet. We suggest mountain mullet shares similar functional niche characteristics as red devil. Mountain mullet occupy the water column and feed on insects, invertebrates, shrimp, and algae (Schneider et al., 1995; Smith & Kwak, 2015). Like the mountain mullet, red devil inhabit the water column and feed on insects, smaller fish, zooplankton, plants and shrimp (Ariasari et al., 2018; Hartel, 1997; Sowersby et al., 2020). Alternatively, bigmouth sleeper are a carnivorous, benthic species occupying slower portions of the river and feed on smaller fish (e.g., gobies), crab, and shrimp (Robins & Ray, 1986). River goby are also a benthic species preferring faster flowing waters; the main diet item of river goby is filamentous algae (Watson, 1996). Based on habitat preference and feeding habits, the assemblage shifts we observed may be partially explained by niche overlap between mountain mullet and red devil. Niche overlap can increase interspecific competition affecting assemblage structure (Ross, 1986). Although we did not investigate the

exact mechanism contributing to red devil success in the Río Piedras; it is clear their presence impacted mountain mullet abundance and overall assemblage structure.

*Assemblage evenness changes with extreme flows and non-native fish*

Mean rank shifts were the highest following years characterized by uneven assemblages, extreme flows, and the presence of red devil. This means assemblage structure shifted the most in response to extreme flows when few species dominated the assemblage. Our results may suggest that uneven assemblages had a detrimental effect on the diversity-stability relationship (Campbell et al., 2011; Filstrup et al., 2019; Villéger et al. 2010). Assemblage stability is characterized by low variance in species abundance (Walter et al., 2021). Higher mean rank shifts signify decreased stability in an assemblage (Jacinto et al., 2023). Shifts in species richness, evenness, and mean species abundance can alter the diversity-stability relationship changing population variability (Thibaut & Connolly, 2013). Decreased assemblage stability resulting from changes to the dominant species and assemblage evenness may explain our mean rank shift results. For example, research has shown that uneven, less stable assemblages may affect ecosystem responses and resistance to environmental disturbances (Villéger et al., 2010). The portfolio effect concept that more diverse ecosystems can better cope with fluctuations in environmental factors may be a factor in the Río Piedras (Schindler et al., 2015). Rivers tend to lack functional redundancy and strong buffering against disturbance (Teichert et al., 2018). Thus, decreases in one species in response to disturbance may strongly influence assemblage response due to a lack of functional redundancy. Our results suggest functional diversity may be lacking in the Río Piedras, affecting the native assemblage's ability to cope with disturbances, particularly in years where one or two species dominate the assemblage.

Non-native species can further alter assemblage stability through shifting functional diversity (Shuai et al., 2018; Toussaint et al., 2018). In our study, red devil may have altered functional diversity, making the fish assemblage more susceptible to large shifts following disturbance. This conclusion aligns with work by Jacinto et al. (2023). The authors found stream restoration efforts to restore the natural flow regime in a California creek decreased mean rank shifts and increased ecosystem stability. They primarily attributed this to an increase in native species and decrease in non-native species following restoration. Other research has shown non-native species increase with increasing disturbance (Larentis et al., 2022; Richardson & Pyšek, 2006). Islands characterized by periodic disturbance tend to be more invadable than the mainland (D'antonio & Dudley, 1995). Thus, island ecosystems may be more susceptible to decreased functional diversity as a result of non-native fish. Although we did not assess functional diversity directly this is an area for future research in Puerto Rico. Our results provide evidence that non-native species coupled with extreme low flow disturbance may alter assemblage evenness and stability and increase the impacts of extreme events.

*High and low flow extremes associated with changepoints in native and non-native dominant assemblages*

We found two quantitative assemblage changepoints over the period of the study (Figure 11). One significant changepoint was characterized by an increase in red devil abundance following and during an extreme drought. Based on changes in relative abundance, the changepoint suggests a significant assemblage transition occurred between native and non-native fishes between 2014 and 2015. Changepoints have also been identified in other river systems. For example, Mahardja et al., (2017) detected changepoints in abundance of 12 of the 23 species analyzed in the Sacramento-San Joaquin Delta. Changepoints were detected for native and non-

native species and showed increased abundance at the changepoint for almost all the species. The majority of changepoints were associated with an abrupt increase in non-native species abundance like we observed in 2015. Our finding provides further evidence to Ramírez et al. (2018), who found extreme drought may have provided an avenue for the establishment of non-native species in Puerto Rico. For example, Ramírez et al. (2018) analyzed a shorter-term dataset from the Río Piedras and found mountain mullet and red devil accounted for 60% of the assemblage dissimilarity and documented a shift from mountain mullet dominant to red devil dominant following the 2015 drought. Building on this finding, we show non-native red devil may have triggered an abrupt shift in the fish assemblage.

Our results suggest the most extreme high flows may be detrimental to red devil in the short-term and positive for mountain mullet, while, the most extreme low flows are detrimental to mountain mullet and beneficial to red devil (Table 3; Figure 11). The second assemblage changepoint, although not significant, occurred in 2018, following Hurricane María. We suggest the extreme flooding from the hurricane allowed mountain mullet abundance to increase following a decreased red devil abundance. Before the shift from a red devil to mountain mullet dominant assemblage in 2018, red devil were present in lower numbers in 2017 compared to 2015-2016. This could have been due to reduced maximum low flow durations in 2017 and higher than average mean annual high flow duration that favored native fishes. Our model results showed native relative abundance decreased with longer low flow durations and increased with longer high flow durations (Figure 11). These flow conditions may have resulted in a high recruitment year in 2017 for mountain mullet and a low recruitment year for red devil. Cichlidae species spawn during low flows, which create shallower areas with submerged habitat (Reiss, 2015). Alternatively, Smith & Kwak (2015) found mountain mullet recruitment increased

following major flood disturbance. The authors also showed mountain mullet density increased following flooding. This trend has been shown in other stream ecosystems. For example, Meffe (1984) observed flooding benefitted native fish species and reduced non-native species in an arid desert tributary. Native species responded quicker behaviorally to high discharge through better positioning. Similar to our results, Bernardo et al. (2003) found non-native centrarchids increased following drought years, but extreme flooding, at least in the short-term, resulted in increases in native species and large decreases (~72%) in the proportion of non-native species. Our results suggest higher mean annual and maximum high flow durations could support mountain mullet populations and control for red devil populations.

High flows negatively affecting red devil was not observed across all years when red devil were present. For example, red devil was dominant in some years with longer duration high flows, suggesting other variables may be more important in driving non-native abundance. For example, we showed non-native abundance decreased significantly with increases in mean daily flow and increased significantly with longer low flow durations. One-off extreme low flows may function as a pathway for non-native species to establish. Extreme flooding and low flows can lead to the establishment of non-native species in ecosystems by reducing biotic resistance of native populations and facilitating dispersal of non-native species, depending on local characteristics (e.g., presence of dams, reservoirs upstream with non-native species) (Diez et al., 2012). Schultz et al. (2003) found decreases in non-native fish abundance in Sonoita Creek after flooding; however, some non-native fish that were present in an upstream of the lake increased in abundance. The authors concluded that high flows facilitated increased movement of non-native fish into the lake. Displacement of native species during extreme high flows may have also contributed to our findings. In Puerto Rico, mountain mullet and bigmouth sleeper were

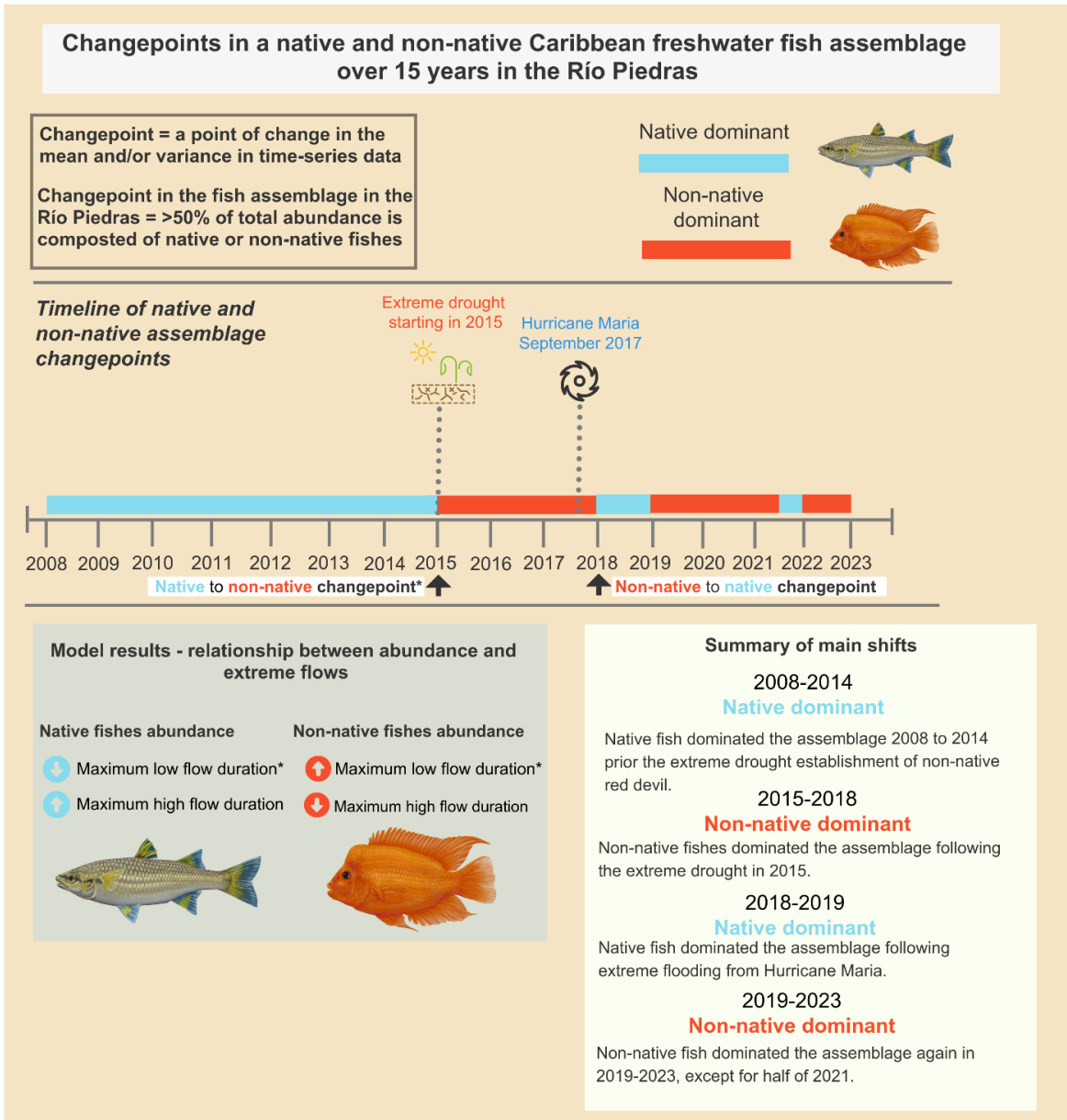
displaced during extreme high flow events (Chabrierie et al., 2019; Smith & Kwak, 2015). Displacement in the Río Piedras may have reduced native species abundance during certain flooding events and allowed red devil to disperse from upstream areas and reestablish quickly.

*Non-native relative abundance affects mountain mullet body size*

Body size may be one factor affecting assemblage structure response to flows. We showed mountain mullet and red devil body size varied inversely at certain points. Our length frequency analysis revealed red devil population size was unstable in the initial years after the 2015 spike in abundance (see Figure 9). Mountain mullet body size decreased after the large increase in red devil abundance. Red devil size distribution was more variable until it stabilized in 2021. Following the reduction of red devil abundance in 2018, our size distribution showed larger individuals of both red devil and mountain mullet comprised the population after Hurricane María. Smaller individuals were most likely displaced by the flood (Valdez et al., 2001). Larger red devil may have outcompeted smaller fish for refugia. This aligned with another study by Chea et al. (2020), which documented higher abundances of larger individuals during a flood followed by a recruitment event in the Tonle Sap. Furthermore, moderate drought in 2021 may have resulted in a successful red devil recruitment year than conditions in the second half of 2018 and 2019. This recruitment may have resulted in a population with a mix of young of the year and adult individuals.

We attempted to identify the first changepoints in native mean lengths with environmental flows and non-native fishes in Puerto Rico. Our segmented regression revealed non-native abundance was the main driver of decreases in native mountain mullet body size. Non-native abundance resulted in a decrease in mountain mullet mean length over time; however, we did not identify a significant changepoint. This finding could be explained by

density-dependent decreases in growth when non-native fish are present (Griffin & Fayram, 2007). Additionally, native species body size decreases when non-native species are present due to increased competitive interactions and increased stress (Houde et al., 2015). In our study, mountain mullet body size was negatively effected by increases in relative abundance of non-native species, particularly red devil, revealing their mulifaceted effects on native species.



**Figure 11:** Quantitative changepoints in fish assemblage over time from 2008-2023 and model results for native and model results for non-native relative abundance as a function of extreme high flow (maximum high flow duration) and extreme low flows (maximum low flow duration). \*signifies significant relationships between relative abundance and the extreme flow variables or a significant changepoint in the fish assemblage (e.g. 2014). Two extreme events are included on the timeline to represent events that may have contributed to the changepoint in the fish assemblage.

### *Research needs: Monitoring for changepoints*

We consider the change from native to non-native-dominated fish assemblages to be a changepoint and not a “true” tipping point, regime shift, or shifts to an alternative stable state as we do not have the data or study design to confidently say a critical ecological threshold passed (Capon et al., 2015). However, our observed shifts between native and non-native assemblages may be considered shifts in ecosystem states. For example, our study methods align with criteria identified by Capon et al. (2015) for identifying different ecosystem states. First, our time series data spans 15 years, which allowed us to observe changes in the historical fish assemblage. Second, we coupled our time series data on fish assemblages with time series flow data. The flow data represented the pressure in the system facilitating the ecosystem state changes between native and non-native assemblages. We assert the flow conditions prompting increases in non-native abundance and decreases in native abundance drove the changepoint in native abundance between 2014 and 2015 (Figure 11). Reynolds (2021) suggests non-native species can prompt an earlier changepoint in response to environmental disturbance. Although we cannot conclude that is the case in our study, this is an area to consider for future research as extreme events increase in Puerto Rico.

Further monitoring to identify future changepoints is an area for future research that could provide important information to managers. Managers could use early warning signals to prevent or manage for environmental thresholds (Selkoe et al., 2015). Early warning signals can indicate an ecosystem approaching a changepoint or future tipping point (Scheffer et al., 2009). Stelzer et al. (2021) synthesized experiments identifying early warning signals and found that 70% successfully signaled a regime shift in the system. Experiments focusing on flow conditions and red devil abundance that causes an irreversible shift in Puerto Rico’s fish assemblages could

be a useful avenue to pursue. This would provide a better understanding of the mechanisms prompting changepoints in native and non-native assemblages. For example, further research on the impacts on functional diversity when non-native species become established under changing flow regimes may give more insight. We showed shifts from native to non-native dominant assemblages are not irreversible in the Río Piedras. This means managers still have the opportunity to conserve and manage native populations before ecosystem transformation occurs.

#### *Conclusions and management implications*

Our results and analyses show extreme flows and presence of red devil shifted assemblage structure in the Río Piedras. Low assemblage evenness coupled with extreme events facilitated the largest changes in assemblage structure indicating instability in the ecosystem. Mountain mullet and red devil are particularly prominent in driving observed shifts in assemblage structure. Temporal shifts in native and non-native fishes in the assemblage are reversible and dependent on flow conditions. Our results serve as an early warning that non-native species abundance coupled with extreme flows may trigger shifts in assemblage structure and changepoints in native assemblages. The most extreme high flows benefit native over non-species species and taxa. Conversely, extreme low flows benefit non-native taxon. These temporal shifts in the Caribbean driven by extreme flows and non-native fish are important in predicting future fluctuations in the assemblage. Our results reveal projected increases in drought and intensity and magnitude of extreme floods coupled with already established populations of non-native species on island ecosystems will be an ongoing issue.

Without management action, the assemblage will continue to be dominated by non-native fishes if projected increases in drought are realized. If managers could reduce red devil abundance and recruitment, future increases in extreme high flows could maintain native species

populations. For example, managers successfully used this strategy in Florida, reducing population size and eradicating two non-native cichlid species in a 1.7 hectare wetland in Florida (Schofield et al., 2019). The managers' initial goal was to reduce the population size of two non-native cichlids. They capitalized on the negative impacts of Hurricane Irma on the cichlid populations and were able to eradicate them (Schofield et al., 2019). (Schofield et al., 2019). The authors along with an interagency team used rotenone in ponds that were isolated due to hurricane effects to remove cichlids. The team then restocked the ponds with native species. Although the systems are different, managers could consider developing rapid response strategies following floods in the Río Piedras and other rivers in Puerto Rico to reduce red devil populations. The situation is complex given the pattern of drought and flooding in Puerto Rico, which may reduce the benefits of extreme flooding.

Shifts in assemblage structure will have consequences on important ecological and cultural services. Decreases in native species abundance may affect ecosystem functioning via impacts on secondary production and functional diversity (Valentine-Rose et al., 2011; Woodward, 2009). Decreased secondary production of native species will impact ecosystems services and is a cause for management concern. Strategies to prevent and manage non-native and invasive species throughout the invasion process is needed (Britton et al., 2023). The best strategy for managers may be to “live with the invasive species”, reduce spread to rivers where red devil are not established, and continue monitoring of the impacts of red devil on assemblage structure (Britton et al., 2023). Managers could also mitigate the impacts of extreme drought through flow management. These strategies fall under the RAD (Resist-Adapt-Direct) framework for managing ecosystem transformations (Rahel, 2022; Thompson et al., 2021). In fisheries, Lynch et al. (2021) outlines three strategies for managing ecosystem transformation. In

this context, managers may be best able to manage the fish assemblage by employing the strategies mentioned to conserve native fish. Resisting the change all together in invaded rivers in Puerto Rico may not be feasible or cost effective. If prior conditions are suitable, extreme flood events may provide an opportunity for native fishes to reestablish dominance in the fish assemblage and allow managers to direct the ecosystem transformation—managers accept the change and strategize to guide the ecosystem to a desirable state.

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## CHAPTER 3

### **Extreme flow effects on competitive interactions, habitat use, and swimming performance of native and non-native Caribbean fishes**

#### **ABSTRACT**

Climate change and non-native species interact to transform freshwater fish assemblages globally. Caribbean fish assemblages are likely to shift with the predicted increases in the magnitude and frequency of floods and drought, and the already-observed increasing presence of non-native species. More research is needed on mechanisms that may be driving changes in fish assemblages such as competition, shifts in behavior, and swimming performance of native and non-native species to effectively manage transformations caused by climate change and species invasions. To address these knowledge gaps, we conducted experiments to better understand interspecific competition, habitat use, behavioral changes, and swimming ability of a native (mountain mullet *Dajaus monticola*) and non-native fish (red devil *Amphilophus* spp.) to high and low flows in the Caribbean island of Puerto Rico. To date, no study has addressed the interactions of mountain mullet and red devil under low and high flows or the baseline swimming performance of these two important species. The objectives of our study were to (1) investigate mountain mullet habitat use in low and high flow treatments, (2) to evaluate if red devil alter mountain mullet habitat use and competitive interactions in low and high flow treatments, and (3) to estimate critical swimming speed and burst swimming of mountain mullet and red devil. We found mountain mullet habitat use decreased significantly in low flow treatments ( $\beta=-1.06$ ;  $p=0.004$ ) compared to high flow treatments and when red devil were present in low ( $\beta=-0.86$ ;  $p=0.018$ ) and high ( $\beta=-1.35$ ;  $p=0.000$ ) densities compared to no red devil present. The combined effects of low flows and high density resulted in increased

aggression towards mountain mullet ( $\beta=1.16$ ;  $p=0.001$ ). Swimming performance tests revealed burst swimming speed and critical swimming speed were significantly faster for mountain mullet than red devil ( $p<0.001$ ). Taken together, these experiments suggest red devil may alter mountain mullet habitat use and behavior during extreme events, which could negatively affect growth, reproductive success, and abundance of mountain mullet. However, mountain mullet may be better adapted for withstanding high flows from extreme flooding than red devil. Our results provide insight into potential mechanisms driving shifts in fish assemblages in Puerto Rico and suggest rivers containing red devil and at risk of extreme drought may be especially vulnerable to shifts in the fish assemblage.

## **INTRODUCTION**

Climate change is an important driver impacting freshwater fish assemblages globally (Kuczynski et al., 2018). Climate change is expected to increase the magnitude and frequency of floods and drought with complex implications for freshwater biota (Gudmundsson et al., 2021; Hirabayashi et al., 2013; Yuan et al., 2023). Precipitation affects the timing, intensity, and frequency of floods (Tabari, 2020). Extended periods of reduced precipitation and warming can cause earlier onset of droughts and more intense periods of drought (Trenberth et al., 2014). Changes to extreme flows can alter freshwater fish assemblage structure (Boucek & Rehage, 2014), physiology (Ficke et al., 2007), biotic interactions (Ummenhofer & Meehl, 2017), habitat use (Lennox et al., 2019), and behavior (Rolls et al., 2017). These changes can have implications for ecosystem functioning and services. The effects will vary regionally with some regions experiencing higher rates of change in extreme events (Sabater et al., 2023; Seneviratne et al., 2021).

Climate change can exacerbate the impacts of non-native species on fish assemblages (Anufriieva & Shadrin, 2018). Extreme flooding may provide pathways for non-native species dispersal and establishment (Thomaz, 2022). In addition, drought may increase competition between native and non-native species due to reduced resource availability (Diez et al., 2012). Increased crowding in limited habitat can cause behavioral changes with potential consequences on fish growth, reproduction, and fitness (Fischer & Öhl, 2005; Lennox et al., 2019). Freshwater rivers and native assemblages in regions projected to experience increases in extreme events coupled with high occurrences of non-native species may be particularly vulnerable to future ecosystem transformation—defined as a novel ecological state that differs from prior ecological structure and function (Lynch et al., 2021).

The mechanisms underlying declines in native fish abundance and biomass in the presence of non-native species are not well known. The introduction of non-native fish can create novel assemblages once dominated by native species (e.g., Goren & Galil, 2005; Pelicice & Agostinho, 2009). Non-native species can shift interspecific competition decreasing native species overall fitness by negatively affecting growth (Cucherousset & Olden, 2011; Gunckel et al., 2002), reproductive success (Cucherousset et al., 2008), and abundance (Turek et al., 2013). These effects can have larger impacts on assemblage structure and ecosystem functioning and services.

Swimming performance influences fish responses to changing flows. Swimming performance can affect fitness, species interactions, habitat selection, and survival (Cano-Barbacid et al., 2020). Critical swimming speed ( $U_{crit}$ ) and burst swimming speed are two metrics traditionally used to assess fish swimming ability (e.g., Beamish, 1978; Dorn et al., 1979; Ojanguren & Brana, 2003; Plaut, 2001). Critical swimming speed is related to the maximum

aerobic capacity of fish and estimates the maximum sustained swimming ability over longer time periods (Jain et al., 1997; Tierney, 2011); burst swimming speed is the maximum speed a fish can swim, can be maintained for much shorter periods, and is related to anaerobic capacity (Beamish, 1978; Cano-Barbacil et al., 2020). Studies have used swimming performance to understand the impacts of anthropogenic stressors, such as climate change (Lyon et al., 2008; Nudds et al., 2020), urbanization (Kern & Langerhans, 2019), and changes in flows on native and non-native species (Ward et al., 2003). A comprehensive database of swimming performance suggests data are limited for most species (Katopodis & Gervais, 2016). No information on swimming performance of our focal species was available in the database, revealing a large gap in understanding baseline swimming performance of these two important species.

Caribbean freshwater rivers may be at particularly high risk of ecosystem transformation caused by extreme events and non-native species (e.g., Jiménez-Segura et al., 2016; Ramírez et al., 2018). Precipitation in the Caribbean is projected to decrease by 2050, resulting in a reduction of wet days and increase in dry days (Vichot-Llano et al., 2021). Bowden et al. (2021) found the Caribbean island of Puerto Rico is projected to experience more substantial decreased precipitation by 2050; however, projections for wetter parts of the island are more uncertain (Bowden et al., 2021). Non-native fishes, particularly red devil *Amphilophus* spp., are also a significant concern in Puerto Rico to the conservation and management of native species (Kwak et al., 2016; Neal et al., 2009; Rodríguez-Barreras et al., 2020). Decreases in mountain mullet *Dajaus monticola* abundance and increases in red devil abundance in response to extreme events have driven shifts in fish assemblages once dominated by native fishes in Puerto Rico (Myers et al. *in prep*).

A review of climate change effects on extreme flooding in tropical regions showed Central America and the Caribbean have been the least researched (Eccles et al., 2019). Data are also lacking on how drought affects fish interactions, highlighting the need for more experimental research (Matthews & Marsh-Matthews, 2003). Combined laboratory and field studies of direct observations of interactions between native and non-native species are relatively uncommon, but are powerful tools to elucidate the effects of non-native species on native species (Almeida & Grossman, 2012). A better understanding of the effects of climate change and non-native species on competition and resource availability is needed (Rolls et al., 2017; Simon & Townsend, 2003). To address these knowledge gaps, our goal was to increase understanding of interspecific competition and swimming ability of a native and non-native fish species to high and low flows in the Caribbean island of Puerto Rico. The objectives of our study were to: (1) investigate mountain mullet habitat use in low and high flow treatments, (2) to evaluate if red devil alter mountain mullet habitat use and competitive interactions in low and high flow treatments, and (3) to estimate  $U_{crit}$  and burst swimming of mountain mullet and red devil. We hypothesized that (1) mountain mullet habitat use decreases in low flow treatments; (2) mountain mullet habitat use decreases when red devil are present in both low and high densities; (3) mountain mullet habitat use decreases more with higher densities of red devil; (4) mountain mullet habitat use decreased with the combined effects of low flow and red devil presence, and (5) mountain mullet have a greater swimming ability compared to red devil. Research on changes to habitat use, behavior, and swimming ability of native species and non-native species can provide a clearer picture to managers tasked with managing for the effects of climate change and invasive species. More broadly, increased understanding of non-native fishes effects on

native fishes habitat use and behavior is useful when identifying mechanisms driving ecosystem transformations on islands in the Caribbean and globally.

## **METHODS**

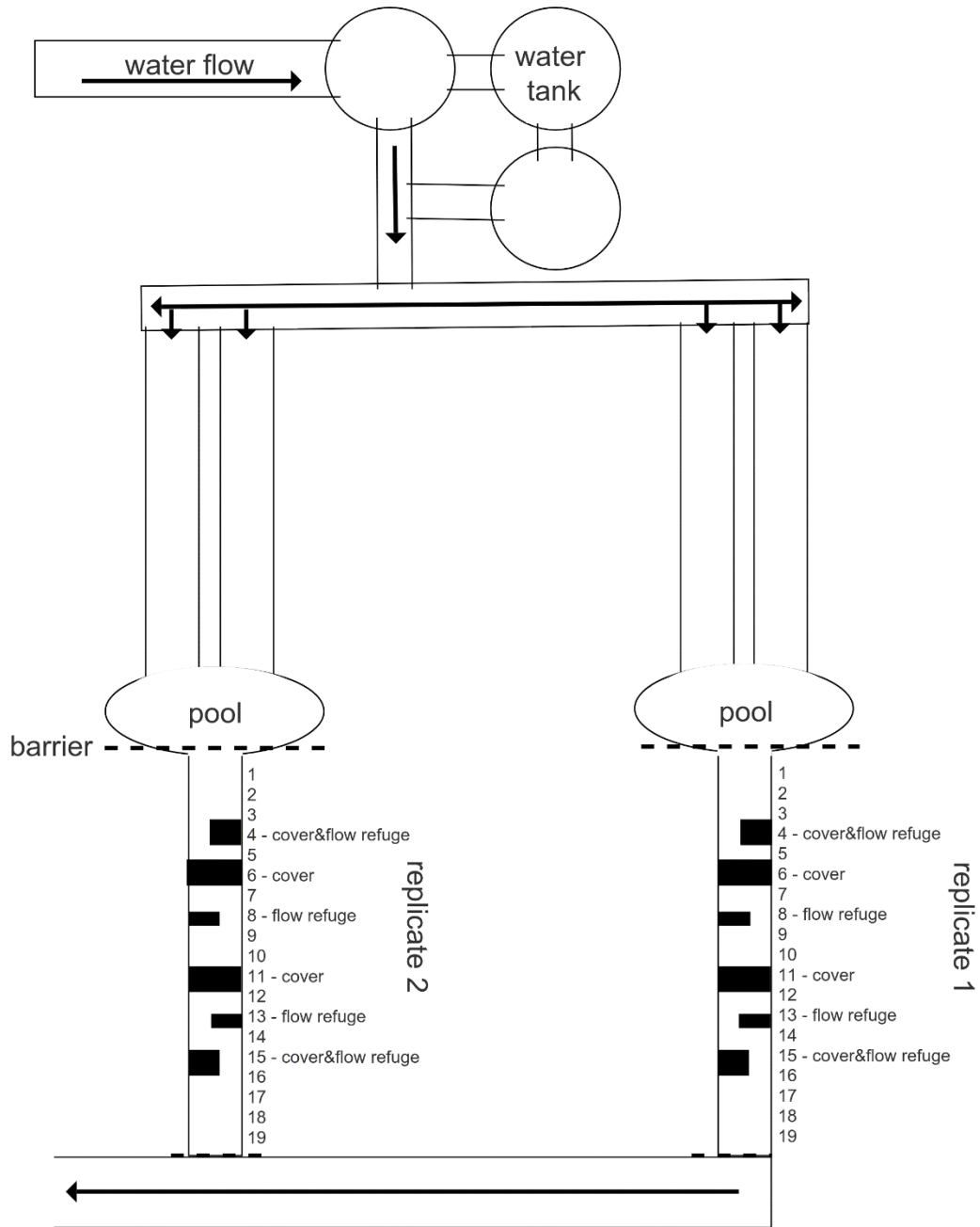
### *Artificial stream setup*

We rehabilitated an artificial stream system at the Luquillo Long-term Ecological Research site in the Luquillo Mountains in Puerto Rico. The artificial streams were constructed using polyvinyl chloride (pvc) pipes that were 0.53 meters wide and six meters long (Kikkert et al., 2009). The pvc pipes were cut in half longitudinally for a previous study (e.g., (Kikkert et al., 2009). A water pump in the Espiritu Santo river pumped water to three cisterns (approximately 1514-L tanks), which then allowed water to flow through 15 cm pvc pipes connecting the cisterns to the artificial stream system. We fitted spouts to the end of the pvc pipes to control water flow during the treatments.

We installed three types of habitat structures in the artificial streams to mimic natural cover, flow refuge, and a combination of flow and cover refuge. We constructed cover habitat using pvc board and placed it across the artificial stream to provide overhead cover. We used cement blocks with a hollow center to provide refuge from flow in combination with cover habitat. We used bricks laid horizontally to provide flow refuge habitat without cover. Two structures of each of the habitat types were placed in the experimental unit for a total of six habitat structures in each unit. Finally, we spread substrate (pebbles and gravel) on the bottom to mimic a streambed.

We employed a gridded system to determine placement of the six habitat structures. We first segmented the artificial stream into a 25 by 30 cm vertical and horizontal grid, respectively. We then stratified the artificial stream into two, 200 cm sections. The streams' first and last 90

cm were open with no habitat structures. We randomly selected three grid locations within each of the two pre-defined sections. We allowed for at least 25 cm between each habitat structure and avoided repeating the same structure in the two sections. The process resulted in placement of two cover structures (60 cm by 50 cm) at grids six and 11, two cover and flow refuge structures (hollow cement blocks) at grids four and 15, and two flow refuge structures at grids 8 and 13 (Figure 1). Habitat structures were placed in identical locations in the two replicates (Figure 1). To prevent fish from migrating out of the experimental unit, we also built a fence using chicken wire downstream of an artificial pool that was part of the existing structure (Figure 1).



**Figure 1:** Schematic of artificial stream setup and habitat locations used to conduct high and low flow experiments with the presence of native and non-native fish species located at the Luquillo Long-term Ecological Research site in Puerto Rico.

### *Treatments*

We tested the effects of high and low flows and the presence, absence, and density levels of red devil with six experimental treatments with two replicates per treatment. The six treatments included the following: (1) high flow – HF; (2) low flow – LF; (3) high flow low density– HF-LD; (4) low flow low density – LF-LD; (5) high flow high density – HF-HD; and (6) low flow high density – LF-HD (Table 1). The flow rate for HF treatment replicates was set at 0.001 cubic meters per second (cms) and the LF treatment replicates was set at 0.0003 cms, which results in HF treatments being 30% faster than LF treatments. In the HF and LF treatments red devil were absent. In the HF-LD and LF-LD treatments red devil were present in equal densities as mountain mullet. In the HF-HD and LF-HD treatments, red devil were present at double the density of mountain mullet (Table 1). All individuals included in the treatments and replicates were unique for a total of 120 mountain mullet and 120 red devil used across all treatments. We ran the experiments from approximately 10am-3pm, conducting one treatment (2 replicates) per day. We measured flow every hour to calculate total discharge for each replicate and monitored temperature, dissolved oxygen, and conductivity throughout the treatments. We ran a two-sample t-test to ensure the flow treatments were significantly different and found that the low flow treatment was significantly lower than the high flow treatment ( $p < 0.0001$ ).

### *Fish collection and acclimation*

All treatments were conducted using mountain mullet and red devil as the target native and non-native fishes, respectively, from two rivers in Puerto Rico. We collected mountain mullet from stream reaches in the Río Mameyes that did not contain red devil and red devil from the Río Piedras located in the northeast in Puerto Rico using a DC-pulsed Smithroot backpack

electrofisher. We conducted fish collection the day prior to each experimental treatment with new individuals collected for each treatment.







We transported individuals back to the experimental site in aerated buckets for acclimation prior to starting the experiments. We acclimated individuals in the artificial streams for at least 24 hours before observation. For experiments with both mountain mullet and red devil, mountain mullet acclimated alone for 10 hours. After the initial acclimation period, we introduced red devil to the artificial stream and both species acclimated together for another 14 hours. We followed an approved care and use protocol outlined in our Institutional Animal Care and Use Committee permit through North Carolina State University.

### *Observations*

We collected data on fish location, activity, and competitive interactions to determine changes in habitat use, behavior, and interspecific competition across the flow and red devil treatments. We recorded the location of each individual and activity displayed, including swimming, sheltering, sitting at the bottom, or guarding at 1-hour intervals for 5 hours for a total of 5 observations per fish. Time points were randomly selected. In addition, we used a GoPro 8 (manufacture details?) to record 12-minute videos once every hour, for a total of 1-hour of video, for each replicate. We analyzed the videos and recorded the number of times individuals engaged in certain behaviors, including displacing, nipping, chasing, or charging. Displacement occurs when one individual supplants another, resulting in the intruder (aggressor) taking over the space previously held by the displaced fish. Aggressive behaviors included nipping, chasing, and charging (Oldfield, 2011). We defined nip as an individual hitting another individual (contact is made), chasing as an individual actively following another individual near a structure for at least two fish lengths, and charging as a lunge toward an individual without making contact. We

recorded the aggressor species, which we defined as the species initiating the behavior, the receiving species, and the habitat type the interaction occurred.

**Table 1:** Characteristics of the flow and red devil treatment groups used to test the effects of high and low flows and the presence of red devil in low and high densities on mountain mullet habitat use, behavior, and competitive interactions at the Luquillo Long-term Ecological Research Site.

Treatment	Treatment code	Flow level	# of mountain mullet	# of red devil
High flow 	HF	High	10	0
Low flow 	LF	Low	10	0
High flow; low density red devil 	HF-LD	High	10	10
Low flow; low density red devil 	LF-LD	Low	10	10
High flow; high density red devil 	HF-HD	High	10	20
Low flow; high density red devil 	LF-HD	Low	10	20

*Statistical analysis of habitat use and behavioral experiments*

We first conducted a chi-squared test using the habitat type (categorical response variable) used by mountain mullet across the six treatment groups to determine if use differed among the treatments. We then built a generalized linear mixed-effects model with a binomial distribution to analyze flow, red devil presence, absence, and density (none, low, high), and the interaction between flow and red devil density effects on the probability of mountain mullet habitat use (Equation 1). We included a nested grouping variable as a random effect in the model

to account for the temporal replication (five observations per treatment) and treatment replication (two replicates per treatment).

*Equation 1:*

$$n_{jkt} \sim \text{Binomial}(p_{jk}, N_{jkt})$$

Where  $n_{jkt}$  is the number of mountain mullet observed using habitat in treatment  $j$ , replicate  $k$ , and at observation time point  $t$ ,  $N_{jkt}$  is the number of mountain mullet in treatment  $j$ , replicate  $k$ , and at time point  $t$ , and  $p_{jk}$  is the probability (0=not using habitat, 1=using habitat) of habitat use in treatment  $j$  and replicate  $k$ , where:

$$\text{logit}(p_{jk}) = \beta_0(\text{reference}) + \beta_1(\text{flow}_j) + \beta_2(\text{red devil density}_j) + \beta_3(\text{flow}_j:\text{red devil density}_j) + 1|t:k + \varepsilon$$

$\beta_0$  is the intercept representing the expected value of the reference categories (high flow, red devil absent),  $\beta_1$  is the effect of low flow in treatment  $j$ ,  $\beta_2$  is red devil density (low, high) in treatment  $j$ ,  $\beta_3$  is the interaction between flow and red devil density in treatment  $j$ ,  $1|t:k$  is the random effect of the nested grouping variable at time point  $t$  within replicate  $k$ , and  $\varepsilon$  is the residual error.

We summarized the behavioral data based on average aggressive interactions per hour by and towards mountain mullet and red devil within each treatment. We built a zero-inflated negative binomial mixed-effects model to analyze aggression towards mountain mullet by red devil and other mountain mullet across the LF-LD, LF-HD, HF-LD, and HF-HD treatments (*package* glmmTMB; Magnusson et al., 2017). The model included the number of interactions per hour as the dependent variable, flow and red devil density (low and high) as fixed effects, the interaction effect between flow and red devil density, and a nested variable of time and replicate as random effects to account for the temporal and treatment replication. We did not include the

LF and HF treatments with no red devil in the analysis as only three interactions occurred across the LF and HF treatments between mountain mullet.

### *Swimming performance experiments and analysis*

We collected 20 mountain mullet and 20 red devil from the Río Piedras to conduct the swimming performance tests. When we could not capture at least 20 mountain mullet from the Río Piedras, we captured additional individuals from the Río Mameyes or Río Cañas. All tests were conducted using a 5-liter Loligo® Systems swim tunnel. Prior to conducting the swimming tests, we acclimated fish for 24 hours in holding tanks and then for 20 minutes in the swim tunnel chamber. We filtered water collected from the Espiritu Santo River into the swim tunnel to remove any larger plants or debris. Prior to the swim tests, we measured total length, depth, and weight of each fish. The program Autoswim was used to automatically increase the velocity at pre-determined intervals and for the solid blocking effect (Bell & Terhune, 1970).

We conducted three swimming tests for each individual to quantify three swimming performance metrics: induced swimming ( $U_{\text{induced}}$ ), burst swimming, and critical swimming speed ( $U_{\text{crit}}$ ). All tests began with a flow rate of five centimeters per second (cms). We determined  $U_{\text{induced}}$  by increasing flow rate by 5 cms every minute and recorded the velocity the fish started actively swimming. We determined burst swimming by increasing the flow rate every minute, 5 cms for red devil, and 10 cms for mountain mullet until the fish could no longer swim and was up against the back grid for at least 20 seconds. We used the ramp  $U_{\text{crit}}$  velocity-increasing method to test critical swimming speed (Tierney, 2011). For red devil, we increased the flow rate by five cm every five minutes for the first five steps. After the first five steps, we increased the velocity by five cms every 20 minutes until the fish could no longer swim. For mountain mullet, we increased the flow rate by 10 cm every five minutes for the first five steps.

After the first five steps, we increased the velocity by 10 cms every 20 minutes until the fish was up against the back grid for at least 20 seconds. We used a larger step increase for mountain mullet based on swim practice tests and time constraints. The tests ended when the fish was touching the back grid for at least 20 seconds. For  $U_{crit}$  estimation, we recorded the time at which the fish swam at its final velocity and the final velocity rate. We calculated  $U_{crit}$  following Brett's equation using Equation 2 (Tierney, 2011). We removed individuals from the analyses entirely when the fish would not swim during the tests and  $U_{crit}$  could not be estimated.

*Equation 2:*

$$U_{crit} = U_f + U_s \times (t_f/t_s)$$

where  $U_{crit}$  = critical swimming velocity;  $U_f$  = speed of the last fully completed step,  $U_s$  = step speed,  $t_f$  = time spent on last step,  $t_s$  = step time.

We conducted statistical tests to determine if the three swimming performance metrics were significantly different between mountain mullet and red devil. We first tested that the data adhered to the normality and equal variance assumption using the Shapiro-wilks test and Levene's test. All tests revealed the data did not violate the assumptions except for the equal variance test for critical swimming speed between mountain mullet and red devil; thus, we conducted a t-test with unequal variance for  $U_{crit}$ . Two sample t-tests were used to test for a significant difference between mountain mullet and red devil induced swimming speed, burst swimming speed, and critical swimming speed (*car* package; Fox et al., 2007). All statistical analyses were conducted in R Studio (2023.06.0 Build 421).

## **RESULTS**

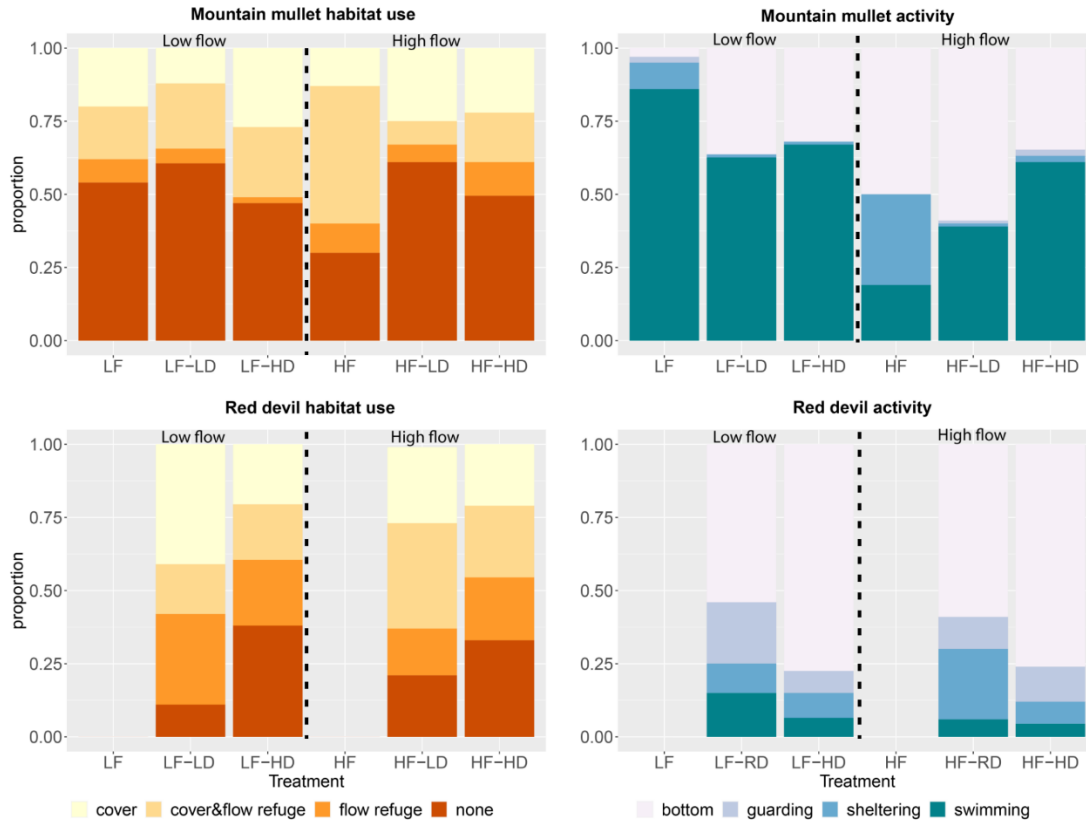
*Habitat use, activity, and behavior experiments*

Mountain mullet habitat use and activity varied across the six HF and LF treatments. Mountain mullet frequently used cover and flow refuge habitat and were inactive in the HF treatment; they used cover and flow refuge habitat 70% of the time and sheltered or positioned at the bottom 50% of the time (Figure 2). Mountain mullet were the least active in HF treatment. We observed them resting at the bottom 60% and using habitat 37.5% of the time (Figure 2). Mountain mullet actively swam  $\geq 50\%$  in all three LF treatments (Figure 2). Mountain mullet were the most active using the least habitat in the LF treatment swimming 87% of the time. Mountain mullet activity decreased and habitat use increased in the LF-HD treatment compared to the LF treatment with red devil absent.

Differences existed between mountain mullet and red devil habitat use and activity. Red devil used the flow refuge habitat type more often than mountain mullet across the RD and HD treatments. Red devil used more habitat and were less active than mountain mullet across all four treatments (Figure 2). Red devil used cover, cover and flow refuge, and flow refuge over 75% of the time in the HF-RD and LF-RD treatments (Figure 2). Red devil were the most inactive, mainly sitting at the bottom or sheltering, in the HD treatments; they also used less habitat and occupied more open water in the HD treatments.

Mountain mullet habitat use decreased significantly in the LF treatment ( $\beta=-1.06$ ;  $p=0.001$ ) compared to the HF treatment, decreased significantly when red devil were present in low densities ( $\beta=-1.35$ ;  $p=0.000$ ) and high densities ( $\beta=-0.86$ ;  $p=0.02$ ) compared to treatments with no red devil. The interaction between low flow and red devil present in low and high densities was significant ( $p=0.03$  and  $p=0.02$ , respectively). Our results revealed mountain mullet habitat use increased with the combination of low flows and red devil at low densities ( $\beta=1.07$ ) and high densities ( $\beta=1.16$ ). The combined effects of low flows and red devil presence had a

positive effect on mountain mullet habitat use even though the individual main effects were negative.

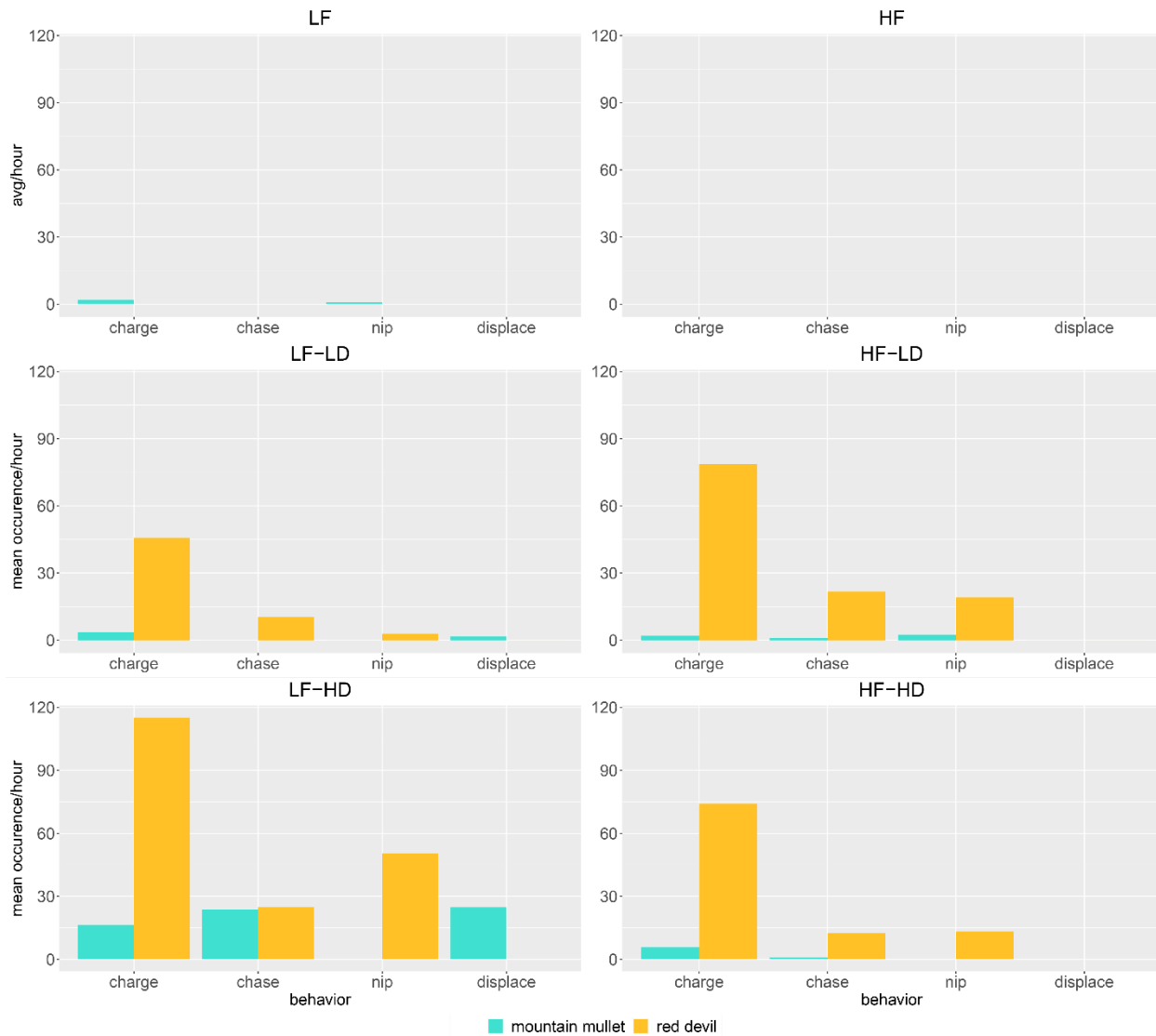


**Figure 2:** Use of different habitat types (proportion) and activity (proportion) of native mountain mullet and non-native red devil across the following six experimental treatments: (1) low flow–LF; (2); (3) low flow low density– LF-LD) low flow high density–LF-HD; (4) high flow–HF; (5) high flow low density – HF-LD; and (6) high flow high density – HF-HD. The black dashed line separates low flow from high flow treatments.

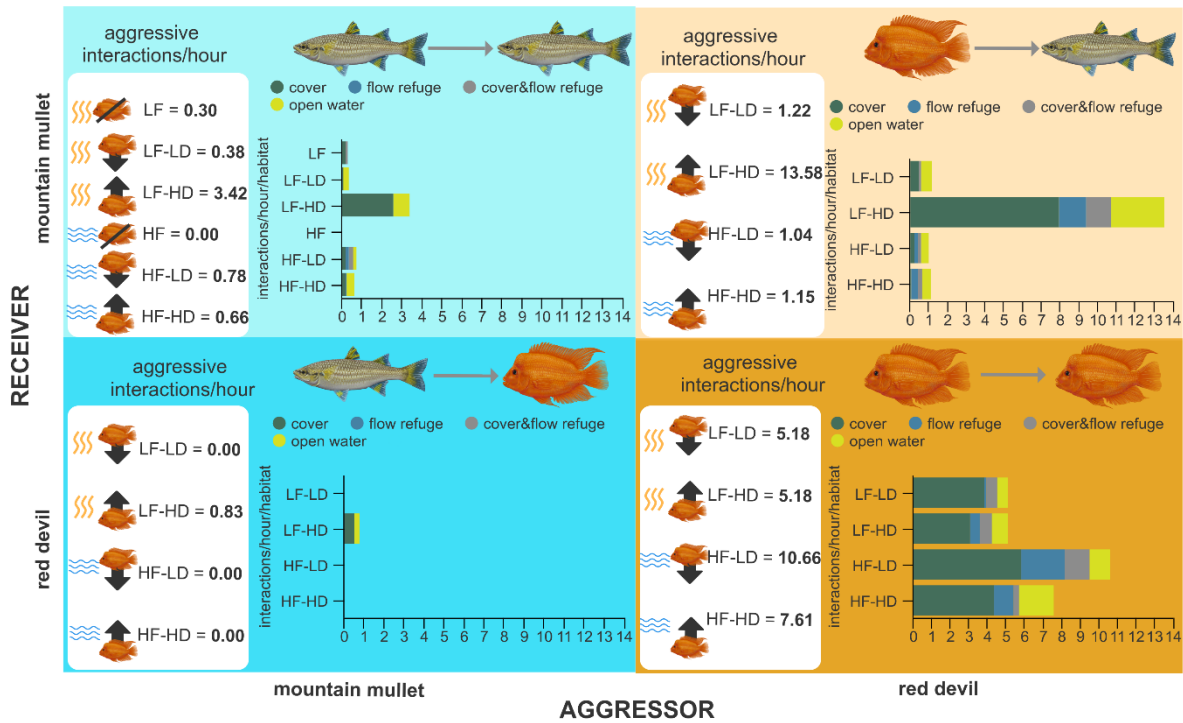
Red devil exhibited more aggressive behavior than mountain mullet across the four treatments (Figure 3). Overall, mountain mullet tended not to initiate aggressive attacks (Figure 3). Red devil and mountain mullet exhibited the most aggressive behaviors (e.g., chasing, charging and nipping) in the LF-HD treatment. The zero-inflated negative binomial mixed-

effects model revealed aggression towards mountain mullet increased in the HD treatments ( $\beta=0.44$ ;  $p=0.10$ ). Aggression towards mountain mullet also increased with the combined effects of low flow and high density red devil ( $\beta=1.16$ ;  $p=0.002$ ).

Red devil and mountain mullet engaged in both interspecific and intraspecific aggressive interactions (Figure 4). Across treatments, red devil aggression was directed to mountain mullet 39% of the time and to other red devil 61% of the time. Red devil aggression toward mountain mullet was greater in the HD treatments compared to the other treatments (Figure 4). Red devil tended to be more aggressive towards other red devil than to mountain mullet in the HF-LD and HF-HD treatments. We found red devil competition increased with other red devil in the HF compared to LF treatments. Aggressive interactions between red devil occurred most often near cover. Competition for flow refuge and the combined cover and flow refuge habitat increased in the HF-LD treatment (Figure 4). Mountain mullet were more aggressive towards other mountain mullet than to red devil across all treatments. Mountain mullet exhibited more aggressive behaviors towards both mountain mullet and red devil in the LF-HD treatments; aggressive behaviors occurred most often in open water or under cover habitat. Overall, the rate of total aggressive interactions initiated by mountain mullet was more than seven times lower than red devil aggression.



**Figure 3:** Proportion of aggressive behaviors (charge, chase, or nip) and displacement of mountain mullet and red devil across the following six experimental treatments: (1) low flow–LF; (2); (3) low flow low density– LF-LD) low flow high density–LF-HD; (4) high flow–HF; (5) high flow low density – HF-LD; and (6) high flow high density – HF-HD.



**Figure 4:** Comparison of observations of mountain mullet and red devil engaged in aggressive interactions across treatments (# of interactions/hour) and the habitat type where the interactions occurred (cover, flow refuge, cover and flow refuge, and open water) across the following experimental treatments: (1) low flow–LF; (2); (3) low flow low density– LF-LD) low flow high density–LF-HD; (4) high flow–HF; (5) high flow low density – HF-LD; and (6) high flow high density – HF-HD. LF and HF treatments are only included on the mountain mullet to mountain mullet interactions, since no red devil were present in those two treatments.

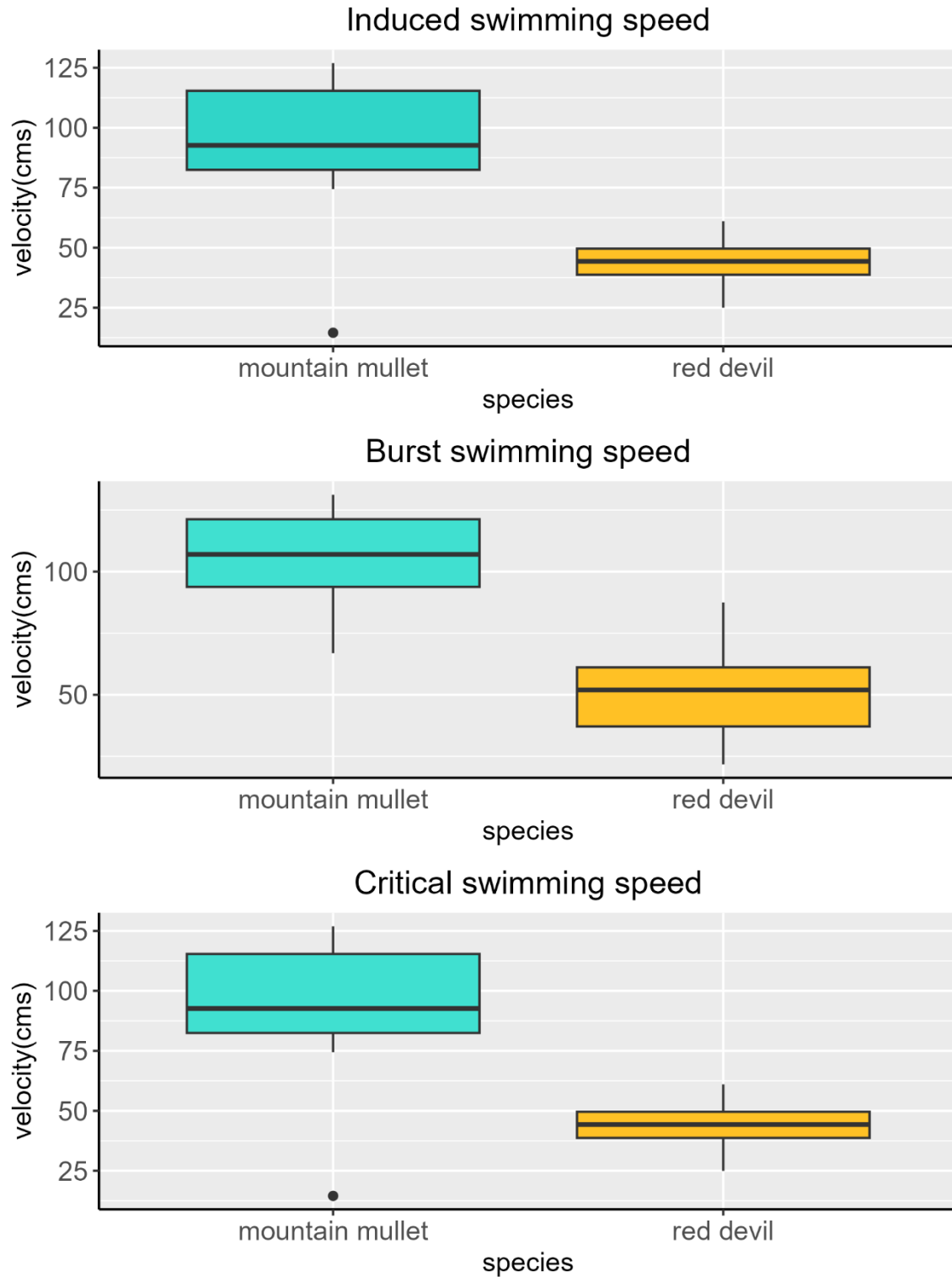
*Swimming performance of mountain mullet and red devil*

Mountain mullet and red devil induced swimming speed did not vary significantly (Table 2; Figure 6). Both fishes started swimming around 23.00 cms. Mountain mullet burst swimming speed was significantly faster (105.10 cms) compared to red devil (51.60 cms) ( $p < 0.0001$ ) (Table 1; Figure 5). Critical swimming speed,  $U_{crit}$ , of mountain mullet was also significantly faster

compared to red devil (Table 1; Figure 5). Mountain mullet mean critical swimming speed was 49.30 cm/s faster than red devil critical swimming speed.

**Table 2:** Mountain mullet and red devil swimming performance metrics, mean length, mean weight, and results of two-sample t-tests for the three swimming performance metrics. Standard deviation for all measurements is in parentheses. \*denotes significant differences at the 0.001 alpha level.

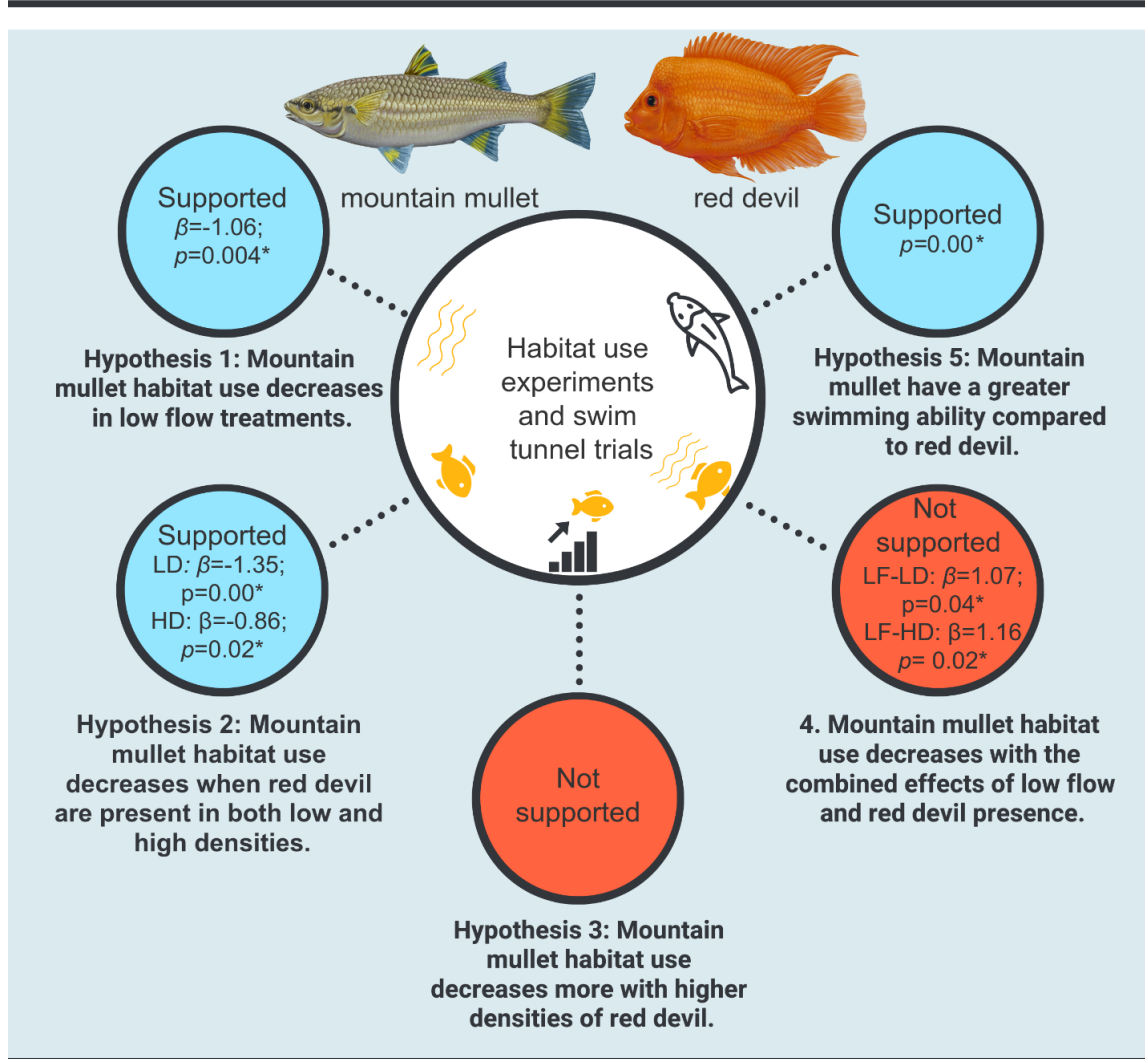
Swimming metric	Species	Mean velocity (cms)	Mean length (cm)	Mean weight (g)	N	p-value
Induced swimming speed	mountain mullet	22.70 (13.20)	15.30 (3.46)	46.8 (35.00)	17	0.90
	red devil	23.00 (14.01)	15.0 (1.71)	66.50 (22.00)	20	
Burst swimming speed	mountain mullet	105.10 (18.30)	14.90 (3.51)	43.50 (34.4)	19	0.00*
	red devil	51.60 (17.60)	15.0 (1.66)	66.60 (21.80)	19	
Critical swimming speed	mountain mullet	92.60 (28.60)	15.30 (3.95)	48.40 (38.90)	14	0.00*
	red devil	43.30 (9.16)	15.50 (1.47)	71.20 (20.00)	17	



**Figure 5:** Box plots representing induced swimming speed, burst swimming speed, and critical swimming speed velocity (cm/s) of mountain mullet (N=17, 19, 14, respectively) and red devil (N=20, 19, 17, respectively).

## DISCUSSION

# Hypotheses and outcomes



**Figure 6:** Summary of hypotheses and model results evaluating the effects of high and low flows (Hypothesis 1), the presence of red devil on mountain mullet habitat use (Hypothesis 2), density effects of red devil (Hypothesis 3) and the combined effects of flow and red devil presence (Hypothesis 4) on mountain mullet habitat use across six experimental treatments including, (1) low flow–LF; (2); (3) low flow low density– LF-LD) low flow high density–LF-HD; (4) high flow–HF; (5) high flow low density – HF-LD; and (6) high flow high density – HF-HD, and comparison of swimming performance between mountain mullet and red devil (Hypothesis 5).

*Habitat use and shifting behaviors of mountain mullet under different flow and non-native red devil treatments*

Mountain mullet habitat use decreases in the presence of the non-native red devil (Figure 6). We suggest this finding demonstrates increased interspecific competition for space in the presence of red devil, which has also been observed in other experiments. For example, Grabowska et al. (2016) found non-native racer gobies *Babka gymnotrachelus* displaced native European bullheads *Cottus gobio* from their preferred shelter habitat. Native species also occupied less desirable habitat in the presence of non-native gobiids in a series of competition experiments (Kessel et al., 2011). Occupying less habitat or decreased access to refugia increases stress on fish, can negatively affect growth, reproductive success and abundance, and exacerbate the effects of disturbance (Dubs & Corkum, 1996). Mountain mullet and red devil both inhabit the water column and have similar diet items; thus, they may compete for space (Schneider et al., 1995; Sowersby et al., 2020). Mountain mullet may have less access to habitat during high flow disturbance due to competition with red devil and altered behavior, which could decrease their ability to cope with disturbance (Magoulick & Kobza, 2003). Access to shelter or refugia during extreme flows, especially low flows, may dampen the negative effects on species richness and abundance (e.g., Penaluna et al., 2021). Competition for habitat by red devil could be one mechanism explaining documented decreases in mountain mullet abundance and biomass in the presence of red devil in Puerto Rico (e.g., Ramírez et al., 2018, Myers et al. *in prep*).

Low flows negatively affect mountain mullet habitat use resulting in increased activity (Figure 6). This result may indicate that low flows triggered mountain mullet to search for refugia, resulting in them actively swimming and using less habitat during low flow treatments. Our finding is supported by research suggesting drought can increase movement of fishes

(Matthews & Marsh-Matthews, 2003), particularly free-swimming species inhabiting the water column (Lennox et al., 2019). To mitigate the effects of low flows some species may behaviorally shift habitat use or disperse to refuge habitats at the onset of drought to escape low water levels and decreased water quality (e.g., low dissolved oxygen) (Magoulick & Kobza, 2003; Rayner et al., 2009). For example, fish increased movement to downstream pools to escape low flow conditions when exposed to experimental drought (Driver & Hoeninghaus, 2016). Fish also move to avoid thermal and oxygen stress that can result in reduced fitness, growth, and recruitment (Bond et al., 2008; Elliott, 2000). Based on our finding and supported research, we conclude decreased mountain mullet habitat use and increased activity suggests individuals may have experienced increased stress and were searching for refuge habitat or better flow conditions during low flows in the artificial streams.

We found higher densities of red devil had a smaller negative effect on mountain mullet habitat compared to low densities, which did not support our original hypothesis (Figure 6). Mountain mullet may have reduced their activity more and used more habitat to avoid aggressive interactions when red devil were present in higher densities. We showed that aggressive interactions towards mountain mullet decreased in the HF treatments (Figure 4). Higher flows can increase habitat complexity which can decrease encounter rates and aggression (Höjesjö et al., 2004; Warnock & Rasmussen, 2013). Higher flows can cast more shadows in the stream and reduce visual cues that can decrease encounter rates (Warnock & Rasmussen, 2013; Wikramanayake, 1990). Nonetheless, higher densities of non-native fish increase competition for space and food, resulting in negative effects on growth and survival (e.g., Parvez et al., 2023). In HD treatments, mountain mullet were confined to the upper and lower sections of the artificial stream, generally attempting to avoid red devil and maintain a position under cover habitat. Our

result illustrates mountain mullet adjust their behavior in the presence higher densities of red devil, which could negatively affect their fitness in natural systems.

The interaction between low flows and the presence of red devil at low and high densities interact to increase mountain mullet habitat use, which did not support our original hypothesis (Figure 6). This could be partially explained by our observations showing increased competition and aggressive interactions in the LF-LD and LF-HD treatments. We found, in the absence of red devil, mountain mullet could freely swim. In a natural system, this would allow them to migrate upstream or downstream to access refugia during drought. Mills et al. (2004) similarly found the native least chub *Iotichthys phlegethontis* spent less time in open water and more time under cover habitat when non-native mosquitofish *Gambusia affinis* were present. Least chub's behavioral change reduced forage opportunities and had a six-fold negative affect on growth. Mountain mullet were also subjected to more aggressive behaviors in the LF-HD treatments (Figure 6). Similarly, they exhibited more aggressive behaviors, particularly chasing, and were able to reestablish in habitat structures or chase red devil out of their space. A similar trend was observed in native brook trout *Salvelinus fontinalis* and non-native bull trout *S. confluentus* high density treatments; both species exhibited increased aggressive behaviors at high densities and were found to be competitively equal (Warnock & Rasmussen, 2013). Increased aggression was also observed in a tropical lake experiment exposed to warmer temperatures; the authors concluded this increased aggression would have consequences on fish energy budget, growth, and fitness (Kua et al., 2020). In the presence of red devil during drought, we suggest mountain mullet may exert more energy to occupy space and engage in aggressive interactions.

#### *Swimming performance*

Mountain mullet exhibited higher burst swimming and critical swimming speed than red devil (Figure 6). Swimming performance impacts fitness; morphology impacts swimming performance (e.g., morphology, performance, fitness paradigm; Arnold, 1983). Steady or sustained swimming speed, which is measured through  $U_{crit}$ , is normally higher in fish with a streamlined body shape; higher sustained swimming speed is normally favored in high flow environments; while, burst (unsteady swimming) is normally more adapted to low flow environments (Langerhans & Reznick, 2010). Mountain mullet have a more streamlined body compared to the deep body of red devil making them more evolutionary adapted to sustaining higher speeds in high flow environments (Echelle et al., 2020; Minckley, 1984). Red devil with deeper body shapes tend to have lower burst and sustained swimming speeds compared to species with more shallow, streamlined bodies (Torres-Dowdall & Meyer, 2021). Our estimate of red devil  $U_{crit}$  was comparable to findings of a similar species with a deep body shape in the same genus (e.g., black midas cichlid, *Amphilophus astorquii*) (Raffini et al., 2020). Our estimates were in the bottom of the range observed for the black midas cichlid, suggesting a lower ability for sustained swimming of red devil. Burst swimming was also lower by almost half (50 cms) compared to black midas cichlid. The closest species to mountain mullet that we could find swimming performance estimates for was *Mugil cephalus* and *Mullus barbatus*. We found mountain mullet exhibited higher burst (~70 cm/s higher) and critical swimming speeds (~90 cms higher) than *Mugil cephalus* (Mitchell, 1989) and lower critical swimming speed (~100cms lower) than *Mullus barbatus*. These comparisons are not ideal as these species vary in their habitat use and are not found in similar ecosystems. Nonetheless, our results reveal that mountain mullet, based purely on swimming performance, are well adapted to swift environments and may be better at resisting extreme high flows compared to red devil.

Swimming performance is a major factor in species ability to move from unfavorable habitats, find food, escape predators, and withstand disturbance (Drucker, 1996; Plaut, 2001). The two types of swimming performance we focused on were burst and critical swimming speed. Burst swimming speed is normally employed to escape predators, catch prey, respond to a sudden disturbance, and navigate through high flows (Reidy et al., 2000); critical swimming speed is a good ecological indicator of fish ability to swim against high flows for migration or spawning (Jain et al., 1997). Some swimming fish may be attracted to periodic increases in turbulent or high flows while others may be behaviorally and physiologically prone to avoid high flows (Liao, 2007). Our study reveals that burst and critical swimming speed performance may be one mechanism facilitating mountain mullet competitive advantage in more extreme high flows over red devil. This suggests maintaining periodic high flows and flashiness in Puerto Rico's streams may benefit mountain mullet populations.

### *Management Implications*

Changing flows combined with the presence of red devil are two mechanisms that alter mountain mullet habitat use and behavior (Figure 6). Red devil shift mountain mullet habitat use and behavior, particularly during low flows, which could negatively affect growth, reproductive success, and abundance of mountain mullet during droughts. Climate projections suggest the Caribbean and Puerto Rico, specifically, will experience increased drying in the future and more extreme droughts (Bowden et al., 2021; Cashman et al., 2010). Managers may expect mountain mullet populations to be at risk with future drought increases and the spread of red devil on the island; our findings show the need to better understand the effects of red devil, particularly under future climate change scenarios. Managers could use these results to manage fish assemblages in Puerto Rico and develop strategies to increase habitat availability, such as cover habitat, and

maintain periodic high flows via dam release in vulnerable rivers where the effects of red devil are already present (e.g., the Río Piedras).

Our results provide potential mechanistic effects of interspecific competition and reveal the negative effects of the non-native red devil. We show that red devil may be considered an invasive species in Puerto Rico. The term non-native tends to be used for introduced fish in the early stages of invasion; species are referred to as invasive where there is a clear impact of the non-native species on native species and increased spread of the non-native species (Blackburn et al., 2011; Colautti & MacIsaac, 2004; Golebie et al., 2022). Our results give clear indications to managers that red devil are invasive and have negative effects on an ecologically and culturally important native species, revealing the need for tailored management or reduction of red devil populations to ensure conservation of native mountain mullet. Managers could consider increasing efforts to prevent future introductions of red devil and to reduce red devil densities through removal practices, such as chemical treatments and physical removal (Rytwinski et al., 2019).

#### *Future research needs*

Future research could address some of the limitations of this study, including increased high flow conditions, incorporation of different sizes and types of species, and varying habitat complexity. First, one limitation is our high flow treatments represented more mean flows than extremes due to limitations in the flow rate we could achieve. Supplemental experiments could better simulate higher extreme flows to compare shifts in habitat use and behavior between native and non-native species under more accurate flood conditions. Second, we qualitatively observed that bigger individuals of both mountain mullet and red devil engaged in more aggressive behavior compared to smaller individuals in the treatments. Although we attempted to

have similar size individuals in all treatments this was not always possible and some variation was present. Future work could assess size effects of mountain mullet and red devil by observing interactions across a range of body sizes and incorporating body size as a variable in the models. The addition of other important native species, bigmouth sleeper *Gobiomorus dormitory* and river goby *Awaous banana*, and non-native species, Amazon sailfin catfish *Pterygoplichthys pardalis*, in experimental treatments could also provide additional information on the effects to the native fish assemblages that were not assessed in this study. Finally, our study did not change habitat complexity across treatments. Additional experiments adjusting habitat complexity could also be a future avenue of research to understand on limiting habitat affects use and behavioral interactions (Warnock & Rasmussen, 2013).

### *Conclusions*

We provide the first experimental testing of mountain mullet habitat use under low and high flows in the presence of the non-native red devil and the first assessment of swimming performance of these two fishes. Our results show mountain mullet activity increases and habitat use decreases during low flows, highlighting the importance of river connectivity and refugia habitat during drought. The combination of low flows and red devil may have negative consequences for mountain mullet. Mountain mullet fitness, growth, and reproduction may be reduced due to the increased energy allocated to competing for space during drought when non-native species are present.

High flows may benefit mountain mullet even in the presence of red devil. Mountain mullet may be able to physiologically withstand higher flows through their burst and critical swimming ability. Further, we showed higher flows may increase intraspecific competition for optimal habitat between red devil compared to interspecific competition. This increased

intraspecific competition may have negative effects on red devil fitness during high flows, which could indirectly benefit mountain mullet (e.g., Oliveira & Bshary 2021).

The presence of red devil coupled with extreme flows alter mountain mullet behavior and competitive interactions, particularly during drought. Mountain mullet must cope with increased aggressive interactions when red devil are present in a river ecosystem, which could negatively affect mountain mullet populations as climate change continues to alter the frequency and magnitude of drought.

We conclude that considering the combined effects of extreme events and presence of non-native species is crucial to the management and conservation of native fish species Puerto Rico. Multiple mechanisms may drive shifts in native fish abundance and biomass in response to extreme events and invasive species; our research highlights altered behavior, competition, and swimming ability may help explain shifts in native mountain mullet in Puerto Rico. Our results could be applied more broadly to similar tropical island species where information is lacking and has the potential to inform management, such as the importance of river connectivity and reducing non-native species density, and future research in tropical islands globally.

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## CHAPTER 4

### **Pilot study to assess ecosystem services and non-market value of rivers in Puerto Rico**

#### **ABSTRACT**

Rivers provide a range of ecosystem services related to aesthetic value, cultural heritage, and fisheries. On islands, there is a particularly strong link between freshwater ecosystem services and people due to insularity combined with dense human population. There is a scarcity of research on the utilization and value of rivers on the island of Puerto Rico, and filling this knowledge gap could help focus attention on the management of river systems vulnerable to extreme events, invasive species, and other drivers of change. To assess the local uses and value of rivers in Puerto Rico, we administered 177 in-person surveys to visitors at 14 rivers in August 2021. The survey instrument posed questions related to the importance of different ecosystem services provided by the rivers (including cultural and provisioning services), visits to the river (including number of visits, transportation cost, travel time, and group size), and demographics. 21% of visitors who were fishing in the river where they were intercepted were asked further questions about their target species, willingness to accept and behavioral response to a hypothetical fishing license. Cultural services, such as aesthetic value, recreation, cultural heritage, and diversity of plants and animals were important ecosystem services, both by visitors who were fishing and by other visitors. Likewise, these were rated most important in both inland and estuarine sites. Swimming and photography were the most common uses of rivers by visitors to inland sites, and fishing and photography by visitors to estuarine sites. Of the visitors who were fishing, 86% accepted the proposition of an annual fishing license. We estimated a zero-truncated negative binomial regression of number of trips as a function of travel cost to estimate the consumer surplus per trip to an inland and estuarine river site (pooling information on visits

to all sites) and to fishers and non-fishers. Reported travel costs (for gas and other transportation) had the expected negative relationship with number of trips, and consumer surplus was \$8.47 per visit to an inland site and \$22.22 to an estuarine site. We found fishers gained more economic benefit from a river visit (consumer surplus = \$21.28) than non-fishers (consumer surplus = \$6.45). Our results provide valuable information on the relative importance of different river ecosystem services, different uses of rivers, and the relative value of visits to rivers among different groups and sites. We also find suggestive evidence that there is an opportunity to generate revenue from licenses to manage the fisheries. These findings provide evidence on the uses and value of rivers in Puerto Rico that may help resource managers make a case for enhancing accessibility and infrastructure, thereby facilitating sustained river utilization. The variation across visitor types and sites underscores the importance of understanding which ecosystem services are of greatest value to different groups and in different sites when planning investments in river systems.

## **INTRODUCTION**

Ecosystem services are the characteristics of natural ecosystems and species therein that support human well-being and provide tangible and intangible benefits to humans (Daily, 1997; Fisher et al., 2009; Millennium ecosystem assessment, 2005a). Rivers provide critical ecosystem services that contribute to human well-being and quality of life (Alan Yeakley et al., 2016; Millennium ecosystem assessment, 2005a). Like other ecosystem types, riverine ecosystem services can be placed into four main categories: provisioning, regulating, supporting, and cultural services. Provisioning services include the provision of food and freshwater; regulating services include flood regulation; supporting services include ecosystem functioning, such as primary production and decomposition; cultural services include aesthetic value and recreational

uses, such as swimming and recreational fishing (Böck et al., 2018; Millennium ecosystem assessment, 2005b). Provisioning and cultural services include both non-material benefits, such as recreational uses and aesthetic views, and material benefits, such as food and medicinal materials (Brauman et al., 2020; Díaz et al., 2018). Understanding the benefits of rivers can help prioritize riverine management and investment (Grizzetti et al., 2016) and quantifying the value of ecosystem services can provide managers and policymakers with a clearer understanding of the importance of resources (De Groot et al., 2012).

There is a strong link between coastal and freshwater ecosystem services and people on islands due to insularity and dense human populations (Millenium ecosystem assessment, 2005c). Cultural services are particularly important; islands provide biodiversity valuable to communities' experiences, aesthetic value, and many recreational uses (Balzan et al., 2018). Despite the apparent importance, a review of studies of island ecosystem services revealed that few have quantified the value of ecosystems to communities. Cultural ecosystem services could be at risk due to multiple direct drivers, including climate change, invasive species, overexploitation, improper management, pollution, and land-use change (Milcu et al., 2013). A clear understanding of the importance of ecosystem services and value of ecosystems is needed to assess the potential impacts of drivers of change on the use and value of ecosystems.

The value of ecosystems and ecosystem services can be classified into multiple categories, including market value, non-market value, intrinsic value, and existence value (Seppelt et al., 2011). For this paper, we focus on non-market values of rivers, including consumptive use (e.g., fishing) and non-consumptive use (e.g., photography, swimming, etc.) values, which are normally measured using non-market valuation and quantified monetarily (Boyer & Polasky, 2004). Non-market value is often characterized by intangible or non-

monetary benefits derived from ecosystems and are not reflected in market prices (Baker & Ruting, 2014; Farnworth et al., 1981). Several valuation methods have been used to quantify the non-market value of rivers and other ecosystems (De Groot et al., 2012).

Revealed preference methods use purchasing habits to assign a value to non-market benefits (Champ et al., 2003). The travel cost method is one of the more common revealed preference methods used to estimate the non-market value of non-market ecosystems (Cetin et al., 2021; Eberle & Hayden, 1991; Ezebilo, 2016; Leh et al., 2018). The travel cost method uses visitors' travel costs and number of trips to estimate a demand curve for a site, allowing the mean value of a visit to that site to be calculated as the consumer surplus at the mean travel cost for gas or other transportation. Other variations on the travel cost model are used to estimate the value of site characteristics by examining travel costs and visits to a location as a proxy for non-market value of a site or multiple sites (Georgiou & Turner, 2012). Stated preference methods can be used to capture the full suite of values associated with a non-market good, e.g., including existence value of native species. The contingent valuation method is the most common stated preference method and uses hypothetical scenarios to elicit users' willing-to-pay for ecosystem services based on their responses to survey questions (Johnston et al., 2017; Loomisa et al., 2018; Mitchell & Carson, 2013). Both the travel cost and contingent valuation methods have potential drawbacks around validity and reliability (Mayor et al., 2007). In this study, we were primarily focused on the value of rivers for visitors (including recreational and subsistence use); thus, we chose to use the travel cost method to estimate the value to those visitors. non-market value, primarily of recreational activities, that require physical visitation to the river (Leh et al., 2018).

Similar to other ecosystems globally, coastal and freshwater rivers on the island of Puerto Rico provide ecosystem services, including recreational fishing and cultural services to the

public (Kwak et al., 2016). Research has demonstrated the importance of freshwater rivers to the island's communities. For example, Puerto Rican residents were willing-to-pay \$11.33 million and \$13.09 million to maintain ecological integrity of two rivers in Puerto Rico (González-Cabán & Loomis, 1997). Specific river characteristics are also valued by communities; the presence of foot trails and waterfalls in El Yunque National Forest rivers increased their economic value (Santiago & Loomis, 2009). Consumer surplus or the economic benefit of recreational visits to rivers in El Yunque National Forest in Puerto Rico were estimated to be between \$11-15 per visit (Gonzalez et al., 2006). A paucity of research exists on the importance of ecosystem services and economic benefit of estuarine and inland rivers across Puerto Rico; however, research has shown their potential to provide important services and benefits to communities.

Researchers have also observed recreational fishing of freshwater, estuarine, and marine species that may be present in lowland and coastal areas of rivers (Kwak et al., 2016). One well-known fishery on the Río Grande de Arecibo is for goby (Gobiidae) postlarvae fish also known as cetí (Engman et al., 2021). Fishers target the goby at the river mouths during the postlarvae run, which occurs over three nights, at relatively low exploitation rates. García-Quijano (2009) assessed traditional ecological knowledge of coastal and ocean small-scale fisheries in southeast Puerto Rico, with a focus mainly on the coast and ocean and not further up the river mouths. It is estimated that 60,000-80,000 fishers participate in freshwater recreational fishing (Kwak et al., in press); thus, a better understanding of the potential for recreational fisheries of native fish consumptive and non-consumptive uses in rivers across Puerto Rico is needed (Kwak et al., 2016).

More information on consumptive and non-consumptive use value of estuarine and inland rivers in Puerto Rico could provide much needed information to managers and

policymakers. Revealing the importance of ecosystem services helps ensure that they are considered in management and decision-making (Willis, 2015). Furthermore, it could help prioritize investments in rivers susceptible to climate change, invasive species, and other drivers of change. To improve management and protection of these important resources and their ecosystem services, we aimed to (1) identify public uses of estuarine and inland rivers across Puerto Rico; (2) investigate the relative importance of different river ecosystem services including heterogeneity between users of estuarine and inland rivers and fishers and non-fishers, (2) to identify consumptive and non-consumptive uses of freshwater rivers between estuarine and inland rivers and fishers and non-fishers, Puerto Rico, (3) quantify the lower-bound economic benefit of recreational use by applying the travel cost method, and (4) investigate whether fishing is a common activity and if fishers encountered would accept and how they might adjust visit frequency in response to their willingness to pay for an annual fishing license.

## **METHODS**

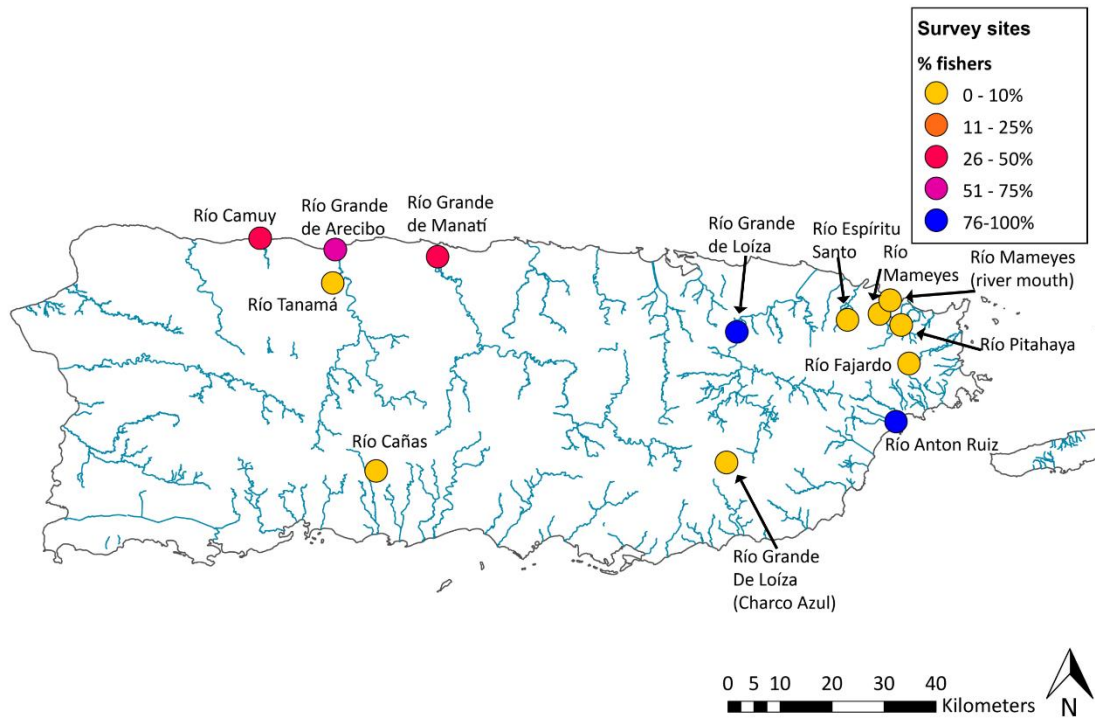
### *Study area and survey sites*

This study was conducted in rivers across Puerto Rico, a tropical island in the Caribbean Region (Figure 1). Puerto Rico is the smallest of the four Greater Antilles islands, Puerto Rico is the smallest by land area but has one of the densest human populations in the Caribbean (Hunter & Arbona, 1995; Kwak et al., 2013; Valle-Esquivel et al., 2011). The majority of the population lives near or around coastal areas (C. G. García-Quijano et al., 2015). Streams originate in the mountains draining into mountain foothills and coastal areas (Kwak et al., 2013). Freshwaters are used for multiple recreational activities, including picnicking, swimming, and fishing (Kartchner, 2003).

Rivers support important native freshwater and estuarine fishes that can support recreational fishing (Neal et al., 2009). Nine native fish species including, mountain mullet *Dajaus monticola* (Mugilidae), bigmouth sleeper *Gobiomorus dormitor* (Eleotridae), smallscaled spinycheek sleeper *Eleotris perniger* (Eleotridae), fat sleeper *Dormitator maculatus* (Eleotridae), river goby *Awaous banana* (Gobiidae), sirajo goby *Sicydium* spp. (Gobiidae), and American eel *Anguilla rostrata* (Anguillidae). All native species inhabit Puerto Rico's rivers. All are considered amphidromous except for the American eel, which is catadromous (Engman, 2016; Neal et al., 2009). Estuarine species found near the river mouths support small-scale fisheries, particularly in the southeastern part of the island (García-Quijano et al., 2015). Common estuarine species include sardines and herrings (Clupeidae), Common snook *Centropomus undecimalis*, tarpon *Megalops atlanticus*, and mojarras *Gerreidae* spp. (Garcia-Quijano, 2007; Kwak et al., 2016).

We identified 14 survey sites through discussions with the Department of Natural Resources that were known recreational areas in the northern, northeastern, and southern parts of Puerto Rico, were accessible, and provided areas to conduct interviews (Figure 1). Our goal was to identify sites that cover different parts of the island and are representative of inland and estuarine rivers. Eight sites were categorized as inland river locations, and six sites were categorized as estuarine locations. Five sites were clustered near El Yunque National Forest, which experiences high levels of recreational use in the streams and rivers (Santiago & Loomis, 2009). Four sites were located in the northern part of the island in the Río Grande de Arecibo, Río Grande de Manatí, Río Camuy, and Río Tanamá. The Río Grande de Arecibo supports both amphidromous fish postlarvae and estuarine fisheries (Engman, 2016). Sites varied in their

accessibility and other characteristics, such as availability of facilities or unique habitat (see Appendix I for site descriptions).



**Figure 1:** Survey sites (N=13) and % fishers of the total survey responses per site across Puerto Rico in 2021. The map excludes one site where we did not encounter any users.

*Survey design*

We designed a 21-question survey in English and Spanish to assess number of trips taken in the last 12 months, travel costs, river uses, and importance of certain aspects of the river. We provide a summary of the questions in Appendix II. Other demographic questions included whether the respondent was a resident, age, sex, and annual income of the respondents. For individuals engaged in fishing activity, we developed questions related to the fish species of interest, the reason for fishing (e.g., catch and release, food for self or family, and for bait). We also asked if fishers would be willing to pay for an annual fishing license if revenue was used to improve access to the site, to manage the fish populations, or improve facilities. We then asked

about the number of trips individuals would take if they had to pay increasing amounts (\$12.50, \$25.00, and \$50.00) for a fishing license annually. Fishers were presented with three dollar amounts in increasing value to capture the lower and higher range fishers would be willing to pay for license and continue to use the river the same amount. Finally, we asked questions related to the effects of COVID-19 and if users took more or less trips in the past year due to COVID. The survey was reviewed and pretested online by seven individuals from Puerto Rico. We obtained approval for human subjects through North Carolina State University's Institutional Review Board.

### *Survey administration*

Sampling was conducted in August 2021 using paper surveys. August was identified as the best month to conduct the surveys based on previous research showing July and August were the months recreational sites were most visited by residents (Santiago & Loomis, 2009). We approached every visitor visiting the site during a six-hour period (~10:00am – 4:00pm) to get a near census of visitors on that day during that time. If visitors were in a group, we approached one adult in the group to take the survey. We conducted sampling on Saturday or Sunday. We approached a total of 223 users and completed a total of 177 surveys (response rate= 79.37%). We assumed that trips were single purpose.

Respondents had a choice to self-administer the survey or to be interviewed. Interviews were conducted by Puerto Rican residents who were native Spanish speakers. Our main population of interest was Puerto Rican residents using the rivers for recreational activities, and secondly, fishers who participate in freshwater recreational, subsistence, or commercial fishing. Respondents were first provided with an introduction and background of the survey. Interviewers communicated how their participation will inform management efforts at the fishery and help the

Puerto Rico Department of Natural and Environmental Resources better understand the value of the freshwater fishery. Furthermore, this survey is unique in Puerto Rico and potentially viewed as a rare opportunity for users to provide their preferences and opinions on how they use the rivers. Informed consent was also requested prior to administering the survey. Respondents were interviewed once. To increase the response rate, we provided a small incentive (e.g., North Carolina State University branded items, such as keychains, drink cozies, and hats) following completion of the survey. One major advantage of in-person interviews is to decrease survey non-response that is common with mail-in surveys and decrease unanswered questions due to survey fatigue (Scheuren, 2004).

#### *Heterogeneity analysis*

We first summarized responses to questions related to the importance of ecosystem services provided by the rivers and activities engaged in while at the river. Users rated the importance of eight different ecosystem services (Appendix II) on a Likert scale of one to five with one being the least important and five being the most important (Allen & Seaman, 2007). We calculated the median importance values to users using the two river types (inland and estuarine) and to fishers and non-fishers to compare the importance of each ecosystem service across river types and users. We also determined the percentage of users engaged in specific activities at the river, including swimming, photography, picnicking, and fishing. Fisher's t-test were used to test differences between users of estuarine and inland rivers and between non-fishers and fishers (Agresti, 2012).

For users whose main activity was fishing (N = 37), we determined the primary reason for recreational fishing (e.g., catch and release, fishing for food for family, fishing for bait, or a combination of the three) and the importance of certain fish species (Appendix II). We defined

recreational fishing as fishing by individuals mainly for sport (catch and release) but with a potential secondary reason for consumption (fishing for food for self or family (Cowx, 2001). We included fishing bait as an option as well as live shrimps *Macrobrachium* spp. are used for bait and can be collected from inland rivers (Greathouse et al., 2006). We first calculated the proportion of fishers engaged in catch and release, fishing for food, and fishing for bait. Users ranked the importance of freshwater and estuarine species on a scale of one to five. To determine the importance of species, we identified the median value of these rankings across fishers fishing in inland and estuarine sites. We report the fraction of fishers who accept the idea of an annual fishing license, and who say that they would continue their same pattern of fishing in rivers at different price points for a fishing license. Elsewhere, we report potential total revenues from fishing licenses using the estimated number of total fishers in Puerto Rico (e.g., Kwak et al. *in press*)

### *Statistical analyses*

We used the individual travel cost method to estimate consumer surplus of the two river types (inland and estuarine) and for non-fishers and fishers based on the number of trips taken annually as a function of the money spent to travel to the river, which included gasoline and other transportation costs. The travel cost method relied solely on our survey data (Ezebilo, 2016). We built four zero-truncated negative binomial regression models to account for overdispersion in the count data of trips. All models included demographic predictors, including gender, annual household income, and age. For annual household income, we created a categorical variable based on the following income ranges and categories: less than \$20,000—lowest, \$20,000 to \$34,999—low, \$35,000 to \$49,999—middle, \$50,000 to \$74,999—mid-high,

\$75,000 to \$99,999—high, \$100,000 or more—highest. Food or equipment costs were excluded in the models.

Negative binomial regression is commonly used to estimate the demand function with count data that suffers from overdispersion (e.g., Loomis et al., 2000; Mayor et al., 2007; Zhang et al., 2015). Negative binomial regression models the log of the expected number of trips taken annually as a function of the predictor variables included in the model. We used a zero-truncated negative binomial regression as all users were interviewed on site resulting in the number of trips all being greater than zero (Dhakal et al., 2012; Oh et al., 2020). The zero-truncated negative binomial regression produces an exponential demand function that can be used to estimate consumer surplus and economic benefits of the river resource. Consumer surplus is considered the net economic benefit to users and estimates users willingness to pay above what they actually pay for a trip to a recreational area (Cesar & van Beukering, 2004; Gordon et al., 1973). We calculated consumer surplus by taking the negative inverse of the travel cost coefficient for the four models (Blackwell et al., 2013; Bowker & Leeworthy, 1998; Zhang et al., 2015).

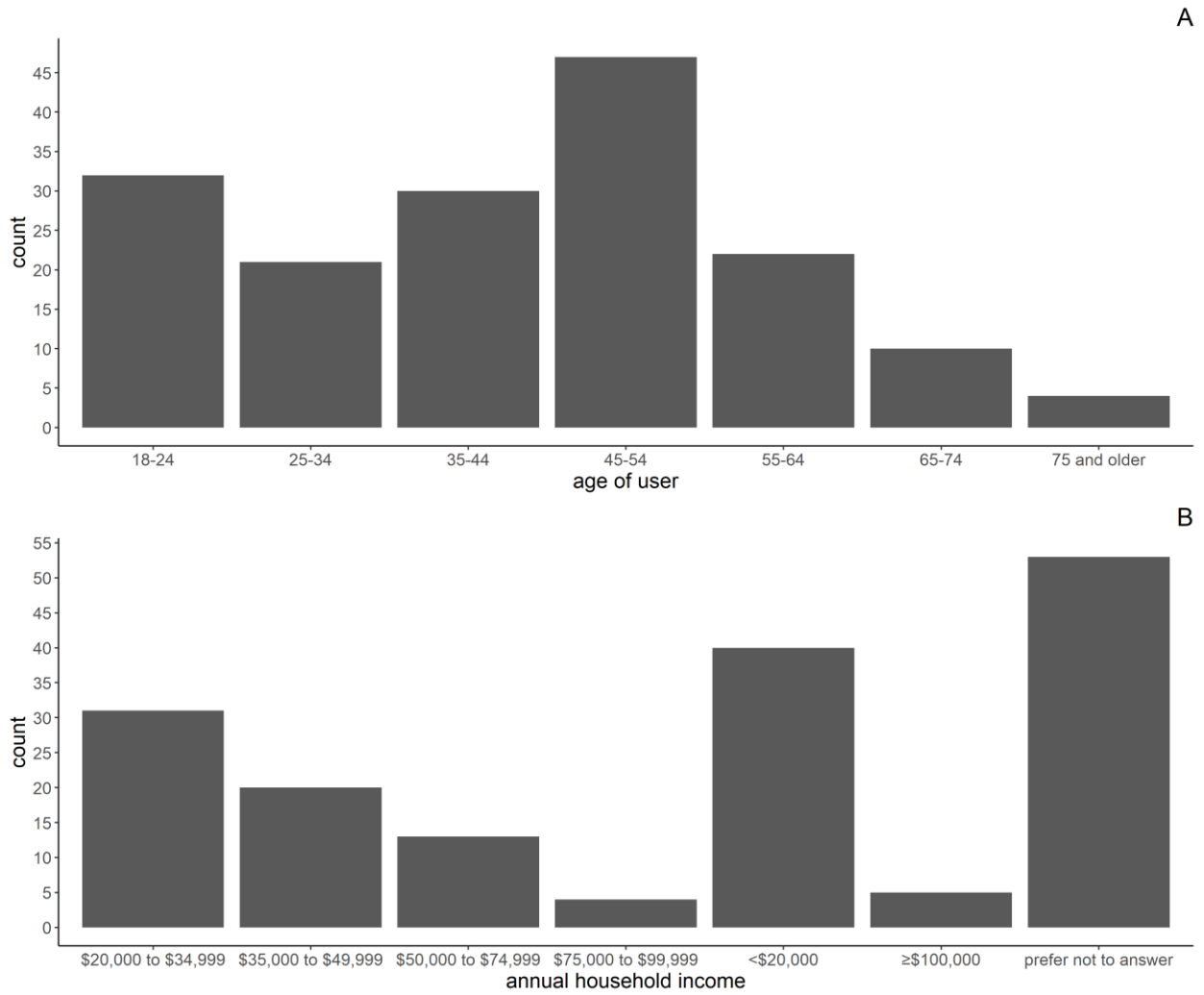
## **RESULTS**

### *Demographics of users*

Of the 177 users surveyed, 63% of respondents were male and were primarily lower-to-middle income (Figure 2). The most common age of respondents was 44-54 years followed by 18-24 years, 35-44 years, 55-64 years, and 25-34 years (Figure 2). Forty-one percent had an annual household income less than \$20,000 or between \$20,000 – \$34,999 and 19% had an annual household income between \$35,000 - \$49, 999 and \$50,000-\$74, 999. Five percent of respondents had an annual income between \$75,000-\$99, 999 or above \$100, 000. A high number of respondents (28%) preferred not to answer the annual household income question. Of

the 177 users we interviewed, 119 were visiting inland river sites and 56 were visiting estuarine river sites.

The number of users and summary of annual visits and mean travel distance and time varied depending on the river location. The most visited sites were the Río Grande de Loíza at Charco Azul, Río Grande de Manatí, Río Fajardo, Río Cañas, and Río Grande de Arecibo, and Río Anton Ruiz (Table 1). The total mean annual trips taken tended to be greater at river locations where users were engaged in fishing (Table 1; Figure 1). Mean travel distance and time tended to be greater at river locations where individuals were engaged most in non-fishing activities, including swimming, picnicking, and photography. These locations included Río Fajardo, Río Grande de Loíza at Charco Azul, and Río Tanama. One outlier was Río Grande de Loiza located below the Carraízo dam, which had a high percentage of fishers and one of the higher mean travel distances to the river. Across all users, fifty two percent of users spent less than three hours, 30% spent three to five hours, and 17% spent the whole day at the river. Out of those who responded, 83% of users revealed they took less trips to the rivers due to the COVID-19 pandemic.



**Figure 2:** Frequency of age (A) and annual household income (B) of users sampled at 14 rivers across Puerto Rico. Non-responses were not included in the counts.

**Table 2:** Summary of river type, the number of respondents, mean number of individuals in the party, mean travel distance, and mean travel time for respondents at each river sampled.

River	river type	# of respondents	mean annual visits	mean # in party (adults and children)	mean travel distance (kilometers)	mean travel time (minutes)
Río Grande De Loíza	inland	7	5.17	2	41.32	28.57
Río Grande de Manatí	estuarine	26	65.08	3	34.08	33.23
Río Tanamá	inland	9	123.22	1	36.49	33.33
Río Camuy	estuarine	2	73.00	4	20.93	30.00
Río Fajardo	inland	15	11.27	3	39.98	51.92
Río Grande de Arecibo	estuarine	13	270.54	1	11.39	15.23
Río Cañas	inland	16	34.47	3	27.00	36.53
Río Grande De Loiza (Charco Azul)	inland	49	5.17	4	54.99	62.92
Río Mameyes	inland	7	7.14	3	22.94	16.00
Río Anton Ruiz	estuarine	13	65.25	1	28.69	32.85
Río Sabana	estuarine	0	0.00	0	0	0.00
Río Mameyes (river mouth)	estuarine	2	188.50	2	64.4	2.50
Río Espíritu Santo	inland	9	18.56	3	28.58	46.88
Río Pitahaya	inland	9	37.22	3	28.52	53.56
<b>Mean across all sites</b>			64.61	3	37.24	41.60
<b>Total # of non-responses</b>			10	7	37	7

### *Heterogeneity analysis*

Fisher's t-tests revealed significant differences between users visiting inland river sites and estuarine sites and between non-fishers and fishers (Table 2). Users visiting estuarine sites had significantly lower annual household income, were significantly older, spent significantly less money and time to travel to the site, and took significantly more trips in the past 12 months. Gender, time spent at the river, and change in the trips taken during COVID-19 did not vary significantly between estuarine and inland river users. Fishers had significantly lower annual household incomes, were significantly older, took more trips annually, and spent more time at the river (whole day or half the day). Fishers were also significantly more likely to be male than non-fishers (Table 2). Transportation cost, travel time, and change in the trips taken during COVID-19 did not vary significantly between non-fishers and fishers.

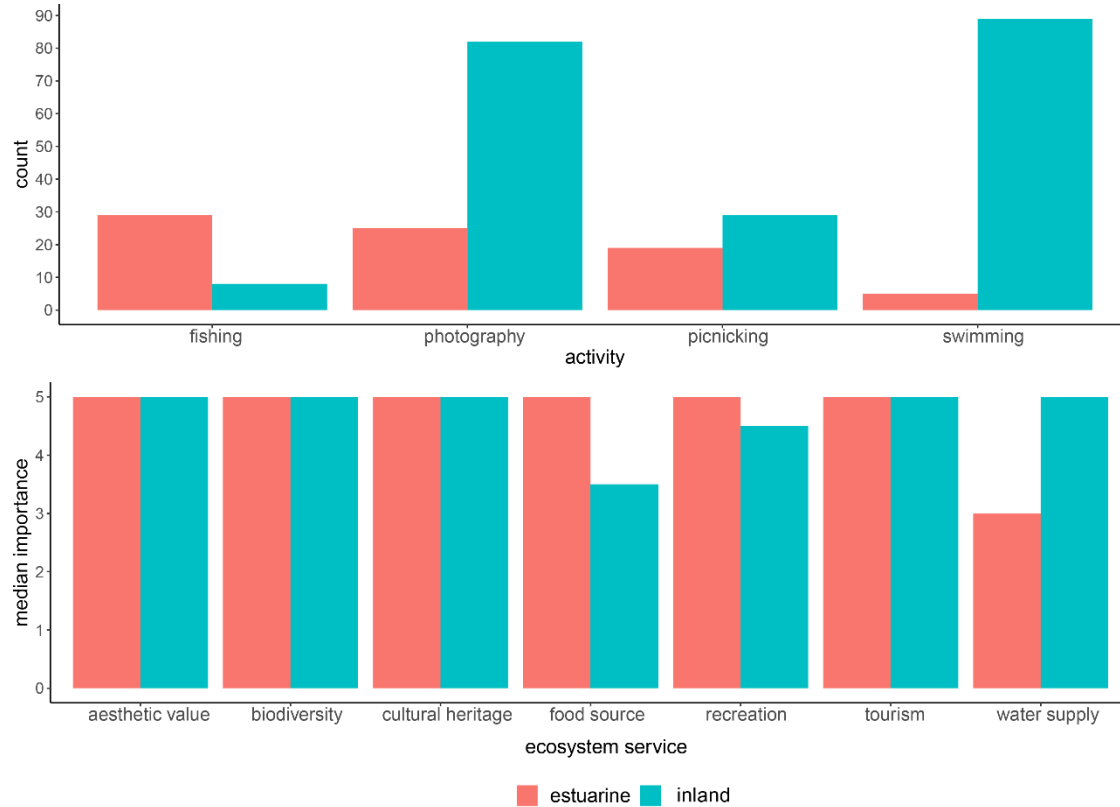
**Table 2:** Heterogeneity analysis results showing Fisher’s t-test results comparing variables between users visiting inland and estuarine sites and non-fishers and fishers.

variable	river type (inland vs. estuarine)	user (non-fisher vs. fisher)
	p-value	p-value
annual household income	0.003*	0.002*
age	0.000*	0.022*
gender	0.176	0.019*
annual trips	0.000*	0.000*
cost	0.047*	0.070
travel time	0.028*	0.091
time spent at the river	0.756	0.019*
change in trips taken during covid	0.605	1.000

\*Significant difference at the 0.05 level

Users visited the rivers for four main uses, including swimming, photography, picnicking, and fishing. We found swimming and photography were the most common river uses followed by picnicking among users of inland river sites (Figure 3). Users of estuarine sites tended to be engaging in fishing more than users at inland river sites. Users of estuarine sites also visited the river for photography and picnicking. We also identified other uses, including jet skiing, walking, meditating, and relaxing with friends and family. Users tended to use the river for multiple uses during the visit.

The mean importance of ecosystem services varied depending on the ecosystem service provided by the rivers. Cultural services were all highly ranked among users of estuarine and inland river sites (Figure 3). The importance of provisioning services, such as food source and water supply, between users of estuarine and inland river sites differed. Fishers, who tended to be encountered in estuarine sites valued the food resources provided by the river more than non-fishers, who tended to be encountered at the inland river sites. The importance of recreation was slightly lower (4.5 compared to 5.0 ranking) for users of inland river sites compared to estuarine sites (Figure 3).



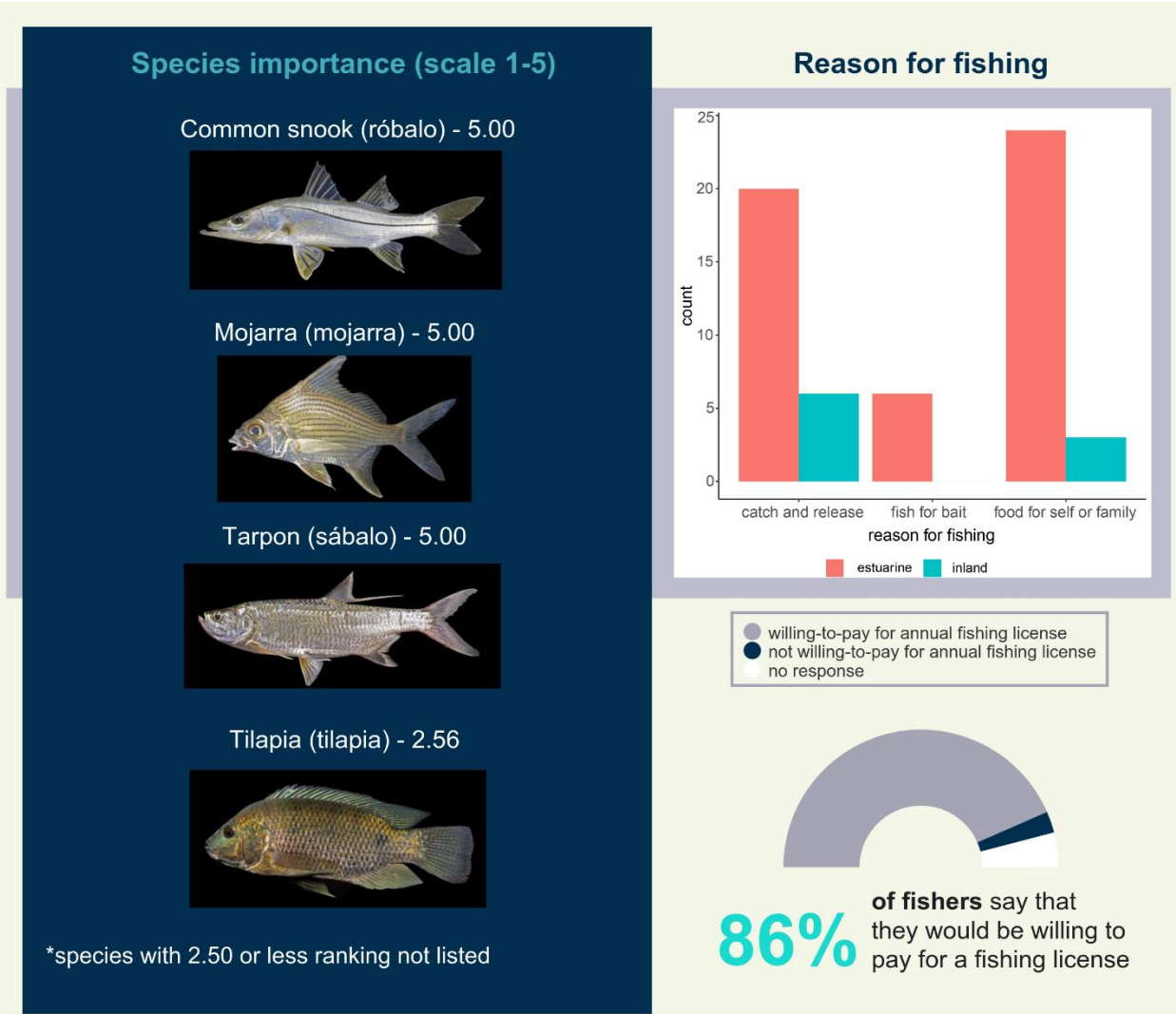
**Figure 3:** The number of users engaged in specific activities and the median importance of seven ecosystem services provided by the water body to users between estuarine and inland river sites.

### *Recreational fishers*

Fishing uses and importance of freshwater and diadromous species were relatively consistent across users engaging in fishing activities. We identified fishers at six of the 14 sites sampled located in the Northwest, Southeast, and more inland in the Northeast (Figure 1). All but one of the sites where fishers were interviewed were classified as estuarine and were not characterized by a swimming hole or waterfall (Appendix I). Fishers were most commonly fishing for catch and release, food for self or their family, or a combination of catch and release and for food (Figure 4). Fishing for bait was the least common reason for fishing. The most important fish species to fishers included estuarine species, including the common snook or róbalo (*Centropomus undecimalis*), striped mojarra or mojarra (*Eugerres plumieri*), and tarpon

or sábalo (*Megalops atlanticus*) (Figure 4). Tilapia (*Oreochromis* spp.), a freshwater non-native fish, had an average importance of 2.56. Bigmouth sleeper or guavina had an importance value of 2.24. It was the only native amphidromous fish species that had a mean rank higher than two. The Puerto Rican freshwater crab or jueye (*Epilobocera sinuatifrons*) had a similar ranking to bigmouth sleeper. Other native riverine species including mountain mullet and American eel had rankings less than two. Freshwater shrimp (Atyidae and Macrobrachium) also had a low importance value at 1.70. Non-native red devil cichlid or ciclido (*Amphilophus* spp.) was not of high importance to fishers.

Even though fishing has been free in Puerto Rico, 86% of fishers say that they would buy an annual fishing license if the revenues were used to manage the fishery and to maintain access points and facilities (Figure 4). Respondents reported that they would not change the number of fishing trips to rivers if they were required to buy an annual license costing \$12.50 or \$25.00, but all said that they would stop fishing in rivers if the annual license cost \$50.00.



**Figure 4:** Summary survey responses from fishers (N=37) on species importance, uses of fishers, and percentage willing to pay for an annual fishing license. Photos credit of Augustin C. Engman and Thomas J. Kwak (Kwak et al., in press). \*Species with an average importance ranking of  $\leq 2$  were not included on the figure.

*Individual travel cost model and economic benefit*

Differences existed in the models results comparing inland and estuarine river sites and fishers and non-fishers. As expected, travel cost was significantly and negatively associated with the annual number of trips taken in the past 12 months across all four models (Table 3). In

addition to travel cost, age was a significant variable in the inland river sites model ( $p=0.003$ ) and the lowest income level was a significant variable in the estuarine river sites models (Table 3). Trips decreased with older users at inland river sites and increased among lower income users at estuarine sites. Our estimated consumer surplus per trip per person was \$8.47 per inland river site trip and \$22.22 per estuarine river site trip. We extrapolated this value to the sum of the actual trips taken by users to inland and estuarine river sites and estimated a consumer surplus of \$19,819 for inland river sites and \$132,476 for estuarine river sites, assuming respondents only visit one site. We assumed rivers included in the models were homogenous and did not include additional site characteristics. For non-fishers, the annual number of trips taken increased significantly when the user was male. For fishers, cost was the only significant predictor variable in the model (Table 3). Our consumer surplus estimates showed the net economic benefit to non-fishers was \$6.45 per trip and to fishers was \$21.28. Based on the total number of visits in the past year of non-fishers and fishers, we estimated the lower bound economic benefit to be \$21,472 for non-fishers and \$111,145 for fishers.

**Table 3:** Model results and comparison across the two models with the lowest AIC, including the marginal effects, for the negative binomial regression model of number of trips predicted by cost and other demographic variables (N=150). The pseudo R<sup>2</sup> and AIC values are included for each model.

Model 1: Inland river sites				Model 2: Estuarine river sites			
variable	coefficient	standard error	P-value	variable	coefficient	standard error	P-value
cost	-0.118**	0.019	0.000	cost	-0.045*	0.023	0.000
age	-0.057**	0.020	0.003	age	0.013	0.025	0.593
male	1.169	0.647	0.071	male	0.971	0.573	0.090
income - lowest	18.256	831.065	0.982	income - lowest	2.246*	0.920	0.027
income - low	18.918	831.065	0.982	income - mid	-11.752	166.869	0.944
income - mid	16.368	831.066	0.984	income - mid to high	0.655	1.318	0.619
income - mid to high	16.705	831.066	0.984	*Significant at the 0.05 level, N=47 respondents			
**Significant at 0.01 level, N=101 respondents				McFadden's Pseudo R-squared=0.305			
McFadden's Pseudo R-squared=0.562							
Model 3: Non-fishers				Model 4: Fishers			
variable	coefficient	standard error	P-value	variable	coefficient	standard error	P-value
cost	-0.155**	0.032	0.000	cost	-0.047*	0.019	0.012
age	-0.019	0.017	0.281	age	0.018	0.020	0.373
male	1.634**	0.585	0.005	male	0.982	0.330	0.108
income - low	14.584	134.808	0.914	income - lowest	1.03	0.972	0.289
income - lowest	14.677	134.809	0.913	income - mid	-1.878	1.423	0.187
income - mid	-11.752	166.869	0.917	*Significant at the 0.05 level, N=37 respondents			
income - mid to high	0.655	1.318	0.919	McFadden's Pseudo R-squared=0.196			
income - highest	-17.422	134.810	0.897				
**Significant at the 0.01 level, N=115 respondents							
McFadden's Pseudo R-squared=0.542							

## DISCUSSION

### *Ecosystem services and uses*

Cultural services were the most important ecosystem services to the people who use Puerto Rico's estuarine and inland rivers. Aesthetic value, cultural heritage of the river location, revenue from tourism, and the diversity of plants and animals provided by the river were of high importance to both estuarine and inland river users. These cultural services are also some of the most studied, with past research highlighting their importance to communities, globally (Milcu et al., 2013). Our findings are similar to those of other studies that have quantified the importance of ecosystem services in other riverine and estuarine areas. For example, Casado-Arzuaga et al.

(2013) found cultural services were the most important across all user groups in a an urban greenbelt in Spain. Specifically, the value provided by biodiversity was the most important ecosystem service in this study. Recreational value, aesthetic value, and cultural heritage were also found to be the most important cultural services provided by a river in Jakarta, Indonesia (Vollmer et al., 2015).

Our findings suggest that conserving native biodiversity may be important to the preservation of the most valued cultural ecosystem services. Three of the main threats to native biodiversity are climate change, particularly changes to the magnitude and frequency of floods and drought, urbanization, and the presence of non-native fish (Dudgeon, 2019; Neal et al., 2009; Ramírez et al., 2009). More extreme drought and non-native species negatively affect native fish biodiversity and abundance and may have cascading effects on ecosystem services outlined in this study (Ramírez et al., 2018). Reducing native species presence, abundance, and biomass affects ecosystem functioning and may alter the delivery of services, such as cultural heritage, wildlife viewing opportunities, and biodiversity in rivers (e.g., Arthington et al., 2010; Petsch et al., 2023). Maintaining native biodiversity is important to conserving ecosystem services and their value under climate change and other threats (Weiskopf et al., 2020). It will be essential to monitor and manage native biodiversity to ensure its continued contribution to ecosystem services in order to mitigate losses in river value.

#### *Recreational fishers and economic benefits*

Recreational fishers made up 21% of the total users surveyed, had unique characteristics compared to non-fishers, and received more economic benefit from visits to the rivers. Estuarine river sites supported the most fishers in our study. Reservoirs in Puerto Rico are known to support recreational (e.g., Bower et al., 2016; Neal et al., 2008) and bait fishing (e.g., Kröger et

al., 2010). However, river fisheries are less developed but have the potential to support recreational and subsistence fishers (Kwak et al., 2016). We provide additional confirmation of the capacity of rivers to support recreational fisheries and the keen interest of fishers in inland and estuarine fishing, specifically fishers who were older and from lower incomes. In addition to the cetí fishery previously documented by Engman et al. (2021), we showed the Río de Arecíbo supports fishing of common estuarine species, specifically common snook (róbalo) and tarpon (sábalo). Río Grande de Manatí and Río Camuy in the North, Río Grande de Loíza below the Carraízo Dam, and Río Anton Luiz in the Southeast were also frequented by recreational and subsistence fishers that targeted estuarine species and non-native tilapia. The two more inland sites in the South did not appear to support fishers (Figure 1). This could be due to the lack of estuarine species located at these sites. Additionally, one of the site's main landscape features was a large swimming hole, which made up most of the recreational activity at this location and may not be conducive for fishing. Importantly, we found native estuarine species were more important to fishers than native freshwater species, such as mountain mullet *Dajaus monticola* and bigmouth sleeper (Figure 4). This is important in the context of fisheries management and suggests the need to monitor and manage estuarine species of interest. The consumer surplus or economic benefit to fishers was greater than for non-fishers. Based on the estimated 60,000 to 80,000 river fishers in Puerto Rico (Kwak et al., in press), we estimated the total consumer surplus could be upwards of \$1,702,400 if fishers made one visit annually. Our findings highlight the importance and economic benefit of five rivers (Río Grande de Arecíbo, Río Grande de Manatí, Río Anton Luiz, Río Grande de Loíza, and Río Camuy) in supporting recreational fisheries and are sites where managers could consider prioritizing and focusing fisheries management efforts.

Currently, the Puerto Rico Department of Natural and Environmental Resources does not require a freshwater recreational fishing license to fish inland waters for residents or tourists. Licenses normally represent a large proportion of a natural resource agency's budget and are vital to effectively managing the fisheries (Hunt et al., 2016, 2017). For islands, revenue from fishing can have a substantial source of capital. For example, fishing access fees comprise the second largest source of government capital in the Pacific Island of Tuvalu (Siaosi et al., 2012). We showed that 86% of fishers' trips to the river would not change if cost of a fishing license was \$12.50 or \$25.00 annually if the license revenue was used to manage the fish populations and improve access and facilities. If a license was applied to the estimated 60,000-80,000 inland fishers (Kwak et al., in press), the natural resource agency has the potential to generate up to \$2,000,000 in revenue. We identified 19 fishers who were not residents of Puerto Rico, suggesting fishing tourism could provide additional revenue. For example, of the 8,400 fishers visiting the Negro River in the Amazon in 2018 and 2019, 20% were tourists generating an additional \$2,855,496 in revenue (Lubich et al., 2023). Initiatives developed to advertise Puerto Rico's river and estuary recreational fisheries to encourage and promote tourism fishing in addition to recreational fishing by residents.

Revenue could be used to understand the effects of water quality—a concern of fishers—on sport fishes, to manage for invasive species, and to monitor the effects of climate change on the fisheries. We documented that fishers in the Río Grande de Arecibo were particularly concerned with water pollution from jetskis that are launched near fishing access points. The fishers were concerned about the oil impacting the fishery; managers may consider evaluating the effects of water pollution on the fish population in the Río Grande de Arecibo.

### *Economic benefits of estuarine and inland rivers sites*

Our consumer surplus estimates suggest estuarine river sites provide greater economic benefit to users compared to inland rivers sites and that fishers receive more economic benefit from trips than non-fishers. Consumer surplus can be extrapolated to represent total user consumer surplus as an average of annual users or households depending on the study (González-Cabán & Loomis, 1997; Mayer & Woltering, 2018). Thus, we relied on our dataset to estimate the annual consumer surplus to the estuarine sites and inland sites based on annual trips taken and did not extrapolate the number out to the total user population. The consumer surplus of approximately \$19, 819 for inland rivers sites and \$132,476 is most likely a gross underestimation of the actual economic benefit to users annually. For example, if 10% of the population size (~3,285,874 people) visited an inland river or estuarine river once a year the annual economic benefit to users would be \$2,783,135 or \$7,301,212, respectively. This shows the potential for estuarine and inland rivers to provide substantial economic benefits to users and supports the need for managing rivers and their ecosystem services.

### *Limitations and future research needs*

Our objectives were to encounter not only fishers but also general recreational users and visitors to the river; thus, we surveyed later in the day (e.g., 10:00am-4:00pm), which may have caused an under sampling of fishers. Repeated sampling at known fishing locations, longer-term sampling, and adjusting the time of day of sampling may greatly increase the encounter rate with fishers. Through communication with fishers on site, most start fishing early in the morning; thus, we may have missed fishers due to our study design. This would allow for more quantitative evaluation of fishers willing to pay for an annual license and provide an opportunity

to estimate the total number of fishers in Puerto Rico and their willingness to pay for a fishing license.

Another limitation of our survey is we did not include a question to estimate cost to a substitute site (Parsons, 2017); however, other research has used the travel cost method without including this variable (Hwang et al., 2021). Future survey efforts could include additional questions to quantify costs to a substitute site to improve model results and provide a more accurate representation of the travel cost and trips relationship. Finally, research to quantify the number of visitors islandwide per year to rivers could provide a more robust estimation of consumer surplus and economic benefit of rivers.

### *Management implications*

Our findings indicate that a range of diverse cultural ecosystem services holds importance and underscore the necessity of incorporating these services into the decision-making process for prioritizing and maintaining river systems in the region. For example, managers could identify specific ecological or geographic features that contribute to cultural heritage, such as cross generational experience at the site, to prioritize conservation of certain ecological features located at the rivers (e.g., Chan, 2011). The value of cultural services can also play an important role in motivating the public in the protection of ecosystems (Daniel et al., 2012). Specifically for Puerto Rico, managers could capitalize on the importance of the value of biodiversity to educate and motivate the public to reduce human introductions of non-native fish species; a prominent issue on the island (Kwak et al., 2016; Neal et al., 2009). Based on the high importance of aesthetic value, protecting and improving the aesthetic value of rivers may also be an important management action to consider. Visual cues can impact users perception of aesthetic quality of ecosystems; these include water flows, the color of the water, litter, and

riparian vegetation (Pflüger et al., 2010). We found one major user complaint, which was documented in our open-ended response, was the lack of trash bins to dispose of waste and lack of consistent removal of litter at the sites. This shows managers could prioritize improving facilities for trash disposal and pickup to reduce unsightly waste to increase aesthetic value. With the increases of extreme events and non-native species on the island, managers could consider managing the effects on native biodiversity to reduce ecosystem service loss.

Our results suggest that fishing licenses may be a viable source of revenue to support fisheries management and their implementation may be accepted by anglers. Revenue could fund projects to understand the effects of water quality—a concern of fishers—on sport fishes, to manage for invasive species, and to monitor the effects of climate change on the fisheries. We documented that fishers in the Río Grande de Arecibo were particularly concerned with water pollution from jet skis that are launched near fishing access points. The fishers were concerned about the oil impacting the fishery; managers may consider evaluating the effects of water pollution on the fish population in the Río Grande de Arecibo. Invasive fish negatively affect native populations through increased competition and predation (Angert et al., 2013; Rahel & Olden, 2008). Thus, managing and controlling non-native populations according to their risk to native species could improve fisheries resources (Britton et al., 2023). Monitoring and effectively managing the fisheries susceptible to extreme events from climate change may also be needed (Hunt et al., 2016, Myers et al. Chapter 2). Particularly in the context of future climate projections for Puerto Rico, which show an increase in dry periods and more extreme droughts (Bowden et al., 2021). Our investigation aligns with previous research and suggests that estuarine fish, rather than native freshwater species, hold greater significance for fishers.

Managers could use this information when prioritizing and conserving fish populations and addressing the most immediate threats to the fisheries.

### *Conclusions*

Cultural ecosystem services are a crucial component of rivers in Puerto Rico with aesthetic value, recreation, cultural heritage, and biodiversity being of particular importance. This finding elucidates the need for managers to consider multiple use and non-use values when managing rivers. With the increases of extreme events and non-native species on the island, it will be important to consider the effects on native biodiversity and important landscape features that provide aesthetic and cultural heritage value (Boger et al., 2019). Based on our findings, managers could focus their efforts on maintaining the aesthetic and cultural value of the rivers by providing more trash bins and periodically removing litter from the rivers and educating the public on activities that may decrease water quality. The value of biodiversity to users is also an important finding; managers could consider leveraging the importance of biodiversity to further educate the public on the importance of preventing non-native species introductions.

Rivers are highly important and provide recreational opportunities and ecosystem services to a range of residents and tourists with varying demographic characteristics. The predominant demographic among users consisted of individuals who identified as male, fell within the lower to middle income bracket, and were aged between 44 and 54 years. This observation underscores the significance of rivers within this user cohort. Considering visits to the rivers decreased during COVID-19, this suggests this user group lost both recreational and economic benefits from the rivers during the pandemic.

Cultural ecosystem services are crucial to the estuarine and inland rivers and users in Puerto Rico and outweighed other services, such as provisioning services. Specifically, the value

of biodiversity to users is an important finding that managers could leverage to educate the public on the importance of preventing non-native species introductions.

Recreational fishers gained greater economic benefit from their visits to the rivers compared to non-fishers, which highlights the importance of the fisheries. We identified specific rivers that provide fishing opportunities and found that males and users from lower income characterized the fishers engaging in recreational fishing (both catch and release and food for self and family). Fishers appeared to be willing to pay for an annual fishing license if revenue was used for fisheries conservation and management. This revenue could be used to control and reduce non-native fish populations that may negatively affect the sport fish and monitor for the effects of extreme events. Further research needs to be conducted to quantify this finding, but the Puerto Rico Department of Natural Resources could use this information to further consider implementing annual fishing licenses for residents and tourists to generate revenue for conserving and managing Puerto Rico's rivers.

Finally, we provided an approximate estimate of the annual consumer surplus for estuarine and inland rivers and fishers and non-fishers in our study, recognizing it is likely an underestimate. These results show the potential economic benefit of rivers for recreation in Puerto Rico and highlight the importance of maintaining access and facilities to foster their use. A more comprehensive survey across the other 91 rivers in Puerto Rico could reveal a broader understanding of the economic benefit of rivers.

Our findings underscore the potential use value of rivers in Puerto Rico and the importance of considering diverse ecosystem services in the prioritization and maintenance of river systems. As climate change, invasive species, and other drivers of change continue to impact rivers, it is crucial to understand the importance of ecosystem services and use value of

rivers to effectively allocate and prioritize funding for continued conservation and management of the freshwater resource.

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## APPENDICES

## Appendix A

**Table S4.1:** Descriptions of each river location, including accessibility, facilities, and important landscape features, if applicable.

River	Description
Río Grande De Loíza	This site is below a dam and is a known site for recreational fishing. Access to the river is difficult and requires users to cut through brush and walk down a steep and large concrete steps to access the river. Users park on the side of the road to access the site.
Río Grande de Manatí	This site is near the river mouth and near a small town with amenities and conveniences. There is a pier and boat ramp at this location. There are also facilities, including toilets and trashbins as well as a sitting area. This site also has areas for picture taking. Fishers were encountered a short walk away from the parking area closer to the river mouth. Other users tended to stay near the pier and other sitting areas by the river.
Río Tanamá	This site is located slightly further inland and not at the river mouth. There is available parking near the river, and a waterfall that is located a short walk (~5 minutes) downstream of the parking area. There are no real facilities here; however, it was a short drive back to town.
Río Camuy	This site is near the river mouth and near a pier and beach promenade area with many facilities, including toilets, trashbins, restaurants, and other conveniences.
Río Fajardo	This site is easily accessible from the road. There are several swimming pool locations and very small waterfalls if users hike upstream some. About one to two miles upstream from this site there is a tourist company that runs rafting tours. Users park on the side of the road to access the river.
Río Grande de Arecibo	This site is easily accessible from the road and is a known recreational fishing location. It is located at the upstream of the river mouth where the river flows into the ocean. There are facilities nearby and benches and gazebos that provide cover from the sun. Users were encountered on both sides of the river near a nearby bridge just upstream of where most users were fishing. There is also a boat ramp nearby where users input jet skis.
Río Cañas	This site is easily accessible; however, the drive to this location is through the mountains and the road is narrow. Users park on the side of the road and walk down to the river. There are a few residential houses nearby and minimal facilities.
Río Grande De Loiza (Charco Azul)	This site is located more inland and in a mountainous area. There is a large parking lot for users. Users have to walk a short distance to access the swimming hole. There are trash bins located at the entrance of the site as well as covered areas for picnicking.
Río Mameyes	This site is slightly hidden from the road by trees and vegetation. There is a small entrance where users can access the river by walking down a small, rocky hill. This site is characterised by a large swimming hole that includes a cliff for jumping and a rope swing. There are no facilities but there is a restaurant nearby. Users can park on the side of the road.
Río Anton Ruiz	This site is located near the river mouth near a bridge. It is easily accessible by car. Users park in a beach area near the river mouth and bridge. It is located near a small town with amenities. There are also areas for camping at the beach near the river mouth.
Río Sabana	This site is located near the river mouth. There is available parking and easy access to the river.
Río Mameyes (river mouth)	This site is located right upstream of the river mouth. It is accessible by walking down a popular beach in Luquillo, Puerto Rico. There are facilities and restaurants nearby.
Río Espíritu Santo	This site is easily accessible and located right off the road. There is an area that charges for parking and to use the facilities. There is a large swimming hole near where users park.
Río Pitahaya	This site is located near El Yunque National Forest. There is ample parking and a water slide nearby that is an attraction for users.

**Table S4.2:** Topic, summary of questions, and variable type summarizing the survey conducted across 14 river locations in Puerto Rico in July-August 2021.

Topic	Summary of question	Response variable
Ecosystem service importance	Importance of each characteristic of this body of water to you. Options included the following: presence of a variety of plant and animal species, natural beauty (e.g., aesthetic value), recreational activities, cultural heritage (e.g., location has been important to generations of your family), tourism, water supply, provides additional food source.	Scale of importance 1-5
River use	Types of activities you are participating. Participants check all of the following options that apply. Options included swimming, photography, picnicking, fishing, and other.	Binary (yes or no)
Fishers questions	Importance of common fish found in the rivers. Options included 8 taxa and the option to insert other fish.	Scale of importance 1-5
	Reason for fishing. Options included catch & release, food for self or family, keep for both, or other.	Binary (yes or no)
	If the revenue from fishing licenses was used to improve the management of the fishery, would you be willing to pay for a yearly freshwater recreational fishing license, if one was required? Types of management include the following: maintaining access points, maintaining and repairing facilities, and managing fish populations.	Binary (yes or no)
	If a yearly fishing licensed increased your fishing trip costs by \$12.50 per year, how many trips would you have taken to the river for fish in the last 12 months?	Number of trips
	If a yearly fishing licensed increased your fishing trip costs by \$25.00 per year, how many trips would you have taken to the river for fish in the last 12 months?	Number of trips
Trip information	If a yearly fishing licensed increased your fishing trip costs by \$50.00 per year, how many trips would you have taken to the river for fish in the last 12 months?	Number of trips
	Open-ended question about the reason for not being willing to pay for an annual fishing license.	Open-ended
Travel cost, distance travelled, and time	Number of trips taken in the past 12 months.	Number of trips
	Length of stay at the waterbody today. Options included all day (6-8 hours), half day (3-5 hours), briefly (less than 3 hours).	Binary (yes or no)
	Mode of transportation. Options provides included personal vehicle, public transportation, walked, and other.	Binary (yes or no)
	Number of adults and children in the group.	Number of people
Demographic questions	Money spent for visit. Options provided included gas, food, and other transportation.	USD\$ amount.
	Approximate distance to travel to site.	Number of miles
	Time in hours and minutes it took to travel to river.	Total time (minutes)
	Resided in Puerto Rico in the past 12 months.	Binary (yes or no)
	Zip code	Value
Covid question	Age range. Options provided included 18-24, 25-34, 35-44, 45-54, 55-64, 65-74, 75 or older years old.	Binary (yes or no)
	Sex. Male or female.	Binary (yes or no)
	Household income. Options provided included less than \$20,000, \$20,000-34,999, \$35,000-49,000, \$50,000-74,999, \$75,000-99,999, greater than \$100,000, or prefer not answer.	Binary (yes or no)
Open-ended question for all users	Less or more trips were taken due to the COVID-19 pandemic.	Binary (more or less)
	Share any additional information or experience regarding use of the river.	Open-ended