Fishery Population and Habitat Assessment in Puerto Rico Streams
Phase 2 Final Report

Federal Aid in Sport Fish Restoration Project F-50



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Cover Photos

Left: A waterfall on Río Lajas in the Grande de Añasco river basin. Such natural waterfalls may limit the dispersal and habitat availability of native diadromous fishes, but several goby species are adapted to ascend waterfalls using their fused pelvic fins that form a suction disc. Photo by Patrick Cooney.

Upper right: A group of mountain mullet or dajao, *Agonostomus monticola*, that were captured, tagged with passive integrated transponders (or microchip tags), and released in Río Mameyes to monitor dispersal patterns of this native sport fish. Photo by Will Smith.

Lower right: A bigmouth sleeper or guavina, *Gobiomorous dormitor*, lurks among the rocky substrate of Río Sabana. This species may be among the most desireable native sport fish, due to its large size, low body lipid content, and minimal contaminant accumulation. Photo by Trey Sherard.

Preface

This document serves as the Final Report for research on Puerto Rico stream fishes and their habitat funded by the Puerto Rico Department of Natural and Environmental Resources, in the form of a grant to the North Carolina Cooperative Fish and Wildlife Research Unit. This research was also conducted to meet the thesis requirement for a Master of Science degree granted to Elissa Buttermore (Chapters 3–4) and the dissertation requirement for a Doctor of Philospophy degree granted to William Smith (Chapters 5–8). Formatting differs among chapters, as each was developed to target a specific scientific journal and to conform to journal style.

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EXECUTIVE SUMMARY

Fish and Habitat Sampling.—During Phase 2 of this project, we continued quantitatively sampling stream fish assemblages extensively among sites using standardized protocols developed in Phase 1. We also conducted habitat surveys that included measurements of instream physical habitat, water quality parameters, and riparian features. Geographic watershed features for each site were quantified using Geographic Information System (GIS) applications and existing databases. In addition to the original 81 sites sampled for fish and crustaceans during the initial phase of this project, 37 sites were sampled during Phase 2 from June 2008 to August 2010 for a total of 118 sampling sites. With the addition of these 37 sites, we have sampled all 46 major drainage basins in Puerto Rico to more thoroughly characterize the fishery and stream resources of the island.

Fish Suitability for Bioassessment.—Biotic assessment and monitoring to indicate ecological integrity of aquatic ecosystems are needed worldwide. The Index of Biotic Integrity (IBI) approach with fish assemblages has been applied predominantly in warmwater streams and rivers, with the vast majority in temperate regions. Tropical island streams differ from corresponding ecosystems in temperate regions in their ecology, geomorphology, and anthropogenic impacts. The need for effective bioassessment methods is urgent in tropical island aquatic systems, but the efficacy of the IBI approach has been rarely tested in these environments. To investigate the efficacy of applying fish assemblage attributes to assessment in Caribbean tropical island streams, we explored the relationships between fish assemblage parameters and stream and watershed characteristics at 118 sites among all 46 drainage basins in Puerto Rico USA. Correlation analyses between fish assemblage parameters and geographic and physical attributes associated with stream size revealed significant expected relationships, no relationship to water nutrient concentrations, and significant relationships with riparian and watershed land cover, but some of the latter were contrary to expectations. Fish assemblages upstream of a high dam and the associated reservoir differed from those assemblages with no downstream reservoir, and native fish were tolerant to watershed and riparian urbanization. We conclude that, because of distinct fish life history, biogeography, stream geomorphology, migration barrier effects, marine influences, and fish tolerance to highly disturbed conditions, fish assemblages in Puerto Rico cannot serve as suitable indicators of ecological integrity without in-depth analysis or integration of additional physical or biotic data.

Contaminants and Land Use.—Manufactured chemicals are continuously released into the environment with a variety of adverse ecological and human health effects. Puerto Rico has a history of anthropogenic chemical usage, and its human population density is among the highest globally, providing a model environment to study human impacts on tropical island stream ecosystems. Our objectives were to quantify occurrences of Polycyclic Aromatic Hydrocarbons (PAHs), historic-use chlorinated pesticides, current-use pesticides, Polychlorinated Biphenyls (PCBs), and metals (mercury, cadmium, copper, lead, nickel, zinc, and selenium) in the habitat and biota of Puerto Rico streams and associate those findings with land-use patterns. We sampled water, sediment, and native fish and shrimp species at 13 sites spanning broad riparian and watershed land-use patterns (e.g., urban, agricultural, industrial, and forested) and conducted intensive sampling at four of these sites. Overall, our findings indicated that stream ecosystems in Puerto Rico were not severely polluted, with the exception of nickel in sediment at sites associated with agricultural watersheds. While nickel concentrations were greatest at agricultural sites, a site with a highly urbanized watershed generally had the greatest concentrations of most classes of contaminants. PCBs may pose human health hazards with some fish concentrations exceeding the EPA consumption limit for 1 meal/month; greatest concentrations were in mountain mullet *Agonostomus monticola* and American eel *Anguilla rostrata*. Bigmouth sleepers *Gobiomorous dormitor* may be the most suitable fish for human consumption with low levels of organic contaminants, but mercury accumulation exceeded EPA's consumption limit for 3 meals/month at 1 of the 13 sites. These results provide public health and natural resource agencies the scientific information required to guide ecosystem and fisheries management and human health risk assessment.

Contaminants in Food Webs.—Fate and effects of pollution are complex processes and many contaminants present in low levels in the environment may increase in concentration from one trophic level to the next, reaching concentrations that are harmful to wildlife and human consumers. Puerto Rico has a history of anthropogenic chemical usage, and its human population density is among the highest globally, providing a model environment to study human impacts on tropical island stream ecosystems. The objective of our research was to quantify occurrences and patterns of aquatic contaminants [polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, and metals] as related to riparian and watershed land-use characteristics and trophic relationships. We used stable isotope analyses of carbon, nitrogen, and sulfur to elucidate contaminant and trophic dynamics within four rivers with differing riparian and watershed land-use patterns (e.g., urban, agricultural, industrial, and forested). Overall, stream ecosystems in Puerto Rico were not severely polluted, with the exception of elevated concentrations of PCBs and mercury in some fish species. Trophic level and contaminant concentrations were poorly correlated in these dynamic systems that are characterized by frequent hydrologic disturbances, nutrient pulses, and marine influences. Calculation of food web magnification factors was complicated by low levels of contaminants, distorted estimates of trophic level due to δ^{15}N enrichment from nutrient pollution, and short food chains. Lipid content of consumers was a better predictor of contaminant concentration than trophic level. These findings enhance understanding of contaminant dynamics in tropical stream ecosystems and provide natural resource managers and public health agencies scientific information to guide ecosystem and fisheries management and human health risk assessment.

Fish Assemblage Response to Flooding.—A combination of deterministic and stochastic processes structures aquatic communities. Periods of stable environmental conditions, favoring development of communities regulated by deterministic processes, are interrupted by random periods of disturbance that may restructure communities. Disturbance may affect populations via habitat alteration, mortality, or displacement. We quantified fish habitat conditions, density, and movement before and after a major flood disturbance in a Caribbean island tropical river using habitat surveys, fish sampling and population estimates, radio telemetry, and passively monitored PIT tags. Native stream fish populations showed evidence of acute mortality and downstream displacement of surviving fish. All fish species were reduced in number at most life stages after the disturbance, but populations responded with recruitment and migration into presumably vacated upstream habitats. Changes in density were uneven among size classes for most species, indicating altered size structures. Rapid recovery processes at the population level appeared to dampen effects at the assemblage level, as fish assemblage parameters (species richness and diversity) changed minimally. The native fish assemblage appeared resilient to flood disturbance, rapidly compensating for mortality and displacement with increased recruitment and recolonization of upstream habitats. In tropical island streams, major flood disturbance may act as a community filter to resist invasion by exotic species with minimal net effect on natives, thereby maintaining relatively stable native stream fish assemblages.

Amphidromous Fish Dispersal.—Characterization of migratory scale is critical to the successful conservation and management of diadromous fishes. We quantified adult movement scale for two tropical diadromous fishes, bigmouth sleeper *Gobiomorus dormitor* and mountain mullet *Agonostomus monticola*, using passive integrated transponders and radio telemetry. Large numbers of fish were tagged in Río Mameyes, Puerto Rico, USA, with passive integrated transponders and monitored at three fixed locations over a 2.5-year period, generating information to estimate transition probabilities between upper and lower elevations and survival probabilities with a multistate Cormack-Jolly-Seber model. A subset of fish was tagged with radio transmitters and tracked at weekly intervals, generating fine-scale spatial information to estimate the scale of dispersal. Changes in spatial and temporal distributions of tagged fishes indicated that neither *G. dormitor* nor *A. monticola* moved into the lowest, estuarine reaches of Río Mameyes during two consecutive reproductive periods, thus demonstrating that both species follow an amphidromous, rather than catadromous, migratory strategy in this system. Further, both species were relatively sedentary, with restricted linear ranges. While substantial dispersal of these species occurs at the larval stage during recruitment to freshwater, our results indicate little dispersal in spawning adults. We conclude that successful conservation of diadromous fauna on Caribbean tropical islands requires management at both broad basin and localized spatial scales.

Fish Otolith Microchemistry and Migration.—Classification of many tropical diadromous fishes as amphidromous or catadromous has not acknowledged that species or populations may follow a range of migratory patterns with full, partial, or no migration to the ocean. Otolith microchemistry is a useful technique to elucidate such migratory patterns and variation within and among species. We applied otolith microchemistry to quantify migratory variation and the proportion of native Caribbean stream fish that undergo full or partial marine migration. Strontium and barium water chemistry in four Puerto Rico U.S.A. rivers was clearly related to a salinity gradient; however, variation in water barium, and thus fish otoliths, was also dependent on river basin. Strontium was the most accurate index of longitudinal migration in tropical diadromous fish otoliths. Among four species examined, bigmouth sleeper *Gobiomorus dormitor*, mountain mullet *Agonostomus monticola*, sirajo goby *Sicydium* spp., and river goby *Awaous banana*, 9–12% of individual recruits were not amphidromous, with no evidence of marine elemental signatures in their otolith core. Populations of one species, *G. dormitor*, may have contained a small contingent of migratory, partially amphidromous adults that temporarily occupied marine habitat (4%); however, adult migratory elemental signatures may have been confounded with those related to diet and physiology. Our findings indicate the plasticity of migratory strategies of tropical diadromous fishes, which may be more variable than simple categorization might suggest.

Fish Life History and Conservation.—Sound natural resource conservation and management rely on quantitative predictions of population response to exploitation and management, but predictive models are frequently limited by a lack of quantitative information on population dynamics. The management of data-limited species can be informed by a general understanding of life history patterns and dynamics and the suitability of common management strategies to particular life history traits. We quantified a suite of life history parameters for native Caribbean amphidromous fishes and compared those to life history parameters of other fish species to define the life history traits of the native fish assemblage. The amphidromous fishes examined follow an intermediate, periodic-opportunistic life history strategy. Deterministic and density-dependent management models are less likely to be effective for periodic and opportunistic populations, relative to models that account for environmental variability. We conclude that the amphidromous fish assemblages examined are robust to low to moderate exploitation of adults, and conservation measures, such as maintenance of stream habitat quality, environmental flows, and ecosystem connectivity may be the optimal approach to conserving native community structure and sustainable amphidromous fisheries.

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CHAPTER 1
FISH ASSEMBLAGE AND HABITAT SAMPLING IN PUERTO RICO STREAMS
(Job 1)

Introduction

During Phase 2 of this project, we continued quantitatively sampling stream fish assemblages extensively among sites using standardized protocols developed in Phase 1 (Kwak et al. 2007). We also conducted habitat surveys that included measurements of instream physical habitat, water quality parameters, and riparian features. Geographic watershed features for each site were quantified using Geographic Information System (GIS) applications and existing databases.

Methods

In addition to the original 81 sites sampled for fish and crustaceans during the initial phase of this project and reported in that final report (Kwak et al. 2007), 37 sites were sampled during Phase 2 from June 2008 to August 2010 for a total of 118 sampling sites (Figure 1). Twenty-nine of the 37 new sites were sampled following the previously established standardized sampling protocol that included a three-pass removal procedure with two backpack electrofishing units. The remaining 9 sites (7C, 24A, 25A, 26A, 27A, 29B, 32D, 39A, and 44B) were sampled in river reaches containing unwadeable pools that were too deep to sample thoroughly with existing sampling equipment. These sampling sites were generally situated in close proximity to river mouths and were sampled with a single electrofishing pass in wadeable sections to yield additional data on fish occurrence (presence/absence and relative abundance). Fish density and biomass were not estimated for these sites, but all other fish and habitat parameters were quantified. This sampling and site selection was designed to complement the results that were gathered during Phase 1 to more completely describe fish occurrence and abundance among river drainage basins and stream sites. With the addition of these 37 sites, we have sampled all 46 major drainage basins in Puerto Rico to more thoroughly characterize the fishery and stream resources of the island. Detailed descriptions of fish sampling, stream survey, GIS, and all analytical methods were reported by Kwak et al. (2007).

Results and Discussion

A total of 21 fish species were captured from new sites; 11 were native species, and 10 were exotic, introduced species. All but three of the 21 species of fish, Atlantic needlefish *Strongylura marina*, shorttail pipefish *Microphis brachyurus*, and fat sleeper *Dormitator maculatus*, were detected at previous sampling sites during Phase 1. The three newly sampled species were detected at only a single site each and are species commonly associated with marine or brackish conditions. Other marine or brackish water species that were detected included fat snook *Centropomus parallelus*, burro grunt *Pomadasys crocro*, and grey snapper *Lutjanus griseus*.

We selected multiple sampling sites in Río Mameyes, as it represents one of the few free-flowing rivers in Puerto Rico that lacks instream dams (Figure 1). No exotic species were detected in this river, suggesting that the physical and biotic conditions in Río Mameyes may provide resistance to establishment of non-native species (see Chapter 3). We detected nine native species in Río Mameyes, with all nine present at the most downstream site (4P). Río Tallaboa (site 29B) was the only other location where nine native species were detected. Both sampling sites were within two kilometers of their respective river mouths, and the fish assemblages included a combination of freshwater and brackish species.

A single site on Río Jueyes (site 20A; Figure 1) was the only site sampled on the entire island where no fish were detected. This river is located in one of the driest regions of the island causing the river to periodically go dry. When the river is flowing, it is generally flashy with strong currents. A combination of factors most likely contributed to the lack of any fish sampled at this location.

All fish assemblage, stream survey, water quality, and geographic results are presented according to site in Tables 1–13. These broad-scale, comprehensive results demonstrate the current distribution and abundance of fish and shrimp populations throughout Puerto Rico. Stream fauna and freshwater resources face increased human demand and disturbance in Puerto Rico, and our data can be applied to aid personnel in management planning and implementation to maintain and enhance the long-term sustainability of these limited resources for the people of Puerto Rico.

Reference

Kwak, T. J., P. B. Cooney, and C. H. Brown. 2007. Fishery Population and Habitat Assessment in Puerto Rico Streams: Phase 1 Final Report. Federal Aid in Sport Fish Restoration Project F-50 Final Report. Marine Resources Division, Puerto Rico Department of Natural and Environmental Resources, San Juan.

Table 1. Site number, drainage basin, river, municipality, location, number of closest route, and GPS coordinates of Puerto Rico stream sites sampled 2008–2010.

Site number	Drainage basin	River name	Municipality	Location	Route number	Latitude (N)	Longitude (W)
4C	Mameyes	Mameyes	Luquillo/Río Grande	1.5 km SE of Palmer	191	18° 21' 25.32"	65° 46' 07.98"
4D	Mameyes	Mameyes	Luquillo/Río Grande	2.4 km W of Sabana	988	18° 19' 21.20"	65° 45' 05.20"
4E	Mameyes	Mameyes	Luquillo/Río Grande	2.5 km W of Sabana	988	18° 19' 21.30"	65° 45' 05.30"
4F	Mameyes	Mameyes	Luquillo/Río Grande	2.6 km WNW of Sabana	988	18° 19' 40.79"	65° 45' 08.10"
4G	Mameyes	Mameyes	Luquillo/Río Grande	2.7 km WNW of Sabana	988	18° 19' 41.30"	65° 45' 10.80"
4H	Mameyes	Mameyes	Luquillo/Río Grande	2.7 km WNW of Sabana	988	18° 19' 41.14"	65° 45' 11.54"
4I	Mameyes	Mameyes	Luquillo/Río Grande	3.4 km SE of Palmer	990	18° 20' 41.40"	65° 45' 23.50"
4J	Mameyes	Mameyes	Luquillo/Río Grande	2.6 km SE of Palmer	191	18° 21' 00.96"	65° 45' 42.23"
4K	Mameyes	Mameyes	Luquillo/Río Grande	2.5 km SE of Palmer	191	18° 21' 01.40"	65° 45' 43.50"
4L	Mameyes	Mameyes	Luquillo/Río Grande	2.4 km SE of Palmer	191	18° 21' 05.42"	65° 45' 47.86"
4M	Mameyes	Mameyes	Luquillo/Río Grande	1.9 km SE of Palmer	191	18° 21' 16.44"	65° 45' 53.33"
4N	Mameyes	Mameyes	Luquillo/Río Grande	1.6 km SE of Palmer	191	18° 21' 22.20"	65° 46' 05.70"
4O	Mameyes	Mameyes	Luquillo/Río Grande	1.2 km SE of Palmer	191	18° 21' 37.00"	65° 46' 06.99"
4P	Mameyes	Mameyes	Luquillo/Río Grande	1.5 km NE of Palmer	3	18° 22' 34.10"	65° 45' 39.65"
4Q	Mameyes	Mameyes	Luquillo/Río Grande	0.5 km E of Palmer	3	18° 22' 13.06"	65° 45' 55.48"
4R	Mameyes	Mameyes	Luquillo/Río Grande	3.9 km SE of Palmer	990	18° 20' 22.23"	65° 45' 15.68"
7C	Fajardo	Fajardo	Fajardo	0.4 km SE of Fajardo	194	18° 19' 18.72"	65° 39' 01.00"
8A	Daguao	Daguao	Nagüabo	0.8 km N of Daguao	973	18° 13' 49.40"	65° 41' 02.60"
9A	Palma	Palma	Naguabo	1.5 km W of El Banco	972	18° 14' 08.14"	65° 42' 54.53"
12A	Anton Ruiz	Collores	Humacao	0.2 km S of Antón Ruíz	926	18° 10' 56.74"	65° 49' 10.91"
12B	Anton Ruiz	Mambiche	Humacao	0.9 km ESE of Antón Ruíz	927	18° 11' 01.52"	65° 48' 41.88"
17A	Jacaboa	Higuera	Patillas	2.2 km NE of Lamboglia	7755	17° 59' 19.10"	65° 57' 58.50"
18A	Patillas	Patillas	Patillas	9.5 km N of Patillas	184	18° 05' 23.30"	66° 02' 11.78"
20A	Jueyes	Jueyes	Salinas	5.0 km NW of Salinas	543	18° 00' 19.29"	66° 20' 06.46"
21A	Coamo	Coamo	Coamo	3.3 km N of Coamo	556	18° 06' 26.82"	66° 20' 48.18"
24A	Jacaguas	Jacaguas	Juana Díaz	2.0 km N of Juana Díaz	570	18° 04' 10.08"	66° 30' 32.88"
24B	Jacaguas	Jacaguas	Villalba	0.9 km E of Villalba	561	18° 07' 45.70"	66° 29' 01.30"
25A	Inabón	Inabón	Ponce	1.0 km E of Coto Laurel	14	18° 03' 00.25"	66° 32' 40.37"
26A	Bucaná	Bucaná	Ponce	7.6 km NE of Ponce	139	18° 03' 12.95"	66° 34' 59.63"
27A	Portugués	Portugués	Ponce	4.9 km N of Ponce	503	18° 02' 27.96"	66° 37' 16.95"
29B	Tallaboa	Tallaboa	Peñuelas	2.1 km NW of Tallaboa	127	18° 00' 17.68"	66° 43' 52.77"
32D	Yauco	Yauco	Yauco	2.5 km W of Indios	335R	17° 59' 12.27"	66° 50' 23.63"
37G	Añasco	Lajas	Maricao	2.1 km E of Maricao	105	18° 10' 47.03"	66° 57' 37.95"

Table 1 continued.

Site number	Drainage basin	River name	Municipality	Location	Route number	Latitude (N)	Longitude (W)
37H	Añasco	Lajas	Maricao	2.2 km E of Maricao	105	18° 10' 59.25"	66° 57' 28.66"
39A	Guajataca	Guajataca	Quebradillas/Isabella	4.4 km SW of Cacao	476	18° 24' 12.56"	66° 56' 53.83"
44B	La Plata	La Plata	Guayama	8.9 km ESE of Cayey	179	18° 05' 42.45"	66° 04' 52.07"
46B	Piedras	Piedras	San Juan	1.9 km W of Cupey	176	18° 22' 01.40"	66° 03' 47.18"

Table 2. Season, date, and electrofishing technique for 37 Puerto Rico stream sites sampled 2008–2010.

Site number	Season	Month	Day	Year	Technique
4C	Summer	6	17	2008	Backpack
4D	Winter	2	8	2010	Backpack
4E	Summer	6	17	2009	Backpack
4F	Summer	6	16	2009	Backpack
4G	Summer	7	4	2009	Backpack
4H	Winter	2	6	2010	Backpack
4I	Summer	7	3	2009	Backpack
4J	Summer	5	18	2009	Backpack
4K	Summer	6	15	2009	Backpack
4L	Winter	2	5	2010	Backpack
4M	Winter	2	3	2010	Backpack
4N	Summer	7	2	2009	Backpack
4O	Summer	6	10	2009	Backpack
4P	Summer	7	1	2009	Backpack
4Q	Summer	8	1	2010	Backpack
4R	Summer	7	10	2010	Backpack
7C	Summer	6	10	2008	Backpack
8A	Summer	6	24	2009	Backpack
9A	Summer	7	14	2010	Backpack
12A	Summer	6	24	2009	Backpack
12B	Summer	6	25	2009	Backpack
17A	Summer	6	25	2009	Backpack
18A	Summer	7	15	2010	Backpack
20A	Summer	7	16	2010	Backpack
21A	Summer	7	17	2010	Backpack
24A	Summer	6	18	2008	Backpack
24B	Summer	6	29	2009	Backpack
25A	Summer	6	16	2008	Backpack
26A	Summer	6	16	2008	Backpack
27A	Summer	6	19	2008	Backpack
29B	Summer	6	6	2008	Backpack
32D	Summer	6	6	2008	Backpack
37G	Summer	7	2	2008	Backpack
37H	Summer	7	2	2008	Backpack
39A	Summer	6	22	2008	Backpack
44B	Summer	7	1	2008	Backpack
46B	Summer	7	9	2009	Backpack

Table 3. Instream habitat and sampling reach characteristics from 37 Puerto Rico stream locations sampled 2008–2010.

Site number	Season	Year	Reach length (m)	Mean width (m)	Area (m^2)	Mean depth (cm)	Mean velocity (m/s)	Dominant substrate	Mean bank angle (°)	% Cover
4C	Summer	2008	100	21.43	2,143	17.4	0.183	Large cobble	154.2	82
4D	Winter	2010	100	26.32	2,632	18.7	0.162	Medium boulder	135.1	86
4E	Summer	2009	100	27.24	2,724	19.6	0.154	Medium boulder	134.3	87
4F	Summer	2009	100	19.41	1,941	28.8	0.145	Medium boulder	140	83
4G	Summer	2009	100	10.36	1,036	23.2	0.171	Bedrock	147.8	84
4H	Winter	2010	100	19.52	1,952	27.8	0.135	Medium boulder	141.1	83
4I	Summer	2009	100	12.4	1,240	25.3	0.214	Small cobble	135.8	78
4J	Summer	2009	100	18.31	1,831	24.7	0.196	Small cobble	143.6	82
4K	Summer	2009	100	18.41	1,841	17.6	0.195	Small cobble	149.8	77
4L	Winter	2010	100	18.51	1,851	18.9	0.185	Small cobble	147.6	79
4M	Winter	2010	100	20.31	2,031	21.2	0.163	Large cobble	142.2	81
4N	Summer	2009	100	14.94	1,494	23.1	0.14	Large cobble	148.8	67
4O	Summer	2009	100	22.63	2,263	31.2	0.068	Large cobble	114.5	67
4P	Summer	2009	100	11.47	1,147	23.3	0.326	Small cobble	151.3	63
4Q	Summer	2010	100	23.67	2,367	16.4	0.168	Small cobble	162.3	67
4R	Summer	2010	100	17.34	1,734	23.5	0.191	Medium boulder	133.2	82
7C	Summer	2008	.	7.08	.	20.3	0.259	Small cobble	152.5	94
8A	Summer	2009	150	3.37	506	17.7	0.027	Very coarse sand	139.8	56
9A	Summer	2010	150	7.72	1,158	9.4	0.073	Very Coarse Sand	167.3	34
12A	Summer	2009	141	4.45	627	12.3	0.069	Medium gravel	127.5	53
12B	Summer	2009	130	3.99	519	20.5	0.08	Pea gravel	146	57
17A	Summer	2009	146	3	438	8.3	0.061	Medium gravel	163.8	52
18A	Summer	2010	150	6.34	951	15.7	0.64	Large cobble	131.4	78
20A	Summer	2010	150	12.1	1,815	0.1	0.001	Sand	173.1	8
21A	Summer	2010	150	6.18	927	16.3	0.114	Large cobble	147.3	74
24A	Summer	2008	.	6.67	.	19.3	0.333	Large cobble	123.3	82
24B	Summer	2009	150	6.06	909	10.3	0.097	Fine gravel	157.3	64
25A	Summer	2008	.	4.87	.	21.6	0.08	Large cobble	141.7	82
26A	Summer	2008	.	4.7	.	16.8	0.279	Medium gravel	161.7	31
27A	Summer	2008	.	5.43	.	12.1	0.189	Very coarse gravel	154.2	86
29B	Summer	2008	.	4.57	.	18	0.146	Coarse gravel	137.5	37
32D	Summer	2008	.	2.6	.	9.4	0.025	Sand	160	43
37G	Summer	2008	200	7.73	1,546	11.2	0.133	Medium gravel	139.3	58

Table 3 continued.

Site number	Season	Year	Reach length (m)	Mean width (m)	Area (m^2)	Mean depth (cm)	Mean velocity (m/s)	Dominant substrate	Mean bank angle (º)	% Cover
37H	Summer	2008	200	6	1,200	19.6	0.184	Sand	155.8	59
39A	Summer	2008	.	6.52	.	34.4	0.073	Coarse gravel	100.8	45
44B	Summer	2008	.	5.73	.	20.2	0.069	Medium boulder	150.8	76
46B	Summer	2009	150	7.2	1,080	29.7	0.163	Small cobble	139	37

Table 4. Geographic characteristics of 37 Puerto Rico stream sampling reaches sampled 2008–2010.

Site number	Elevation (m)	Gradient %	Distance to river mouth (km)	Road density (km/ha)	Watershed area (km^2)	Downstream reservoir
4C	14	0.56	4.44	0.025	24.681	No
4D	115.6	6.17	10.12	0.002	15.857	No
4E	116	6.13	10.47	0.002	15.829	No
4F	86	3.14	9.38	0.004	17.759	No
4G	82	3.13	9.27	0.004	17.902	No
4H	82	3.17	8.94	0.004	17.958	No
4I	37	2.70	6.76	0.007	23.136	No
4J	27	1.86	5.78	0.008	23.867	No
4K	25	1.83	5.6	0.008	23.879	No
4L	24.1	1.77	5.37	0.009	24.334	No
4M	19.2	1.65	4.99	0.013	24.587	No
4N	16	0.54	4.42	0.015	24.669	No
4O	12	0.25	4.13	0.014	28.579	No
4P	3	0.06	1.77	0.046	38.855	No
4Q	5.8	0.32	2.61	0.032	37.927	No
4R	54.9	2.77	7.25	0.006	21.806	No
7C	4.5	0.67	3.45	0.03	58.538	No
8A	27	1.05	5.69	0.028	5.067	No
9A	33.2	1.09	7.15	0.004	1.010	Yes
12A	45	1.32	10.96	0.021	2.469	Yes
12B	31	1.37	10.06	0.032	4.010	Yes
17A	20	1.54	2.21	0.018	3.655	No
18A	597.8	3.67	20	0.012	3.887	No
20A	47	1.34	4.14	0.026	19.462	Yes
21A	179	2.45	24.48	0.021	5.676	Yes
24A	54.8	2.00	17.17	0.035	124.086	No
24B	168	2.64	27.34	0.037	14.234	Yes
25A	57.7	1.83	11.6	0.025	31.535	No
26A	5.4	1.34	10.27	0.019	49.427	Yes
27A	56.3	0.97	10.45	0.035	30.356	Yes
29B	4.3	0.09	2.01	0.028	82.746	No
32D	10.2	0.08	5.74	0.042	115.871	No
37G	341	3.17	66.14	0.016	15.720	Yes
37H	326	3.45	67.05	0.017	28.623	Yes
39A	142	2.23	16.13	0.024	54.206	Yes
44B	572	3.67	93.9	0.016	6.932	Yes
46B	27	1.44	9.98	0.095	20.649	No

Table 5. Fishes detected at 37 sites in Puerto Rico stream reaches sampled 2008–2010, including 21 species from 14 families. Fish species with an asterisk (*) are not native to Puerto Rico. The sirajo goby *Sicydium plumieri* has been split into four *Sicydium* species (*S. buscki, S. gilberti, S. plumieri, and S. punctatum*), which are combined here as *S. plumieri*.

Family	Scientific name	Common Name	4C	4D	4E	4F
Anguillidae	*Anguilla rostrata*	American Eel	X	X	X	X
Belonidae	*Strongylura marina*	Atlantic Needlefish	.	.	.	.
Centrarchidae	*Micropterus salmoides**	Largemouth Bass	.	.	.	.
Centropomidae	*Centropomus parallelus*	Fat Snook	.	.	.	.
Cichlidae	*Oreochromis mossambicus**	Mozambique Tilapia	.	.	.	.
Cichlidae	*Tilapia rendalli**	Redbreast Tilapia	.	.	.	.
Cyprinidae	*Puntius conchonius**	Rosy Barb	.	.	.	.
Eleotridae	*Dormitator maculatus*	Fat Sleeper	.	.	.	.
Eleotridae	*Eleotris perniger*	Smallscaled Spinycheek Sleeper	X	.	.	X
Eleotridae	*Gobiomorus dormitor*	Bigmouth Sleeper	X	X	X	X
Gobiidae	*Awaous banana*	River Goby	.	.	.	.
Gobiidae	*Sicydium plumieri* [a]	Sirajo Goby	X	X	X	X
Haemulidae	*Pomadasys crocro*	Burro Grunt	.	.	.	.
Ictaluridae	*Ictalurus punctatus**	Channel Catfish	.	.	.	.
Loricariidae	*Pterygoplicthys pardalis**	Amazon Sailfin Catfish	.	.	.	.
Lutjanidae	*Lutjanus griseus*	Grey Snapper	.	.	.	.
Mugilidae	*Agonostomus monticola*	Mountain Mullet	X	X	X	X
Poeciliidae	*Poecilia reticulata**	Guppy	.	.	.	.
Poeciliidae	*Poecilia sphenops**	Mexican Molly	.	.	.	.
Poeciliidae	*Xiphophorus hellerii**	Green Swordtail	.	.	.	.
Syngnathidae	*Microphis brachyurus*	Shorttail Pipefish	.	.	.	.
Total			5	4	4	5

Table 5 continued.

Scientific name	4G	4I	4J	4K	4H	4L	4M	4N	4O	4P	4Q
Anguilla rostrata	X	X	X	X	X	X	X	X	X	X	X
Strongylura marina	.	.	.	.	.	.	.	.	.	X	.
*Micropterus salmoides**	.	.	.	.	.	.	.	.	.	.	.
Centropomus parallelus	.	.	.	.	.	.	.	.	.	X	.
*Oreochromis mossambicus**	.	.	.	.	.	.	.	.	.	.	.
*Tilapia rendalli**	.	.	.	.	.	.	.	.	.	.	.
*Puntius conchonius**	.	.	.	.	.	.	.	.	.	.	.
Dormitator maculatus	.	.	.	.	.	.	.	.	.	.	.
Eleotris perniger	X	X	X	X	X	X	X	X	X	X	X
Gobiomorus dormitor	X	X	X	X	X	X	X	X	X	X	X
Awaous banana	.	.	.	.	.	.	.	X	.	X	.
Sicydium plumieri [a]	X	X	X	X	X	X	X	X	X	X	.
Pomadasys crocro	.	.	.	.	.	.	.	.	.	.	.
*Ictalurus punctatus**	.	.	.	.	.	.	.	.	.	.	.
*Pterygoplicthys pardalis**	.	.	.	.	.	.	.	.	.	.	.
Lutjanus griseus	.	.	.	.	.	.	.	.	.	X	.
Agonostomus monticola	X	X	X	X	X	X	X	X	X	X	X
*Poecilia reticulata**	.	.	.	.	.	.	.	.	.	.	.
*Poecilia sphenops**	.	.	.	.	.	.	.	.	.	.	.
*Xiphophorus hellerii**	.	.	.	.	.	.	.	.	.	.	.
Microphis brachyurus	.	.	.	.	.	.	.	.	.	.	.
Total	5	5	5	5	5	5	5	6	5	9	4

Table 5 continued.

Scientific name	4R	7C	9A	8A	12A	12B	17A	18A	20A	21A	24A
Anguilla rostrata	X	X	X	X	.	.	X	.	.	.	X
Strongylura marina	.	.	.	.	.	.	.	.	.	.	.
*Micropterus salmoides**	.	.	.	.	.	.	.	.	.	.	.
Centropomus parallelus	.	.	.	.	.	.	.	.	.	.	.
*Oreochromis mossambicus**	.	.	.	X	.	X	.	.	.	.	.
*Tilapia rendalli**	.	.	X	.	.	.	.	.	.	.	.
*Puntius conchonius**	.	.	.	.	.	.	.	.	.	.	.
Dormitator maculatus	.	.	.	.	.	.	.	.	.	.	.
Eleotris perniger	X	X	.	X	.	X	X	.	.	.	X
Gobiomorus dormitor	X	X	X	X	X	X	X	.	.	.	X
Awaous banana	.	.	.	X	X	X	X	.	.	.	X
Sicydium plumieri [a]	X	.	.	.	.	.	X	.	.	.	X
Pomadasys crocro	.	.	.	.	.	.	.	.	.	.	X
*Ictalurus punctatus**	.	.	.	.	.	.	.	.	.	.	.
*Pterygoplicthys pardalis**	.	.	.	.	.	.	.	.	.	.	X
Lutjanus griseus	.	.	.	.	.	.	.	.	.	.	.
Agonostomus monticola	X	X	X	X	.	.	X	.	.	.	X
*Poecilia reticulata**	.	.	.	X	X	.	.	X	.	.	.
*Poecilia sphenops**	.	.	X	.	X	X	.	X	.	X	.
*Xiphophorus hellerii**	.	.	.	.	.	.	.	X	.	.	.
Microphis brachyurus	.	.	.	.	.	.	.	.	.	.	.
Total	5	4	8	7	4	5	6	3	0	1	8

Table 5 continued.

Scientific name	24B	25A	26A	27A	29B	32D	37G	37H	39A	44B	46B	Total
Anguilla rostrata	.	.	.	.	X	X	.	.	X	.	X	25
Strongylura marina	.	.	.	.	.	.	.	.	.	.	.	1
*Micropterus salmoides**	.	.	.	.	.	.	.	.	X	X	.	2
Centropomus parallelus	.	.	.	.	.	.	.	.	.	.	.	1
*Oreochromis mossambicus**	.	.	.	.	.	.	.	.	.	.	.	2
*Tilapia rendalli**	.	X	X	.	.	X	.	.	.	.	.	4
*Puntius conchonius**	X	.	.	.	.	.	.	.	.	.	.	1
Dormitator maculatus	.	.	.	.	X	.	.	.	.	.	.	1
Eleotris perniger	.	.	.	.	X	X	.	.	.	.	X	22
Gobiomorus dormitor	.	X	.	.	X	X	.	.	.	X	X	28
Awaous banana	.	X	X	.	X	X	X	X	.	.	X	14
Sicydium plumieri	.	X	X	X	.	X	X	X	X	.	X	25
Pomadasys crocro	.	.	.	.	X	.	.	.	.	.	.	2
*Ictalurus punctatus**	.	.	.	.	.	.	.	.	.	X	.	1
*Pterygoplicthys pardalis**	.	.	.	.	.	X	.	.	.	.	X	3
Lutjanus griseus	.	.	.	.	X	.	.	.	.	.	.	2
Agonostomus monticola	.	X	.	.	X	X	X	X	X	.	X	28
*Poecilia reticulata**	X	.	X	.	.	.	X	.	X	.	.	7
*Poecilia sphenops**	X	X	X	X	.	X	.	.	.	.	.	10
*Xiphophorus hellerii**	.	.	.	.	.	.	.	.	.	X	X	3
Microphis brachyurus	.	.	.	.	X	.	.	.	.	.	.	1
Total	3	6	5	2	9	9	4	3	5	4	8	

Table 6. Assemblage variables for all species of fish collected among 37 Puerto Rico stream sampling reaches 2008–2010. Density and biomass were estimated according to species and summed for totals presented here.

Site number	Species richness	Diversity (*H'*)	Density (fish/ha)	Biomass (kg/ha)
4C	5	1.12	1,107.20	13.50
4D	4	0.80	845.40	11.80
4E	4	0.83	1,882.70	20.80
4F	5	1.08	1,157.20	13.60
4G	5	0.96	3,871.80	43.20
4H	5	0.98	429.00	4.50
4I	5	1.24	1,977.30	24.20
4J	5	1.16	851.20	8.50
4K	5	1.08	2,793.10	33.60
4L	5	0.93	768.20	20.50
4M	5	0.88	1,121.20	21.70
4N	6	1.52	3,502.10	74.60
4O	5	1.37	1,141.40	24.30
4P	9	1.60	7,037.50	218.10
4Q	4	0.98	492.60	19.10
4R	5	0.66	222.20	4.90
7C	4	0.85	.	.
8A	7	1.30	7,700.70	210.90
9A	5	1.37	6,397.60	200.50
12A	4	1.06	2,989.30	13.40
12B	5	1.37	14,238.30	197.40
17A	6	1.41	13,709.80	67.70
18A	3	0.97	8,203.70	28.50
20A	0	0.00	0.00	0.00
21A	1	0.00	8,476.50	34.60
24A	8	1.34	.	.
24B	3	0.98	6,389.10	14.60
25A	6	1.09	.	.
26A	5	1.43	.	.
27A	2	0.38	.	.
29B	9	1.86	.	.
32D	9	1.27	.	.
37G	4	0.46	1,656.20	8.40
37H	3	1.04	3,011.50	207.70
39A	5	1.34	.	.
44B	4	0.72	.	.
46B	8	1.65	3,479.20	256.20

Table 7. Assemblage variables for native species of fish collected among 37 Puerto Rico stream sampling reaches 2008–2010. Density and biomass were estimated according to species and summed for totals presented here.

Site number	Species richness	Diversity (H')	Density (fish/ha)	Biomass (kg/ha)
4C	5	1.15	1,107.2	13.5
4D	4	0.67	845.4	11.8
4E	4	0.80	1,882.7	20.8
4F	5	1.09	1,157.2	13.6
4G	5	1.05	3,871.8	43.2
4H	5	1.01	429.0	4.5
4I	5	1.12	1,977.3	24.2
4J	5	1.01	851.2	8.5
4K	5	1.06	2,793.1	33.6
4L	5	0.98	768.2	20.5
4M	5	0.99	1,121.2	21.7
4N	6	1.45	3,502.1	74.6
4O	5	1.36	1,141.4	24.3
4P	9	1.54	7,037.5	218.1
4Q	4	1.03	492.6	19.1
4R	5	0.71	222.2	4.9
7C	4	N/A	N/A	N/A
8A	5	1.04	7,098.8	126
9A	3	0.95	2,477.1	99
12A	2	0.69	422.6	10.5
12B	3	0.83	4,802.7	56.1
17A	6	1.31	13,709.8	67.7
18A	0	.	0	0
20A	0	.	0	0
21A	0	.	0	0
24A	7	N/A	N/A	N/A
24B	0	.	0	0
25A	4	N/A	N/A	N/A
26A	2	N/A	N/A	N/A
27A	1	N/A	N/A	N/A
29B	9	N/A	N/A	N/A
32D	6	1.00	99	3105.5
37G	3	0.49	218.2	8.1
37H	3	1.02	3,011.5	207.7
39A	3	N/A	N/A	N/A
44B	1	N/A	N/A	N/A
46B	6	1.47	3,275.5	143.1

Table 8. Assemblage variables for introduced species of fish collected among 37 Puerto Rico stream sampling reaches 2008–2010. Density and biomass were estimated according to species and summed for totals presented here.

Site number	Species richness	Density (fish/ha)	Biomass (kg/ha)
4C	0	0	0
4D	0	0	0
4E	0	0	0
4F	0	0	0
4G	0	0	0
4H	0	0	0
4I	0	0	0
4J	0	0	0
4K	0	0	0
4L	0	0	0
4M	0	0	0
4N	0	0	0
4O	0	0	0
4P	0	0	0
4Q	0	0	0
4R	0	0	0
7C	0	N/A	N/A
8A	2	601.9	84.9
9A	2	3920.5	101.5
12A	2	2566.7	2.9
12B	2	9435.6	141.3
17A	0	0	0
18A	3	8203.7	28.5
20A	0	0	0
21A	1	8476.5	34.6
24A	1	N/A	N/A
24B	3	6389.1	14.6
25A	2	N/A	N/A
26A	3	N/A	N/A
27A	1	N/A	N/A
29B	0	N/A	N/A
32D	3	8	959.4
37G	1	1438	0.3
37H	0	0	0
39A	2	N/A	N/A
44B	3	N/A	N/A
46B	2	203.7	113.1

Table 9. Density and biomass estimates of all fish species sampled among 37 Puerto Rico stream reaches 2008–2010. Standard error (SE) estimates with an asterisk indicate species for which the removal criteria failed; density and biomass estimates for those populations represent actual capture converted to the standardized area (ha). Those with an ‘a’ for SE of density and ‘b’ for SE of biomass represent sites that were spot sampled; density and biomass estimates represent actual capture.

Site number	Species	Density (fish/ha)	SE	Biomass (kg/ha)	SE
4C	Bigmouth Sleeper	82.1	4.3	4.7	0.3
	Mountain Mullet	567.5	36.7	6.8	0.5
	Sirajo Goby	329.1	3.3	1.1	0.1
	Smallscaled Spinycheek Sleeper	128.5	83.8	0.9	0.6
4D	Bigmouth Sleeper	26.5	7.7	1.4	0.9
	Mountain Mullet	622.3	288.6	10.3	6.1
	Sirajo Goby	196.6	57.3	0.1	0.1
4E	Bigmouth Sleeper	60.2	26.4	6.2	3.4
	Mountain Mullet	774.6	409.3	12.9	8.9
	Sirajo Goby	1047.9	272.9	1.7	1.0
4F	American Eel	86.0	36.1	2.0	1.2
	Bigmouth Sleeper	78.5	29.3	5.9	0.7
	Mountain Mullet	305.7	152.4	4.7	3.0
	Sirajo Goby	673.7	278.1	0.7	0.4
	Smallscaled Spinycheek Sleeper	13.3	4.5	0.3	0.1
4G	American Eel	255.1	89.2	5.3	1.4
	Bigmouth Sleeper	126.3	60.0	5.7	1.5
	Mountain Mullet	1646.9	605.9	28.0	3.3
	Sirajo Goby	1811.7	541.4	2.9	0.7
	Smallscaled Spinycheek Sleeper	31.8	16.9	1.3	0.4
4H	Bigmouth Sleeper	27.4	5.3	1.9	0.5
	Mountain Mullet	226.4	102.0	1.9	1.3
	Sirajo Goby	157.3	46.2	0.1	0.1
	Smallscaled Spinycheek Sleeper	17.9	6.3	0.6	0.4
4I	American Eel	141.4	32.5	5.2	3.3
	Bigmouth Sleeper	94.1	21.1	3.2	0.9
	Mountain Mullet	1188.5	701.3	13.3	2.7
	Sirajo Goby	465.5	299.5	1.3	0.7
	Smallscaled Spinycheek Sleeper	87.8	22.3	1.2	0.6
4J	Bigmouth Sleeper	82.2	21.1	1.7	0.9
	Mountain Mullet	541.5	0*	5.4	0*
	Sirajo Goby	175.4	65.0	0.3	0.2
	Smallscaled Spinycheek Sleeper	52.1	0*	1.1	0*

Table 9 continued.

Site number	Species	Density (fish/ha)	SE	Biomass (kg/ha)	SE
4K	American Eel	102.3	54.4	3.2	0.8
	Bigmouth Sleeper	241.0	86.9	12.1	4.7
	Mountain Mullet	734.3	0*	16.6	0*
	Sirajo Goby	1665.0	677.3	1.2	0.3
	Smallscaled Spinycheek Sleeper	50.5	19.2	0.5	0.2
4L	Bigmouth Sleeper	152.3	90.1	7.5	4.1
	Mountain Mullet	477.6	0*	10.9	0*
	Sirajo Goby	11.4	7.7	0.1	0.1
	Smallscaled Spinycheek Sleeper	126.9	0*	2.0	0*
4M	American Eel	28.5	7.6	0.6	0.1
	Bigmouth Sleeper	280.6	0*	9.3	0*
	Mountain Mullet	715.8	489.6	10.6	5.5
	Sirajo Goby	27.7	0*	0.0	0*
	Smallscaled Spinycheek Sleeper	68.6	0*	1.2	0*
4N	American Eel	460.8	70.3	21.6	12.3
	Bigmouth Sleeper	721.1	387.4	38.7	7.1
	Mountain Mullet	883.3	600.0	9.3	4.2
	River Goby	22.7	14.6	0.3	0.1
	Sirajo Goby	1305.9	0*	3.0	0*
	Smallscaled Spinycheek Sleeper	108.3	41.0	1.7	0.3
4O	Bigmouth Sleeper	353.7	0*	18.5	0*
	Mountain Mullet	219.7	91.2	2.6	0.5
	Sirajo Goby	210.8	92.4	0.3	0.1
	Smallscaled Spinycheek Sleeper	357.2	130.0	2.9	1.3
4P	American Eel	2596.1	1182.8	148.6	62.2
	Atlantic needlefish	13.6	6.8	0.2	0.1
	Bigmouth Sleeper	703.8	234.0	42.4	18.8
	Gray snapper	10.4	1.8	1.4	0.6
	Mountain Mullet	1716.1	433.1	17.0	4.6
	River Goby	17.7	9.1	0.1	0.1
	Sirajo Goby	804.5	212.8	0.5	0.3
	Fat Snook	15.4	10.6	4.6	3.2
	Smallscaled Spinycheek Sleeper	1159.9	705.8	3.3	1.6
4Q	Bigmouth Sleeper	226.2	122.4	15.5	9.5
	Mountain Mullet	178.4	61.1	3.1	1.0
	Smallscaled Spinycheek Sleeper	88.0	0*	0.5	0*
4R	Bigmouth Sleeper	31.2	0*	1.1	0*
	Mountain Mullet	174.7	64.3	3.7	2.5
	Sirajo Goby	9.0	0*	0.0	0*
	Smallscaled Spinycheek Sleeper	7.3	4.7	0.1	0.1

Table 9 continued.

Site number	Species	Density (fish/ha)	SE	Biomass (kg/ha)	SE
7C	American Eel	17	a	760.8	b
	Bigmouth Sleeper	89	a	7348.8	b
	Mountain Mullet	5	a	141.3	b
	Smallscaled Spinycheek Sleeper	11	a	104.2	b
8A	American Eel	266.8	71.7	35.1	118.1
	Bigmouth Sleeper	1708.7	814.7	32.5	206.8
	Guppy	182.5	24.7	0.1	0.1
	Mountain Mullet	831.5	536.3	37.7	13.3
	Mozambique Tilapia	419.4	41.9	84.8	17.8
	River Goby	20.4	3.2	1.3	0.3
	Smallscaled Spinycheek Sleeper	4271.4	0*	19.4	0*
9A	American Eel	253.9	62.1	33.8	52.5
	Bigmouth Sleeper	1247.2	437.5	22.3	43.2
	Mexican molly	2174.2	357.9	3.1	1.4
	Mountain Mullet	976.0	227.6	42.9	29.5
	Redbreast Tilapia	1746.3	748.2	98.4	49.2
12A	Bigmouth Sleeper	221.6	38.6	7.3	1.4
	Guppy	449.8	233.5	0.1	0.1
	Mexican molly	2116.9	362.4	2.8	0.7
	River Goby	201.0	131.6	3.2	1.5
12B	Bigmouth Sleeper	699.4	127.8	13.7	3.8
	Mexican molly	7523.2	5081.2	31.2	8.1
	Mozambique Tilapia	1912.4	912.6	110.1	58.5
	River Goby	765.0	305.5	12.0	6.1
	Smallscaled Spinycheek Sleeper	3338.3	1010.5	30.4	4.3
17A	American Eel	192.7	81.7	5.4	3.5
	Bigmouth Sleeper	509.3	114.7	11.3	2.5
	Mountain Mullet	3093.7	597.1	27.9	10.0
	River Goby	881.9	325.2	3.1	0.5
	Sirajo Goby	7068.9	3591.7	12.1	5.6
	Smallscaled Spinycheek Sleeper	1963.3	1245.3	7.9	2.9
18A	Guppy	1542.1	231.5	1.8	0.2
	Mexican Molly	6453.9	1347.1	25.4	5.3
	Green Swordtail	207.7	44.2	1.3	0.1
20A	.	.	.	.	.
21A	Mexican Molly	8476.5	2347.2	34.6	9.7

Table 9 continued.

Site number	Species	Density (fish/ha)	SE	Biomass (kg/ha)	SE
24A	Amazon sailfin catfish	12	a	7433.6	b
	American Eel	2	a	543.6	b
	Bigmouth Sleeper	16	a	1622.7	b
	Burro Grunt	3	a	401.3	b
	Mountain Mullet	75	a	1676.1	b
	River Goby	3	a	29.2	b
	Sirajo Goby	18	a	37.1	b
	Smallscaled Spinycheek Sleeper	1	a	30.9	b
24B	Guppy	1129.8	128.8	0.3	0.1
	Mexican molly	3323.5	470.6	4.4	1.0
	Rosy Barb	1935.8	414.5	9.9	6.9
25A	Bigmouth Sleeper	10	a	500.4	b
	Mexican molly	6	a	11.4	b
	Mountain Mullet	132	a	4747.1	b
	River Goby	13	a	134.1	b
	Sirajo Goby	60	a	303.1	b
	Redbreast Tilapia	1	a	69	b
26A	Guppy	31	a	6.2	b
	Mexican molly	35	a	50.3	b
	River Goby	3	a	46.4	b
	Sirajo Goby	31	a	110.2	b
	Redbreast Tilapia	16	a	187.4	b
27A	Mexican molly	56	a	62.8	b
	Sirajo Goby	8	a	17.6	b
29B	American Eel	9	a	1184.2	b
	Bigmouth Sleeper	19	a	1823.1	b
	Burro Grunt	21	a	1454.7	b
	Fat Sleeper	4	a	1.9	b
	Grey snapper	2	a	58.6	b
	Mountain Mullet	11	a	68.2	b
	Pipe Fish	1	a	1.1	b
	River Goby	5	a	40.1	b
	Smallscaled Spinycheek Sleeper	3	a	33.9	b
32D	Amazon sailfin catfish	2	a	701.9	b
	American Eel	4	a	954	b
	Bigmouth Sleeper	12	a	1837.6	b
	Mexican molly	4	a	1.7	b
	Mountain Mullet	70	a	149	b
	River Goby	1	a	57.6	b
	Sirajo Goby	3	a	1.9	b
	Smallscaled Spinycheek Sleeper	9	a	105.4	b
	Redbreast Tilapia	2	a	255.8	b

Table 9 continued.

Site number	Species	Density (fish/ha)	SE	Biomass (kg/ha)	SE
37G	Guppy	1438.0	899.9	0.3	0.1
	Mountain Mullet	21.0	12.4	3.2	0.8
	River Goby	9.7	1.2	1.0	0.3
	Sirajo Goby	187.5	106.8	3.9	0.6
37H	Mountain Mullet	1432.9	834.5	126.8	72.0
	River Goby	501.7	131.8	52.7	18.3
	Sirajo Goby	1076.9	653.4	28.2	7.9
39A	American Eel	21	a	3635.7	b
	Guppy	1	a	0.2	b
	Largemouth Bass	5	a	20.2	b
	Mountain Mullet	14	a	1389.2	b
	Sirajo Goby	10	a	31	b
44B	Bigmouth Sleeper	21	a	1741.5	b
	Channel Catfish	1	a	709.1	b
	Green Swordtail	4	a	28.6	b
	Largemouth Bass	1	a	21.3	b
46B	Amazon sailfin catfish	118.6	43.9	112.7	46.9
	American Eel	103.9	28.5	18.0	2.6
	Bigmouth Sleeper	1086.2	352.2	51.7	14.4
	Green Swordtail	85.1	23.3	0.4	0.1
	Mountain Mullet	732.5	365.9	37.1	10.2
	River Goby	807.8	361.5	30.1	10.1
	Sirajo Goby	9.3	2.6	0.2	0.1
	Smallscaled Spinycheek Sleeper	535.8	350.6	6.0	1.2

Table 10. Shrimp, crab, and crayfish species sampled at 37 Puerto Rico stream reaches 2008–2010.

Species	4C	4D	4E	4F	4G	4H	4I	4J	4K	4L	4M
Atya innocous	X	X	X	X	X	X	X	.	.	X	X
Atya lanipes	.	.	.	.	.	.	.	.	.	.	.
Atya scabra	.	.	.	X	X	.	X	.	.	.	.
Jonga serrei	.	.	X	.	X	.	.	X	X	.	.
Macrobrachium acanthurus	.	.	.	.	.	.	.	.	.	.	.
Macrobrachium carcinus	X	X	X	X	X	X	X	X	X	X	X
Macrobrachium crenulatum	.	.	X	.	.	.	.	.	X	.	.
Macrobrachium faustinum	X	X	X	X	X	.	X	X	.	X	.
Macrobrachium heterochirus	.	.	X	X	X	.	X	.	.	.	.
Micratya poeyi	.	X	X	.	X	.	.	X	X	X	X
Xiphocaris elongata	X	X	X	X	X	X	X	.	X	X	X
Armases roberti	.	.	.	.	.	.	.	.	.	.	.
Callinectes sapidus	.	.	.	.	.	.	.	.	.	.	.
Epilobocera sinuatifrons	X	.	X	.	X	X	X	X	.	X	.
Total	5	5	9	6	9	4	7	5	5	6	4

Table 10 continued.

Species	4N	4O	4P	4Q	4R	7C	8A	9A	12A	12B	17A
Atya innocous	.	.	.	X	X	.	.	X	X	X	X
Atya lanipes	.	.	.	.	.	.	.	.	.	.	.
Atya scabra	.	.	.	.	.	.	.	X	X	.	X
Jonga serrei	.	X	.	.	.	.	.	.	X	.	X
Macrobrachium acanthurus	.	.	.	.	.	X	X	X	.	X	.
Macrobrachium carcinus	X	X	X	X	X	X	.	X	X	.	X
Macrobrachium crenulatum	.	X	.	.	.	X	.	.	.	X	.
Macrobrachium faustinum	X	.	X	X	X	X	X	X	X	X	X
Macrobrachium heterochirus	X	.	.	.	.	.	X	.	X	.	X
Micratya poeyi	X	X	.	.	.	.	X	.	X	.	X
Xiphocaris elongata	X	.	X	X	X	.	.	X	X	X	X
Armases roberti	.	.	.	.	.	X	.	.	.	.	.
Callinectes sapidus	.	.	.	.	.	.	.	.	.	.	.
Epilobocera sinuatifrons	X	.	.	.	X	.	X	.	X	X	X
Total	6	4	3	4	5	5	5	6	9	6	9

Table 10 continued.

Species	18A	20A	21A	24B	24A	25A	26A	27A	29B	32D	37G
Atya innocous	.	.	.	X	X	.	.	X	.	.	X
Atya lanipes	.	.	.	X	.	.	X	.	.	.	X
Atya scabra	.	.	.	X	X	.	X	X	.	.	X
Jonga serrei	.	.	.	X	X	X	.	X	.	.	.
Macrobrachium acanthurus	.	.	.	.	.	.	.	.	X	X	.
Macrobrachium carcinus	.	.	.	.	X	.	.	X	X	.	.
Macrobrachium crenulatum	.	.	.	.	X	X	X	X	.	.	X
Macrobrachium faustinum	.	.	.	.	X	X	X	X	.	X	X
Macrobrachium heterochirus	.	.	.	.	X	X	X	X	.	.	.
Micratya poeyi	.	.	.	X	X	X	.	X	.	.	.
Xiphocaris elongata	.	.	.	X	X	X	X	X	.	X	X
Armases roberti	.	.	.	.	.	.	.	.	.	.	.
Callinectes sapidus	.	.	.	.	.	.	.	.	X	.	.
Epilobocera sinuatifrons	X	.	.	X	.	.	.	X	.	.	X
Total	1	0	0	7	9	6	6	10	3	3	7

Table 10 continued.

Species	37H	39A	44B	46B
Atya innocous	X	.	.	.
Atya lanipes	.	.	.	.
Atya scabra	X	.	.	.
Jonga serrei	.	.	.	.
Macrobrachium acanthurus	.	.	.	.
Macrobrachium carcinus	.	.	.	.
Macrobrachium crenulatum	X	.	.	X
Macrobrachium faustinum	X	.	.	X
Macrobrachium heterochirus	X	.	.	.
Micratya poeyi	.	.	.	.
Xiphocaris elongata	.	.	.	.
Armases roberti	.	.	.	.
Callinectes sapidus	.	.	.	.
Epilobocera sinuatifrons	X	.	X	.
Total	6	0	1	2

Table 11. Water quality analyses from 37 Puerto Rico river sampling sites during 2008-2010 surveys.

Site	Season	Year	Water temperature (°C)	Total dissolved solids (g/L)	Conduct-ivity (µS/cm)	Salinity (ppt)	Nitrate (mg/L as NO_3^-)	Nitrite (mg/L as NO_2^-)	Ammonia (NH_3)	Phos-phorous (mg/L as PO_4^-)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	Turbidity (FAU)	pH	Dissolved oxygen (mg/L)
4C	Summer	2008	28.99	0.085	140	0.06	2.7	0.01	0	2.06	46	49	3	7.64	7.8
4D	Winter	2010	24.57	0.089	131	0.06	0	0.011	0	0.07	127	125	2	7.6	8.27
4E	Summer	2009	24.52	0.085	129	0.06	0	0.013	0	0.05	124	123	2	7.54	8.25
4F	Summer	2009	24.69	0.079	121	0.06	1.8	0.006	0	0.02	33	38	4	7.27	8.26
4G	Summer	2009	24.99	0.096	147	0.07	1.5	0.005	0	0.18	38	38	4	7.59	8.28
4H	Winter	2010	24.87	0.098	143	0.07	1.3	0.007	0	0.17	42	43	4	7.63	8.34
4I	Summer	2009	25.13	0.111	171	0.08	2.6	0.017	0.05	0.88	42	51	4	7.48	8.8
4J	Summer	2009	26.58	0.116	180	0.08	0.2	0.008	0	0.16	107	110	3	7.5	8.86
4K	Summer	2009	27.99	0.115	186	0.08	0	0.007	0	0	117	115	0	7.55	9.05
4L	Winter	2010	26.63	0.118	173	0.08	0.32	0.008	0	0.18	97	99	3	7.57	8.87
4M	Winter	2010	27.08	0.113	187	0.09	0.73	0.001	0	0.51	63	67	1	7.32	7.97
4N	Summer	2009	28.08	0.123	192	0.09	0.8	0.001	0.07	0.31	57	52	1	7.46	7.86
4O	Summer	2009	28.62	0.118	194	0	2	0.018	0.08	0.15	113	112	3	7.4	8.4
4P	Summer	2009	28.77	0.134	209	0.1	1.8	0.007	0	0.26	52	61	3	7.42	8.17
4Q	Summer	2010	28.79	0.137	213	0.11	1.9	0.008	0	0.28	56	58	3	7.47	8.04
4R	Summer	2010	25.57	0.111	174	0.07	1.7	0.014	0.04	0.73	48	51	3	7.48	8.73
7C	Summer	2008	30.34	0.124	211	0.09	3.9	0.041	0.01	2.06	38	43	3	8.9	10.9
8A	Summer	2009	27.55	0.324	522	0.24	2.1	0.01	0	1.83	94	115	6	7.07	7.08
9A	Summer	2010	27.89	0.103	159	0.07	0.9	0.011	0.11	0.42	35	41	0	7.63	7.22
12A	Summer	2009	27.1	0.331	530	0.24	5.4	0.035	0.02	0.39	112	114	5	7.51	7.86
12B	Summer	2009	27.36	0.31	498	0.23	12.7	0.367	0.01	0.94	106	132	18	7.33	6.25
17A	Summer	2009	27.64	0.272	439	0.2	1.1	0.011	0.01	1.45	88	99	2	7.52	8.57
18A	Summer	2010	22.5	0.132	104	0.05	0.23	0.006	0	0.31	41	42	1	7.62	6.79
20A	Summer	2010	28.33	0.232	483	0.27	1.4	0.013	0.02	0.95	137	107	1	7.38	6.73
21A	Summer	2010	27.37	0.273	322	0.17	1.7	0.036	0.01	0.67	88	93	0	7.45	7.23
24A	Summer	2008	29.95	0.171	289	0.12	0.3	0.011	0.01	0.5	97	103	2	8.38	9.76
24B	Summer	2009	27.35	0.331	532	0.24	3.3	0.005	0	0	147	149	0	7.9	9.97
25A	Summer	2008	31.98	0.196	342	0.14	5	0.012	0.01	2.06	120	119	1	8.6	8.79

Table 11 continued

Site	Season	Year	Water temperature (°C)	Total dissolved solids (g/L)	Conduct-ivity (µS/cm)	Salinity (ppt)	Nitrate (mg/L as NO3-)	Nitrite (mg/L as NO2-)	Ammonia (NH3)	Phos-phorous (mg/L as PO4-)	Alkalinity (mg/L as CaCO3)	Hardness (mg/L as CaCO3)	Turbidity (FAU)	pH	Dissolved oxygen (mg/L)
26A	Summer	2008	26.57	0.166	263	0.12	5.8	0.019	0.01	1.16	100	113	2	8.59	8.85
27A	Summer	2008	29.29	0.201	334	0.15	2.4	0.012	0.01	2.75	131	137	3	8.72	8.12
29B	Summer	2008	27.46	0.175	298	0.13	0.9	0.023	0	0.31	314	208	1	7.81	9.08
32D	Summer	2008	27.43	0.7	1,125	0.53	4.6	0.012	0.01	0.3	139	140	2	7.76	4.34
37G	Summer	2008	22.47	0.15	220	0.11	1.1	0.01	0.01	0.53	106	112	4	7.98	7.07
37H	Summer	2008	23.34	0.148	233	0.11	1	0.009	0.01	0.55	105	115	5	8.2	8.76
39A	Summer	2008	26.37	0.244	376	0.17	5.6	0.023	0.01	1.38	144	163	1	8.83	7.15
44B	Summer	2008	22.7	0.13	106	0.05	0.3	0.007	0	0.33	39	44	3	7.62	6.79
46B	Summer	2009	27.82	0.353	572	0.26	3.8	0.036	0	0.57	102	133	6	7.54	8.78

Table 12. Land cover composition for 37 Puerto Rico stream sampling sites. Riparian and watershed percentages were calculated for the entire riparian zone and watershed upstream of each site.

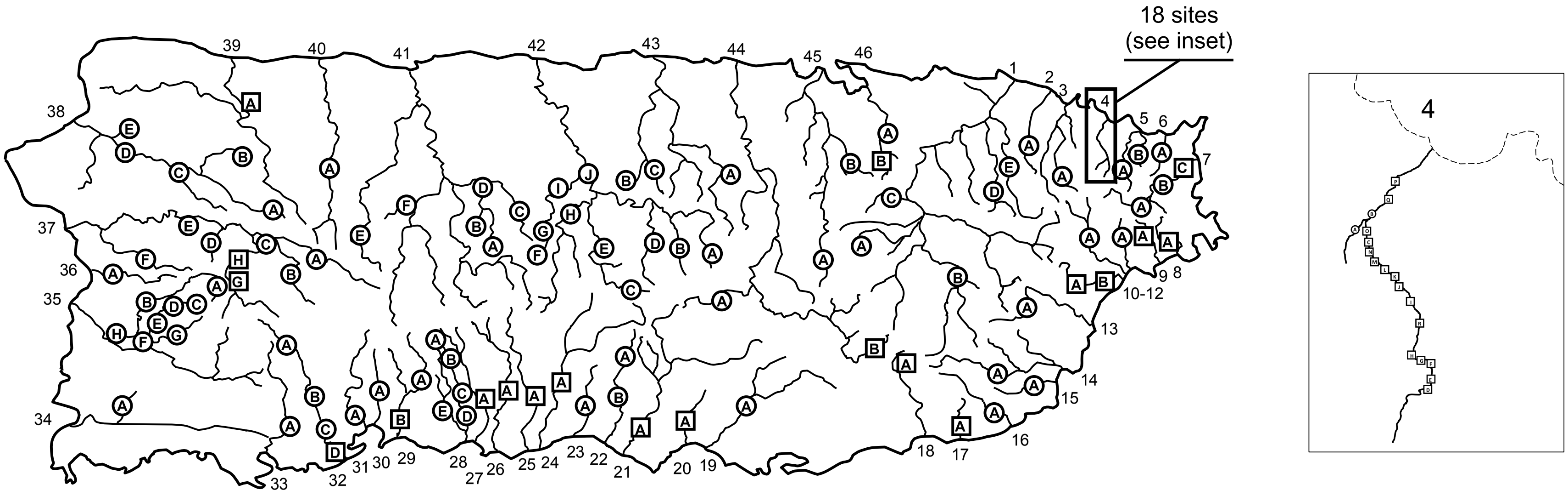
	30-m riparian buffer land cover (%)				100-m riparian buffer land cover (%)				Watershed land cover (%)				
Site	Agriculture	Forest	Shrub and woodland	Urban	Agriculture	Forest	Shrub and woodland	Urban	Agriculture	Forest	Freshwater	Shrub and woodland	Urban
4C	3.2	94.8	2	0	5.2	92.8	1.9	0.1	5.1	92.9	0.4	1.4	0.2
4D	0.3	99	0.7	0	0.4	99	0.6	0	0.4	99	0	0.6	0
4E	0.5	98.9	0.6	0	0.6	98.8	0.6	0	0.3	99.1	0	0.6	0
4F	0.2	99	0.8	0	0.2	99	0.8	0	0.3	99	0	0.7	0
4G	0.6	98.7	0.7	0	0.6	98.7	0.7	0	0.3	99	0.1	0.6	0
4H	0.3	99	0.7	0	0.5	98.8	0.7	0	0.3	99	0.1	0.6	0
4I	3	95.5	1.5	0	3.1	95.5	1.3	0.1	3	95.6	0.1	1.1	0.2
4J	3.8	94.6	1.6	0	3.8	94.6	1.5	0.1	3.6	94.8	0.2	1.2	0.2
4K	3.7	94.7	1.6	0	3.7	94.7	1.5	0.1	3.6	94.8	0.2	1.3	0.1
4L	4.7	93.6	1.7	0	4.7	93.6	1.6	0.1	4.4	93.9	0.2	1.3	0.2
4M	5.1	93.2	1.7	0	5.1	93.1	1.7	0.1	5	93.2	0.3	1.4	0.1
4N	4.3	93.4	2.1	0.2	5.2	92.8	1.8	0.2	5.1	92.9	0.4	1.4	0.2
4O	6.5	90.7	2.1	0.7	6.9	90.3	2.1	0.7	6.9	90.3	0.4	1.6	0.8
4P	9.8	82.7	4.8	2.7	17.6	74.9	4.8	2.7	17.5	75	1.6	3.2	2.7
4Q	14.2	79.3	4	2.5	17	76.5	4	2.5	16.8	76.7	0.9	3.1	2.5
4R	0.5	98.6	0.8	0.1	1.5	97.6	0.8	0.1	1.5	97.6	0.1	0.7	0.1
7C	30.8	58.3	8.3	2.6	34.5	54.6	8.3	2.6	34.5	53.8	1.5	7.4	2.8
8A	39.4	49.1	9.4	2.1	45.9	42.6	9.4	2.1	46.1	41.9	0.4	9.3	2.3
9A	51.6	35.9	11.8	0.7	57.3	30.2	11.7	0.8	57.3	30.1	0.1	11.7	0.8
12A	25.6	60	9.4	5	30.7	54.9	9.2	5.2	31	54.2	0	9.6	5.2
12B	56.2	26.1	9.7	8	61.3	21	9.3	8.4	61.6	20.9	0.2	8.8	8.5
17A	6.8	88.8	3.8	0.6	11.4	84.2	3.7	0.7	11.7	83.4	0	4.2	0.7
18A	0	99.5	0.4	0.1	0	99.5	0.4	0.1	0	99.5	0.1	0.4	0
20A	31.6	35.1	26.4	6.9	34.8	31.9	26.5	6.8	34.9	31.4	2.6	24.3	6.8
21A	50.5	14.7	34.7	0.1	56	9.2	34.7	0.1	56	8.7	0.3	34.7	0.3

Table 12 continued.

Site	30-m riparian buffer land cover (%)				100-m riparian buffer land cover (%)				Watershed land cover (%)				
	Agriculture	Forest	Shrub and woodland	Urban	Agriculture	Forest	Shrub and woodland	Urban	Agriculture	Forest	Freshwater	Shrub and woodland	Urban
24A	32.4	34.1	28.9	4.6	36.7	29.8	28.7	4.8	36.9	29.4	2.6	26.3	4.8
24B	37.9	38.8	20.2	3.1	44.6	32.1	20	3.3	44.6	31.4	0	20.4	3.6
25A	23	47.2	27.8	2	29.6	40.6	27.4	2.4	29.8	40.4	0.8	26.5	2.5
26A	10.8	33.5	54.7	1	15.1	29.2	54.9	0.8	15.3	28.4	3.1	52.4	0.8
27A	14.2	30.1	54.3	1.4	20.6	23.7	53.7	2	20.9	23.1	0.1	53.6	2.3
29B	15.9	53.9	25.2	5	21.8	48	25.2	5	22	47.1	0.2	25.4	5.3
32D	21.3	35.4	37.2	6.1	24.9	31.8	37.1	6.2	24.9	31.5	0.8	36.6	6.2
37G	2.1	28.9	68.7	0.3	3.2	27.8	68.1	0.9	3.4	27.5	0	68.2	0.9
37H	1.9	21.7	75.7	0.7	5.8	17.8	75.6	0.8	5.8	17.2	0	76.2	0.8
39A	32.2	31.5	30.8	5.5	38	25.7	30.8	5.5	38.3	24.8	6.1	25.1	5.7
44B	3.4	92.5	3.7	0.4	9.9	86	3.6	0.5	9.9	85.8	0	3.8	0.5
46B	19.1	36.2	10.8	33.9	25.8	29.5	10.4	34.3	26.1	29	0.8	9.7	34.4

Table 13. Upstream riparian zone and watershed ownership for 37 Puerto Rico stream sampling reaches.

Site	100-m riparian buffer ownership (%)			Watershed ownership (%)		
	Private	Public	Utility and NGO	Private	Public	Utility and NGO
4C	24.7	75.3	0	19.9	80.1	0
4D	0	100	0	0	100	0
4E	0	100	0	0	100	0
4F	0	100	0	0	100	0
4G	0	100	0	0	100	0
4H	0	100	0	0	100	0
4I	15.2	84.8	0	13.4	86.6	0
4J	20.9	77.1	0	16.9	83.1	0
4K	21.3	78.7	0	17.2	82.8	0
4L	21.5	78.5	0	17.4	82.6	0
4M	22.8	77.2	0	18.3	80.7	0
4N	24.6	75.4	0	19.8	80.2	0
4O	25.7	74.3	0	20.8	79.2	0
4P	37.3	62.7	0	33.4	66.6	0
4Q	35.6	64.4	0	32.7	67.3	0
4R	8.2	91.8	0	7.3	92.7	0
7C	91.7	8.3	0	91.1	8.9	0
8A	100	0	0	100	0	0
9A	100	0	0	100	0	0
12A	100	0	0	100	0	0
12B	100	0	0	100	0	0
17A	100	0	0	100	0	0
18A	0	100	0	0	100	0
20A	32.1	67.9	0	31.4	68.6	0
21A	100	0	0	100	0	0
24A	100	0	0	100	0	0
24B	100	0	0	100	0	0
25A	57.4	42.6	0	54.7	45.3	0
26A	54.3	45.7	0	51.1	48.9	0
27A	100	0	0	100	0	0
29B	100	0	0	100	0	0
32D	99.4	0.2	0.4	99.6	0.1	0.3
37G	17.8	82.2	0	16.3	83.7	0
37H	17.7	82.3	0	16.4	83.6	0
39A	100	0	0	100	0	0
44B	17.1	82.9	0	16.7	83.3	
46B	100	0	0	100	0	0



1 - Loíza
2 - Herrera
3 - Espíritu Santo
4 - Mameyes
5 - Sabana
6 - Juan Martín
7 - Fajardo
8 - Dagüao
9 - Palma
10 - Santiago
11 - Blanco
12 - Antón Ruiz
13 - Humacao
14 - Guayanés
15 - Caño de Santiago
16 - Maunabo
17 - Jaraboa
18 - Patillas
19 - Salinas
20 - Jueyes
21 - Coama
22 - Descalabrado
23 - Cañas
24 - Jacaguas
25 - Inabón
26 - Bucaná
27 - Portugés
28 - Matilde
29 - Tallaboa
30 - Macana
31 - Guayanilla
32 - Yauco
33 - Loco
34 - Cartagena
35 - Guanajibo
36 - Yagüez
37 - Añasco
38 - Culebrinas
39 - Guajataca
40 - Camuy
41 - Arecibo
42 - Manatí
43 - Cibuco
44 - La Plata
45 - Bayamón
46 - Piedras

Figure 1. Fish, instream habitat, and water quality sampling sites (*N* = 118) spanning all 46 drainage basins in Puerto Rico. Sites denoted by circles were sampled during project Phase 1 (2005–2007, *N* = 81); those denoted by squares were sampled during Phase 2 (2008–2010, *N* = 37).

CHAPTER 2

SUITABILITY OF CARIBBEAN ISLAND STREAM FISH ASSEMBLAGES AS INDICATORS OF ECOLOGICAL INTEGRITY

(Job 2)

Abstract

Biotic assessment and monitoring to indicate ecological integrity of aquatic ecosystems are needed worldwide. The Index of Biotic Integrity (IBI) approach with fish assemblages has been applied predominantly in warmwater streams and rivers, with the vast majority in temperate regions. Tropical island streams differ from corresponding ecosystems in temperate regions in their ecology, geomorphology, and anthropogenic impacts. The need for effective bioassessment methods is urgent in tropical island aquatic systems, but the efficacy of the IBI approach has been rarely tested in these environments. To investigate the efficacy of applying fish assemblage attributes to assessment in Caribbean tropical island streams, we explored the relationships between fish assemblage parameters and stream and watershed characteristics at 118 sites among all 46 drainage basins in Puerto Rico USA. Correlation analyses between fish assemblage parameters and geographic and physical attributes associated with stream size revealed significant expected relationships, no relationship to water nutrient concentrations, and significant relationships with riparian and watershed land cover, but some of the latter were contrary to expectations. Fish assemblages upstream of a high dam and the associated reservoir differed from those assemblages with no downstream reservoir, and native fish were tolerant to watershed and riparian urbanization. We conclude that, because of distinct fish life history, biogeography, stream geomorphology, migration barrier effects, marine influences, and fish tolerance to highly disturbed conditions, fish assemblages in Puerto Rico cannot serve as suitable indicators of ecological integrity without in-depth analysis or integration of additional physical or biotic data.

Introduction

Biotic assessment and monitoring approaches to indicate ecological integrity of aquatic ecosystems are needed worldwide, and that need may be most pressing among developing countries. Protocols and indices that are socially relevant, quantitative and sensitive, but simple in their application and interpretation, are useful in guiding resource use and management to comply with environmental policy and regulations (O'Connor and Dewling 1986; Kwak and Freeman 2010). Fishes are especially well suited as indicators of environmental quality (Karr et al. 1986; Simon 1999b). They are widely distributed and can accurately reflect environmental conditions at multiple scales; life history and geographic distribution information is extensive for many species; and effective techniques are available for sampling. Additionally, fishes are socially relevant, visible, understood, and valued by regulators, politicians, and the general public.

Fish assemblages, guilds, and taxa have been applied as biotic indicators in multiple ecosystem types throughout the United States. The primary application of a formal biotic index based on fishes is the Index of Biotic Integrity (IBI; Karr et al. 1986). Since its development for wadeable, warmwater streams in the midwestern United States, the IBI has been modified for application in coldwater streams, large rivers, lakes, wetlands, estuaries, and highly-modified habitats, such as reservoirs and tailraces, in various regions of the United States and other countries (Simon 1999a). Most of these modified IBI applications have followed a generally similar framework, in which the fish assemblage sample is interpreted in terms of its compositional, structural, and functional attributes, relative to samples from reference sites or conditions.

The IBI approach has been applied predominantly in warmwater streams and rivers, but the vast majority of those applications are for temperate regions, with most in the United States. Hughes and Oberdorff (1999) reviewed IBI development and application in wadeable streams and small rivers among six continents, with varying modifications for regional fish assemblages and conditions. Some of these worldwide applications span tropical regions, but IBI approaches to assess ecological integrity remain most widely and frequently applied in temperate lotic ecosystems.

Tropical island streams differ from corresponding ecosystems in temperate regions in their ecology, geomorphology, and anthropogenic impacts. Basal production sources and food web processes of tropical islands vary dramatically from those in temperate regions (March and Pringle 2003; Greathouse and Pringle 2006; Covich et al. 2009). Fish assemblages may be dominated by exotic fishes and a few native diadromous species, and the occurrence of endemism is widely variable among islands (Briggs 1984; Kwak et al. 2007; Neal et al. 2009). Streams are often high-gradient, flashy, and spatially condensed (García-Martinó et al. 1996).

Tropical islands may be characterized by periods of rapid human population growth, a general lack of land use planning, deteriorated water quality, and extensive hydrological alteration of flowing waters (Hunter and Arbona 1995; Pringle et al. 2000; Brasher 2003; Fitzpatrick and Keegan 2007; Martinuzzi et al. 2007). Thus, the need for effective biotic assessment methods is urgent in tropical island aquatic systems, but the efficacy of the IBI approach has been rarely tested in these environments.

A biotic assessment protocol based on fish and macroinvertebrate assemblages was recently developed for the stream ecosystems of the Hawaiian Islands (Kido 2012). Kido (2012) suggested that the Hawaiian stream IBI is indicative of human impact on stream ecosystems, and it may be adapted to other Pacific tropical island streams where pristine habitat exists. No such biotic assessment approach based on fish assemblages, however, has been developed or validated for tropical Atlantic or Caribbean island stream ecosystems.

To investigate the efficacy of applying fish assemblage attributes to biotic and ecological assessment in Caribbean tropical island streams, we explored the relationships between fish parameters and stream and watershed characteristics. Identifying candidate biotic metrics and estimating their variability, predictability, and response to physical conditions and human impact is among the first steps in IBI development (Hughes et al. 1998; Barbour et al. 1999). Therefore, we compared fish assemblage parameters with geographic, physical, and chemical parameters to evaluate the suitability of typical fish attributes as biocriteria to indicate ecological integrity of Caribbean island stream ecosystems. Our primary objective in this research was to assess the feasibility of applying fish assemblage attributes from these ecosystems into a standardized IBI or similar bioassessment protocol for Puerto Rico USA and other islands in the Caribbean region.

Study Area

Puerto Rico is the fourth largest of the Antilles Islands. Puerto Rico represents the densest human population in the Caribbean, and ranks amongst the densest populations in the world (Hunter and Arbona 1995) and therefore, may serve as a discrete model system for evaluating the effects of tropical development and human impact on aquatic environments. Agricultural, industrial, and municipal needs led to high demand for water and subsequent damming of almost all of the 46 major rivers in Puerto Rico (Hunter and Arbona 1995, Cooney and Kwak 2013). Stream networks originate in high-elevation mountains and form high-gradient rivers with rocky substrates, waterfalls, and cascades that drain water to mountain foothills and a restricted coastal plain. The stream macrofauna is dominated by decapod crustaceans and fish (Holmquist et al. 1998; Neal et al. 2009). Of the approximately 82 species of fish found on the island (14 orders, 29 families), 26 are primarily freshwater inhabitants (Neal et al. 2009). As few as 10 native freshwater fish species inhabit Puerto Rico, including gobies (Gobiidae, up to five species), sleepers (Eleotridae, three species), one mullet species (Mugilidae), and an eel (Anguillidae). All of these native species are diadromous; the American eel (*Anguilla rostrata*) is catadromous, and the others are amphidromous.

Methods

We surveyed fish assemblages from 118 river reaches in all 46 major river drainages of Puerto Rico from 2005 to 2010 (Figure 1). In 109 of these reaches that were wadeable, fish were sampled quantitatively, and population sizes were estimated. Reaches ranged from 100 to 150 m, incorporating riffle, run, and pool habitats, and were blocked at each terminus with block nets. Three-pass removal methods were conducted with either a barge or two backpack electrofishers, proceeding in an upstream direction, allowing high detection for Caribbean amphidromous fish species (Kwak et al. 2007). All fish were weighed, measured, and identified to species. In nine of the sampling reaches, water depths precluded thorough fish sampling, and fish population estimates were not attained. In these reaches, wadeable areas were sampled thoroughly, and the total catch was quantified to characterize the fish assemblage.

Fish assemblage indices were estimated based on density estimates or total catch for each sampling site to facilitate comparison with physicochemical site attributes. Species richness, the total number of species sampled, was derived for all sites, and further stratified into native and introduced richness. Shannon's species diversity index (*H'*; Krebs 1998; Kwak and Peterson 2007), which accounts for number of species and their relative abundance in a sample, was calculated for each site based on all species and separately for native species. Fish density, biomass, and associated variance were estimated for all species using a three-pass removal method (Seber 1982; Hayes et al. 2007). We stratified population estimates by size group to minimize size bias associated with electrofishing (Kwak 1992; Pine et al. 2003). Population estimates were standardized to units of fish/ha for density and kg/ha for biomass according to species and total for a site. Total, native, and introduced fish species density and biomass estimates were each calculated by combining respective species estimates.

We conducted instream habitat surveys at the 109 stream reaches that were quantitatively sampled for fish following a line-transect survey method to measure physical instream habitat characteristics. We also collected a 1-L water sample at each site from an area of laminar flow and placed it on ice for subsequent analyses. The sample was returned to the lab and analyzed for water quality parameters using a Hach CEL/850 Portable Aquaculture Laboratory. Watershed and riparian attributes, including land cover and geographic characteristics, were attained using existing data analyzed with ArcHydro 1.2 and ArcGIS 9.1 spatial analysis software. Details of instream habitat surveys, water quality procedures, and spatial analyses were reported by Kwak et al. (2007). Site parameters included in this analyses were elevation above sea level (m), watershed area (ha), distance to the ocean (river km), mean stream width (m), water nitrate concentration (mg/L NO_3^-), water orthophosphorus concentration (mg/L PO_4), watershed road density (km/ha), and the percent urban, agricultural, and forest land cover over the site watershed and for the 30-m riparian zone of the upstream network.

We examined the general relationship between fish assemblage parameters and geographic, physical, and chemical parameters by calculating Pearson correlation coefficients for parameter pairs and determining their statistical significance (Zar 1999). We conducted one-way analyses of variance (ANOVA) to detect differences in the means of fish assemblage parameters between stream reaches upstream of a reservoir to those without a downstream reservoir. All statistical comparisons were considered significant at a probability (α) of 0.05.

Results

We sampled 28 fish species representing 16 families among 118 stream reaches and all 46 drainage basins in Puerto Rico (Table 1). Thirteen of these species are native to the island; seven of these are primarily freshwater inhabitants with diadromous life histories, and six are marine species that sporadically ascend freshwater streams. The seven native freshwater species were the most widespread; the sirajo goby, mountain mullet, river goby, and bigmouth sleeper were each collected at over one-half of the sampling sites. The most prevalent exotic fishes were three poeciliids, the guppy, Mexican molly, and green swordtail, which were each sampled at over 30 sites.

The fish assemblages sampled were generally low in species richness and diversity, but frequently included a high density and biomass of fish (Table 2). Mean species richness was 5 species, associated with a mean diversity under 1.0. Fish assemblage density averaged about 9,000 fish/ha, corresponding with a mean biomass of 77 kg/ha. On average, native species occurred at higher frequency and were more abundant by weight, but exotic fishes dominated the average assemblage by number. This trend reflects the occurrence of high numbers of small exotic fishes at sites where they occurred. No fish was detected at one site (20A; Figure 1) in a reach that is known to periodically dry, reducing minimum assemblage statistics to zero.

Geographic, physical, and chemical attributes of stream sampling sites varied widely with spatial location on the island (Table 3). The stream sampling reaches represent a continuum from small, high-elevation headwaters to larger lowland reaches that vary in the degree of human disturbance reflected by nutrient loading, watershed and riparian land-cover, and associated density of roads. Among the most pristine watersheds and stream reaches were found in Río Mameyes in the northeastern region of the island (Figure 1), that rises in the mountain rainforest of El Yunque National Forest. The most degraded stream sampled was Río Piedras, which flows through the San Juan metropolitan area, resulting in the highest watershed and riparian urban land use on the island.

Correlation analyses between fish assemblage parameters and geographic and physical attributes revealed strong relationships between parameters (Table 4). All assemblage parameters showed significant ($P < 0.05$) correlations with one or more of the four physical variables examined, with the exception of exotic fish biomass, which did not correlate with any variable. These consistent findings reflect an expected relationship between fish assemblages and stream size, which is common in biotic assessments using fish, including IBIs. Measures of assemblage richness and diversity generally increased with stream size, and density and biomass decreased with size. Correlations for native and exotic species with geographic and physical attributes were opposite, with exotic fish more diverse and abundant in higher, smaller streams and native fishes more diverse and abundant in downstream, larger reaches.

We detected no significant correlation between any fish assemblage parameters and stream water nutrient concentrations (Table 5). This lack of relationship was not due to low variation in the chemical parameters, as standard deviation values exceeded the mean in both nitrate and orthophosphorus measures, and the maximum measurements of both parameters exceeded corresponding mean values by over four times (Table 3). In comparisons of 11 fish assemblage parameters with 2 chemical parameters (i.e., 22 comparisons), one would expect at least one significant correlation by chance alone with an alpha of 0.05, so this absence of correlation clearly indicates a lack of fish assemblage response to these water quality measures at this spatial scale.

Fish assemblage parameters were significantly correlated with land cover and use attributes at both watershed and riparian scales (Table 6). The relationships for assemblage species richness and diversity were contrary to expectations, with positive correlations with urban land cover and road density, parameters reflecting human impact. Relationships according to native and exotic assemblage components, however, followed expected trends. Native species richness and diversity were inversely correlated with agricultural land cover and positively to forest, while exotic richness and diversity were directly related to road density, urban, and agricultural land uses and inversely to forest cover. Total fish density and biomass followed similar trends to those found and expected for exotic fishes, while native fish density and biomass were not significantly correlated with any watershed or riparian attribute. These findings indicate that the assemblage-level, ecological correlations with land cover and use are driven by exotic fishes.

Comparison of fish assemblages upstream of a high dam and the associated reservoir relative to those assemblages with no downstream high dam and reservoir show clear differences, highlighting the influence of dams on tropical island fish assemblages (Table 7). The influence of a high dam and reservoir was significant in 8 of 11 of the fish assemblage parameters compared. Assemblages upstream of a reservoir were significantly lower in total and native species richness, native diversity, native density; they were higher in exotic richness, total density, exotic density, and exotic biomass. This finding reflects fish assemblages upstream of reservoirs that are dominated by exotic species that complement those with no downstream reservoir where native species are prevalent, suggesting a replacement of native fish by exotic species upstream of high dams and reservoirs.

Examination of fish assemblage and land cover parameter bi-plots indicate inconsistent relationships and fish response to human impacts (Figure 2). While correlation trends may in some cases follow expected fish response, example plots comparing species richness and fish density to urban land cover at the watershed scale show inconsistent relationships. If a fish assemblage is responsive to an independent parameter reflecting human impact, then a wedge-shaped distribution might be expected. And we should expect lower maximum values of native species parameters and higher values for exotic species as human impact increases. The only plot that follows expectations is that for native fish density (Figure 2e), and the five sites with the highest density included at least three native species and were not located upstream of a reservoir. Sites upstream of a reservoir were common outliers to expected distributions.

Discussion

Correlations between fish assemblage parameters and geographic and physical parameters reflecting stream size generally followed expectations, but those relations between fish parameters and chemical and land use parameters conformed to expectations only in some cases. In most stream networks, fish species richness and diversity increase with stream and watershed size (Fausch et al. 1984; Karr et al. 1986). In Puerto Rico streams, however, this trend is reversed for exotic species, which we found can be very abundant in small upstream reaches (this study; Kwak et al. 2007). The lack of correlation of fish assemblage attributes with water nutrient concentrations is a surprising result, as nutrient pollution and ecosystem eutrophication is a common anthropogenic impact to lotic ecosystems and is known to alter the fish assemblage (Carpenter et al. 1998). This unexpected result suggests that other water quality or physical habitat attributes exert a greater effect on fish occurrence than nutrient pollution.

Correlations of fish assemblage richness and diversity with land cover were contrary to expectations, as we found a positive relationship with road density and urban land use. This appeared to be a dominant response by the exotic fish component of the assemblage. The consequences of urbanization are known to negatively affect stream function, habitat, and fish assemblages, in both tropical and temperate regions (Allan 2004; Walsh et al. 2005; Daga et al. 2012), but our correlational findings revealed a more complex relationship, which is also revealed in fish–land cover bi-plots (Figure 2). For example, the two stream reaches that we sampled in Río Piedras with the highest watershed urban land cover (39.4%, 46A; 34.4%, 46B) flowing through metropolitan San Juan are clearly impacted by urbanization at multiple scales, yet these sites both supported six species of native freshwater fish, virtually the entire freshwater fish fauna of the island. The only urbanized stream habitat that appears to show a reduced native fish species richness response is concrete-channelized reaches that support fish assemblages with few to no native species and abundant, tolerant exotic species (Engman and Ramírez 2012).

The inconsistent response by Puerto Rico stream fish assemblages to geographic and physicochemical attributes known to influence fish may be due to multiple biological and ecological factors unique to these tropical island assemblages. These include fish biogeography and life history, stream geomorphology and network characteristics, climate and disturbance regime, instream barriers to fish migration, and biotic interactions among species.

Bioassessment with fishes is most effective in regions and ecosystems with species rich and diverse assemblages (i.e., high variation). The low number of native freshwater fish species (7–10 species; only 7 can be identified in the field) that occur in Puerto Rico reduces the variation and utility of this parameter for bioassessment. The distribution of some of these species is related to habitat affinity; for example, two native freshwater fishes, the smallscaled spinycheek sleeper and fat sleeper, are generally restricted to low-elevation, low-gradient, downstream reaches or in brackish water (Corujo Flores 1980; Kwak et al. 2007). Further, numerous primarily marine and estuarine native fishes periodically ascend rivers (5 species in our sampling; Table 1), adding variation to any species richness or diversity measure.

All native freshwater fishes are diadromous and require connectivity between freshwater stream and marine habitats to complete their life cycle (Kwak et al. 2007; Neal et al. 2009). All but one of the native freshwater species follow an amphidromous life history; the exception is the catadromous American eel. Amphidromous fish spawn in the stream environment, larvae drift to the estuary or ocean to develop, and ascend the river as postlarvae (McDowall 2007). Ocean currents may transport early life stages and mix amphidromous fish populations among islands and among river basins within islands to create dynamic source–sink mechanisms (McDowall 2007; Cook et al. 2009). Thus, a river that may lack the habitat to support viable populations of native fishes or is subject to frequent disturbance, may be recolonized periodically through marine dispersal of larval stages (McDowall 2010). Diadromy in native freshwater fishes may reduce their discriminatory ability for bioassessment applications.

Stream barriers play an important role in diadromous fish distribution and abundance, with limitations depending on the ability of each species to ascend or descend an obstruction (McDowall 2010). Diadromous fish species that lack strong jumping or climbing abilities are restricted to lower elevation stream reaches downstream of major migration impediments

(Holmquist et al. 1998; Cooney and Kwak 2013). Cooney and Kwak (2013) identified 335 artificial instream barriers (dams and road crossings) in Puerto Rico and empirically modeled a pattern of native freshwater species loss from river mouth to headwater streams, depending on the type and size of barrier, with complete extirpation upstream of high dams and reservoirs. The native species are commonly replaced by exotic fishes in habitats where they have been extirpated. Two of the native goby species on the island have fused pelvic fins that form a suction disc, allowing ascension of steep, wetted gradients and waterfalls, but not dry, high dams (Schoenfuss et al. 2011; Cooney and Kwak 2013). Therefore, it is common to find only a single goby species or no native fish upstream of an artificial reservoir, but the same may occur upstream of a large waterfall in a pristine stream; both would result in the same native species richness in a biotic assessment.

The topography, geomorphology, and hydrology of Puerto Rico streams are rather unique and complicate application of fish bioassessment methods. The Luquillo Mountains of Puerto Rico are of volcanic origin and reach heights greater than 1,000 m within 20 km of the island coast (Pike et al. 2010). The steep topography interacts with northeasterly trade winds and frequent tropical storms to produce extremely high rainfall (averaging nearly 5 m annually; Lugo et al. 2012) and flood-dominated rivers, typical in hydrology to other Antillean rivers. Spates 50 times greater than base flow are common in the region, and river hydrographs are flashy, often peaking and returning to near base flows within 24 hours.

Bioassessment Implications

Our findings in this investigation suggest that a tropical Caribbean island may be one of the few exceptional regions where lotic fish assemblages are not suitable indicators of human impacts and ecological integrity. The IBI concept was originally developed for application in wadeable midwestern U.S. warmwater streams, but has been applied widely across the globe in other aquatic ecosystems (Karr et al. 1986; Hughes and Oberdorff 1999; Simon 1999a). Tropical insular stream ecosystems differ dramatically from temperate mainland streams in their physical surroundings, ecology, fish faunas, and human impacts, so it is logical that direct application of many metrics developed elsewhere would not apply on tropical islands (Pringle et al. 2000; Neal et al. 2009). This direct incompatibility does not necessarily preclude application of the concept of indicator taxa to biotic assessment on the island. Further exploration of these physical–biotic relationships and classification of streams or stream reaches may identify criteria thresholds that were not apparent in our analyses.

Definitions of integrity and one's philosophical approach may affect conclusions on the suitability and utility of fish or other biota as indicators for bioassessment. Biotic integrity of an ecosystem is the capability of supporting and maintaining an integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of a natural habitat of the region (Karr and Dudley 1981). And following that, ecological integrity is the summation of chemical, physical, and biological integrity and extends beyond fish to represent a holistic approach for ecosystem management (Kwak and Freeman 2010). If native diadromous fish are extirpated from a pristine mountain tropical rainforest stream by the presence of a high dam in downstream waters, has that stream lost all biotic integrity or ecological integrity? A logical response is that if the native fishes are extirpated, then the ecosystem has no biotic or ecological integrity. Most government agencies and other constituents, however, apply bioassessment with fish or invertebrates to reflect the physical and chemical environmental conditions to inform conservation and management planning. In that context, when a single stressor (e.g., a dam or other migration barrier) dramatically alters the fish biota, but local water quality and physical habitat remain in a relatively unaltered state, the question becomes more complex, as other biota and ecological processes may also remain intact. If the presence of an unaltered fish assemblage is the single biocriterion for conservation planning and implementation, then the fish indicator and IBI approach is not appropriate without additional physical and biotic assessment.

One approach that warrants additional consideration is combining fish and macroinvertebrate biota into ecological assessment of tropical island streams. Diadromous shrimp are abundant in Puerto Rico streams and serve important ecological functions (March and Pringle 2003). Yet they are subject to the same influences of artificial and natural instream barriers as diadromous fish, and their distribution in streams may not reflect habitat quality at the reach scale (Covich et al. 2009; Crook et al. 2009). Other non-crustacean macroinvertebrates are known to be responsive to habitat quality and human influences, and benthic macroinvertebrate multimetric indices have been developed and applied widely (Barbour et al. 1999; Rosenberg et al. 2008). The only published and validated IBI based on fish for tropical island streams was developed for Hawaiian island streams and includes metrics for both benthic fishes and macroinvertebrates (Kido 2012). Such an approach may be similarly applicable to Caribbean island streams.

Ecological and local knowledge of the fauna, habitat, and data upon which bioassessment indices are based is critical to their effective development. Development of biocriteria from existing data by investigators unfamiliar with the sampling protocol, habitat requirements and sensitivities of taxa, physical and biotic interactions and processes, and local distinctive features of the instream environment and landscape risk development of an erroneous protocol. In the case of Puerto Rico fish assemblages, it would be possible to naively incorporate our findings into a multimetric index and proceed to misapply it, but with in-depth knowledge of the local fishes, stream environment, and human influences, we advise caution.

In this investigation to assess the feasibility of applying fish assemblage attributes from freshwater streams into a standardized IBI or similar bioassessment protocol for Puerto Rico and other islands in the Caribbean region, we conclude that, because of distinct fish life history, biogeography, stream geomorphology, migration barrier effects, marine influences, and fish tolerance to highly disturbed conditions, fish assemblages in Puerto Rico cannot serve as suitable indicators of ecological integrity without in-depth analysis or integration of additional physical or biotic data. We consider our findings and conclusions to be a first step that reveals the unique features and attributes of Caribbean streams and their fauna that differ from other temperate systems in a bioassessment context. We look forward to future advancements in bioassessment approaches and methods in Caribbean tropical island streams to further understanding of these systems and inform conservation policy and resource management in the region.

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Table 1. Fishes sampled and their occurrence among 118 sites in the freshwater streams and rivers of Puerto Rico. Exotic species are denoted with an asterisk. The sirajo goby *Sicydium plumieri* has been split into four *Sicydium* species (*S. buscki, S. gilberti, S. plumieri, and S. punctatum*; Watson [2000]), which are combined here as *S. plumieri*.

Family	Common name	Scientific name	Number of sites
Anguillidae	American eel	*Anguilla rostrata*	57
Belonidae	Atlantic needlefish	*Strongylura marina*	1
Centrarchidae	Redbreast sunfish	*Lepomis auritus**	4
	Bluegill sunfish	*Lepomis macrochirus**	1
	Largemouth bass	*Micropterus salmoides**	6
Centropomidae	Fat snook	*Centropomus parallelus*	1
Cichlidae	Convict cichlid	*Archocentrus nigrofasciatus**	2
	Mozambique tilapia	*Oreochromis mossambicus**	27
	Nile tilapia	*Oreochromis niloticus**	1
	Redbreast tilapia	*Tilapia rendalli**	5
Cyprinidae	Rosy barb	*Puntius conchonius**	9
Eleotridae	Fat sleeper	*Dormitator maculatus*	1
	Smallscaled spinycheek sleeper	*Eleotris perniger*	44
	Bigmouth sleeper	*Gobiomorus dormitor*	63
Gobiidae	River goby	*Awaous banana*	66
	Sirajo goby	*Sicydium plumieri*	74
Gyrinocheilidae	Chinese algae eater	*Gyrinocheilus aymonieri**	1

Table 1 concluded.

Family	Common name	Scientific name	Number of sites
Haemulidae	Burro grunt	*Pomadasys crocro*	9
Ictaluridae	Channel catfish	*Ictalurus punctatus**	7
Loricariidae	Amazon sailfin catfish	*Pterygoplicthys pardalis**	8
Lutjanidae	Grey snapper	*Lutjanus griseus*	3
Mugilidae	Mountain mullet	*Agonostomus monticola*	69
	White mullet	*Mugil curema*	1
Poeciliidae	Sailfin molly	*Poecilia latipinna**	2
	Guppy	*Poecilia reticulata**	53
	Mexican molly	*Poecilia sphenops**	38
	Green swordtail	*Xiphophorus hellerii**	32
Syngnathidae	Shorttail pipefish	*Microphis brachyurus*	1

Table 2. Puerto Rico stream fish assemblage statistics. Sample sizes were 118 sites for assemblage richness and diversity parameters and 109 for fish density and biomass parameters.

Parameter	Mean	SD	Minimum	Maximum
Species richness	4.97	2.08	0	11.00
Native species richness	3.31	2.32	0	9.00
Exotic species richness	1.66	1.87	0	11.00
Species diversity (H')	0.888	0.434	0	1.860
Native species diversity (H')	0.638	0.521	0	1.860
Total density (number/ha)	8,997	14,125	0	83,101
Native density (number/ha)	2,696	4,241	0	28,140
Exotic density (number/ha)	6,301	14,602	0	83,101
Total biomass (kg/ha)	76.71	99.56	0	621.90
Native biomass (kg/ha)	55.52	93.75	0	621.90
Exotic biomass (kg/ha)	21.19	45.45	0	235.10

Table 3. Puerto Rico stream geographic, physical, and chemical statistics for 118 sampling sites.

Parameter	Mean	SD	Minimum	Maximum
Elevation (m)	143.7	166.2	3.0	702.4
Watershed area (km^2)	21.52	23.24	1.01	124.09
Distance to ocean (rkm)	23.66	20.82	1.77	93.90
Mean stream width (m)	7.65	5.42	2	27.24
Nitrate (mg/L NO_3^-)	3.27	3.84	0	25.80
Orthophosphorus (mg/L PO_4)	0.66	0.73	0	2.75
Watershed				
Road density (km/ha)	0.034	0.018	0.001	0.098
Urban (%)	3.78	5.91	0	39.40
Agricultural (%)	33.33	21.65	0	82.30
Forest (%)	48.68	28.28	5.40	99.80
30-m riparian				
Urban (%)	3.21	5.52	0	33.90
Agricultural (%)	30.51	22.23	0	82.00
Forest (%)	50.98	27.65	4.20	99.50

Table 4. Pearson correlation coefficients (*r*) comparing fish assemblage parameters with corresponding geographic and morphological parameters. Sample sizes were 118 sites for assemblage richness and diversity parameters and 109 for fish density and biomass parameters. Bold statistics are significant ($P < 0.05$).

Fish assemblage parameter	Elevation (m)	Watershed area (km^2)	Distance to ocean (rkm)	Mean stream width (m)
Species richness	**-0.421**	**0.492**	**-0.217**	0.055
Native species richness	**-0.612**	**0.461**	**-0.556**	**0.292**
Exotic species richness	**0.292**	-0.025	**0.447**	**-0.302**
Species diversity (*H'*)	**-0.428**	**0.351**	**-0.344**	0.119
Native species diversity *(H'*)	**-0.619**	**0.376**	**-0.566**	**0.291**
Total density (number/ha)	**0.266**	**-0.191**	**0.327**	**-0.275**
Native density (number/ha)	**-0.260**	0.029	**-0.301**	-0.107
Exotic density (number/ha)	**0.333**	**-0.194**	**0.404**	**-0.235**
Total biomass (kg/ha)	**-0.230**	0.104	-0.162	-0.184
Native biomass (kg/ha)	**-0.289**	0.139	**-0.260**	-0.111
Exotic biomass (kg/ha)	0.092	-0.056	0.181	-0.172

Table 5. Pearson correlation coefficients (*r*) comparing fish assemblage parameters with corresponding stream water nutrient concentration parameters. Sample sizes were 118 sites for assemblage richness and diversity parameters and 109 for fish density and biomass parameters. No *r* values were significant ($P > 0.05$).

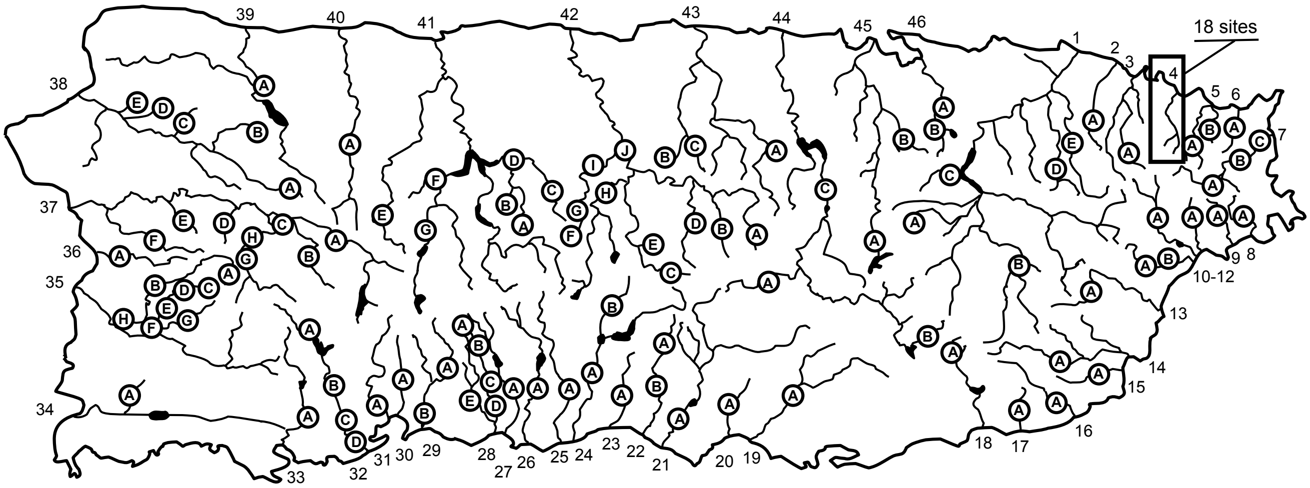
Fish assemblage parameter	Nitrate (mg/L NO_3^-)	Orthophosphorus (mg/L PO_4)
Species richness	-0.041	-0.142
Native species richness	-0.173	-0.142
Exotic species richness	0.170	0.018
Species diversity (*H′*)	-0.040	-0.077
Native species diversity *(H′*)	-0.138	-0.068
Total density (number/ha)	0.139	0.038
Native density (number/ha)	-0.041	-0.003
Exotic density (number/ha)	-0.173	-0.142
Total biomass (kg/ha)	-0.018	-0.063
Native biomass (kg/ha)	-0.052	-0.086
Exotic biomass (kg/ha)	0.066	0.037

Table 6. Pearson correlation coefficients (*r*) comparing fish assemblage parameters with corresponding stream watershed and riparian parameters. Sample sizes were 118 sites for assemblage richness and diversity parameters and 109 for fish density and biomass parameters. Bold statistics are significant ($P < 0.05$).

	Watershed				30-m riparian		
Fish assemblage parameter	Road density (km/ha)	Urban (%)	Agricultural (%)	Forest (%)	Urban (%)	Agricultural (%)	Forest (%)
Species richness	**0.261**	**0.309**	0.026	-0.046	**0.380**	0.065	-0.079
Native species richness	-0.104	-0.012	**-0.291**	**0.283**	0.157	**-0.263**	**0.255**
Exotic species richness	**0.418**	**0.359**	**0.390**	**-0.403**	**0.228**	**0.399**	**-0.404**
Species diversity (*H'*)	0.045	**0.205**	-0.140	0.084	**0.212**	-0.095	0.078
Native species diversity (*H'*)	-0.118	0.052	**-0.274**	**0.230**	0.171	**-0.263**	**0.221**
Total density (number/ha)	**0.269**	**0.274**	**0.292**	**-0.297**	**0.247**	**0.229**	**-0.276**
Native density (number/ha)	-0.031	-0.093	-0.042	0.072	0.012	-0.019	0.044
Exotic density (number/ha)	**0.269**	**0.293**	**0.294**	**-0.308**	**0.236**	**0.227**	**-0.279**
Total biomass (kg/ha)	0.153	**0.191**	0.095	-0.187	**0.363**	0.075	**-0.191**
Native biomass (kg/ha)	0.039	0.015	-0.031	-0.049	0.186	-0.033	-0.059
Exotic biomass (kg/ha)	**0.255**	**0.386**	**0.273**	**-0.309**	**0.411**	**0.234**	**-0.298**

Table 7. Means of fish assemblage parameters from Puerto Rico stream reaches upstream of a reservoir and high dam and those with no downstream reservoir or high dam compared using one-way analysis of variance and resulting statistics.

Fish assemblage parameter	Mean among sites with no downstream reservoir	Mean among sites with downstream reservoir	*F*	*P*
Species richness	5.18	4.05	5.51	0.0207
Native species richness	3.84	0.96	36.14	<0.0001
Exotic species richness	1.33	3.09	18.16	<0.0001
Species diversity (H')	0.920	0.748	2.85	0.0942
Native species diversity (H')	0.729	0.242	17.89	<0.0001
Total density (number/ha)	6,476.6	21,740.1	20.76	<0.0001
Native density (number/ha)	3,108.9	607.3	5.44	0.0215
Exotic density (number/ha)	3,367.8	21,132.8	27.76	<0.0001
Total biomass (kg/ha)	75.17	84.49	0.13	0.7182
Native biomass (kg/ha)	62.31	21.19	2.94	0.0891
Exotic biomass (kg/ha)	12.86	63.31	22.13	<0.0001



1. Loíza
2. Herrera
3. Espíritu Santo
4. Mameyes
5. Sabana
6. Juan Martín
7. Fajardo
8. Dagüao
9. Palma
10. Santiago
11. Blanco
12. Antón Ruiz
13. Humacao
14. Guayanés
15. Caño de Santiago
16. Maunabo
17. Jacaboa
18. Patillas
19. Salinas
20. Jueyes
21. Coama
22. Descalabrado
23. Cañas
24. Jacaguas
25. Inabón
26. Bucaná
27. Portugés
28. Matilde
29. Tallaboa
30. Macaná
31. Guayanilla
32. Yauco
33. Loco
34. Cartagena
35. Guanajibo
36. Yagüez
37. Añasco
38. Culebrinas
39. Guajataca
40. Camuy
41. Arecibo
42. Manatí
43. Cibuco
44. La Plata
45. Bayamón
46. Piedras

Figure 1. Fish, instream habitat, and water quality sampling sites (*N* = 118) spanning all 46 drainage basins in Puerto Rico.

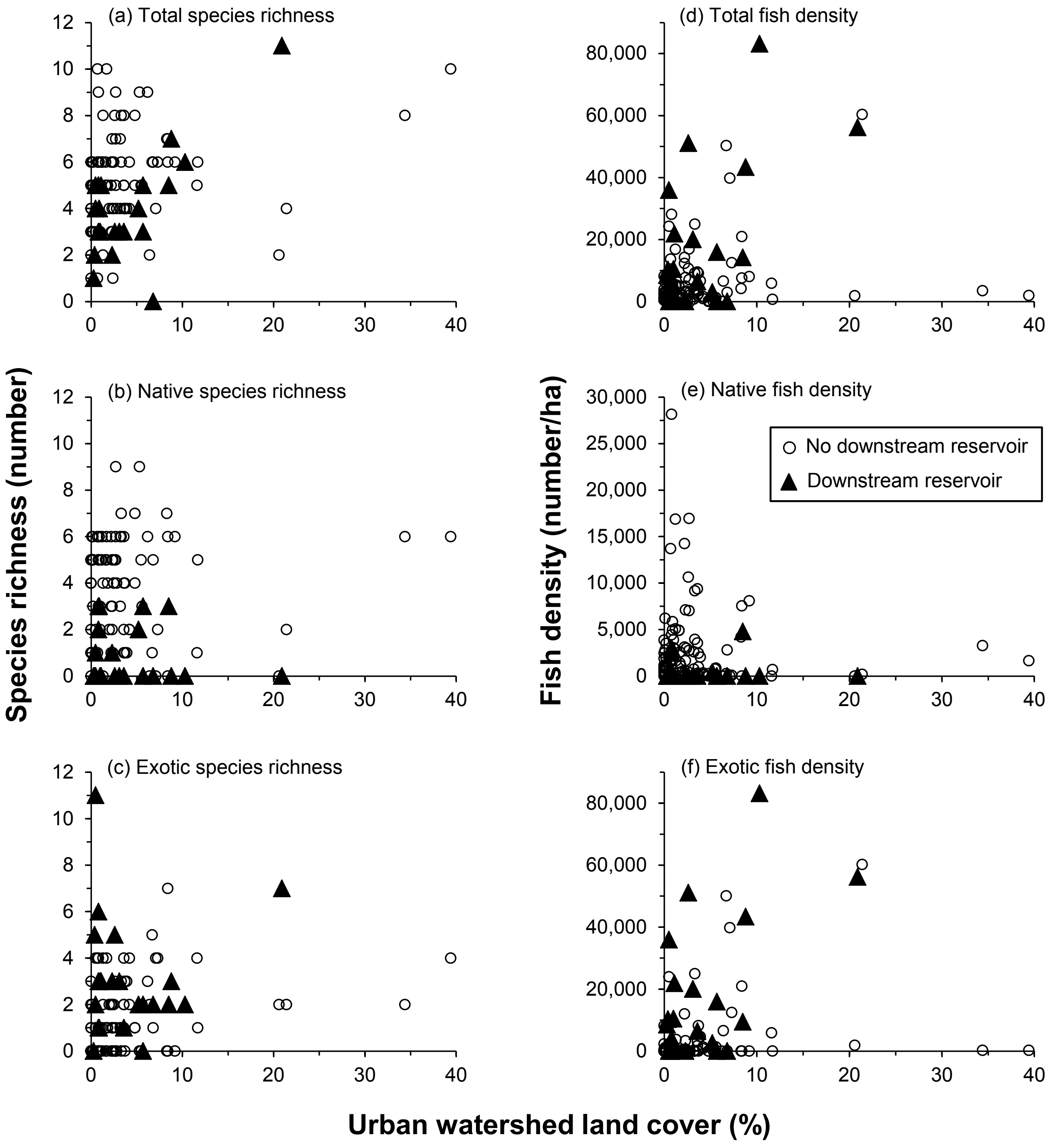


Figure 2. Bi-plots of fish species richness (a–c) and fish density (d–f) versus urban watershed land cover according to the presence of a downstream reservoir for Puerto Rico stream sampling sites. Sample sizes were 118 sites for species richness parameters and 109 for fish density parameters.

CHAPTER 3
WATER, SEDIMENT, AND BIOTA CONTAMINATION OF TROPICAL ISLAND STREAM ECOSYSTEMS IN RELATION TO LAND USE
(Job 3)

Abstract

Manufactured chemicals are continuously released into the environment with a variety of adverse ecological and human health effects. Puerto Rico has a history of anthropogenic chemical usage, and its human population density is among the highest globally, providing a model environment to study human impacts on tropical island stream ecosystems. Our objectives were to quantify occurrences of Polycyclic Aromatic Hydrocarbons (PAHs), historic-use chlorinated pesticides, current-use pesticides, Polychlorinated Biphenyls (PCBs), and metals (mercury, cadmium, copper, lead, nickel, zinc, and selenium) in the habitat and biota of Puerto Rico streams and associate those findings with land-use patterns. We sampled water, sediment, and native fish and shrimp species at 13 sites spanning broad riparian and watershed land-use patterns (e.g., urban, agricultural, industrial, and forested) and conducted intensive sampling at four of these sites. Overall, our findings indicated that stream ecosystems in Puerto Rico were not severely polluted, with the exception of nickel in sediment at sites associated with agricultural watersheds. While nickel concentrations were greatest at agricultural sites, a site with a highly urbanized watershed generally had the greatest concentrations of most classes of contaminants. PCBs may pose human health hazards with some fish concentrations exceeding the EPA consumption limit for 1 meal/month; greatest concentrations were in mountain mullet (*Agonostomus monticola*) and American eel (*Anguilla rostrata*). Bigmouth sleepers (*Gobiomorous dormitor*) may be the most suitable fish for human consumption with low levels of organic contaminants, but mercury accumulation exceeded EPA's consumption limit for 3

meals/month at 1 of the 13 sites. These results provide public health and natural resource agencies the scientific information required to guide ecosystem and fisheries management and human health risk assessment.

Introduction

Over 100,000 anthropogenic chemicals are released into the environment annually (Shea 2010). Some aquatic organisms may accumulate contaminants that are present in low concentrations in the environment to levels that are harmful to the organism and also exceed human health guidelines (Thrower and Eustace 1973). These contaminants exert a variety of adverse effects on organisms, including alterations in behavior (Chura and Stewart 1967) and morphology (Park et al. 2001), reproductive abnormalities (Guillette 1999), and mortality. Water pollution is especially problematic for tropical islands with dense human populations (Hunter and Arbona 1995). Few contaminant studies have been conducted in the Caribbean (Rodríguez and Pérez de González 1981; Neal et al. 2005), and no study has examined the effects of contamination in stream ecosystems there. More knowledge is needed on the occurrence and patterns of contaminants in Caribbean stream ecosystems to inform ecosystem and fisheries management and human health risk assessments.

Contaminant issues are of global importance because pollutants are known to demonstrate long-range, trans-continental, transport (Oehme 1991; Welch et al. 1991; Iwata et al. 1993; Burkow and Kallenborn 2000). Garrison et al. (2006) suggest that dust air masses from the Sahara Desert, Africa, are likely a source of atmospheric persistent organic pollutants (POPs) at downwind Caribbean sites. In the Caribbean, surface currents move rapidly, transporting pollutants from other areas and spreading pollution from island to island (Ross and DeLorenzo 1997). Several deep ocean basins in the Caribbean receive very little renewal or flushing and coastal upwelling could potentially cause release of high contaminant concentrations (Rawlins et al. 1998). These oceanographic features in combination with locally high human population densities and associated activities make the Caribbean especially susceptible to the accumulation of contaminants (Hunter and Arbona 1995; Ross and DeLorenzo 1997).

Puerto Rico is a densely populated Caribbean island, supporting nearly 440 people per square kilometer, providing an appropriate model to study aquatic contaminant dynamics (Martinuzzi et al. 2007; Neal et al. 2009). During the past century, rapid industrialization and the subsequent human population growth have strained the limited natural resources of the island (Hunter and Arbona 1995). Water is a scarce resource in Puerto Rico because there are no natural lakes. Most of the rivers have been transformed by dam construction or other structures that are conducive for water collection (Cooney and Kwak 2010). However, the Puerto Rican people are becoming increasingly aware of the benefits associated with conserving stream ecosystems. González-Cabán and Loomis (1996) demonstrated that citizens would be willing to pay a total of $11.33 million to prevent dam construction on Río Mameyes, the last remaining pristine river in Puerto Rico.

The streams of Puerto Rico provide many services for local populations, including water for drinking, recreation, irrigation, and as a source of fish and crustaceans for consumption. Therefore, good water quality is necessary to protect human health as well as ecological integrity. However, the island has experienced an era of rapid human population growth leading to deteriorated water quality (Hunter and Arbona 1995; Fitzpatrick and Keegan 2007). The streams have a history of die-offs of fish, shellfish, shrimp, and domesticated animals, which were contaminated by industrial, agricultural, and municipal wastes (Hunter and Arbona 1995). Epidemiological evidence also suggests that water contamination has jeopardized human health (Colón et al. 2000). For example, Puerto Rico has the highest incidence of premature breast development (thelarche) in girls, with some affected patients younger than two years of age (Colón et al. 2000). High levels of endocrine-disrupting chemicals have been found in the serum of Puerto Rican girls with premature thelarche. Yet, there is a notable lack of research and available information on the degree and effects of water contamination in Puerto Rico.

Contaminants and land use

Rivers and streams are influenced by their surrounding landscapes (Hynes 1975; Vannote et al. 1980; Allen 1994). Direct correlations have been clearly demonstrated between land use and water quality (Lenat and Crawford 1994; Bolstad and Swank 1997; Fisher et al. 2000; Tong and Chen 2002). Surface runoff, especially after a drought, is a major contributor to non-point source pollution because it transports sediment and associated chemicals into aquatic ecosystems. Runoff from varying types of land use is enriched with different contaminants; for example, runoff from urban areas may be enriched with rubber fragments and heavy metals, whereas runoff from agricultural lands may be enriched with fertilizers and pesticides (Lenat

1984; Osborne and Wiley 1988; Cooper 1993; Johnson et al. 1997; Tong and Chen 2002). Further, vegetation modifies land surface characteristics, water balance, and the hydrologic cycle through evapotranspiration, interception, infiltration, percolation, and absorption (Tong and Chen 2002). Human-altered land use also transforms the hydrological system by changing runoff dynamics and composition and quality of receiving water bodies (Changnon and Demissie 1996; Mander et al. 1998; Warne 2005).

Puerto Rico has undergone a number of anthropogenic alterations to its landscape as a result of agriculture, deforestation, stream channelization, industrial and municipal pollution, urbanization, and impoundment of rivers (Neal et al. 2009). Historically, Puerto Rico's economy was predominantly agricultural, but in the early 1900s, global markets changed and the economy shifted toward industry and tourism (Warne et al. 2005). While rapid industrialization of Puerto Rico most likely lead to an increase in contaminant volume and diversity, tourism relies upon oceans, beaches, and other minimally disturbed areas such as the El Yunque National Forest (Warne et al. 2005). Therefore, it is imperative that contaminants and water quality be quantified in Puerto Rico stream ecosystems to guide natural resource planning and economic development.

Objective

The purpose of this study was to quantify contaminants in water, sediment, and biota in the stream ecosystems of Puerto Rico across a spectrum of watershed land-use patterns. This was accomplished by first surveying habitat and biota extensively island-wide for contaminants and then conducting more intensive studies in selected areas. Associations among land use and contaminant occurrence in stream ecosystems may then suggest relationships for future investigations.

Methods

Field survey sites were selected based on presence of target species and watershed land use. Prior knowledge of target species distribution and abundance was provided by Kwak et al. (2007). Thirteen of the 46 major river drainages in Puerto Rico were sampled, and sites were categorized based on primary watershed land-use patterns or distinctive riparian features as one reference site within a primarily forested watershed [Río Mameyes (1R)], two industrial sites [Río Tallaboa (2I), Río Cañas (3I)], two urban sites [Río Piedras (4U), Río Bayamon (5U)], seven agricultural sites [Río Añasco (6A), Río Yauco (7A), Río La Plata (8A), Río Jacaguas (9A), Río Guanajibo (10A), Río Cartagena (11A), Río Arecibo (12A)], and one site with substantial recreational fishing effort [Río Fajardo (13A)] (Table1; Fig. 1).

Data from the initial extensive contaminant sampling (13 sites) were used to select sites and contaminants for additional intensive sampling, to include more species and replicate samples. Four sites, among those sampled in the extensive contaminant survey, were selected for intensive contaminant investigation to represent specific water quality or watershed land-use effects. These sites are 1R (reference), 7A (agricultural), 3I (industrial), and 4U (urban). Water, sediment, and biota were collected at each sampling site.

Water chemistry is known to affect bioavailability and degradation of contaminants. For example, hardness influences bioavailability of metals as explained by the free ion activity model (Morel 1983). Thus, physicochemical water parameters were measured using a Yellow Springs Instrument (YSI) 556 multi-probe system and a Hach CEL/850 Portable Aquaculture Laboratory and included temperature, pH, alkalinity (mg/L $CaCO_3$), total hardness (mg/L $CaCO_3$), conductivity (µS/cm), nitrate concentration (µg/L NO_3^-), nitrite concentration (mg/L NO_2^-), and orthophosphorus concentration (mg/L PO_4). Water was collected using a 1-L container, rinsed repeatedly with site water, and then was submersed 0.25-0.50 m beneath the water surface, filled, and stored on ice in a cooler.

Universal passive sampling devices (uPSDs)

Time-integrated contaminant concentrations in water were sampled using Universal Passive Sampling Devices (uPSDs). Passive sampling devices are a less labor-intensive method for sampling and measuring water contaminants (Heltsley et al. 2005). They estimate ecologically relevant, chronic contaminant exposure (Hirons 2009) and bioconcentration for aquatic species (Heltsley et al. 2005). UPSDs offer advantages over traditional grab sampling because they represent exposure of the bioavailable portion and they collect transient contaminants at trace levels (Hirons 2009).

Two types of uPSDs were used in this study. Fiber passive sampling devices (fPSDs) were used for extensive sampling and cartridge passive sampling devices (cPSDs) were used for intensive sampling. FPSDs have a surface area of 5.8 cm^2, and cPSDs have an internal surface area of 6.2 cm^2 (Hirons 2009). The fPSDs are hollow, polyethersulfone fibers filled with Waters

Oasis HLB® sorbent, with a diameter of 1 mm and pore size of 0.2 µm. CPSDs are incased in porous, stainless steel and filled with the same polymeric sorbent, Oasis HLB®. Three fPSDs were deployed at each site during the extensive survey, and 6 cPSDs were deployed at each site during the intensive study. They stayed submersed in the water for 3-4 weeks. Each uPSD was wrapped in aluminum foil immediately upon retrieval and placed inside a plastic bag with a label, indicating the retrieval date and time, sampling location, and condition of the uPSD. The uPSDs were kept on ice inside a cooler until they could be transferred to a -20˚C freezer.

Sediment

One composite sediment sample was collected from each site for the extensive study, and three per site for the intensive study, using a stainless steel scoop, rinsed with site water prior to use. Each sample consisted of 3 to 5 scoops from depositional areas, within the site area, totaling approximately 0.75 L. Only sediment from the biologically-active, surface layer (top 5 cm) was collected and any rocks, debris, or biota were removed. Each sample was sealed in a plastic bag, stored on ice in a cooler, and then transferred to a -20˚C freezer as soon as possible.

Measurements of sediment contaminant concentrations are influenced by a number of covariates and require careful interpretation (Hoffman et al. 2003; Luoma and Rainbow 2008). Thus, we measured total organic carbon, particle-size, and iron concentration of sediment samples in this study for normalization purposes. Aliquots from sediment samples were dried at 60°C and sent to the Environmental and Agricultural Testing Service Laboratory in the Department of Soil Science at North Carolina State University in Raleigh, North Carolina, for analysis of total carbon content of each sample and to the Soil Physical Properties Laboratory in the Department of Soil Science at North Carolina State University for particle size analysis, using the hydrometer method (Gee and Or 2002). If organic matter exceeded 2%, samples were treated with hydrogen peroxide. Freeze-dried sediment aliquots were analyzed for iron concentration by Environmental Conservation Laboratories in Cary, North Carolina, using EPA Method 6010C (www.epa.gov/sam).

Fish and shrimp

Few native fish inhabit the streams of Puerto Rico and other islands in the Caribbean and Greater Antilles because these volcanic islands are relatively newly formed and are isolated from potential sources of colonizing species (Neal et al. 2009). Only six freshwater native fish are commonly found in Puerto Rico and all share common specialized life history traits, specifically they are diadromous (Kwak et al. 2007; Neal et al. 2009; Cooney and Kwak 2010). Samples of all native freshwater fish species were analyzed for contaminants, with the exception of the fat sleeper (*Dormitator maculatus*), which was not collected at any site. Native species sampled included bigmouth sleeper (*Gobiomorous dormitor*), smallscaled spinycheek sleeper (*Eleotris perniger*), American eel (*Anguilla rostrata*), mountain mullet (*Agonostomus monticola*), sirajo goby (*Sicydium* spp.), river goby (*Awaous banana*), and *Macrobrachium* shrimp. Exotic species, introduced by anglers, the aquaculture industry, and aquarium owners, are commonly found in Puerto Rico. Although, this study focused on the native species, because of their natural heritage value and because indigenous and exotic species have different distributions, some exotic species were collected including Nile tilapia (*Oreochromis mossambicus*), redbreast sunfish (*Lepomis auritus*), and channel catfish (*Ictalurus punctatus*). Target species represent different taxa and feeding strategies and are consumed by humans, except for the smallscaled spinycheek sleeper. Fish and shrimp were collected using backpack electrofishing (Kwak et al. 2007). Specimens were sorted by species into labeled plastic bags, cooled, and then transferred as soon as possible to a -20˚C freezer.

Fish and shrimp were analyzed as composite samples using whole body or muscle tissue. The whole body of sirajo gobies was analyzed because the local people consume the whole body of these fish, as do instream and avian predators. The whole body of river gobies and spinycheek sleepers was analyzed for contaminants, for similar reasons. The edible muscle, excluding skin or scales, of American eel, Nile tilapia, bigmouth sleeper, redbreast sunfish, and channel catfish, was analyzed. Abdominal muscle tissue was analyzed for *Macrobrachium* shrimp.

Laboratory analyses and quality control

Selected toxicants were analyzed in water, sediment, and biota to describe how they were compartmentalized within each part of the ecosystem. Only sediment and biota were analyzed for metals (mercury, selenium, copper, nickel, zinc, cadmium, and lead) because the passive sampling devices, used in this study, do not accumulate metals. Passive sampling devices and sediment samples were analyzed for 34 current-use pesticides, 26 chlorinated pesticides, 48 polycyclic aromatic hydrocarbons (PAHs), and 20 polychlorinated biphenyl congeners (PCBs). Current-use pesticides and PAHs were not tested in biota because they are rapidly metabolized (Eisler 1987; Cope et al. 2011).

Analysis of organic contaminants in uPSDs, sediment, and biota was performed at the North Carolina State University Department of Environmental and Molecular Toxicology Chemical Exposure Assessment Laboratory in Raleigh, North Carolina, using a gas chromatograph-mass spectrometer. Sediment and biota samples were freeze dried and sent to Environmental Conservation Laboratories in Cary, North Carolina, for inorganic toxicant analyses. Cadmium, copper, lead, nickel, selenium, and zinc were analyzed using EPA Method 6010B for the extensive study and EPA Method 6010C for the intensive study (www.epa.gov/sam). Mercury was analyzed using EPA Method 7471A for the extensive study and EPA Method 7471B for the intensive study (www.epa.gov/sam).

A rigorous quality assurance protocol was followed during analyses. For metal analyses, quality assurance included blanks, laboratory control samples (LCS), matrix spike, matrix spike duplicates, post spikes, and surrogate internal standards. The blanks were clean (i.e., no target analytes were detected), with the exception of one detection of iron (3.6 mg/kg) and a detection of copper (0.07 mg/kg) and 2 detections of lead (<0.13 mg/kg) below the method reporting limit (MRL). The relative percent difference (RPD) of duplicate samples averaged 15% and ranged from 0.4-64%. For the few RPD values that were out of range, the batch was accepted based on percent recoveries for these samples that were within range. Overall, percent recoveries averaged 95%. All LCS percent recoveries were within range (mean = 99%, range = 85-110%). Results were not corrected for recoveries, due to acceptable accuracy and precision revealed by this protocol.

Procedural blanks, uPSD blanks (for uPSD batches), matrix spikes, and surrogate internal standards (SIS) were used to assess organic contaminant data quality. Procedural blanks were clean with few exceptions. Five PAHs were detected during sediment analysis (<8 ng/g). PCB 138 was detected in a procedure blank during sediment analysis (2 ng/g) and during fish analysis (<2 ng/g). Mean RPD values were 6% (range, 0-17%). Average surrogate recoveries were 80% (range, 48-115%) for uPSDs, 61% (range, 13-137%) for sediment, and 75% (range, 25-178%) for fish. Results were not corrected for recoveries, due to acceptable accuracy and precision revealed by this protocol.

Duplicate samples were also analyzed for quality assurance of lipid content of fish and shrimp and organic carbon content and particle size composition of sediment samples. The mean RPD value for lipid data was 9% (range, 0-18%). RPD values for sediment total organic carbon averaged 3% (range, 0-5%) and percent clay averaged 3% (range, 0-7%).

Contaminant criteria and guideline exceedance

Established criteria and guidelines are useful to assess the hazard of chemicals measured in water, sediment, and fish. We consulted EPA national recommended water quality criteria, EPA Office of Pesticide Program's aquatic life benchmarks, consensus based sediment guidelines, and EPA consumption limit tables (http://water.epa.gov/scitech/swguidance/waterquality/standards/current/index.cfm; http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm; MacDonald et al. 2000; U.S. Environmental Protection Agency 2000). EPA aquatic life benchmarks are estimates of concentrations below which chemicals are not expected to harm aquatic life and are based on the most sensitive toxicity endpoint for taxa. The consensus-based Threshold Effect Concentration (TEC) reflects sediment concentrations below which harmful effects to benthic organisms are unlikely to be observed, and the consensus-based Probable Effect Concentration (PEC) represents a threshold that if exceeded, harmful effects are likely to be observed. The Severe Effect Level (SEL) represents a threshold where adverse effects of the majority of sediment-dwelling organisms are expected if exceeded (McDonald et al. 2000). Consumption limit tables (U.S. Environmental Protection Agency 2000) are useful to infer human risk associated with consumption of fish and shrimp. They list contaminant concentration ranges and the associated limited numbers of meals per month and are based on an adult body weight of 70 kg and a meal of size of 0.227 kg. Some contaminant consumption limits are based only on noncancer endpoints, or chronic, systemic effects, but others include both noncancer and cancer endpoints.

Results

Water quality

Water quality measurements varied widely among sampling sites. Value ranges for water quality variables were, temperature, 22.7-34.7°C; total dissolved solids, 0.08-0.90 g/L; conductivity, 106-1451 µS/cm; salinity, 0.05-0.69 ppt; nitrate as NO_3^-, 0.3-10.0 mg/L; nitrite as

NO_2^-, 0.006-0.670 mg/L; ammonia, 0.00-0.69 mg/L; phosphorus as PO_4^-, 0.02-2.06 mg/L; alkalinity, 33-317 mg/L; hardness, 43-235 mg/L; turbidity, 1-22 FAU; pH, 7.18-8.90; dissolved oxygen, 4.34-12.36 mg/L (Tables 2 and 3). Stream water from the reference site, with a primarily forested watershed, was low in ionic and nutrient content. Measurements of total dissolved solids, conductivity, salinity, nitrogen, phosphorus, alkalinity, and hardness were generally low at the reference site, while the agricultural sites generally had greater measurements of total dissolved solids, conductivity, salinity, ammonia, phosphorus, alkalinity, and hardness.

Water contaminants

Low concentrations of contaminants were estimated in water by uPSDs at all sites (Tables 4 and 5). No PCBs were detected at any site. The only chlorinated pesticides detected were chlordane compounds. Current use pesticides (CUP) detected in water included butylate, carbaryl, trifluralin, simazine, prometon, atrazine, metolachlor, and malathion. Prometon was detected at the greatest concentrations at an urban site (4U), but was not detected at any other site. Trifluralin was the most frequently detected CUP and was found at all tested sites, with the exception of an industrial site (2I). An urban site (4U) generally had the greatest water contaminant concentrations, including chlordanes, total CUP, and total PAHs.

Sediment

Total organic carbon was generally low among sites. It was less than 4% at all sampling sites, with the exception of an industrial sampling site (3I) that had a mean total organic carbon content of approximately 8%. Clay composition and iron concentration was variable among sites. Clay composition ranged from 3 to 20%, and iron concentration ranged from 23.9 to 60.3 g/kg dry (Figs. 2 and 3).

Organic contaminants were at low concentrations in sediment at all sites (Tables 6 and 7). Chlordanes (cis-chlordane, trans-chlordane, and transnonachlor), DDTs (4,4’-DDE and 4, 4’-DDD), and hexachlorobenzene were the only chlorinated pesticides detected in sediment. DDTs were detected at greatest concentrations at agricultural sites. Chlordanes had greatest concentrations at an industrial (3I) and an agricultural (4U) site. Tebuthiuron, carbaryl-1, carbofuran-1, cyhalothrin (lambda), and bifenthrin were the only CUPs found in sediment.

Overall, PCBs in sediment were greatest at urban sites. During extensive sampling, the greatest concentrations of total PAHs (235.1 ng/g dry) were also found at the urban site (4U), but the greatest total PAHs were found at the industrial site (3I) (mean = 493.0 ng/g dry) during intensive sampling. The reference site (1R) appeared to be the least contaminated site, with no detections of OCs or CUPs and the lowest level of PAHs. However, low levels PCBs were detected at site 1R.

Cadmium, lead, mercury, selenium, and zinc were found at low concentrations in sediment samples (Tables 6 and 7). Copper concentrations were at moderately high levels (38.3-103 mg/kg dry) in sediment and was at greatest concentrations at agricultural sites. Nickel concentrations in sediment were variable among sites ranging from 4.63 to 336 mg/kg dry weight, and also had elevated concentrations at agricultural sites (10A, 9A, and 7A).

Fish and shrimp

Relatively high concentrations of PCBs and low levels of chlorinated pesticides, with the exception of dieldrin, were detected in fish tissue (Tables 8 and 9; Figs. 4 and 5). Mountain mullet and American eels were generally the most contaminated species and an urban site (4U) was the most contaminated site in terms of organic pollution. Chlordane, DDT, hexachlorobenzene, dieldrin, and gamma-BHC were the only chlorinated pesticides detected in fish tissue. High concentrations of dieldrin were found in American eels at the urban site (4U). PCBs were in greatest concentrations in mountain mullet, American eels, and river gobies from an urban site (4U). DDT was greatest in river gobies and American eels from an agricultural site (7A). Hexachlorobenzene was greatest in mountain mullet samples from an urban site (4U).

Fish tissue generally contained low concentrations of metals. Cadmium, nickel, mercury, and lead concentrations were generally below the method reporting limit (MRL) and method detection limit (MDL) for fish samples (Cd: <MDL = 59%, <MRL = 90%; Ni: <MDL = 57%, <MRL = 74%; Hg: <MDL = 75%, <MRL = 87%; Pb: <MDL = 77%, <MRL = 97%; *N* = 115). Copper and selenium concentrations of several samples were below the MRL and MDL (Cu: <MDL = 35%, <MRL = 48%; Se: <MDL = 33%, <MRL = 43%; *N* = 115), while zinc was above the MRL for most samples (Zn: <MDL = 0%, <MRL = 6%; *N* = 115) and varied among species and sites. Selenium concentrations were similar among all biota, and observed concentrations were low, ranging from 0.298 to 0.934 mg/kg wet weight. Cadmium and copper were found at

greatest concentrations in *Macrobrachium* spp. (Fig. 6 and 7). Mercury was greatest in channel catfish from site 8A, but bigmouth sleepers from site 4U had the greatest concentrations of mercury among native fish species (Fig. 8 and 9).

Mountain mullet and American eel had the greatest concentrations of organic contaminants, explained by their greater lipid content. Fish and shrimp lipid content and size within species varied significantly among sites ($P < 0.05$), with the exception of lipid content in sirajo gobies ($P = 0.34$, $N = 6$) and total length of river gobies ($P = 0.079$, $N = 9$) and spinycheek sleepers ($P = 0.230$, $N = 6$) (Figs. 10-13). Thus, PCB and mercury fish and shrimp concentrations were normalized by fish and shrimp lipid content and size (total length) to provide a comparative basis among sites (Figs. 14-16). An urban site (4U) had the greatest mercury and PCB concentrations after size and lipid normalization.

Contaminant criteria and guideline exceedance

All estimated contaminant concentrations that we measured in Puerto Rico streams were below the available EPA national recommended water quality criteria and the Office of Pesticide Programs' aquatic life benchmarks for the protection of plants, invertebrates, and fish. All organic contaminants, mercury, lead, selenium, cadmium, and zinc that we measured in sediment were below available consensus-based guidelines (MacDonald et al. 2000). Sediment analyzed from all sites exceeded the consensus-based TEC for copper, but were below the consensus-based PEC (MacDonald et al. 2000; Figs. 17 and 18). Nickel sediment concentrations exceeded the consensus based-TEC at five agricultural and both industrial sites, with four of these sites also exceeding the consensus-based PEC (MacDonald et al. 2000) (Fig. 19). Two agricultural sites exceeded the consensus-based SEL by 4 orders of magnitude, and intensive sampling at another agricultural site revealed a SEL exceedance (Persaud et al.1993; MacDonald 2000; Fig. 20).

Some mercury, cadmium, chlordane, DDT, dieldrin, and PCB concentrations that we measured in fish and shrimp in Puerto Rico rivers exceeded EPA consumption limits (Tables 8 and 9). EPA human consumption limits are not available for zinc, nickel, lead, or copper. Mercury had the most consumption exceedances of all contaminants, and all are based on noncancer endpoints with most at the 16 meals per month limit (noncancer). Bigmouth sleepers from an urban site had the greatest mercury concentrations among native species and exceeded the EPA consumption limit recommended for 3 meals per month. However, the greatest mercury fish tissue concentration was a channel catfish from an agricultural site, also exceeding the 3 meals per month limit. Chlorinated pesticides were generally detected at low levels in fish tissue, however, dieldrin concentrations in American eels from the urban site (4U) were above all human consumption limits for cancer endpoints (i.e., 0 meals per month). There were few threshold exceedances for DDT; river gobies from an agricultural site (7A) and American eels from an urban site (4U), both exceeded the EPA consumption limits recommended for 16 meals per month for cancer endpoints. Mountain mullet from site 4U had the greatest concentrations of PCBs, exceeding the recommended consumption limit for 1 meal per month for cancer endpoints (4 meals for noncancer endpoints). No bigmouth sleepers, sirajo gobies, or *Macrobrachium* spp. exceeded human consumption limits for PCBs.

Overall, Puerto Rico streams are relatively less polluted than water bodies of other tropical regions and the United States (Table 10). The maximum advisable concentration for mercury (300 ppb wet) was never exceeded in our study; a study of mercury in a Cuba river revealed only 4% of the samples exceeding the criterion for mercury (Rosa et al. 2009). Other researchers found that 27% of streams and 49% of predatory fish in lakes in the United States exceeded this criterion (Stahl et al. 2009; Scudder et al. 2009). PCBs were lower in Puerto Rico streams relative to other regions with 8% of all samples exceeding 12 ppb wet; in comparison, 50-75% benthic-feeding fish from lakes in the United States exceeded the same benchmark (Stahl et al. 2009). Only 5% of our samples exceeded the NAWQA benchmark$_{\mathrm{low}}$ (6 ppb wet) for DDT; however, 63% and 76% of all samples for Hawaii and U.S. streams exceeded this concentration, respectively.

Discussion

Our results indicate that pollution in the stream ecosystems of Puerto Rico is not severe or widespread with several notable exceptions. Nickel concentrations in sediment at three agricultural sites exceeded the severe effect level. An urban site generally had the greatest concentrations of contaminants, including the greatest concentrations of PAHs and PCBs in sediment and the greatest mercury, PCB, and dieldrin concentrations in native fishes. An urban site (4U) had the greatest concentrations of current use and chlordane pesticides and PAHs in water and the greatest concentrations of PAHs, chlordanes, and PCBs in sediment. Site 5U, the other urban site, also seemed to be relatively contaminated. The sediment was relatively low in organic carbon (Table 6), but it had the third greatest concentration of PAHs and chlordane in water and second greatest concentrations of PCBs and PAHs in sediment. Native fish species were not available for comparison with the other urban site (5U) because they were not present. In urban areas, there may be a greater input of pesticides for mosquito control, maintenance of right-of-ways, golf courses, and domestic lawns (Miles and Pfeuffer 1997). A greater amount of contaminants may also be released into urban streams as a result of impervious surfaces (Klein 1979; Holland et al. 2003).

Nickel and copper were the only contaminants to exceed sediment quality guidelines. Nickel was highly elevated at three agricultural sites, even after normalization by iron concentration, total organic carbon, and percent clay. These high levels could be due to applications of nickel-based fungicides or from illegal disposal of various electroplated items (Rowell 1968; Tandon et al. 1977; Hunter and Arbona 1995; Eisler 1998). Elevated levels of copper could possibly be due to the natural geology of the island, pesticide applications, or they may be associated with vehicle brake pads. Copper is a major component of automobile brake pads, and all of our sampling sites were near road stream crossings (Gasser et al. 2009).

Our findings at three sites were contrary to expectations considering their land use and potential pollution sources. The reference site (1R) was relatively contaminated with metals, which was unexpected because it was located within a predominately forested watershed of the El Yunque National Forest. This unexpected finding may be attributed to persistent legacy effects of past land use or management practices (Harding et al. 1998; Kwak and Freeman 2010). This area was historically mined for gold, copper, and chalcopyrite and was also farmed as a coffee plantation (Cardona 1984). We also revealed unexpected findings at two industrial sampling sites. Site 2I was downstream of a nonoperational oil refinery and was expected to contain greater concentrations of PAHs in sediment and water, but levels of both media were low. Site 3I was located near a cement production facility and was expected to be a significant source of mercury, yet low mercury levels were detected there. The unexpected findings at industrial stream sites may be related to hydrology.

Contaminants and hydrology

Although streams of Puerto Rico receive substantial amounts of pollution from a variety of sources (Hazen 1988; Hunter and Arbona 1995; Stallard 2001; Neal et al 2008), our results indicate that these stream ecosystems are not severely polluted, with the sporatic exception of nickel in sediment and PCBs and dieldrin in fish tissue. Hydrology may be an important factor limiting contaminant bioaccumulation in the stream ecosystems of Puerto Rico. If so, we would expect lentic ecosystems in Puerto Rico to show better evidence of pollution. Lentic and lotic environments differ in chemistry, hydrology, and ecology, which consequently can affect bioaccumulation. For example, fish from lentic systems have been shown to bioaccumulate selenium at a rate 10 times greater than fish from lotic environments exposed to similar concentrations (Adams et al. 2000). Lentic systems form sediment from organic matter that is constantly being recycled within the system along with associated contaminants due to long hydraulic retention times (Jefferies and Mills 1990; Simmons and Wallschläger 2005). In contrast, lotic systems create high flushing rates that prevent sedimentation of contaminated organic matter and exposure of benthos and detritral components of the ecosystem, thus reducing bioaccumulation (Lillebo et al. 1988; Van Derveer and Canton 1997; Adams et al. 2000; Simmons and Wallschläger 2005). In addition, lotic systems have a greater redox potential than lentic systems, due to constant aeration from flowing water (Simmons and Wallschläger 2005). Reducing conditions can form metal species that are less bioavailable than those more oxidized metal species (Lenz and Lens 2009). Additionally, the streams that we studied were shallow, facilitating chemical degradation by photolysis.

Streams in Puerto Rico tend to be well incised and narrow and may have less of a potential for bioaccumulation because they lack connections to environments similar to lentic systems, such as floodplains. Most of the sites that we sampled also lack a connection to reservoirs because the native species that we targeted for sampling are only found downstream of reservoirs due to their diadromous life history. Conversely, non-native channel catfish from site 8A collected upstream of Dos Bocas reservoir had the greatest concentrations of mercury in our study. It is likely that channel catfish from this site migrated upstream from the reservoir where they had been exposed to more contaminants. Relationships with benthic habitats, as illustrated by this channel catfish example, and other microhabitat affinities likely play a minimal role in these streams because they are shallow and the substrate appears low in organic content and thus, organic pollutants.

Similarly, bioaccumulation in coastal areas is also influenced by habitat type because sheltered intertidal shores and creeks allow for accumulation of fine sediments, providing sinks for contaminants (Rawlins et al. 1998). Although, most Caribbean islands have little continental shelf with effective mixing and dispersal of terrestrial-based pollution (Rawlins et al. 1998), several deep oceanic basins in the Caribbean receive little flushing, making them vulnerable to contaminant accumulation (Ross and DeLorenzo 1997). Impoundments and estuaries of Puerto Rico may be more polluted than the stream habitats that we sampled, as suggested by other studies. For example, a study of coastal sediments revealed that Guanica Bay, Puerto Rico, had elevated levels of PCBs and DDT (Pait et al. 2008). In contrast, another study generally found low concentrations of mercury in biota at three estuaries in Puerto Rico (Burger et al. 1992). High levels of contaminants in fish tissue were found in marine and some reservoir fish of Puerto Rico (Rodríguez and González 1981). Although, a contaminant survey of redear sunfish and sediment in the Dos Bocas Reservoir, Puerto Rico, showed little evidence of contamination problems (Neal et al. 2005).

General conclusions

Stream ecosystems in Puerto Rico were not severely polluted especially when compared with other water bodies in tropical ecosystems and the United States; several exceptions were nickel in sediment at agricultural sites and PCBs and dieldrin at an urban site. All fish species contained variable concentrations of contaminants, but among those sampled, bigmouth sleepers may be the most suitable fish for human consumption. They have low levels of organic contaminants and rare occurrences of mercury. This is the first study examining contaminants and resulting trends in the freshwater ecosystems of Puerto Rico. Results of this project will assist water and natural resource agencies in identifying areas of concern, planning to improve ecological and human health, and development of freshwater fisheries.

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Table 1. Puerto Rico stream sampling site characteristics, including location, distance from river mouth, elevation, gradient, and watershed attributes.

Site	Designated Land Use	River Name	Latitude	Longitude	Distance to River Mouth (km)	Site Elevation (m)	Watershed Area (km^2)	Gradient (%)	Watershed Land Use: Agriculture (%)	Watershed Land Use: Forest (%)	Watershed Land Use: Shrub and Woodland (%)	Watershed Land Use: Urban (%)
1R	Reference	Mameyes	N18°21'23.7"	W65°46'06.9"	4.4	14.0	21.3	1.4	2.7	95.6	1.0	0.1
2I	Industrial	Tallaboa	N18°00'17.3"	W66°43'53.0"	1.8	6.1	82.7	0.1	22.0	47.1	25.4	5.3
3I	Industrial	Cañas	N18°01'23.2"	W66°38'26.4"	5.0	30.2	20.6	0.4	30.0	27.4	32.3	9.8
4U	Urban	Piedras	N18°23'02.8"	W66°03'31.2"	8.6	13.4	23.2	0.2	24.8	26.3	8.8	39.4
5U	Urban	Bayamon	N18°19'51.7"	W66°06'04.2"	19.1	58.8	22.1	1.5	25.0	50.0	13.4	11.6
6A	Agricultural	Añasco	N18°14'14.9"	W67°02'42.6"	25.7	201.6	9.2	1.3	77.9	7.9	13.4	0.8
7A	Agricultural	Yauco	N17°59'12.9"	W66°50'25.7"	5.7	10.1	115.9	0.1	24.9	31.5	36.6	6.1
8A	Agricultural	La Plata	N18°13'28.4"	W65°12'58.9"	43.4	188.4	146.5	0.4	44.2	21.6	25.5	8.1
9A	Agricultural	Jacaguas	N18°04'11.1"	W66°30'33.5"	19.2	52.4	124.1	1.0	36.9	39.4	26.3	4.8
10A	Agricultural	Guanajibo	N18°09'29.8"	W67°05'06.7"	23.6	48.8	48.2	2.0	53.5	34.4	10.2	1.8
11A	Agricultural	Cartagena	N18°01'42.5"	W67°06'48.6"	7.1	20.1	10.6	0.3	40.4	41.8	13.6	4.2
12A	Agricultural	Arecibo	N18°15'34.9"	W66°43'20.4"	40.8	146.3	112.2	0.5	13.5	25.0	58.7	2.4
13A	Agricultural	Fajardo	N18°19'20.5"	W65°38'59.3"	3.58	4.0	58.5	0.1	34.5	53.8	7.4	2.8

Table 2. Water quality parameter values measured at each site during the extensive study.

	Site												
Parameter	1R	2I	3I	4U	5U	6A	7A	8A	9A	10A	11A	12A	13A
Temperature (°C)	29.0	27.5	29.8	28.3	28.5	24.9	27.4	22.7	30.0	29.3	25.6	27.7	30.3
Total dissolved solids (g/L)	0.09	0.18	0.27	0.20	0.25	0.10	0.70	0.13	0.17	0.17	0.28	0.17	0.12
Conductivity (µS/cm)	140	298	434	442	411	152	1125	106	289	265	430	280	211
Salinity (ppt)	0.06	0.13	0.20	0.20	0.18	0.07	0.53	0.05	0.12	0.12	0.20	0.13	0.09
Nitrate (mg/L as NO_3-)	2.7	0.9	6.2	2.7	0.8	0.3	4.6	0.3	0.3	0.8	10.0	3.9	3.9
Nitrite (mg/L as NO_2-)	0.01	0.02	0.01	0.01	0.06	0.05	0.01	0.01	0.01	0.04	0.06	0.07	0.04
Ammonia/nitrogen (mg/L as NH_3)	0.00	0.00	0.00	0.14	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.11	0.01
Phosphorus (mg/L as PO_4-)	2.06	0.31	0.17	2.06	1.21	0.40	0.30	0.33	0.50	2.26	0.69	2.06	2.06
Alkalinity (mg/L as $CaCO_3$)	46	314	121	123	123	70	139	39	97	123	317	98	38
Hardness (mg/L as $CaCO_3$)	49	208	153	145	149	72	140	44	103	129	191	113	43
Turbidity (FAU)	3	1	1	14	22	4	2	3	2	1	4	10	3
pH	7.64	7.81	8.47	8.04	8.43	7.79	7.76	7.62	8.38	8.64	7.43	8.43	8.90
Dissolved oxygen (mg/L)	7.80	9.08	8.70	7.92	8.09	7.30	4.34	6.79	9.76	7.60	4.75	8.03	10.90

Table 3. Water quality parameter values measured at each site during the intensive study.

	Site			
Parameter	1R	3I	4U	7A
Water temperature (°C)	34.7	31.7	27.8	27.6
Total dissolved solids (g/L)	0.079	0.489	0.386	0.899
Conductivity (µS/cm)	121	844	628	1451
Salinity (ppt)	0.06	0.36	0.28	0.69
Nitrate (mg/L as NO_3-)	1.8	1.5	4.4	3.5
Nitrite (mg/L as NO_2-)	0.006	0.006	0.046	0.015
Ammonia/nitrogen (mg/L as NH_3)	<0.01	0.08	0.02	0.69
Phosphorus (mg/L as PO_4-)	0.02	0.95	0.59	0.83
Alkalinity (mg/L as $CaCO_3$)	33	131	118	248
Hardness (mg/L as $CaCO_3$)	38	185	182	235
Turbidity (FAU)	4	1	3	4
pH	7.27	8.10	7.29	7.18
Dissolved oxygen (mg/L)	8.26	12.36	8.23	5.43

Table 4. Estimated contaminant concentrations (ng/L) in fiber PSDs for each extensive study site. A dash symbolizes concentrations that were below detection limits.

	Site											
Analyte and Duration	1R	2I	3I	4U	5U	6A	7A	8A	10A	11A	12A	13A
Butylate	–	–	–	–	–	–	–	–	–	–	–	2.0
Carbaryl	–	1.3	–	–	–	–	–	–	–	–	–	–
Trifluralin	0.3	–	0.3	0.5	0.6	0.4	0.4	0.5	0.3	0.3	0.2	0.4
Simazine	–	–	–	5.6	–	–	–	–	–	–	–	–
Prometon	–	–	–	42.9	–	–	–	–	–	–	–	–
Atrazine	–	–	4.2	10.1	–	–	1.0	2.8	–	–	–	–
Metolachlor	–	–	–	–	–	1.1	–	–	–	–	–	–
Chlordanes	0.1	–	–	0.3	0.2	0.2	–	0.2	–	–	–	0.2
Total PAHs	101.9	41.7	148.9	293.2	216.0	83.4	94.4	226.0	79.8	67.3	108.1	148.1
Deployment duration (days)	25.9	28.9	27.9	22.2	22.1	26.9	29.2	21.2	29.9	30.3	24.9	26.0

Table 5. Mean estimated contaminant concentrations in cartridge PSDs for intensive sites. A dash symbolizes concentrations that were below detection limits.

	Site			
Analyte (ng/L)	1R	3I	4U	7A
Atrazine	–	0.1	0.9	–
Malathion	–	0.8	–	–
Total PAHs	7.0	12.1	2.1	12.4
Deployment duration (days)	26.8	27.7	34.8	28.9

Table 6. Contaminant concentrations in sediment for each site during the extensive study. A dash symbolizes concentrations that were below detection limits.

	Site												
Analyte	1R	2I	3I	4U	5U	6A	7A	8A	9A	10A	11A	12A	13A
Organics (ng/g dry)													
Hexachlorobenzene	–	0.072	–	0.073	–	–	–	–	–	–	–	–	–
Chlordanes	–	–	0.962	0.621	–	–	–	0.061	–	–	–	–	–
DDTs	–	–	–	–	0.145	–	0.423	0.623	–	–	–	–	–
PCBs	0.156	–	–	1.840	1.156	–	–	–	–	–	–	–	0.259
Total PAH	2.4	38.1	170.0	235.1	204.7	10.1	42.1	88.7	20.8	17.0	13.4	16.6	26.7
Tebuthiuron	–	–	–	–	8.7	–	–	–	–	–	–	–	–
Metals (mg/kg dry)													
Cadmium	–	0.14	0.09	–	0.04	0.14	0.05	0.23	0.12	0.08	0.22	0.26	–
Copper	53.2	59.4	38.3	51.9	94.1	76.4	52.5	103	50.9	49.8	44.5	84.7	65.3
Lead	4.36	10.20	5.79	9.55	10.90	7.97	9.45	9.91	7.19	6.58	5.44	11.8	5.21
Mercury	0.020	0.030	0.050	0.060	0.010	0.050	0.040	0.070	0.030	0.008	0.010	0.009	0.030
Nickel	19.5	33.2	31.9	22.1	10.2	38.9	50.1	52.1	327	336	19.9	6.3	4.6
Zinc	61.8	65.6	87.6	94.8	82.1	99.7	60.3	79.1	57.7	56.1	70.7	102.0	65.3

Table 7. Mean contaminant concentrations in sediment for each site during the intensive study. A dash symbolizes concentrations that were below detection limits. An asterisk indicates contaminants that were detected, but could not be quantified.

	Site			
Analyte	1R	3I	4U	7A
Organics (ng/g dry)				
Hexachlorobenzene	–	–	0.092	–
Chlordanes	–	0.236	1.17	–
DDTs	–	–	0.170	0.877
PCBs	–	0.244	0.834	0.539
Bifenthrin	–	3.34	1.93	0.280
Carbaryl-1	–	*	–	–
Carbofuran-1	–	*	–	–
Cyhalothrin (lambda)	–	–	–	0.29
Total PAHs	2.9	493.0	171.4	87.7
Metals (mg/kg dry)				
Cadmium	0.184	0.426	0.144	0.290
Copper	46.3	43.3	48.3	63.9
Lead	6.2	10.6	13.4	15.3
Mercury	0.005	0.043	0.049	0.051
Nickel	13.8	30.7	12.2	148
Zinc	55.1	97.7	100.3	88.9

Table 8. Contaminant concentrations exceeding EPA consumption limit recommendations for extensive study sites. 'NA' represents contaminant's number of meals per month that are not applicable because consumption limits for cancer endpoints have not been established. 'UR' indicates an unrestricted number of meals per month (U.S. Environmental Protection Agency 2000).

Analyte	Site	Taxon	Concentration (ppm wet)	EPA noncancer consumption limit (meals/month)	EPA cancer consumption limit (meals/month)
DDTs	7A	River goby	0.0093	UR	16
PCBs	12A	Channel catfish	0.0029	UR	12
	13A	American eel	0.0046	UR	8
	9A	American eel	0.0037	UR	12
	2I	American eel	0.0086	16	4
	7A	American eel	0.0065	16	4
	4U	Mountain mullet	0.0189	8	2
	4U	River goby	0.0134	12	3
	7A	River goby	0.0049	UR	8
Cd	9A	*Macrobrachium*	0.1838	12	NA
	1R	*Macrobrachium*	0.2043	12	NA
	2I	*Macrobrachium*	0.1942	12	NA
Hg	3I	Bigmouth sleeper	0.0344	16	NA
	1R	Bigmouth sleeper	0.0363	16	NA
	4U	Bigmouth sleeper	0.0773	12	NA
	2I	Bigmouth sleeper	0.0390	16	NA
	8A	Channel catfish	0.2823	3	NA
	11A	American eel	0.0585	16	NA
	13A	American eel	0.0396	16	NA
	1R	American eel	0.0361	16	NA
	13A	*Macrobrachium*	0.0379	16	NA
	9A	Mountain mullet	0.0651	12	NA
	8A	Redbreast sunfish	0.0743	12	NA
	6A	River goby	0.0630	12	NA
	10A	River goby	0.0405	16	NA
	4U	River goby	0.0920	8	NA
	7A	River goby	0.0470	16	NA
	1R	Sirajo goby	0.0500	16	NA

Table 9. Contaminant concentrations exceeding EPA consumption limit recommendations for intensive study sites. 'NA' represents contaminant's number of meals per month that are not applicable because consumption limits for cancer endpoints have not been established. 'UR' indicates an unrestricted number of meals per month (U.S. Environmental Protection Agency 2000).

Contaminant	Site	Species	Concentration (ppm-wet)		EPA noncancer consumption limit (meals/month)		EPA cancer consumption limit (meals/month)	
			Mean	SD	Mean	Range	Mean	Range
PCBs	3I	American eel	0.0050	0.0027	UR	UR-16	8	4-12
		Mountain mullet	0.0044	0.0013	UR	UR-16	8	4-12
	4U	American eel	0.0250	0.0059	4	4-8	1	1-2
		Mountain mullet	0.0275	0.0032	4	4-4	1	1-1
		River goby	0.0062	0.0013	16	UR-16	4	4-8
		Spinycheek sleeper	0.0108	0.0033	16	12-16	4	3-4
	7A	American eel	0.0065	0.0024	16	UR-16	4	4-8
		Mountain mullet	0.0038	0.0020	UR	UR-16	12	4-16
		River goby	0.0020	0.0006	UR	UR-UR	16	16-16
		Spinycheek sleeper	0.0015	0.0013	UR	UR-UR	16	UR-16
Chlordanes	4U	American eel	0.0104	0.0037	UR	UR-UR	16	UR-16
		Mountain mullet	0.0135	0.0029	UR	UR-UR	16	16-16
DDTs	4U	American eel	0.0080	0.0050	UR	UR-UR	UR	UR-16
Dieldrin	4U	American eel	0.0142	0.0125	UR	UR-16	none	UR-none
Mercury	4U	Bigmouth sleeper	0.1679	0.0561	4	3-4	NA	NA
		American eel	0.0679	0.0651	12	UR-4	NA	NA
		Spinycheek sleeper	0.0775	0.0251	12	8-16	NA	NA
	7A	American eel	0.0391	0.0153	16	UR-16	NA	NA
		Mountain mullet	0.0281	0.0238	UR	UR-16	NA	NA
		Spinycheek sleeper	0.0406	0.0106	16	16-16	NA	NA
	3I	Bigmouth sleeper	0.0252	0.0239	UR	UR-16	NA	NA

Table 10. Comparison of aquatic contaminants in fish tissue among aquatic ecosystems, including U.S. lakes (Stahl et al. 2009), U.S. streams (Gilliom et al. 2007, Scudder et al. 2009), Puerto Rico streams (this study), one Cuba river (Rosa et al. 2009), and Hawaii streams (Gilliom et al. 2007). Benchmark exceedance concentrations are 300 ppb for mercury, 12 ppb for PCBs, 67 ppb for chlordane, and 69 ppb for DDT (U.S. Environmental Protection Agency 2000). A dash indicates data not available; 'ND' indicates that the concentration was below detection limits.

	Mercury			PCBs			Chlordane			DDT		
Water body	Median (ppb)	Max (ppb)	Threshold Exceeded (%)	Median (ppb)	Max (ppb)	Threshold Exceeded (%)	Median (ppb)	Max (ppb)	Threshold Exceeded (%)	Median (ppb)	Max (ppb)	Threshold Exceeded (%)
U.S. lakes												
Predators	285	6,605	49	2	705	17	ND	100	<1	2	1,481	<2
Benthic feeders	69	596	<5	14	1,266	50-75	27	378	<5	13	1,761	10-25
U.S. streams												
Overall	169	1,950	27	–	–	–	ND	1,790	13	33	9,494	34
Urban	–	–	–	–	–	–	46	1,790	39	63	2,200	47
Agricultural	–	–	–	–	–	–	5	445	12	64	9,494	49
Undeveloped	–	–	–	–	–	–	<1	634	2	7	2,148	13
Mixed	–	–	–	–	–	–	13	586	17	48	7,200	40
Hawaii streams	–	–	–	–	–	–	17	1,790	30	10	361	15
PR streams												
Overall	13	282	0	<1	31	8	ND	15	0	0	12	0
Reference	13	50	0	<1	1	0	ND	ND	0	0	<1	0
Urban	19	233	0	7	31	8	<1	15	0	0.6	12	0
Industrial	13	53	0	<1	9	0	ND	2	0	0	2	0
Agricultural	18	282	0	<1	9	0	ND	3	0	0	10	0
Cuba river	–	375	4	–	–	–	–	–	–	–	–	–

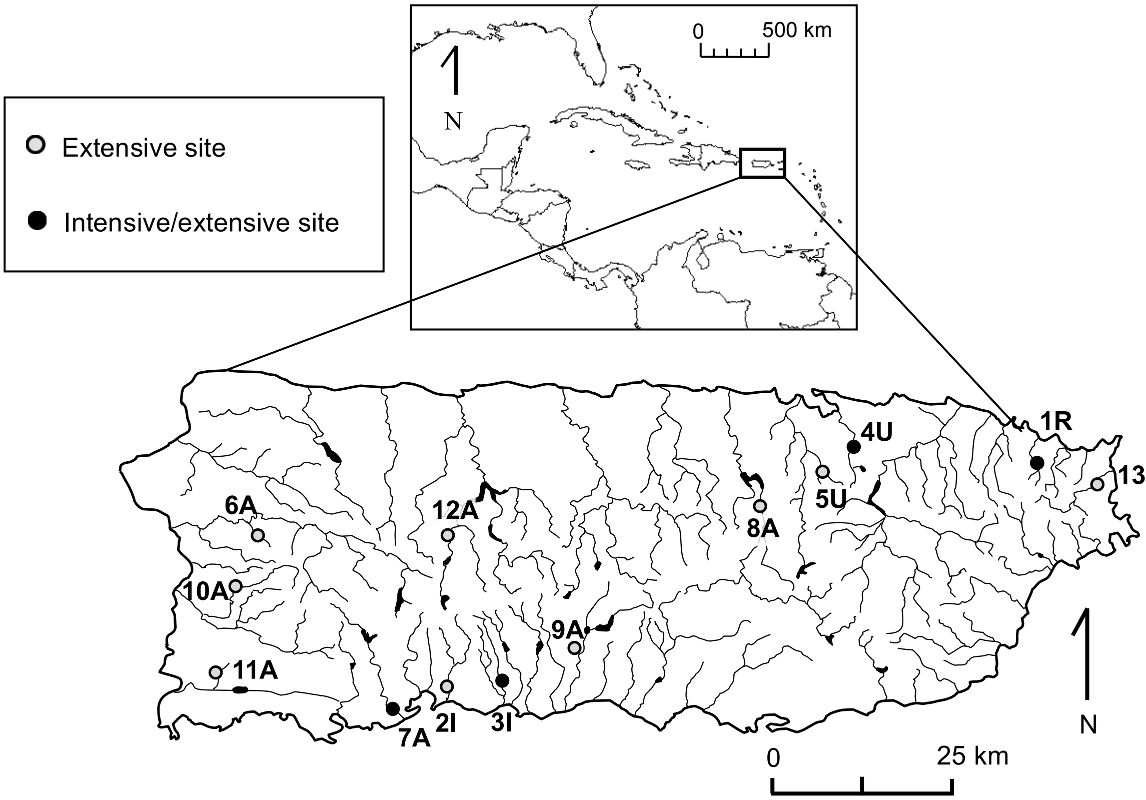

Figure 1. Map of Puerto Rico indicating stream study sites.

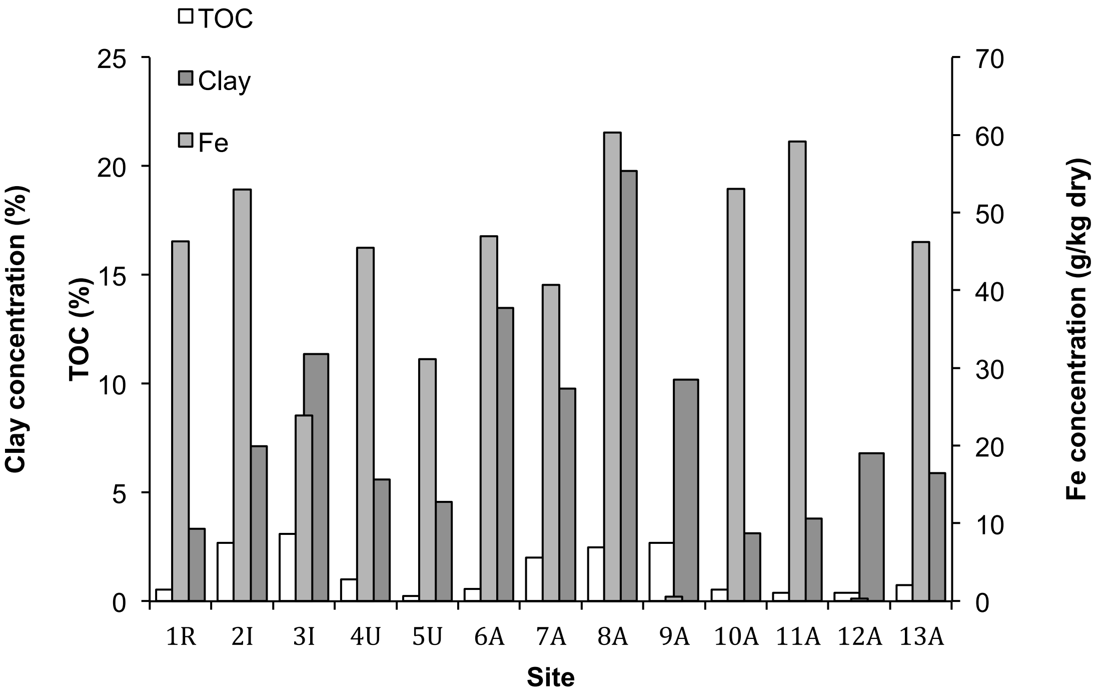

Figure 2. Sediment normalization factors total organic carbon (TOC), clay, and iron (Fe) concentration for extensive study sites.

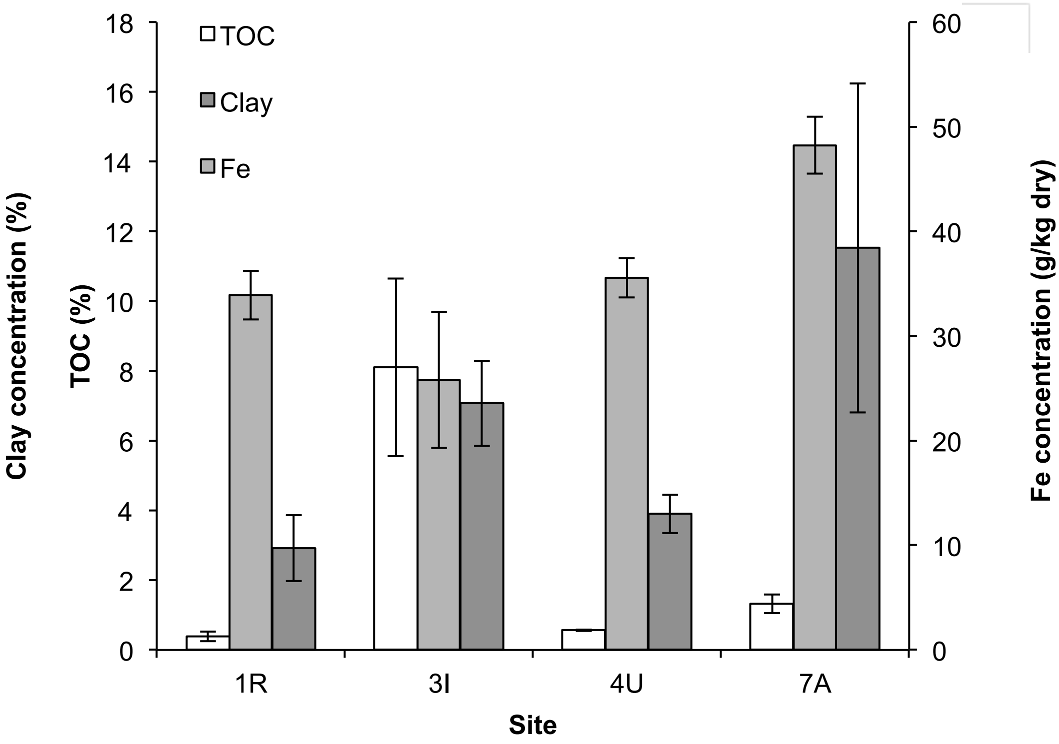

Figure 3. Mean (± SD) sediment normalization factors total organic carbon (TOC), clay, and iron (Fe) concentration for intensive study sites.

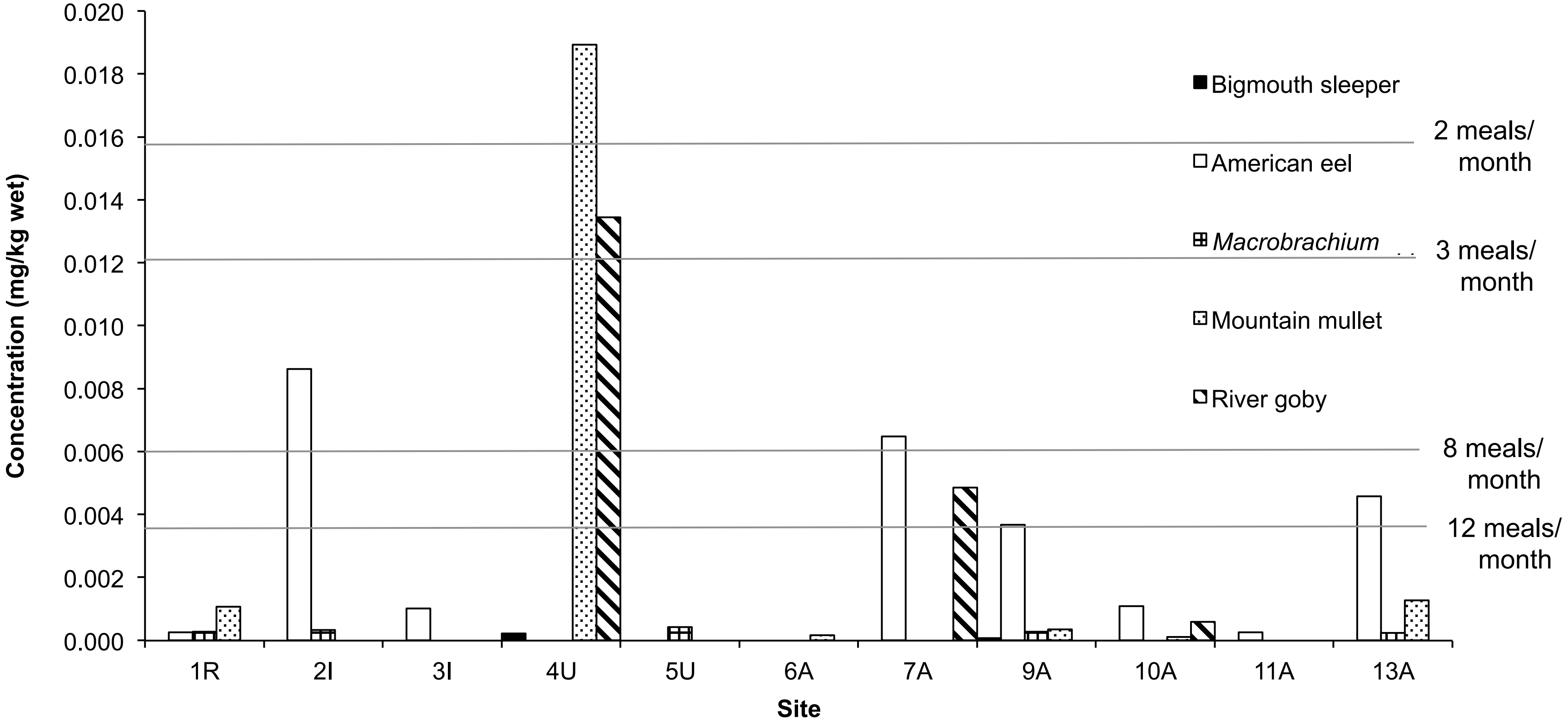

Figure 4. PCB concentrations in fish and shrimp at extensive sampling sites with EPA monthly human consumption limits indicated (U.S. Environmental Protection Agency 2000).

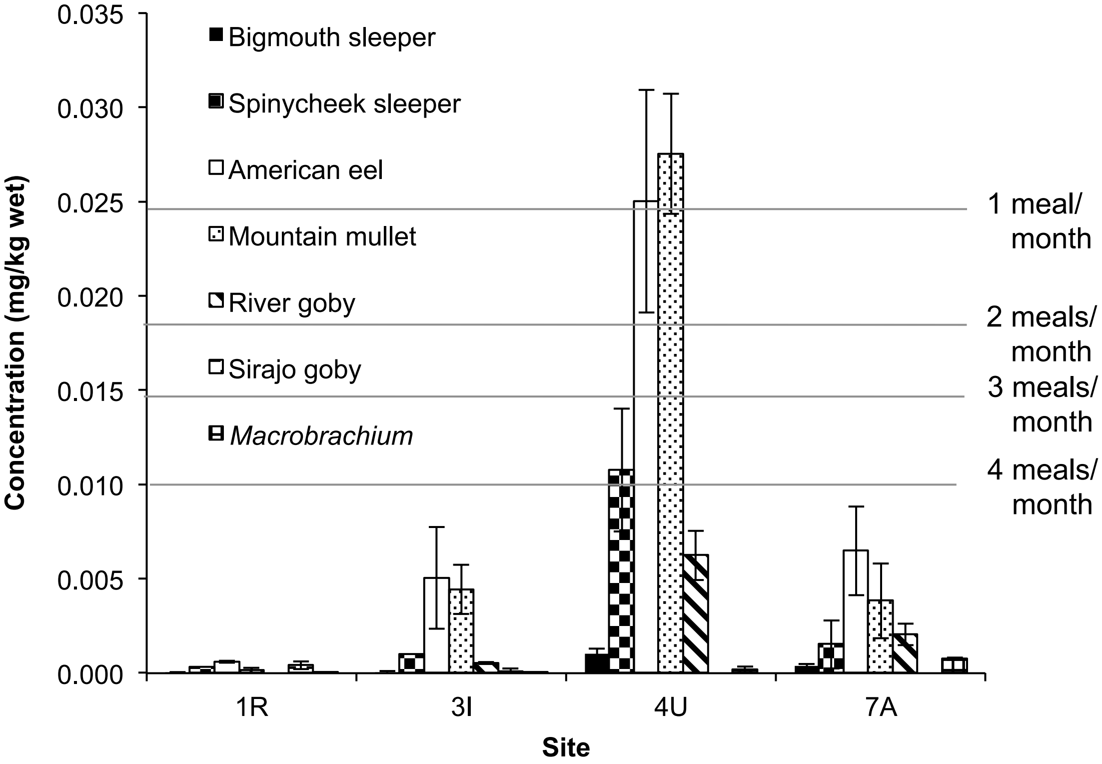

Figure 5. Mean (± SD) PCB concentrations in fish and shrimp at intensive sampling sites with EPA monthly consumption limits indicated (U.S. Environmental Protection Agency 2000).

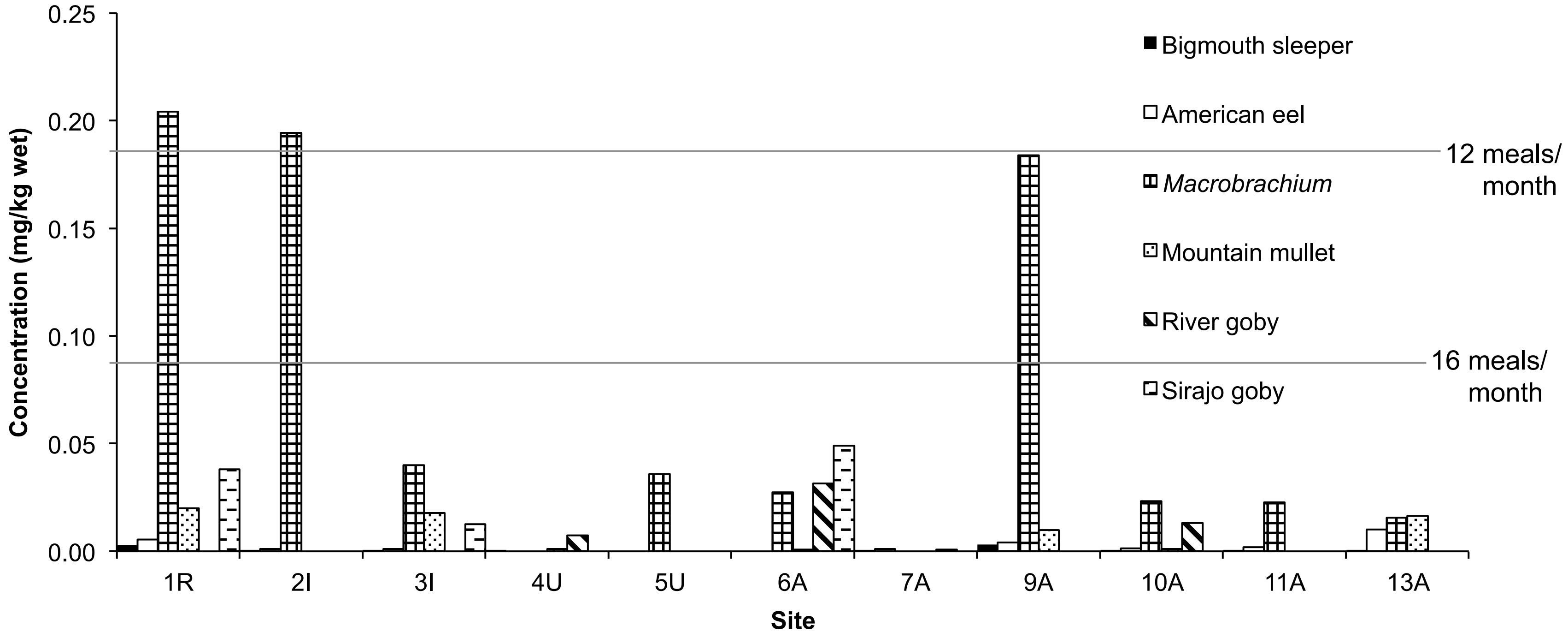

Figure 6. Cadmium concentrations in fish and shrimp at extensive sampling sites with EPA monthly human consumption limits (U.S. Environmental Protection Agency 2000).

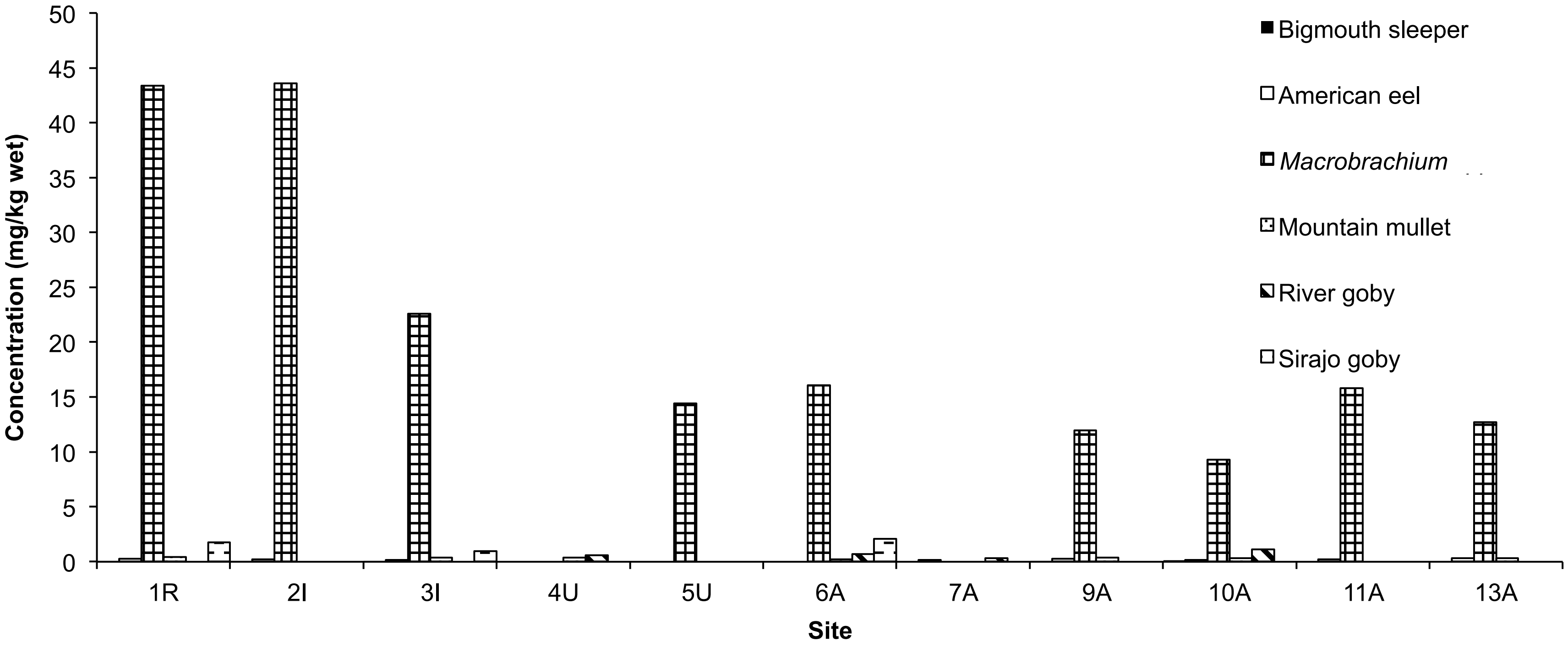

Figure 7. Copper concentrations in fish and shrimp at extensive sampling sites.

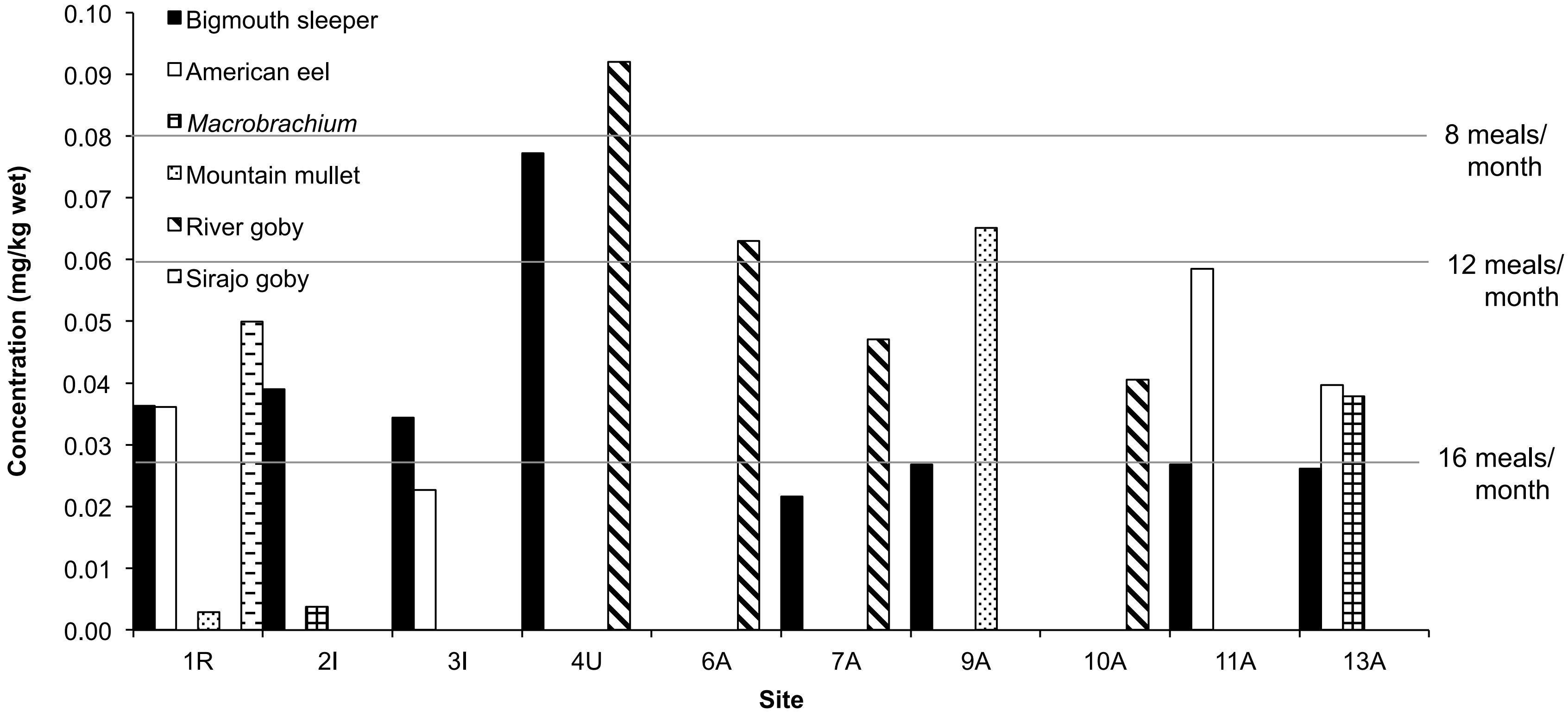

Figure 8. Mercury concentrations in composite fish and shrimp samples at extensive sampling sites with EPA monthly consumption limits indicated (U.S. Environmental Protection Agency 2000).

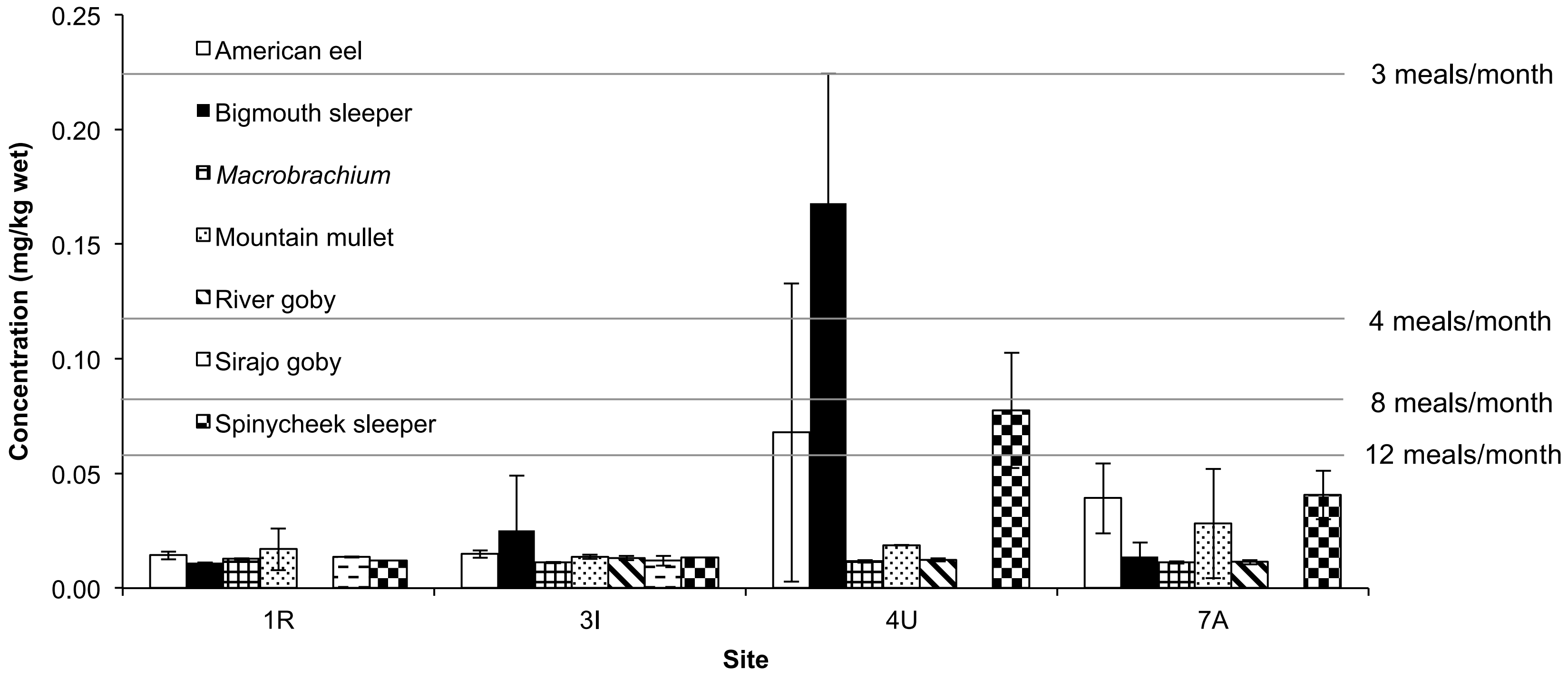

Figure 9. Mean (±SD) mercury concentrations in fish and shrimp at intensive sampling sites and EPA monthly fish consumption limits indicated (U.S. Environmental Protection Agency 2000).

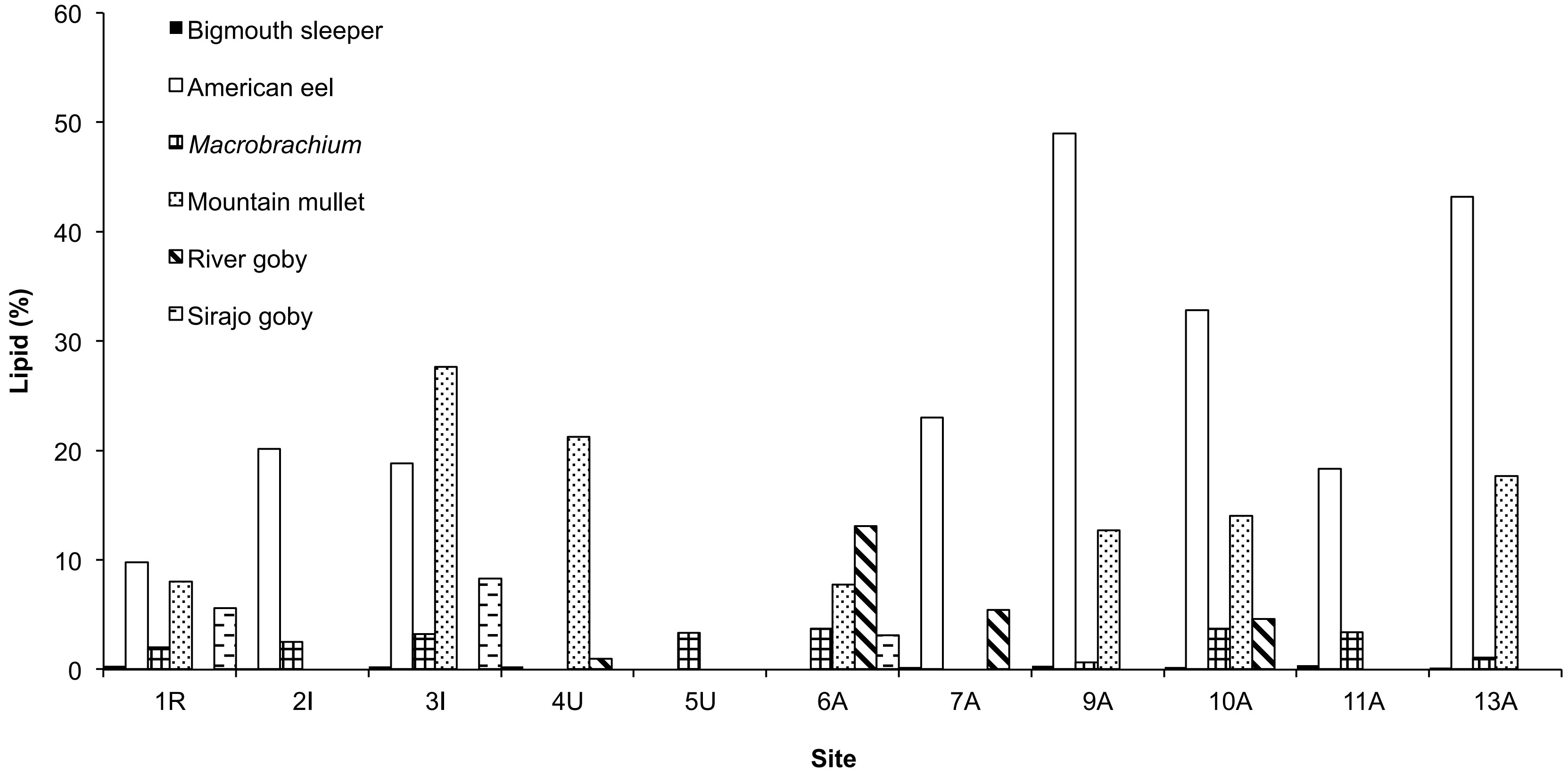

Figure 10. Lipid content in fish and shrimp samples at extensive sampling sites.

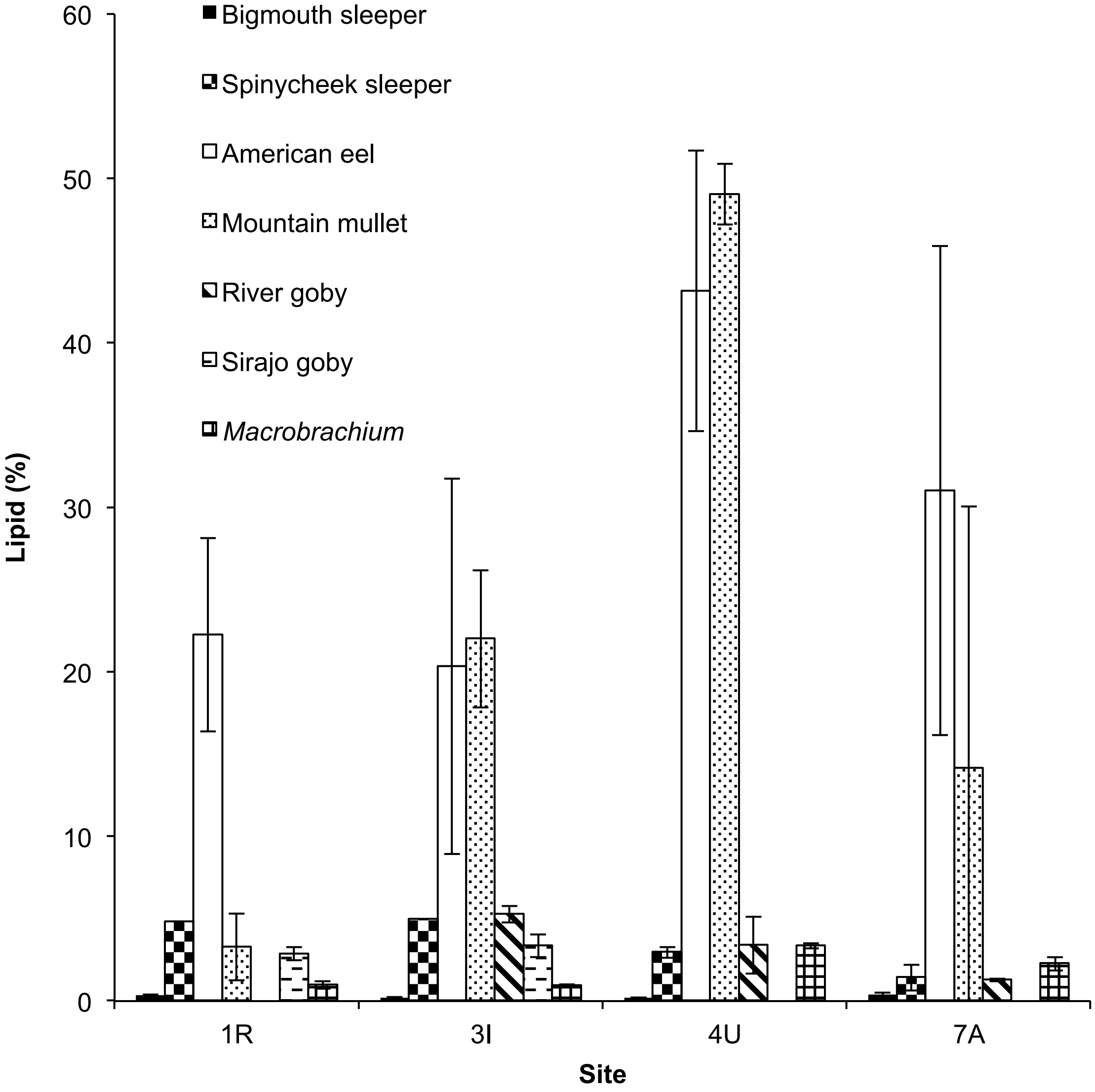

Figure 11. Mean (± SD) lipid content for fish and shrimp at intensive sampling sites.

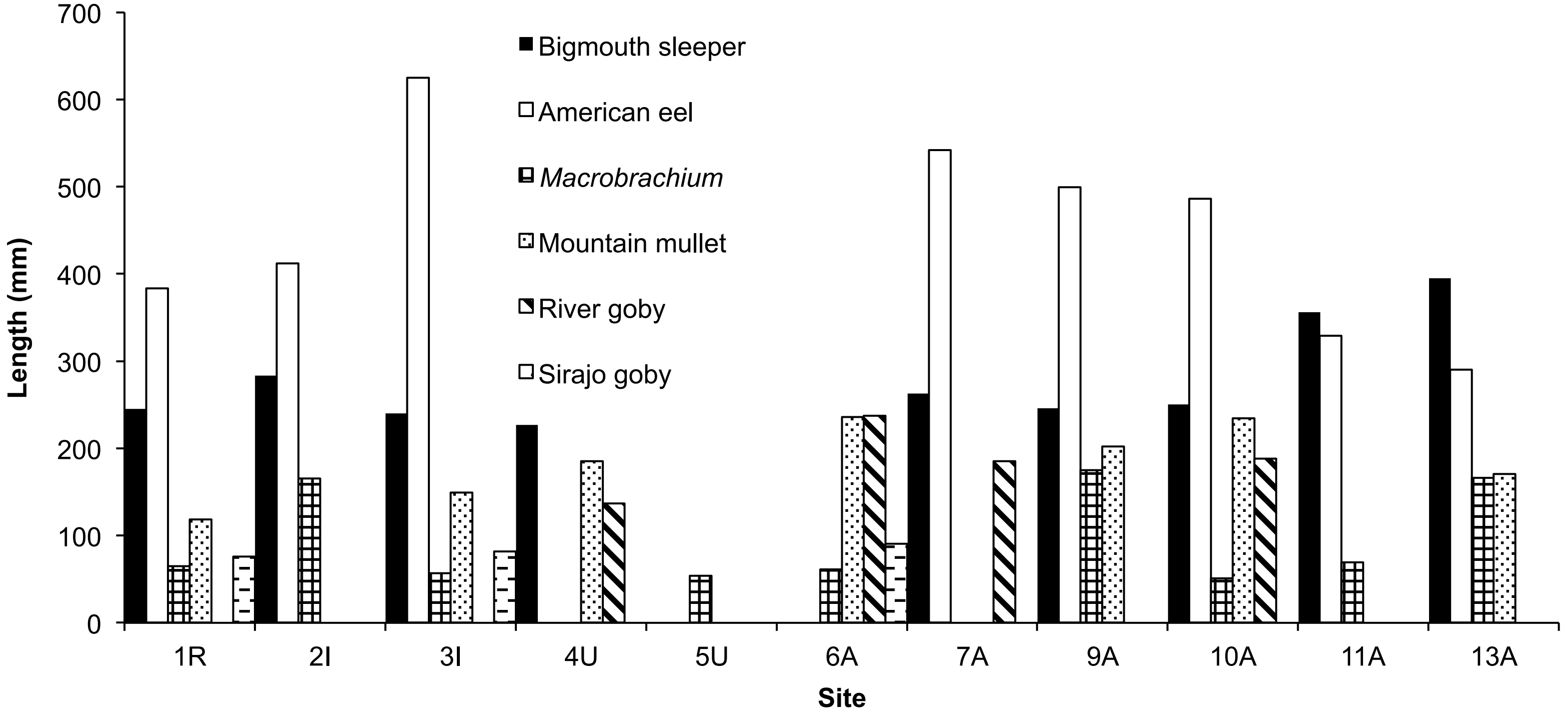

Figure 12. Length of fish and shrimp sampled at extensive sites.

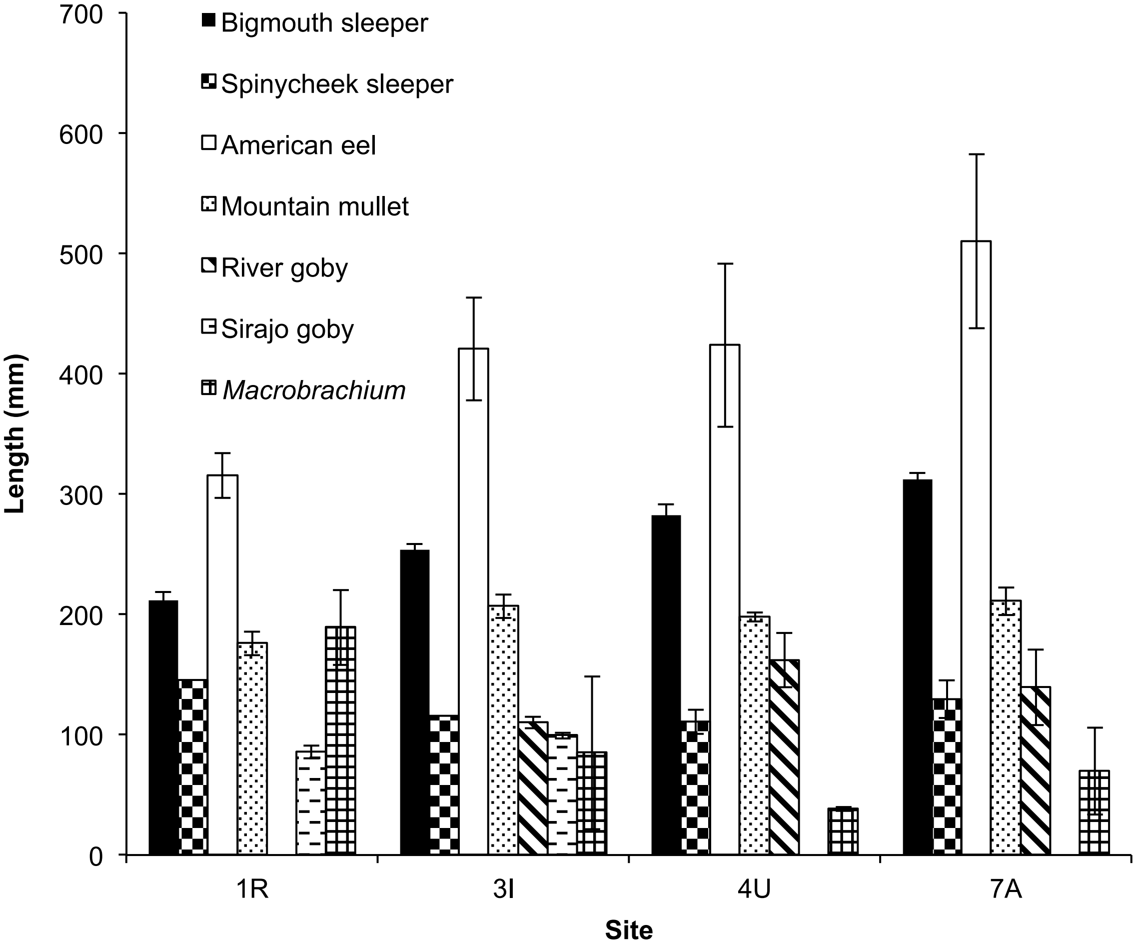

Figure 13. Mean (± SD) length of fish and shrimp sampled at intensive sites.

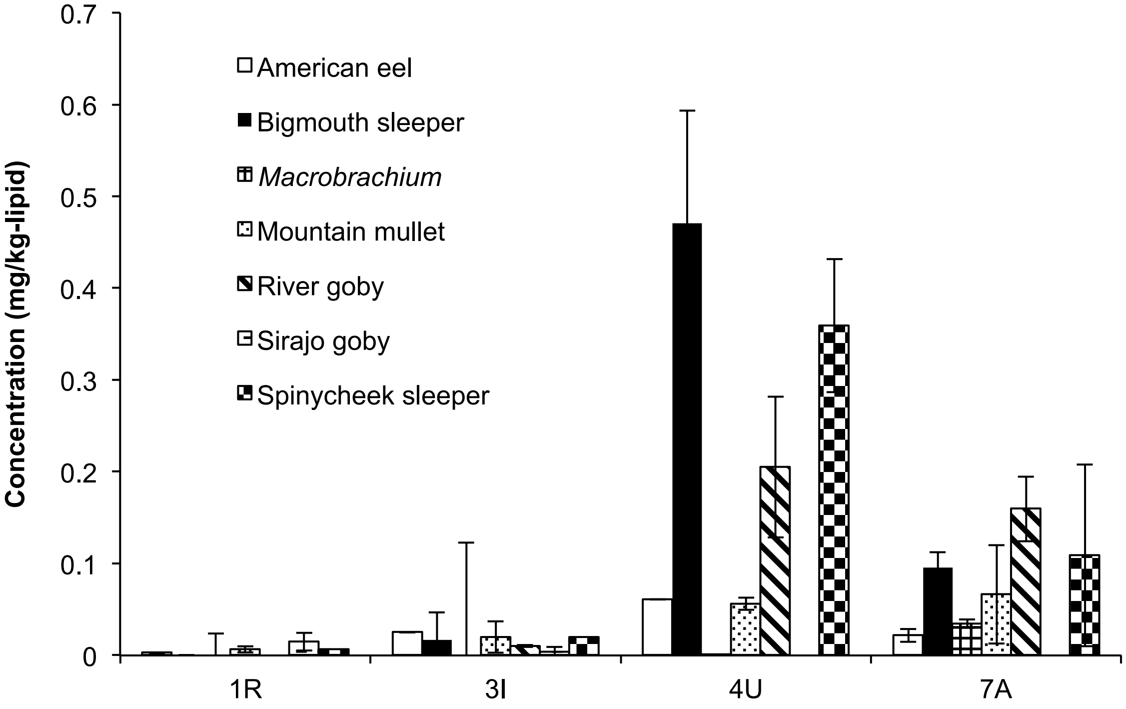

Figure 14. Mean (± SD) PCB concentrations normalized by lipid content for fish and shrimp sampled at intensive sites.

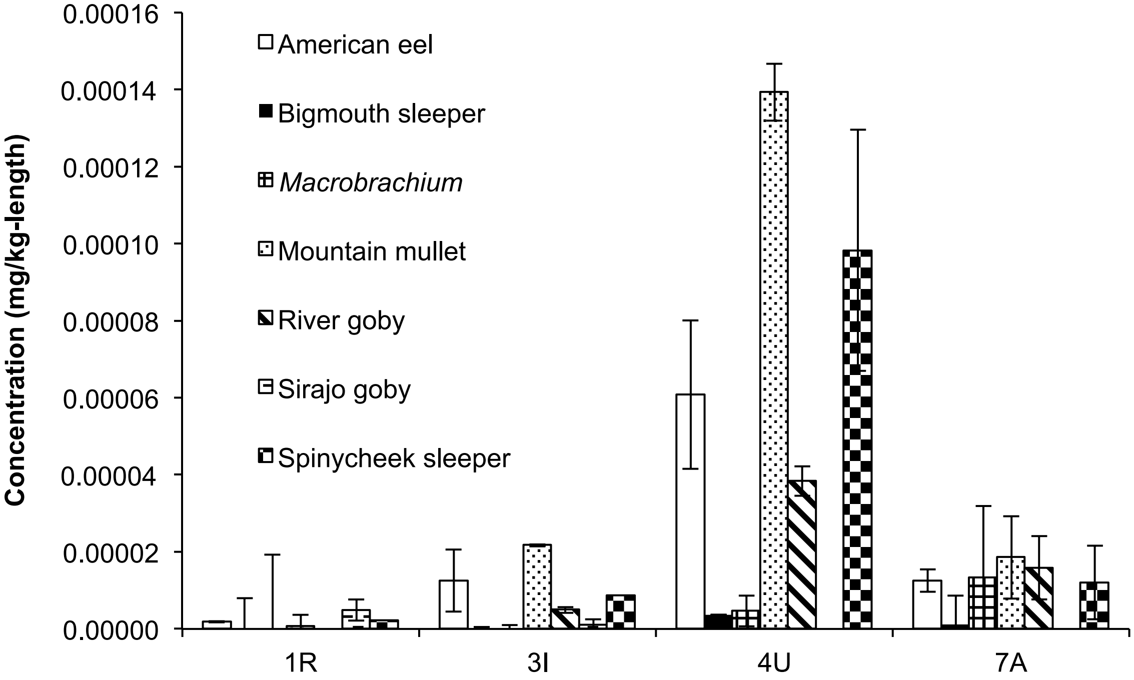

Figure 15. Mean (± SD) PCB concentrations normalized by organism length for fish and shrimp sampled at intensive sites.

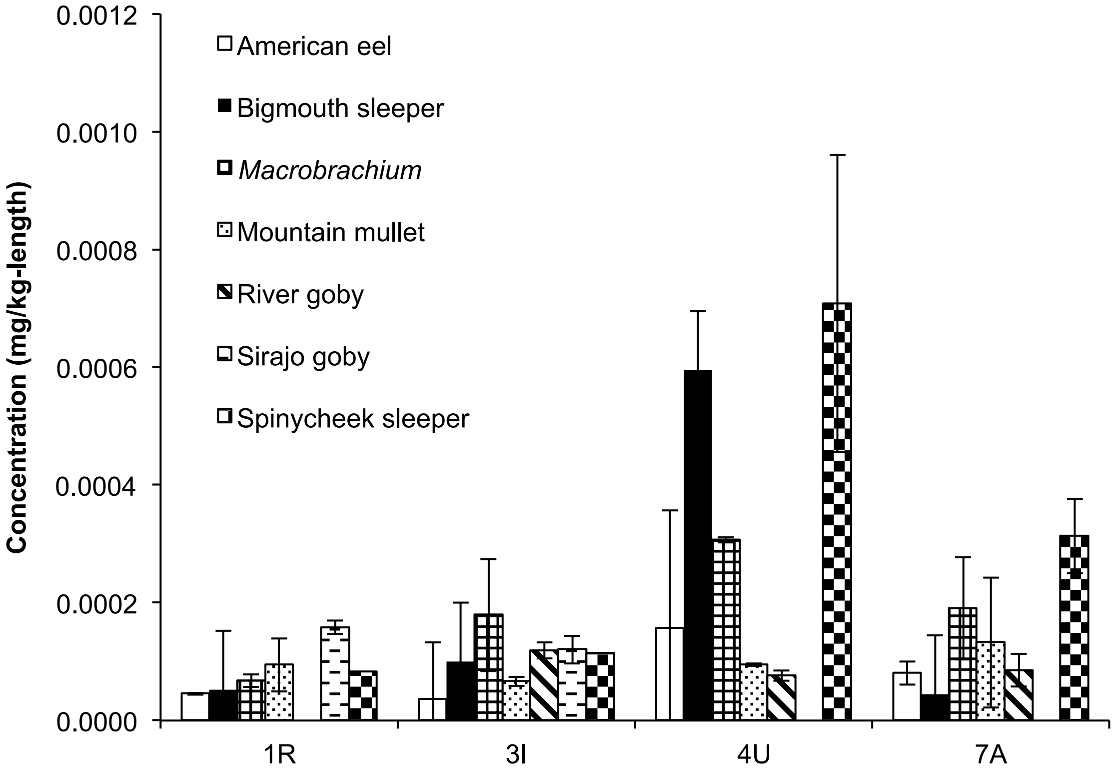

Figure 16. Mean (± SD) mercury concentrations normalized by organism length for fish and shrimp sampled at intensive sites.

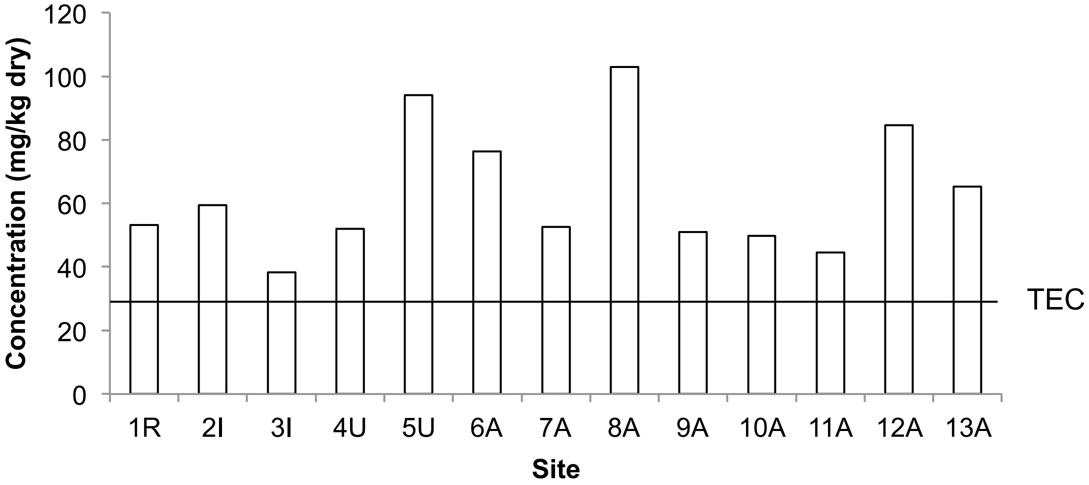

Figure 17. Copper concentrations in sediment with guideline exceedances for extensive sampling sites. TEC indicates the consensus based-threshold effect concentration (MacDonald et al. 2000).

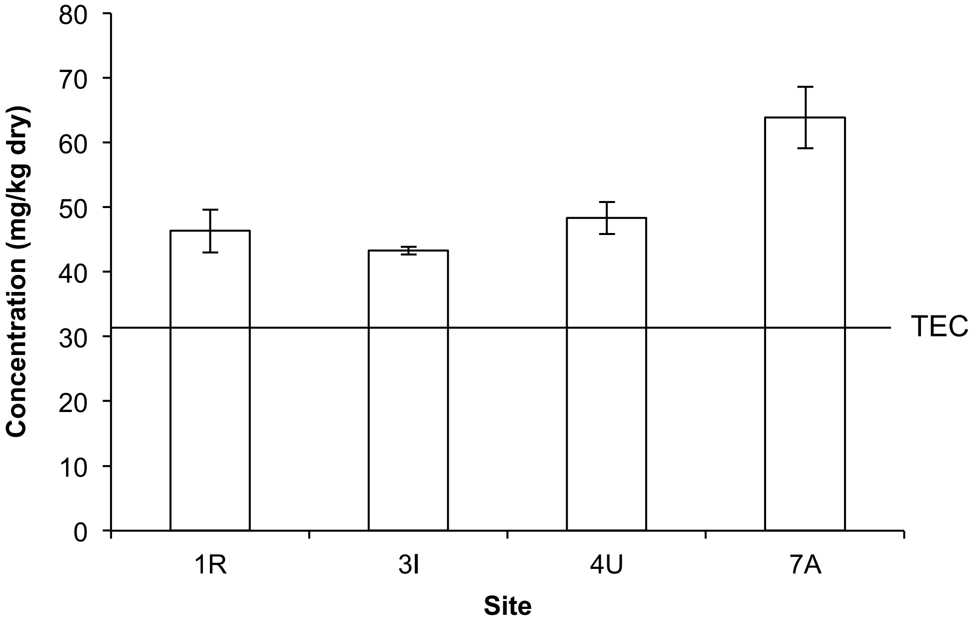

Figure 18. Mean (± SD) copper concentrations in sediment for intensive sampling sites. TEC indicates the consensus based-threshold effect concentration (MacDonald et al. 2000).

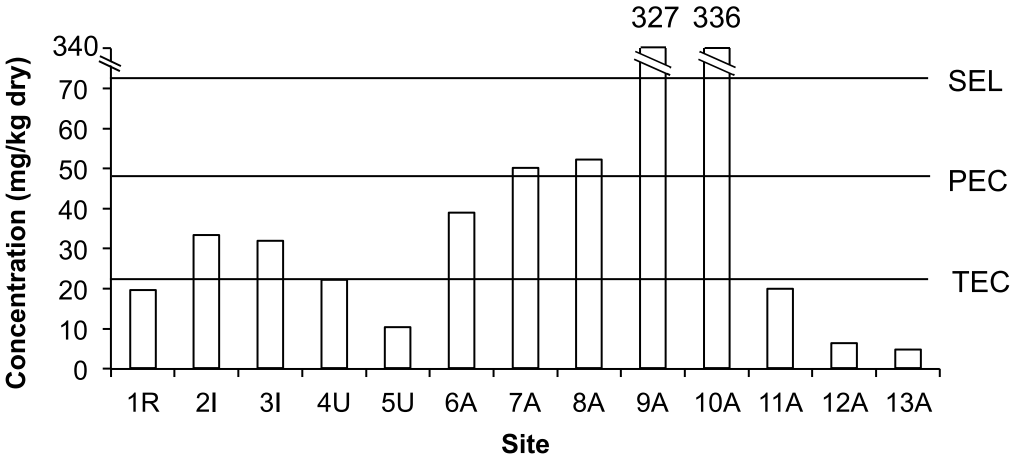

Figure 19. Nickel concentrations in sediment for extensive sampling sites. TEC indicates the consensus based-threshold effect concentration; PEC is the consensus based-probable effect concentration; and SEL is the severe effect level (MacDonald et al. 2000).

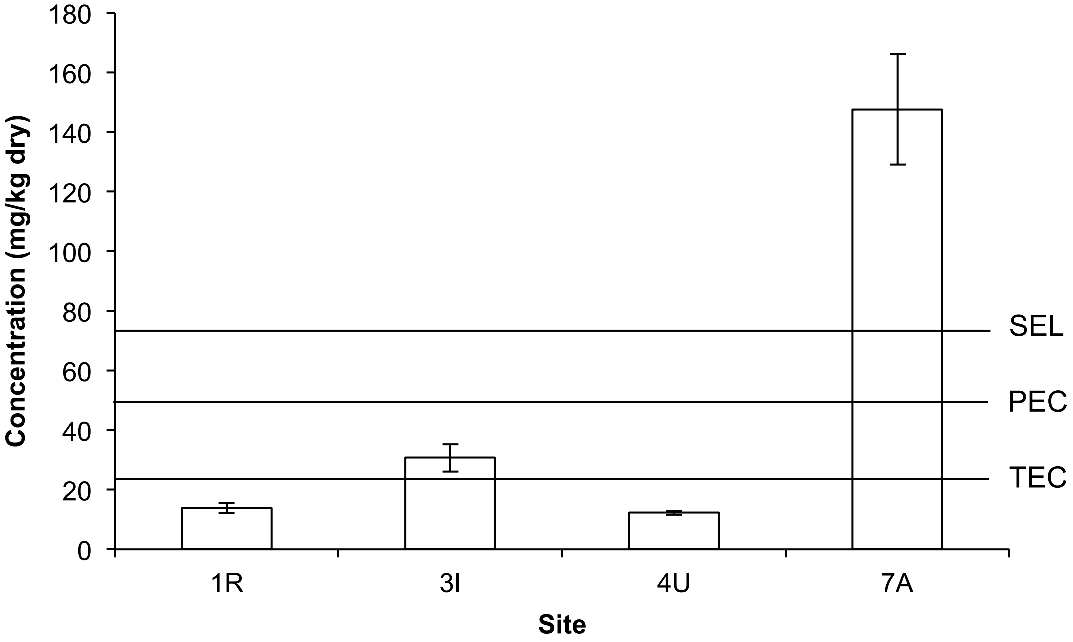

Figure 20. Mean (± SD) nickel concentrations in sediment for intensive sampling sites. TEC indicates the consensus based-threshold effect concentration; PEC is the consensus based-probable effect concentration; and SEL is the severe effect level (MacDonald et al. 2000).

CHAPTER 4

POLLUTION OF TROPICAL ISLAND STREAM ECOSYSTEMS: RELATION OF BIOTIC ACCUMULATION TO LAND USE AND TROPHIC DYNAMICS

(Job 3)

Abstract

Fate and effects of pollution are complex processes and many contaminants present in low levels in the environment may increase in concentration from one trophic level to the next, reaching concentrations that are harmful to wildlife and human consumers. Puerto Rico has a history of anthropogenic chemical usage, and its human population density is among the highest globally, providing a model environment to study human impacts on tropical island stream ecosystems. The objective of our research was to quantify occurrences and patterns of aquatic contaminants [polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, and metals] as related to riparian and watershed land-use characteristics and trophic relationships. We used stable isotope analyses of carbon, nitrogen, and sulfur to elucidate contaminant and trophic dynamics within four rivers with differing riparian and watershed land-use patterns (e.g., urban, agricultural, industrial, and forested). Overall, stream ecosystems in Puerto Rico were not severely polluted, with the exception of elevated concentrations of PCBs and mercury in some fish species. Trophic level and contaminant concentrations were poorly correlated in these dynamic systems that are characterized by frequent hydrologic disturbances, nutrient pulses, and marine influences. Calculation of food web magnification factors was complicated by low levels of contaminants, distorted estimates of trophic level due to $\delta^{15}N$ enrichment from nutrient pollution, and short food chains. Lipid content of consumers was a better predictor of contaminant concentration than trophic level. These findings enhance understanding of contaminant dynamics in tropical stream ecosystems and provide natural resource managers and public health agencies scientific information to guide ecosystem and fisheries management and human health risk assessment.

Introduction

Fate and effects of pollution are complex processes and many contaminants present in low levels in the environment may increase in concentration from one trophic level to the next (i.e., biomagnify), reaching concentrations that are harmful to wildlife and human consumers (Thrower and Eustace 1973; Rasmussen et al. 1990). Puerto Rico stream ecosystems may serve as an optimal model to study impacts and processes of pollution because of the island's dense human population and history of anthropogenic chemical usage. During the past century, rapid industrialization and human population growth have strained the limited natural resources of Puerto Rico (Hunter and Arbona 1995). Freshwater is an especially scarce resource, because no natural lakes occur on the island; consequently, most of the rivers have been transformed for water collection (e.g., construction of impoundments; Cooney and Kwak 2010). Streams in Puerto Rico provide many services for local populations, including water for drinking, recreation, irrigation, and as a source of fish and crustaceans for consumption (Neal et al. 2009). Therefore, a common conundrum exists where good water quality is necessary to protect human health and ecological integrity, but rapid human population growth has led to deteriorated water quality (Fitzpatrick and Keegan 2007). The streams have a history of die-offs of fish, shellfish, shrimp, and domesticated animals, caused by contaminated industrial, agricultural, and municipal wastes (Hunter and Arbona 1995). Yet, research is lacking on the degree and effects of contamination, and to our knowledge, no studies have applied multiple stable isotope analyses to elucidate trophic processes associated with contaminants in tropical island streams.

Stable isotope ratios of carbon ($^{13}C/^{12}C$ or $\delta^{13}C$), nitrogen ($^{15}N/^{14}N$ or $\delta^{15}N$) and sulfur ($^{34}S/^{32}S$ or $\delta^{34}S$) are often used to trace organic matter pathways in aquatic ecosystems (Peterson and Fry 1987; Kwak and Zedler 1997). Generally, nitrogen isotopic signatures may be used to estimate trophic position, carbon isotopes determine trophic pathways, and sulfur isotopes can identify migrant populations (Jardine et al. 2006). $\delta^{13}C$ signatures can determine the relative importance of different diet items by measuring the amount of carbon assimilated from each food source (Peterson and Fry 1987). Nitrogen indicates trophic position because consumers are enriched in $\delta^{15}N$ compared to their diet (Fry 1991). Sulfur stable isotope analysis is useful as a source tracer because amino acids containing sulfur are essential to animals, and it eliminates fractionation in the food chain, even revealing feeding relationships in complex estuarine systems that are inhabited by diadromous fish populations (Hesslein et al 1991).

An approach that integrates stable isotope and contaminant analyses can elucidate dietary exposure and biomagnification of contaminants, because diet is the major route of exposure for many persistent pollutants (Thomann et al. 1984; Hall et al. 1997). Stable isotope studies are a more powerful approach than assigning organisms to discrete trophic level classifications to predict contaminant concentrations of top predators because they account for large δ^{15}N variations, sometimes exhibited in omnivores or consumers of complex food webs, by representing a continuous, integrative measure of trophic level (Rasmussen et al. 1990; Cabana et al. 1994; Cabana and Rasmussen 1994,1996; Jardine et al. 2006). Estimating the food web magnification factor of contaminants is important, because contaminants with a high potential to biomagnify pose a greater threat to the health of humans and wildlife that rely on aquatic biota as a food source (Jardine et al. 2006). The biomagnification potential of contaminants in aquatic ecosystems is also useful information for guiding environmental policy and regulations, such as the maximum total release of industrial pollutants or other chemicals into the environment (Mackay and Fraser 2000).

The objectives of this study were to (1) measure stable isotope compositions of aquatic food web components, (2) examine patterns in isotopic composition of organic matter sources and consumers to identify trophic pathways, (3) estimate trophic position of consumers using δ^{15}N, and (4) describe relationships among isotope ratios, trophic level, lipid content, and contaminant concentrations to enhance understanding trophic dynamics of contaminants in tropical island rivers.

Methods

To examine anthropogenic effects on native fauna in stream ecosystems, sites were selected based on presence of target species and predominant riparian and watershed land uses. Prior knowledge of target species distribution and abundance was found in Kwak et al. (2007). Four stream sites were selected to represent varying predominant riparian and watershed land uses including a reference site (1R) downstream of a protected forest area within the El Yunque National Forest on Río Mameyes; an urban site (2U) within a densely populated region of San Juan on Río Piedras; an industrial site (3I) downstream of a cement plant on Río Cañas; and an agricultural site (4A) on Río Yauco, surrounded by banana plantations (Table 1; Fig 1).

Only four native freshwater fish families (consisting of less than 10 species) and three decapod families are found in Puerto Rico streams, and most of these species require a connection to the ocean to complete their life cycle (Kwak et al. 2007; Neal et al. 2009). *Xiphocaris elongata*, Atyid shrimp, and *Macrobrachium* spp. feed on algae, microbes, plant matter, and small insects (Pringle et al. 1993; Covich and McDowell 1996). Additionally, *Macrobrachium* shrimp consume mollusks, small fish, and other shrimp (Covich and McDowell 1996). *Neritina punctulata* and *Thiara granifera* are algae-grazing snails that are also known to consume terrestrial material (March et al. 2001, 2002). Mountain mullet (*Agonostomus monticola*) consume insects, shrimp, fruit, and algae (Phillip 1993; Aiken 1998; March and Pringle 2003). River gobies (*Awaous banana*) consume mostly periphyton and algae (Debrot 2003; Coat et al. 2009). Sirajo gobies (*Sicydium* spp.) rely mostly on algal sources and have been described as strict herbivores (Erdman 1961; Erdman 1986; Watson 2000), but others suggest that they may also consume insects (Parenti and Maciolek 1993; March and Pringle 2003). The diet of American eels (*Anguilla rostrata*) is dominated by the most abundant macroinvertebrates available (Tesch 1977). Smallscaled spinycheek sleeper (*Eleotris perniger*) diets are comprised mostly of biofilm, macroinvertebrates, and fish (Coat et al. 2009). Bigmouth sleepers (*Gobiomorous dormitor*) are generalist predators including a wide range of diets items (Hildebrand 1938; Winemiller and Ponwith 1998). In rivers, they consume fish, shrimp, crabs, snails, ostracods, insects, arachnids, terrestrial invertebrates, and reptiles (Bachelor et al. 2004) (Fig. 2).

Sample Collection

Fish were sampled during the summers of 2009-2010 and analyzed for contaminants and stable isotope ratios of C, N, and S. Native species sampled included bigmouth sleeper, smallscaled spinycheek sleeper, American eel, mountain mullet, sirajo goby, river goby, and *Macrobrachium* shrimp. Exotic species, introduced by the aquaculture industry, aquarium owners, anglers, and by agencies for fishing opportunities, are common throughout Puerto Rico (Neal et al. 2009). Our study focused on native species, but a reduced number of exotic species were sampled for stable isotope analyses to further describe the stream food web. Exotic fishes sampled included Nile tilapia (*Oreochromis mossambicus*), Amazon sailfin catfish (*Pterygoplichthys pardalis*), green swordtail (*Xiphophorus hellerii*), and guppy (*Poecilia* *reticulata*). The coastal river sites are inhabited by marine fish species that were also collected, including fat snook (*Centropomus parallelus)* and burro grunt (*Pomadasys crocro*). Fish, shrimp, and crabs were collected using backpack electrofishing following methods described by Kwak et al. (2007). Other macroinvertebrates were collected from instream cobble, bedrock, leaf packs, and macrophytes using a sweep net. Leaves and macrophytes were thoroughly rinsed of all invertebrates and fine particulate organic matter and then placed in a labeled plastic bag. Algae were collected using a scalpel to remove them from their substrate. Invertebrates and detritus were removed from algae and sorted under a dissecting scope. Aquatic snails were collected by hand. All samples were stored in a -20˚C freezer until they could be further processed.

Water Quality

A suite of water quality parameters was measured at each site during organism sampling procedures. Water was collected using a 1-L container that was submersed 0.25-0.50 m beneath the water surface, in laminar flow, and stored on ice in a cooler. Physicochemical water variables were measured using a calibrated Yellow Springs Instrument (YSI) 556 multi-probe system and a Hach CEL/850 Portable Aquaculture Laboratory and include temperature, pH, alkalinity (mg/L $CaCO_3$), total hardness (mg/L $CaCO_3$), conductivity (µS/cm), nitrate concentration (µg/L NO_3^-), nitrite concentration (mg/L NO_2^-), and reactive phosphorus concentration (mg/L PO_4).

Contaminant Analyses

Fish and shrimp tissue was analyzed for contaminant concentrations as composite samples using whole body or muscle tissue. The whole body of sirajo gobies was analyzed because humans consume the whole body of these fish, as do instream and avian predators. The whole body of river gobies and spinycheek sleepers was analyzed for contaminants, for similar reasons. The edible muscle, excluding skin or scales, of American eel and bigmouth sleeper was analyzed. Abdominal muscle tissue was analyzed for *Macrobrachium* shrimp.

Organic contaminant analyses of 26 chlorinated pesticides and 20 polychlorinated biphenyl congeners (PCBs) were performed at the North Carolina State University Department of Environmental and Molecular Toxicology Chemical Exposure Assessment Laboratory in

Raleigh, North Carolina, using a gas chromatograph-mass spectrometer. For metal analyses, biota samples were freeze dried and transported to Environmental Conservation Laboratories in Cary, North Carolina, for analyses of mercury, selenium, copper, nickel, zinc, cadmium, and lead. Cadmium, copper, lead, nickel, selenium, and zinc were analyzed using EPA Method 6010C and mercury was analyzed using EPA Method 7471B (www.epa.gov/sam).

Stable Isotope Analyses

Freeze-dried aliquots of fish and shrimp samples analyzed for contaminants were also used for stable isotope analyses. Stable isotope samples were dried at 60˚C to a constant weight. All samples were comprised of multiple individuals, and insect samples may have consisted of more than one species, but only one family. Only the muscle tissue of crustaceans, mollusks, and fish was analyzed for stable isotopes. Dried samples were sent to the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University in Flagstaff, Arizona, and were combusted to gases (CO_2, N_2, and SO_2), which were analyzed with an isotope mass spectrometer for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$. Snail samples were acid fumigated to remove any shell fragments, which consist of non-dietary derived carbonates that would bias results.

Isotopic ratios were calculated as $\delta X = (R_{sample}/R_{standard} - 1) \times 1000$; where X is the heavier isotope, R_{sample} is the ratio of the heavy to light isotope in the sample, and $R_{standard}$ is the ratio of the heavy to light isotope in the standard (Peterson and Fry 1987, Fry 1991). Analytical standards included atmospheric air for nitrogen, Vienna Pee Dee Belenite for carbon, and Canyon Diablo Triolite for sulfur (Fry 2006).

$\delta^{13}C$ Lipid Correction Techniques

Plant and animal species vary in lipid content, which is related to differences in life history constraints and foraging behavior (Schultz and Conover 1997; Post et al. 2007). Studies have shown lipid content is negatively correlated with their $\delta^{13}C$ values (Tieszenel et al. 1983; Focken and Becker 1998; Doucett et al. 1999) because lipids consist of a greater amount of the lighter isotopes as a result of oxidation of pyruvate to acetyl-CoA, during lipid formation (DeNiro and Epstein 1977). Two methods are used to correct for variation in $\delta^{13}C$ caused by lipid content—chemical extraction and mathematical normalization. Lipid normalization involves an adjustment of $\delta^{13}C$ based on the relationship between lipid content and C:N in tissues

(McConnaughy and McRoy 1979). It offers some advantages over lipid extraction because it requires less sample preparation and preserves the integrity of $\delta^{15}N$, but its accuracy is questionable (Post et al. 2007). It is most important to correct for lipids in species that have highly variable lipid content to correctly estimate food source (Post et al. 2007). Therefore, subsamples of fish and shrimp were analyzed for $\delta^{13}C$ and $\delta^{15}N$ both before and after lipid extraction treatment to evaluate the degree that lipids affect stable isotope signatures of these samples and to better understand how to account for $\delta^{13}C$ variation introduced by lipids.

Estimation of Trophic Level

Natural and anthropogenic inputs of nutrients are common in many aquatic food webs (Polis et al. 1997; Carpenter et al. 1998), which influence stable isotope signatures (Wayland and Hobson 2001). Thus, we estimated the base of the food web using primary consumers instead of primary producers because variation in $\delta^{15}N$ of primary producers can produce variation in $\delta^{15}N$ within consumers, leading to erroneous interpretations of trophic levels and food chain length (Kline et al. 1993; Cabana and Rasmussen 1996). Trophic level (TL) was estimated as: $TL_{organism} = (\delta^{15}N_{organism} - \delta^{15}N_{baseline})/\Delta 15N + 2$, where $\delta^{15}N_{baseline}$ is the measured $\delta^{15}N$ of a long-lived primary consumer (TL = 2). $\Delta 15N$ is a selected constant that represents the increase in $\delta^{15}N$ from one trophic level to the next (i.e., trophic enrichment) and is considered to be 3 to 4‰ (Minagawa and Wada 1984; Fry 1988). For this study, we set $\Delta 15N$ at 3‰, because ammonia-excreting organisms, as in stream ecosystems, have lower $\Delta 15N$ (Vanderklift and Ponsard 2003).

Bivalves (Gustafson et al. 2007), gastropods (Kidd et al. 1998; Post 2002), copepods (Fisk et al. 2001; Moisey et al. 2001; Fisk et al. 2003; Campbell et al. 2005) and other invertebrates (Vander Zanden and Rasmussen 1999) have been used to standardize the base of food webs. However, small organisms show greater temporal variability in $\delta^{15}N$ signatures than larger organisms (Cabana and Rasmussen 1996). Therefore, large primary consumers are optimal for site comparisons of isotopic signatures because of their slower nitrogen turnover rate; thus, we used snails as the baseline organism for this study.

Quality Control

A rigorous quality assurance protocol was followed during analyses. For metal analyses quality assurance included blanks, laboratory control samples (LCS), matrix spike, matrix spike duplicates, post spikes, and surrogate internal standards. The blanks were clean, with the exception of a few detections below the method reporting limit (MRL) including one detection of copper (0.07 mg/kg) and two detections of lead (<0.13 mg/kg). The relative percent difference (RPD) averaged 15% and ranged from 0.4-64%. For the few RPD values that were out of range, the batch was accepted based on percent recoveries for these samples that were within range. Overall, percent recoveries averaged 95% and ranged 2-185%. Very few matrix spike samples had percent recoveries that were out of range and all LCS percent recoveries were within range (mean = 99%, range = 85-110%), so range-exceeding samples were accepted based on the corresponding LCS percent recoveries. Procedural blanks, matrix spikes, and surrogate internal standards were used to assess organic contaminant data quality. Procedure blanks were clean with a few exceptions of PCB 138 in procedure blanks (<2 ng/g). Mean RPD values were 2% (range 0-4%) and average surrogate recoveries were 79% (range 28-176%). Results were not corrected for recoveries. Duplicate samples were also analyzed for quality assurance of lipid content of fish and shrimp and for stable isotope data. The mean RPD value for lipid data was 2% (range 0-4%).

Results

Water Quality

Water quality measurements varied widely among sampling sites, with notable differences between the reference site and other sites. Study site value ranges for water quality variables were, temperature, 27.6-34.7°C; total dissolved solids, 0.079-0.899 g/L; conductivity, 121-1,451 μS cm^{-1}; salinity, 0.06-0.69 ppt; nitrate as NO_3^-, 1.5-4.4 mg/L; nitrite as NO_2^-, 0.006-0.046 mg/L; ammonia, 0.00-0.69 mg/L; phosphorus as PO_4^-, 0.02-0.95 mg/L; alkalinity, 33-248 mg/L; hardness, 38-235; turbidity, 1-4 FAU; pH, 7.18-8.10; dissolved oxygen, 5.43-12.36 mg/L (Table 2). Stream water from the reference site, with a primarily forested watershed, was the lowest in ionic and nutrient content. Measurements of total dissolved solids, conductivity, salinity, nitrogen, phosphorus, alkalinity, and hardness were lowest at the reference site, while the agricultural site had the greatest measurements of total dissolved solids, conductivity, salinity, ammonia, phosphorus, alkalinity, and hardness. The agricultural site also had the lowest dissolved oxygen.

Stable Isotopes

One-way ANOVA for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, and pairwise comparisons between sites revealed that the baseline values of each site were significantly different from all other sites for all isotope ratios ($P < 0.05$; Figs 3 and 4). The reference site had the greatest baseline $\delta^{13}C$ lipid uncorrected values (mean = -23.3‰, SD = 0.36) and lowest baseline $\delta^{15}N$ values (mean = 4.6‰, SD = 0.22), while the agricultural site had the lowest baseline $\delta^{13}C$ (lipid corrected mean = -26.5‰, SD = 0.29; uncorrected mean = -24.7‰, SD = 0.26) and the greatest baseline $\delta^{15}N$ values (mean = 19.5‰, SD = 0.37). The urban site had the greatest baseline $\delta^{34}S$ values (mean = 6.3‰, SD = 0.30) and the lowest baseline $\delta^{34}S$ values were found at the industrial site (mean = 0.2‰, SD = 0.56). Samples collected from the agricultural site were approximately 15‰ enriched in ^{15}N compared to the reference site.

Studies have shown lipid content affects $\delta^{13}C$ values (Tieszenel et al. 1983; Focken and Becker 1998; Doucett et al. 1999) and that it is most important to correct for lipids in species that have highly variable lipid content (Post et al. 2007), which are mountain mullet and American eels in our study (Fig 5). Lipid extraction prior to stable isotope analyses resulted in a $\delta^{15}N$ average difference of 0.31‰ that ranged from 0.03‰ to 0.72‰ with a significant difference between extracted and unextracted samples ($N = 39$; $P < 0.0001$). We also detected a significant difference in $\delta^{13}C$ between lipid-extracted and unextracted samples averaging 0.82‰ with a range of -0.05‰ to 3.60‰ ($N = 39$; $P < 0.0001$). There was also a strong relationship between lipid content and C:N ($P < 0.01$, $R^2 = 0.82$; Fig 6) and change in $\delta^{13}C$ and C:N ($P < 0.01$, $R^2 = 0.63$; Fig. 7), demonstrating that mathematical normalization is appropriate. McConnaughy and McRoy (1979) and Post et al. (2007) both developed equations for lipid normalization that were compared to this study (Fig 8). The McConnaughy and McRoy (1979) correction technique underestimated most of the data while Post et al. (2007) was a better fit. Our results demonstrate species-specific differences in the relationship between change in $\delta^{13}C$, due to lipids, and C:N (Fig 9).

Stable isotope ratios did not consistently reveal food web interactions and structure, likely due to the complex and dynamic temporal variation of lotic systems. For example, lipid corrected and uncorrected $\delta^{13}C$ and $\delta^{13}S$ results did not indicate dietary sources. Secondly, predicted trophic levels of the agriculture site's food web were clearly inaccurate (e.g., algae were most enriched in ^{15}N), although, at the other sites, $\delta^{15}N$ exhibited similar trends, with top predators having more enriched ^{15}N values and producers being the most depleted in ^{15}N. In addition, microbial processes were revealed by significant negative correlations found between δ^{34}S and δ^{15}N at all sites ($P < 0.05$), except for at the reference site where there was a significant positive correlation ($P < 0.001$), possibly indicating trophic fractionation.

Contaminant Patterns

We generally found low contaminant concentrations at our sample sites (Table 3). Few organochlorine pesticides and few metals were consistently detected in fish samples. Although, bigmouth sleeper samples from the urban site had high levels of mercury (average = 0.17 mg/kg wet, range = 0.13-0.23 mg/kg wet), most samples (78%) contained mercury concentrations that were below detection limits. Food Web Magnification Factors were not calculated because no correlation was detected between lipid corrected contaminant concentrations and trophic level ($P > 0.05$; Table 3; Fig 10), with the exception of DDT concentration that was negatively correlated ($P < 0.001$). Trophic level, derived by δ^{15}N values, did not explain variance in lipid corrected concentrations of PCBs, chlordane, and mercury ($P > 0.05$) (Table 3). Contaminant concentrations were highly correlated with lipid content for all contaminants ($P < 0.001$), with the exception of mercury ($P > 0.05$; Figs 11-13).

Trophic level, δ^{15}N, and δ^{13}C were not correlated with contaminant concentrations, but δ^{34}S was correlated with PCB concentration, chlordane concentration, and mercury concentration when all sites were included in a single regression analyses ($P < 0.01$). However, when stratified by site, these variables were not significantly correlated ($P > 0.05$). No significant correlation of δ^{13}C with any contaminant concentration (PCBs, chlordane, DDT, and Hg) was detected after adjusting for lipid content ($P > 0.05$).

Discussion

Stable Isotope Variation Among Sites

Many factors influence isotopic composition of organic matter resulting in variation both among and within species and sites; such influences may include lipid content, salinity, hydrology, climate, and anthropogenic nutrient inputs (DeNiro and Epstein 1977; Gustafson et al. 1997; Finlay and Kendall 2007). Our results indicated significantly different baseline δ^{13}C, δ^{15}N, and δ^{34}S values among sites and a significant lipid extraction effect on δ^{15}N and δ^{13}C.

After adjusting for lipid content, our results indicated that lipid content is not a primary cause for $\delta^{13}C$ variation among sites, because baseline $\delta^{13}C$ values adjusted for lipids remained significantly different among sites. Our results confirm the need to account for lipid-related variation in ^{13}C, using techniques such as chemical lipid extraction or mathematical normalization, for biota with variable and high lipid content. The magnitude of the lipid-extraction effect on isotopic composition (i.e., difference between corresponding extracted and unextracted samples) was significantly explained by lipid content of organisms for $\delta^{13}C$, indicating potential for misinterpretation of dietary source if not lipid corrected. One disadvantage of chemical lipid extraction is that it is known to affect ^{15}N (Post 2007). We found a significant difference between the relative percent difference of quality control duplicates and the relative percent difference of the difference between ^{15}N lipid extracted and unextracted samples with the lipid extraction having a larger RPD than that associated with analytical error (i.e., RPD of duplicate samples) ($P < 0.001$). However, we found a maximum ^{15}N enrichment of 0.72‰, which would only result in an erroneous increase of 0.24 of a trophic level. We compared our results on lipid effects to the findings of other researchers, McConnaughy and McRoy (1979) and Post et al. (2007) and found that their equations did not fit our data. In addition, our data demonstrates that species-specific differences exist in the relationship between enrichment in ^{13}C due to lipids and C:N. Therefore, one generalized lipid correction equation cannot predict lipid correction of $\delta^{13}C$ as accurately as one developed for a single species.

Another factor that may influence isotopic composition is water salinity. Coastal ecosystems are typically enriched in ^{13}C, because C_4 saltmarsh grasses form the base of the food web (Kwak and Zedler 1997; Garcia et al. 2007; Winemiller et al. 2010). Our baseline $\delta^{13}C$ data conform to this trend, with baseline values decreasing with increasing salinity. Coastal ecosystems with higher salinities are also more enriched in ^{15}N than forested ecosystems (Ambrose and DeNiro 1987; Heaton 1987; Ambrose 1991; Hebert and Wassenaar 2001). Indeed, our agricultural site, with the most enriched $\delta^{15}N$ values, had the highest salinity, but it is unlikely that this variation accounted for the 15‰ difference observed between the reference and agricultural sites.

Stable isotope ratios of ^{13}C and $\delta^{34}S$ did not reveal diet sources of food web components, likely due to the complex and dynamic temporal variation of lotic systems associated with hydrology. We expected to observe a marine influence on these food webs as an explanation for the missing food web end members, due to the amphidromous nature of these shrimp and fish, and as suggested by stable isotope studies in other tropical island streams (Coat et al. 2009). However, all food web components $\delta^{34}S$ values ranged from -1.6 to 8.4‰, which is consistent with a range typical in freshwater ecosystems (Fry 2006). In contrast, marine $\delta^{34}S$ producers typically range from 17- 21‰ (Fry 2006). Animals that migrate between ecosystems with isotopically distinct food webs may retain isotope signatures from other feeding areas (Hobson 1999), but this probably does not explain our difficulty predicting dietary sources, because we sampled non-migratory adult consumers. Another possibility is that we did not sample the true organic matter sources, but other researchers indicated algae as a primary dietary source supporting a Puerto Rico stream food web (March and Pringle 2003).

Stable isotope ratios did not consistently indicate food web structure, which may also be attributed to the complex temporal variation of lotic systems associated with hydrology. $\delta^{15}N$ trends exhibited similar patterns for each site, with top predators having more enriched ^{15}N values and producers being the most depleted in ^{15}N, with the exception of the predicted trophic levels of the agriculture site's food web, which were clearly inaccurate (e.g., algae were most enriched in ^{15}N). Riverine food webs have high spatial and temporal complexity and variability relative to other ecosystems. Streams typically contain a complex mixture of allochthonous and autochthonous organic matter, as a result, food web interactions are difficult to elucidate by traditional approaches (Finlay and Kendall 2007). Isotopic conditions of aquatic plants are more variable and consequently less predictable than terrestrial plants, mainly because of the large variation in the concentrations stable isotope ratios of dissolved inorganic carbon, nitrogen, and sulfur in freshwater ecosystems and the physiological diversity of aquatic producers (Finlay 2004; Finlay and Kendall 2007). Physical and biogeochemical processes, such as assimilation/uptake, nitrification-denitrification, carbon dissolution, degassing exchange, photosynthesis, respiration, methane oxidation, may cause large variations in isotopic compositions of dissolved inorganic species and lead to variability in isotopic compositions of aquatic plants (Finlay and Kendall 2007).

Stream hydrology strongly influences the $\delta^{13}C$ signature of dissolved inorganic carbon ($\delta^{13}C_{DIC}$) with fluctuations in $\delta^{13}C$ during high-flow events, because of shifts in the relative proportion of flow from surface water versus groundwater contributing to discharge (Finlay and Kendall 2007). It is especially problematic for the use of stable isotope techniques when stochastic processes are involved, such as floods, which render stable isotope values unpredictable (Finlay and Kendall 2007). Flood events may mobilize new and distinctly different sources of carbon, nitrogen, and sulfur than those during baseflow conditions sources, resulting in isotope values within food webs that vary temporally and $\delta^{13}C$ values that constantly change making it difficult to determine carbon source (Finlay and Kendall 2007; Buda and DeWalle 2009). Other researchers demonstrated a negative correlation between discharge and $\delta^{13}C$ and $\delta^{13}C_{DIC}$, with most of the temporal variation in algal $\delta^{13}C$ explained by discharge (Finlay 2004; Finlay and Kendall 2007).

Hot, arid environments tend to be associated with higher nitrogen isotope ratios than cool, wet environments (Heaton 1987; Ambrose 1991), because denitrification rates are related to temperature (Kendall 1998), and precipitation can affect riverine N export (Hebert and Wassenaar 2001; Showers et al. 2006). A mountain range in central Puerto Rico forms a barrier to northeast tradewinds causing a rainshadow effect over much of the southern coast, which receives less than 1,140 mm of rain annually, whereas northern Puerto Rico averages about 2,030 mm (Hunter and Arbona 1995). However, this climatic trend does not explain the differences in $\delta^{15}N$ that we observed among sites. As expected, the reference site located in the northern, rainforest region of the island had the lowest $\delta^{15}N$ values and those of the agricultural site in the southern, dry forest region were the greatest, but the industrial and urban sites had similar $\delta^{15}N$ values even though the industrial site is located on the southern mountain slope and the urban site is on the northern mountain slope. Thus, factors other than water availability must influence spatial variation in ^{15}N enrichment.

Pollution and anthropogenic nutrient enrichment can affect trophic state and alter aquatic food webs in watersheds exposed to urbanization or agriculture (Fogg et al. 1998; Clements et al. 2000; deBruyn and Rasmussen 2002). $\delta^{15}N$ differences in the base of the food web among ecosystems may indicate anthropogenic nitrogen inputs from sewage or agriculture (Anderson and Cabana 2005; Cabana and Rasmussen 1996). $\delta^{15}N$ nitrate values of commercial fertilizers generally range from -2.5 to 2.0‰; organic soil nitrate ranges from -2 to 9‰; and human and animal waste range from 10 to 20‰ (Kreitler and Jones 1975; McClelland et al. 1997; Kwak and Zedler 1997), but may vary further due to fractionation (Harrington et al. 1998). For instance, tracing nitrate sources with $\delta^{15}N$ values can be complicated by various sources of fractionation, such as volatile loss of ammonia from animal wastes (Heaton 1986; Macko and Ostrom 1994;

Wayland and Hobson 2001), denitrification in soil and ground water (Bottcher et al. 1990; Aravena et al. 1993), and uptake of nitrate by microbes and algae (Estep and Vigg 1985). Our $\delta^{15}N$ values were elevated at the agricultural site, likely indicating nitrogen enrichment from animal waste applied to the land as fertilizer or from confined animal operations. An animal production facility is located approximately 7.2 km upstream of this site. Our finding is similar to that of Winemiller et al. (in press), who also found a site near a banana plantation with most enriched $\delta^{15}N$ values of their stream sites at about 10‰ greater than their reference site.

Nutrient and fecal pollution in river water are threats to the integrity of aquatic ecosystems in Puerto Rico (Hunter and Arbona 1995; Warne et al. 2005). Large-scale land use changes of the island have caused aqueous nitrogen and phosphorus concentrations to increase 10-fold (McDowell and Ashbury 1994; Warne et al. 2005), a pattern consistent with our results, with greatest nutrient loads at the agriculture and urban site. Urbanization has increased rapidly in Puerto Rico, creating a lag in infrastructural services and a supply of human waste that exceeds treatment capacity, and consequently, raw sewage is sporadically discharged into streams (Hunter and Arbona 1995). In 1984, U.S. Geological Survey reported that 81 percent of their sampling stations exceeded maximum microbiological contaminant levels for recreational water, suggesting widespread fecal contamination, but tropical temperatures and humidity may promote high coliform counts, even in pristine forested sites (Lopez et al. 1987; Hazen 1988). Bacterial densities are positively correlated with water nutrient levels, particularly nitrates and phosphates, with high bacterial densities found at sewage outfalls (Carrillo et al. 1985). Wet, anoxic, and carbon-rich hydric soils, are associated with enriched groundwater nitrate ^{15}N, most likely due to denitrification (Showers et al. 2006). For example, $\delta^{15}N$ and $\delta^{18}O$ studies indicated that denitrification occurs in groundwater under biosolid and poultry litter application fields, but only in hydric soils (Showers et al. 2006). $\delta^{15}N$ of NO_3 and $\delta^{18}O$ could be measured in future research of these Puerto Rico streams to better identify NO_3 sources entering the food web (Fogg et al 1998).

Variation in $\delta^{34}S$ measurements in streams can be attributed to ocean proximity, bedrock geology, redox conditions, and pollution (Finlay and Kendall 2007). An unexpected finding was that the agricultural and industrial sites located closest to the coast were relatively depleted in ^{34}S. Competing microbial processes likely influence $\delta^{34}S$ variation among streams. We found negative correlations between $\delta^{34}S$ and $\delta^{15}N$ values at all sites, except for the reference site, where there was a significant positive correlation. The negative relationship may be explained by sulfate reduction inhibition of denitrification. Elevated levels of ^{15}N are created by denitrification, and suggest that high levels of sulfide (produced by sulfate-reducing bacteria) are not widely distributed because denitrification inhibits sulfate-reduction (Joye and Hollibaugh 1995). Negative $\delta^{34}S$ values are indicative of sulfate reduction (Evans and Crumley 2005) and were not observed in most of our samples; however, nitrogen isotope enrichment was observed at the agricultural, industrial, and urban sites. The positive correlation of $\delta^{34}S$ with $\delta^{15}N$ at the reference site may be attributed to trophic fractionation. Sulfur isotopes are generally considered to remain unaltered with trophic transfers (Peterson 1999); however, McCutchan et al. (2003) demonstrated that trophic fractionation in $\delta^{34}S$ may reach 2‰.

Food Web Magnification

The calculation of food web magnification factors (FWMF) for the streams we studied was not feasible because the studied stream ecosystems were not severely polluted, food chain length was short, and nutrient loads varied temporally. FWMF were not calculated because no correlation existed between most lipid corrected contaminant concentrations and trophic level ($P > 0.05$); this was the case for lipid corrected concentrations of PCBs, chlordane, and mercury. However, DDT concentrations were negatively correlated with trophic level, contrary to expectations. This trend is likely an artifact of erroneous trophic level estimates at the agricultural site. Stream food chains in Puerto Rico are short, consisting of less than 3 consumer trophic levels. Other studies have shown biomagnification increases with food chain length (Rasmussen et al. 1990). In the tropical streams that we studied, lipid content is a better predictor of organic contaminant concentration than trophic level. Another stable isotope study examining an ecosystem with a short food chain showed that lipid and carbon dietary source were more dominant factors affecting biota contaminant concentrations than trophic level (Campbell et al. 2000). Our study predicted very strong positive correlations between contaminant concentrations and lipid content, with the exception of mercury. Because PCBs and chlorinated pesticides are lipophilic, the greater the lipid content of an organism, the greater the capacity for contaminant accumulation. Mercury has a different bioaccumulation mechanism than highly lipophilic organic contaminants. Mercury binds to sulphydryl groups in protein causing it to accumulate in muscle, gill, liver, and brain tissue (Spry and Wiener 1991), and thus, we detected variably low correlations between lipid content and mercury concentrations among sites.

Contaminant and Other Stable Isotope Relationships

Ecological characteristics that promote contaminant bioavailability are similar to those that support microbial fractionation processes of stable isotopes. δ^{34}S was correlated with PCB, chlordane, and mercury concentrations when all sites were included in the analyses ($P < 0.01$). However, when each site was examined separately, these variables were not correlated ($P > 0.05$). These variable results may simply be an artifact of sample size and test power. DDT was below detection limits for a majority of samples, and low sample sizes may partially explain the lack of significant correlations with δ^{34}S ($P > 0.05$). Sulfate reduction and contaminant availability are both associated with organic matter, which varies temporally due to hydrological variation, a dynamic influence on our results.

Our finding that mercury concentrations were correlated with δ^{34}S ($P < 0.01$) support the hypothesis that there are significant biological linkages between bacterial sulfate reduction, methylmercury production, sulfur isotope fractionation, and methylmercury accumulation in fish, as also suggested by other studies (Evans and Crumley 2005; Ethier et al. 2008). A major source of mercury in fish muscle tissue is methylmercury produced by sulfur-reducing bacteria at near-anoxic conditions (Spry and Wiener 1991; Regnell et al. 2001). Increasing water sulfate concentrations, and thus δ^{34}S enrichment, can be associated with both increased sulfur-reducing bacterial activity and elevated methylmercury concentrations in fish (King et al. 2002). δ^{34}S may be correlated with fish mercury concentration, because sulfur is known to fractionate in the environment by sulfate-reducing bacteria (Ethier et al. 2008). Methylmercury production and subsequent food chain transfer is influenced by a number of site-specific factors, including sediment mercury concentrations, watershed area, water temperature, and water chemistry, in particular pH, alkalinity, concentrations of dissolved organic carbon, total nitrogen, and sulfate (Bodaly et al. 1993; Chen et al. 2005; Ethier et al. 2008). Other parameters that may influence fish mercury concentration are fish size, trophic level (as indicated by δ^{15}N), and dietary carbon source (as indicated by δ^{13}C) (Atwell et al. 1998; Weech et al. 2004).

Dietary carbon source (δ^{13}C) may reflect an influence of feeding strategies (e.g., littoral, benthic, pelagic) on contaminant accumulation in some ecosystems (Kidd et al. 2001; Kidd et al.

2003; Jardine et al. 2006). However, we did not find significant correlations of $\delta^{13}C$ with contaminant concentrations (PCBs, chlordane, DDT, and Hg) after adjusting for lipid content. Our study streams are shallow systems that do not include disjunct macrohabitats like those in lentic systems where other researchers have shown relationships among pelagic, benthic, and littoral habitats and contaminant concentrations. Furthermore, considering that $\delta^{13}C$ did not indicate dietary source, it is expected that it would not predict contaminant accumulation, because diet is the major route of contaminant exposure.

Future Studies

Additional studies could improve understanding of contaminant trophic dynamics in tropical island streams. A one-time collection and analysis of food web components may not adequately represent food webs that are temporily variable due to periodic influxes in nutrients, organic matter, and contaminants from adjacent systems (Polis et al. 1997). Species-specific diet-tissue fractionation (i.e., trophic fractionation) and estimates of turnover rate would enhance mechanistic and ecological understanding (Post 2002; Gustafson et al. 2007). An approach using multiple tissue types would more precisely trace the flow of organic matter and contaminants through an ecosystem and could elucidate temporal dynamics (MacNeil et al. 2005), because different tissues have different tissue-isotopic ratio fractionation and different turnover rates (Hobson and Clark 1992). Stream flow data may be used to account for temporal variation of stable isotopes because of the strong effect of discharge on parameters influencing stable isotope signatures of food web components in lotic systems (Finlay and Kendall 2007).

Conclusions

Pollution in Puerto Rico streams is not severe, with only a few localized exceptions, and consequently, it was challenging to elucidate contaminant trophic dynamics. PCBs were detected most frequently of all the contaminants tested, but results based on $\delta^{15}N$ did not indicate a correlation to trophic level, instead there was a stronger relationship with lipid content. Tropical island streams are complex systems with constant species movement and multiple food and energy sources. A better understanding of nutrient and organic matter exchange within these ecosystems may be important in assessing contaminant transfer. Identification of nitrogen inputs contributing to water quality degradation is crucial for management planning and regulation of water quality uses. Our results suggest that the δ^{15}N signature of primary consumers provides a useful tool for monitoring anthropogenic watershed impacts in Puerto Rico stream ecosystems. Landscape alteration for agriculture, urban development, and other uses can have important effects on the ecological integrity of rivers, including eutrophication and loss of biodiversity (Allan 2004; Kwak and Freeman 2010). These findings enhance understanding of contaminant dynamics in tropical stream ecosystems and provide natural resource managers and public health agencies scientific information to guide ecosystem and fisheries management and human health risk assessment.

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Table 1. Puerto Rico stream sampling site characteristics, including location, distance from river mouth, elevation, gradient, and watershed attributes.

Site	Designated Land Use	River Name	Latitude	Longitude	Distance to River Mouth (km)	Site Elevation (m)	Watershed Area (km^2)	Gradient (%)	Watershed Land Use: Agriculture (%)	Watershed Land Use: Forest (%)	Watershed Land Use: Shrub and Woodland (%)	Watershed Land Use: Urban (%)
1R	Reference	Mameyes	N18°21'23.7"	W65°46'06.9"	4.4	14.0	21.3	1.4	2.7	95.6	1.0	0.1
2I	Industrial	Cañas	N18°01'23.2"	W66°38'26.4"	5.0	30.2	20.6	0.4	30.0	27.4	32.3	9.8
3U	Urban	Piedras	N18°23'02.8"	W66°03'31.2"	8.6	13.4	23.2	0.2	24.8	26.3	8.8	39.4
4A	Agricultural	Yauco	N17°59'12.9"	W66°50'25.7"	5.7	10.1	115.9	0.1	24.9	31.5	36.6	6.1

Table 2. Water quality parameters measured at each Puerto Rico stream sampling site.

	Site			
Parameter	1R	3I	4U	7A
Water temperature (°C)	34.7	31.7	27.8	27.6
Total dissolved solids (g/L)	0.079	0.489	0.386	0.899
Conductivity (µS/cm)	121	844	628	1451
Salinity (ppt)	0.06	0.36	0.28	0.69
Nitrate (mg/L as NO_3-)	1.8	1.5	4.4	3.5
Nitrite (mg/L as NO_2-)	0.006	0.006	0.046	0.015
Ammonia/nitrogen (mg/L as NH_3)	<0.01	0.08	0.02	0.69
Phosphorus (mg/L as PO_4-)	0.02	0.95	0.59	0.83
Alkalinity (mg/L as $CaCO_3$)	33	131	118	248
Hardness (mg/L as $CaCO_3$)	38	185	182	235
Turbidity (FAU)	4	1	3	4
pH	7.27	8.10	7.29	7.18
Dissolved oxygen (mg/L)	8.26	12.36	8.23	5.43

Table 3. Most frequently detected mean contaminant concentrations with standard deviation (SD), summarized by site and species. Organic contaminant concentrations are expressed as parts per billion wet weight and mercury (Hg) concentrations are expressed as parts per million wet weight. 'ND' indicates no detection, 'NA' indicates not applicable, an asterisk indicates that $N = 1$.

		PCBs		Chlordanes		DDT		Hg	
Site	Species	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1R	*Gobiomorus dormitor*	ND	NA	ND	NA	ND	NA	ND	NA
	Anguilla rostrata	0.594	0.051	ND	NA	0.244	0.231	ND	NA
	Macrobrachium spp.	ND	NA	ND	NA	ND	NA	ND	NA
	Agonostomus monticola	0.135	0.135	ND	NA	ND	NA	0.017	0.009
	Sicydium spp.	0.410	0.206	ND	NA	ND	NA	0.014	<0.001
	*Eleotris perniger**	0.321	NA	ND	NA	ND	NA	ND	NA
2U	*Gobiomorus dormitor*	1.027	0.268	ND	NA	ND	NA	0.168	0.056
	Anguilla rostrata	25.017	5.902	10.424	3.690	8.039	5.024	0.068	0.065
	Macrobrachium spp.	0.182	0.159	ND	NA	ND	NA	ND	NA
	Agonostomus monticola	27.529	3.182	13.467	0.274	4.866	0.228	ND	NA
	Awaous banana	6.247	1.308	0.399	0.084	ND	NA	ND	NA
	Eleotris perniger	10.762	3.263	2.205	1.394	0.274	0.475	0.077	0.025
3I	*Gobiomorus dormitor*	0.041	0.071	ND	NA	ND	NA	0.025	0.024
	Anguilla rostrata	5.047	2.693	0.547	0.323	0.721	0.378	ND	NA
	Macrobrachium spp.	ND	NA	ND	NA	ND	NA	ND	NA
	Agonostomus monticola	4.442	1.302	1.073	1.029	0.971	0.477	ND	NA
	Awaous banana	0.539	0.064	ND	NA	ND	NA	ND	NA
	Sicydium spp.	0.090	0.155	0.232	0.058	ND	NA	ND	NA
	*Eleotris perniger**	0.989	NA	0.222	NA	ND	NA	ND	NA
4A	*Gobiomorus dormitor*	0.375	0.108	ND	NA	0.130	0.028	0.014	0.015
	Anguilla rostrata	6.490	2.356	1.119	0.471	6.095	2.990	0.039	0.006
	Macrobrachium spp.	0.764	0.056	ND	NA	ND	NA	ND	NA
	Agonostomus monticola	3.841	1.988	0.373	0.470	2.954	1.552	0.028	0.024
	Awaous banana	2.050	0.562	ND	NA	0.793	0.252	ND	NA
	Eleotris perniger	1.525	1.281	0.082	0.071	0.657	0.584	0.041	0.011

Table 4. Correlation matrix (*R* values) of most commonly detected contaminants (PCBs, chlordane, DDT, and Hg) versus stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$-derived trophic level (TL), $\delta^{34}S$) and lipid content, for all sites and within each site. Significant correlations are indicated by '*' ('*' = $P < 0.05$; '**' = $P < 0.01$; '***' = $P < 0.001$). $\delta^{13}C$ were lipid corrected, and contaminant concentrations were also lipid corrected, but only when examining correlations with $\delta^{13}C$ and trophic level. 'NA' indicates that the number of detections for that contaminant were inadequate to evaluate a correlation.

Biotic Parameter	PCBs	Chlordane	DDT	Hg
		All Sites		
$\delta^{13}C$	0.149	0.081	0.190	0.154
$\delta^{34}S$	0.343**	0.290*	0.120	0.318**
Trophic level	0.007	0.033	-0.745***	0.029
Lipid content	0.713***	0.728***	0.717***	0.173
		Reference		
$\delta^{13}C$	0.136	NA	NA	NA
$\delta^{34}S$	0.329	NA	NA	NA
Trophic level	0.258	NA	NA	NA
Lipid content	0.705**	NA	NA	NA
		Industrial		
$\delta^{13}C$	0.432	0.439	0.493*	NA
$\delta^{34}S$	0.330	0.314	NA	NA
Trophic level	0.290	0.161	0.332	NA
Lipid content	0.979***	0.769***	0.936***	NA
		Urban		
$\delta^{13}C$	0.185	0.137	0.357	0.326
$\delta^{34}S$	0.268	0.196	NA	0.238
Trophic level	0.134	0.209	0.265	0.087
Lipid content	0.788***	0.868***	0.726***	0.374
		Agricultural		
$\delta^{13}C$	0.034	0.189	0.420	0.226
$\delta^{34}S$	0.476	0.395	0.400	0.056
Trophic level	0.149	0.384	-0.529*	0.415
Lipid content	0.666**	0.869***	0.875***	0.054

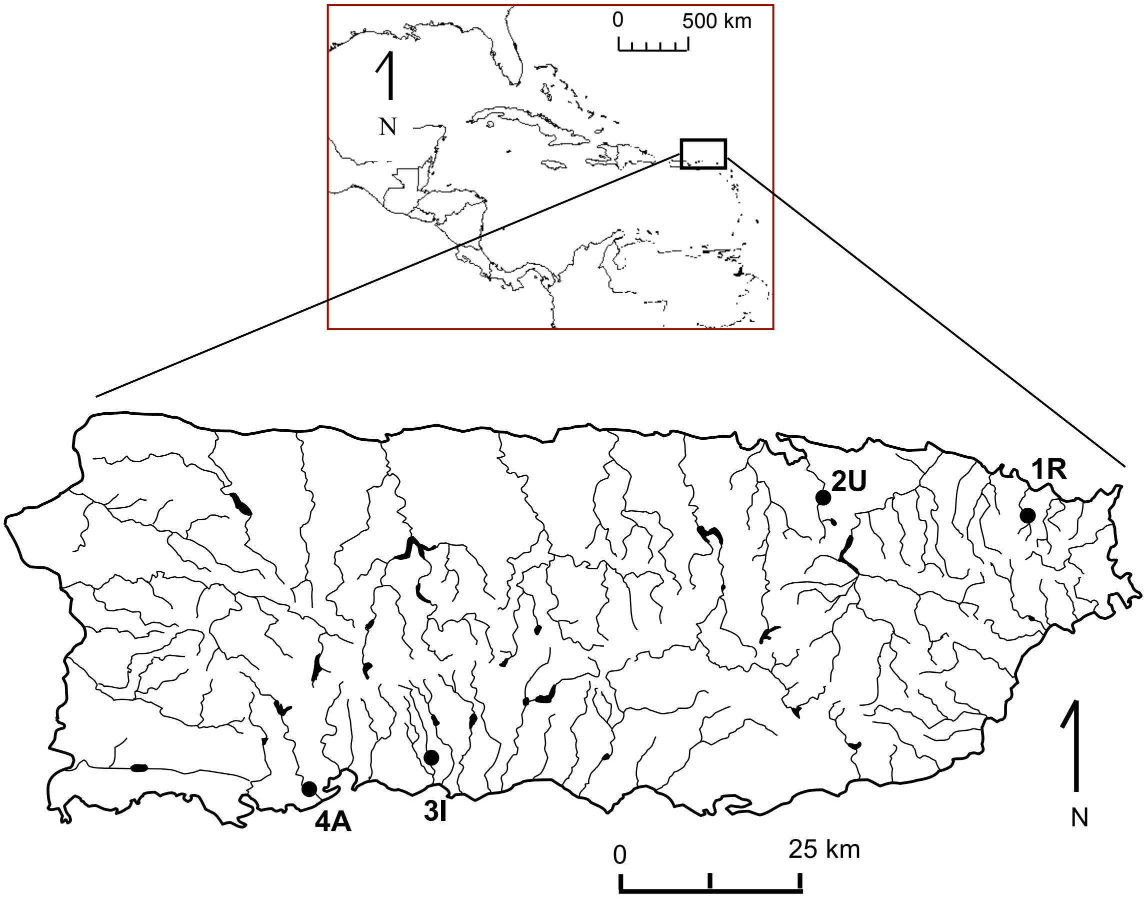

Figure 1. Map of Puerto Rico indicating locations of study sites and streams.

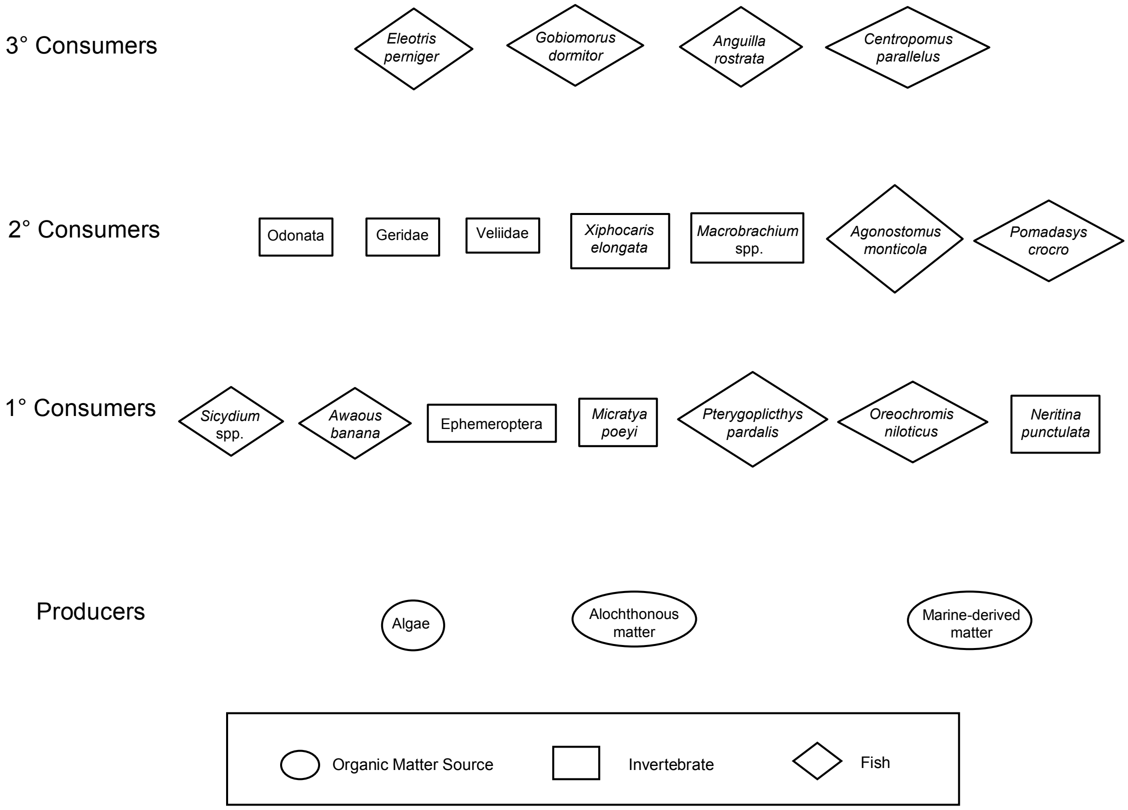

Figure 2. A generalized food web diagram of a stream ecosystem in Puerto Rico.

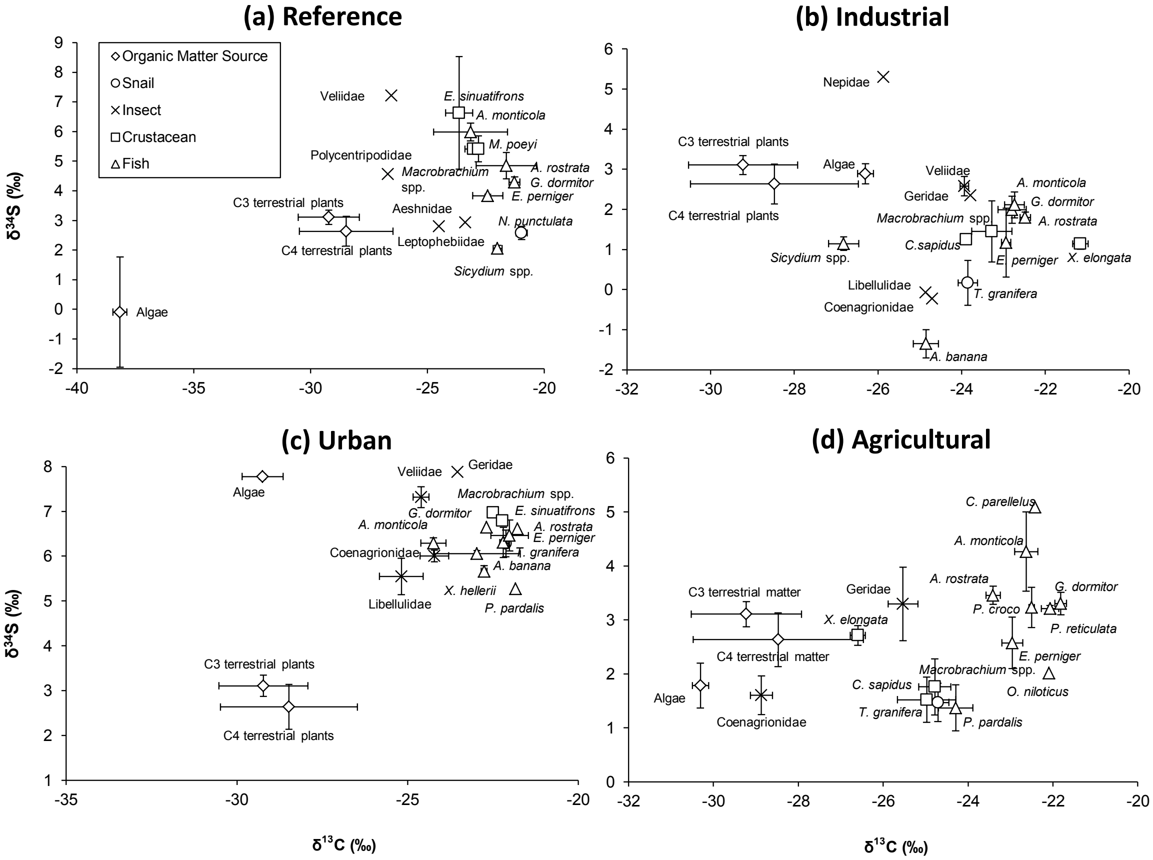

Figure 3. Mean (± SD) lipid corrected $\delta^{13}C$ and $\delta^{34}S$ biplot for stream sites (a-d). General taxa categories of samples are indicated by symbols. C3 and C4 terrestrial-derived organic matter points represent a mean among all sites.

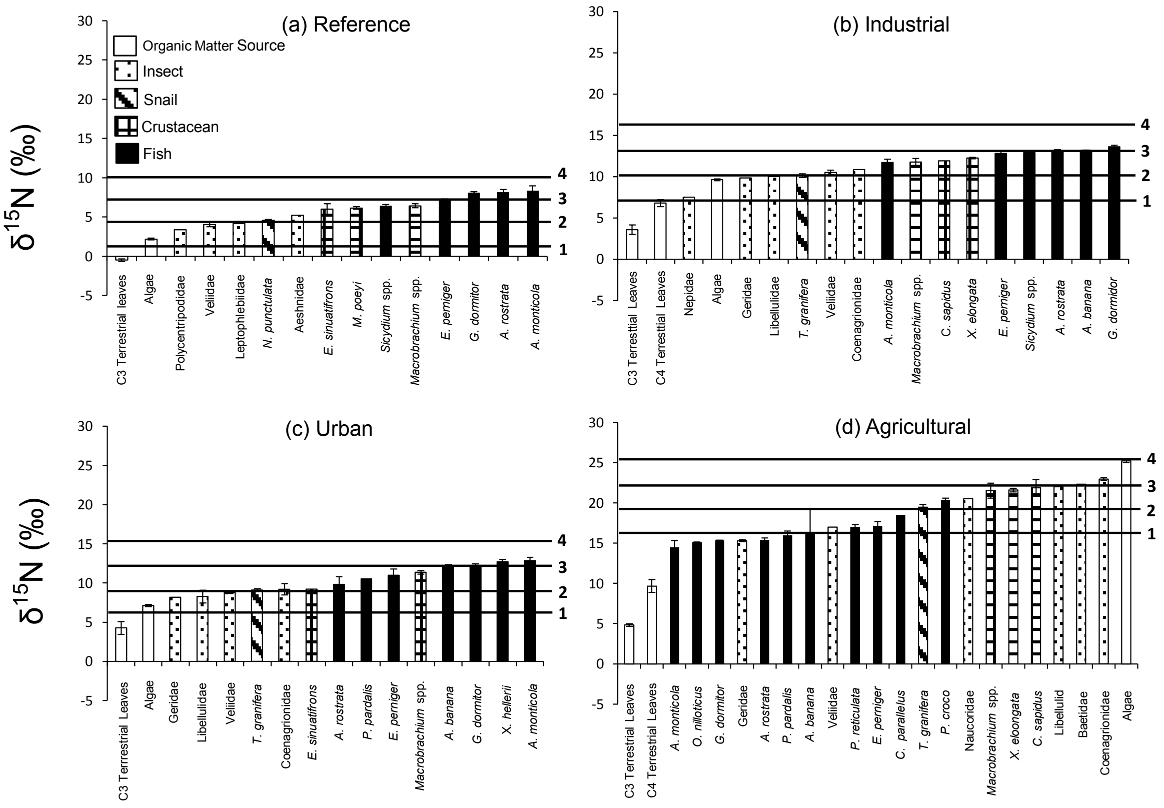

Figure 4. Mean (± SD) δ^{15}N values of food web components, categorized into general taxa, for each stream site (a-d) with trophic levels indicated by horizontal black lines.

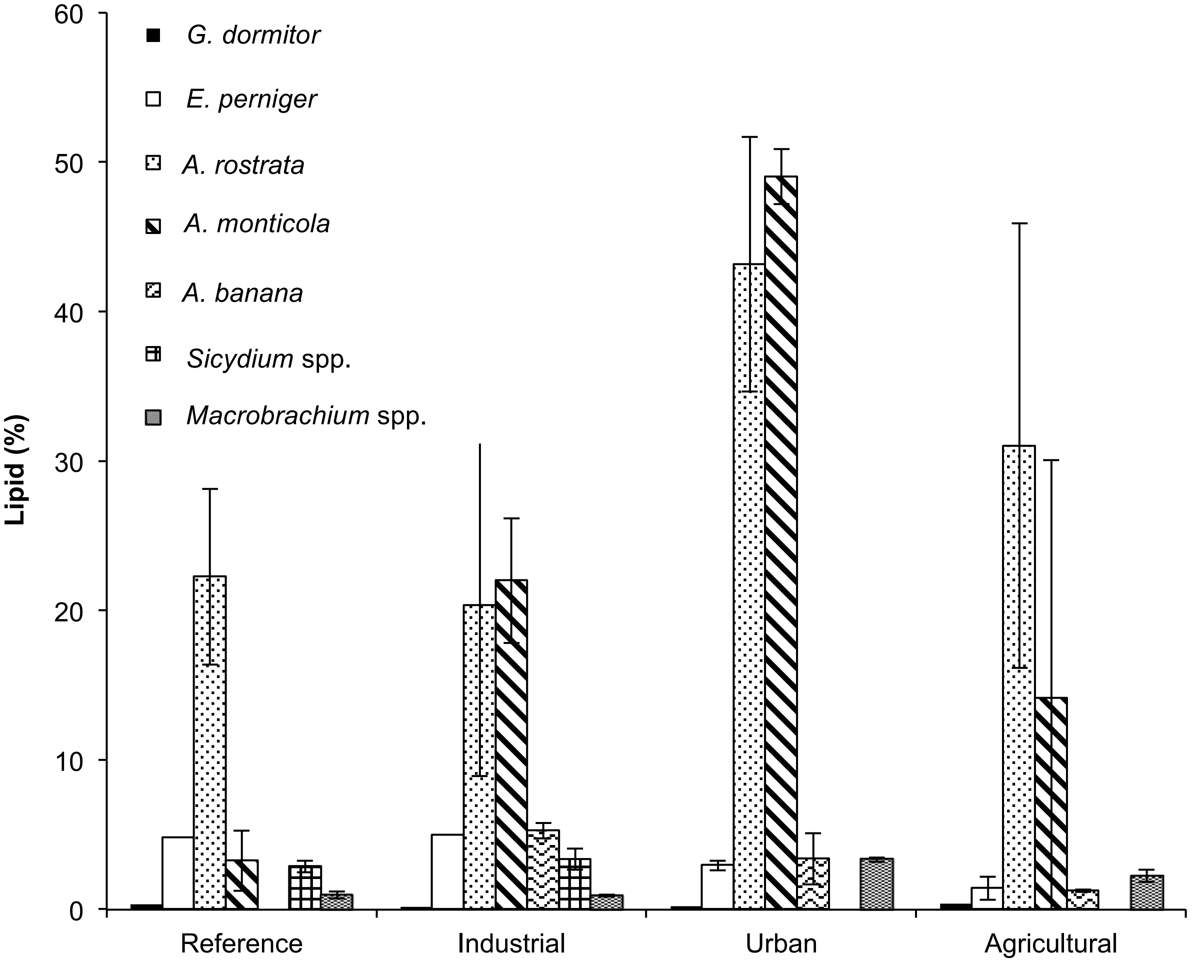

Figure 5. Mean (± SD) lipid content for consumers sampled at each stream site.

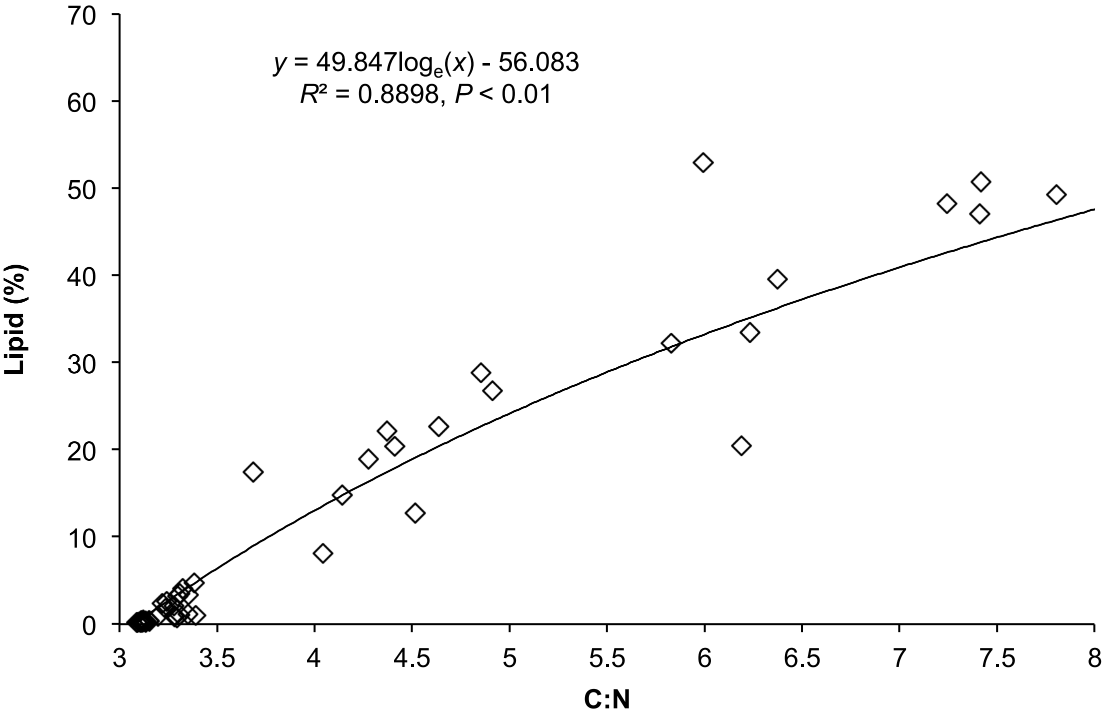

Figure 6. Lipid content of fish and shrimp sampled from four stream sites related to C:N (without the lipid extraction treatment).

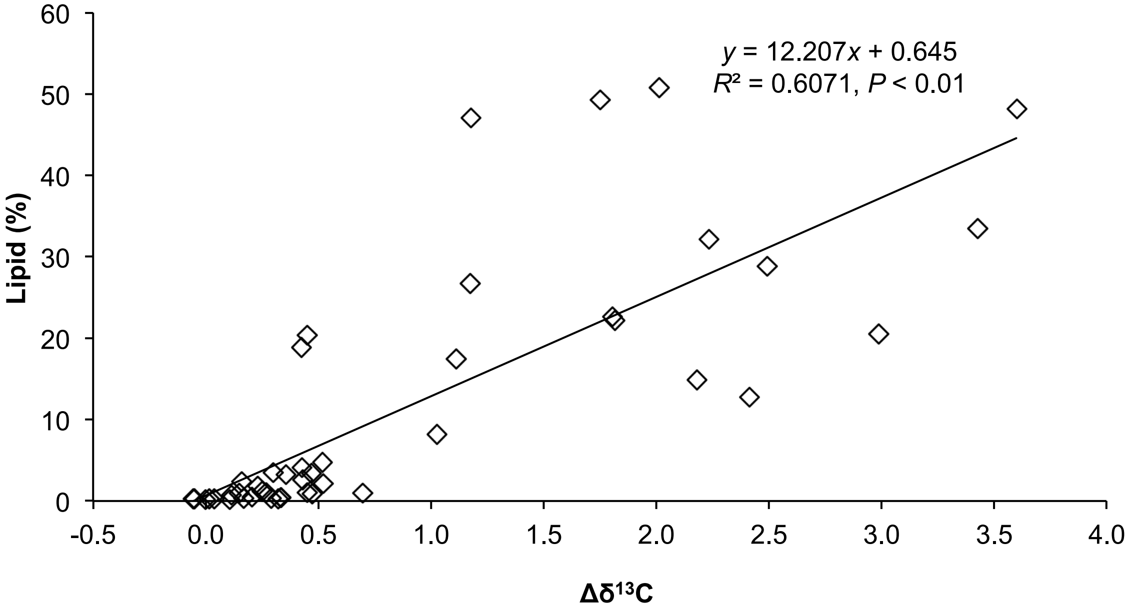

Figure 7. Lipid content of fish and shrimp sampled from four stream sites related to the difference between lipid extracted $\delta^{13}C$ and unextracted $\delta^{13}C$ ($\Delta\delta^{13}C$ **).**

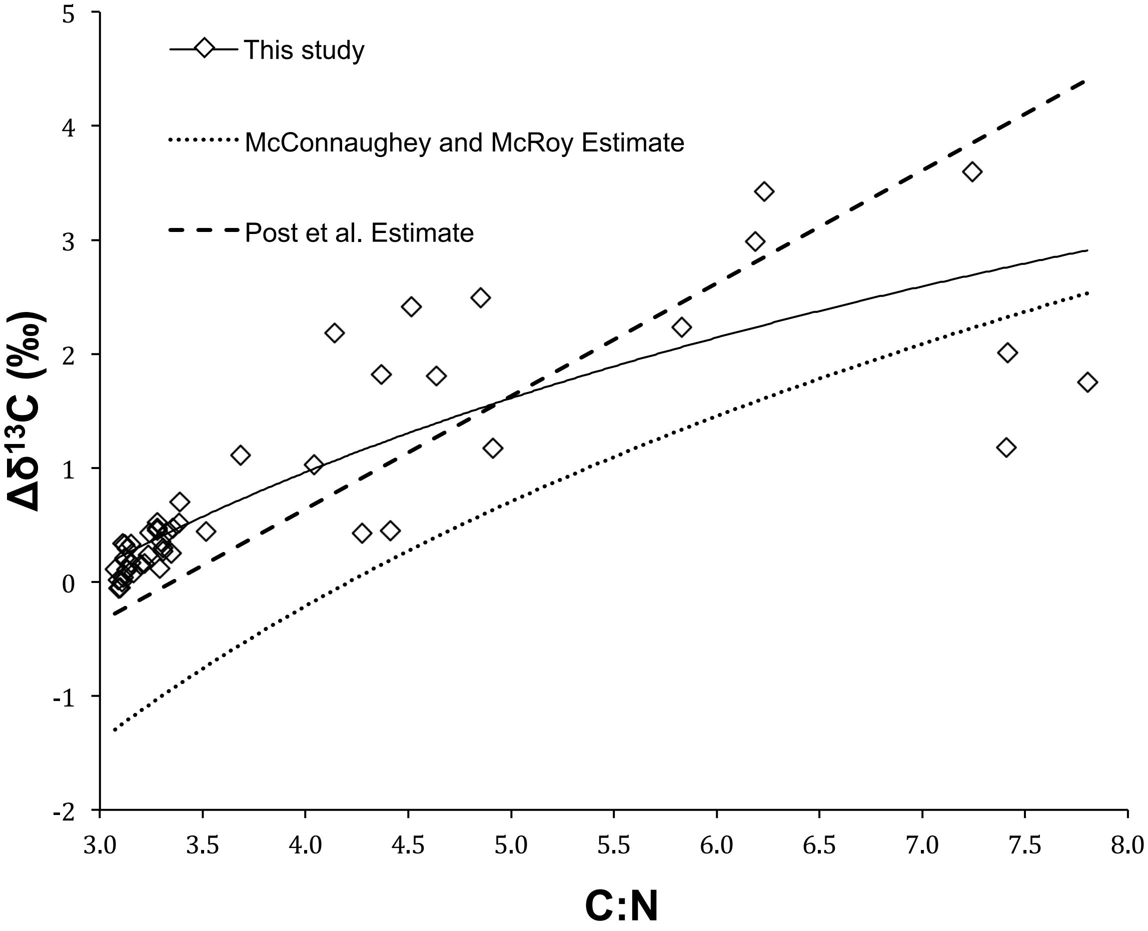

Figure 8. The relationship between C:N and the difference between lipid extracted $\delta^{13}C$ and untreated $\delta^{13}C$ ($\Delta\delta^{13}C$), in comparison with McConnaughey and McRoy (1979) $\delta^{13}C$ correction estimate prediction and Post et al. (2007) $\delta^{13}C$ correction estimate prediction of our data from Puerto Rico streams.

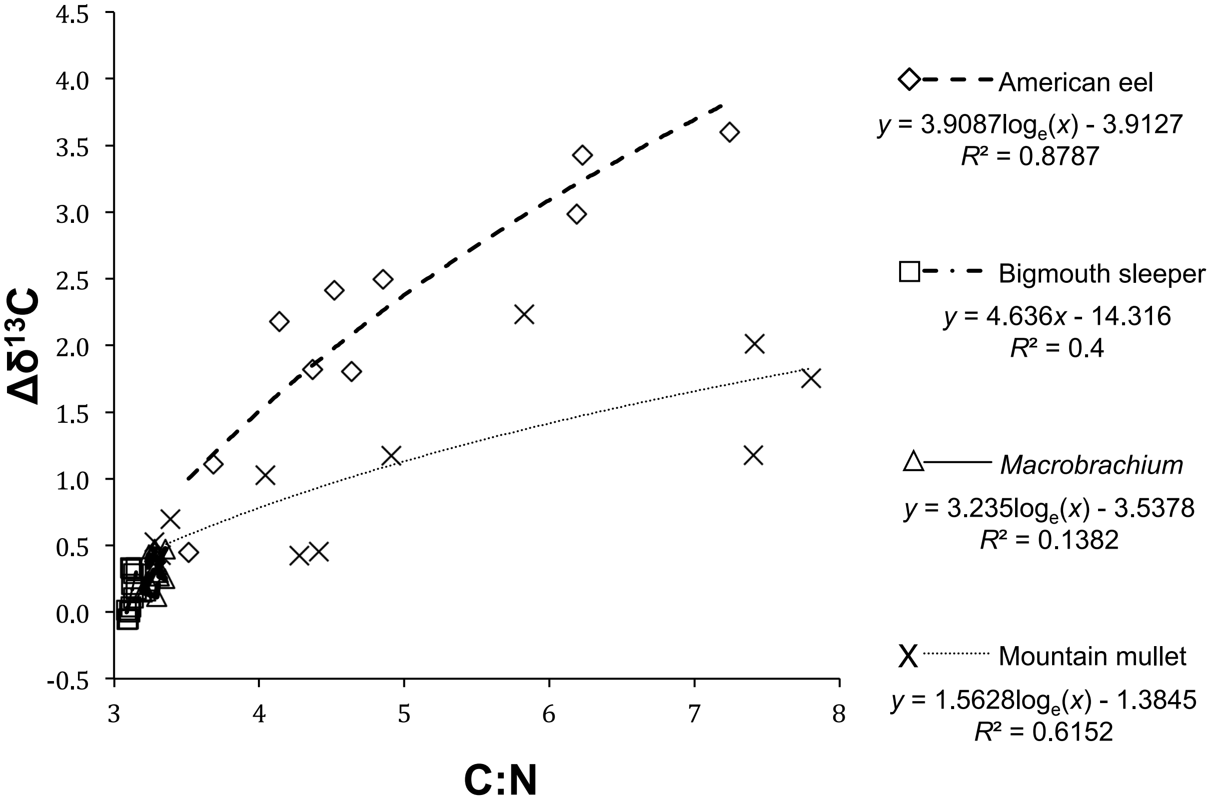

Figure 9. The relationship of C:N and species differences in the difference of δ^{13}C between lipid extracted and non-lipid extracted samples ($\Delta\delta^{13}$C) of fish and shrimp from four stream sites.

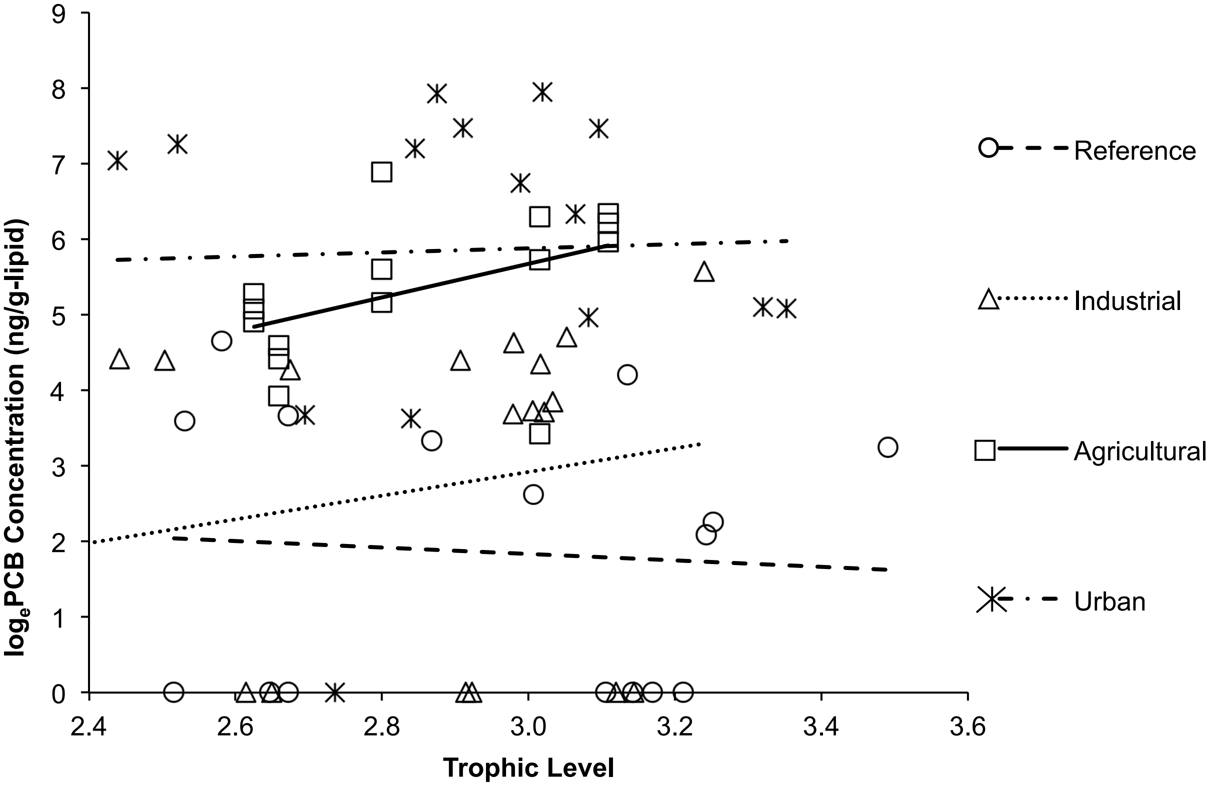

Figure 10. PCB concentration as a function of trophic level of consumers at four stream sampling sites of varying dominant riparian and watershed land use (forested, industrial, agricultural, or urban). All linear relationships plotted were not statistically significant ($P > 0.05$).

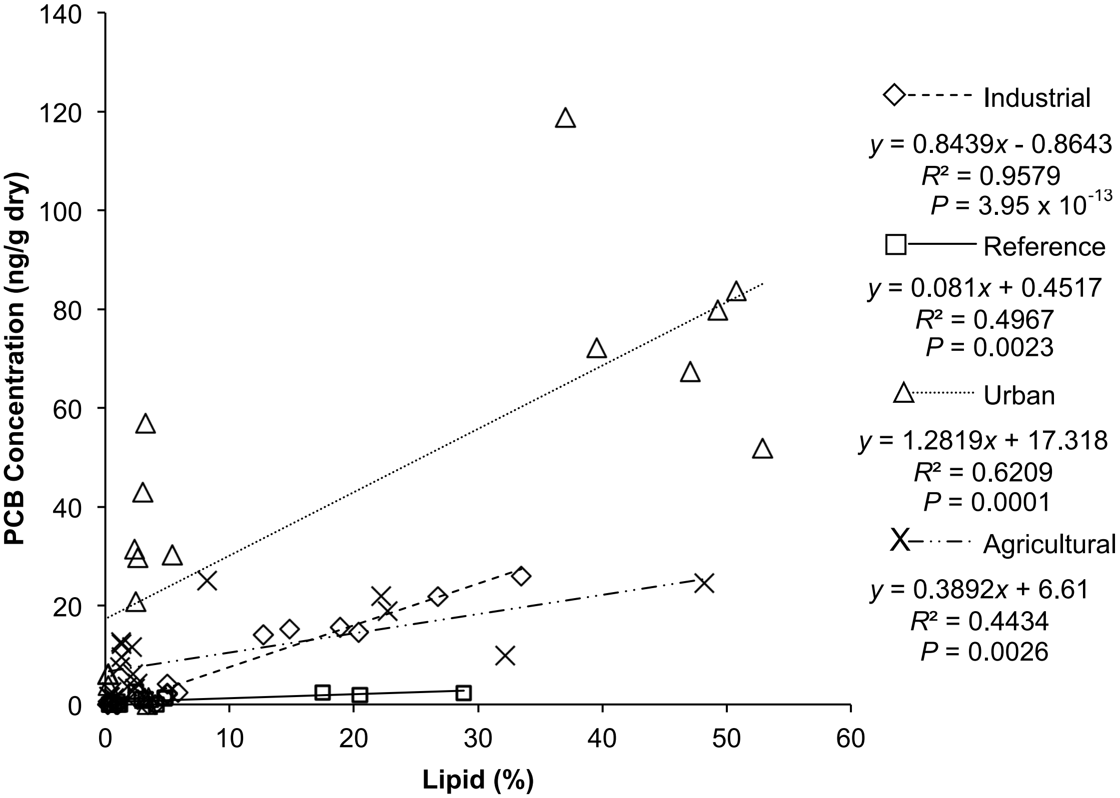

Figure 11. PCB concentration as a function of lipid content of consumers at four stream sampling sites of varying dominant riparian and watershed land use (forested, industrial, agricultural, or urban).

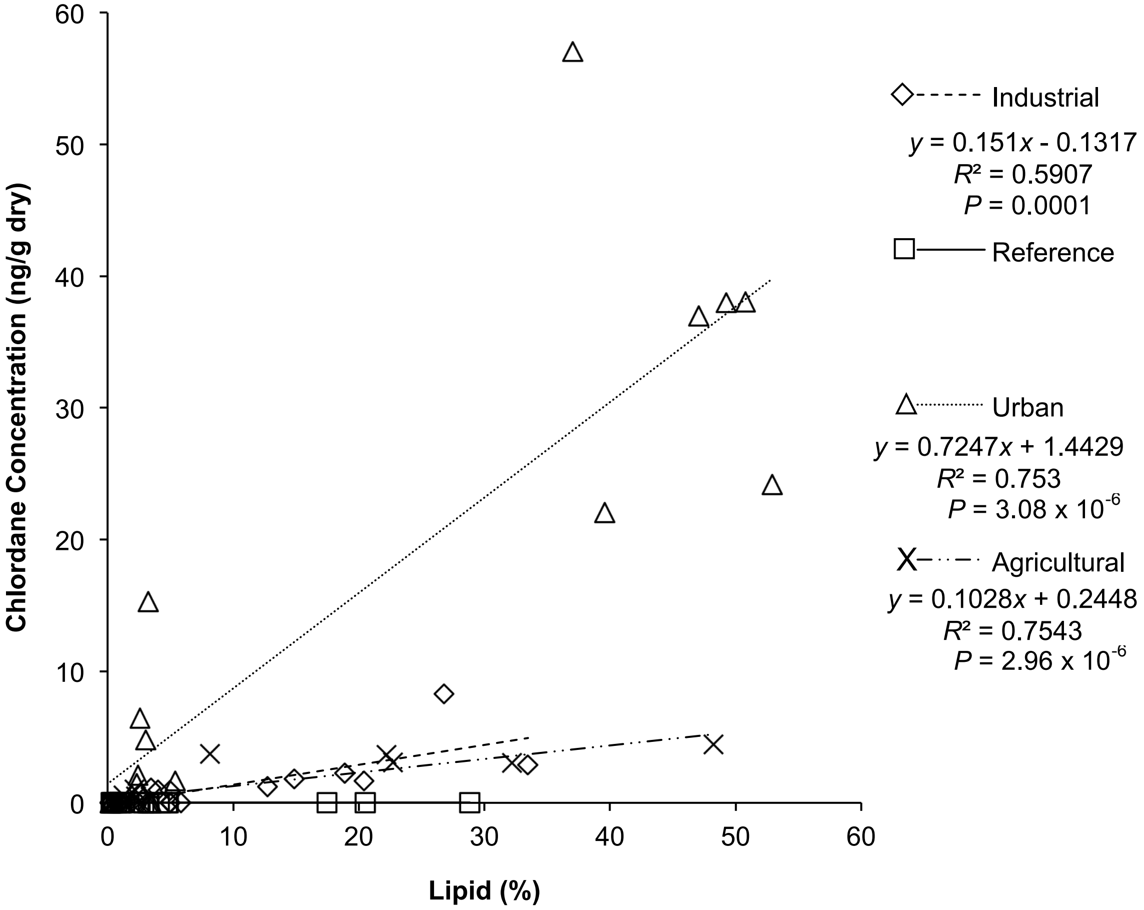

Figure 12. Chlordane concentration and lipid content of each sample with a regression line representing the relationship for each site. Chlordane was not detected in any sample (*N* = 16) from the reference site.

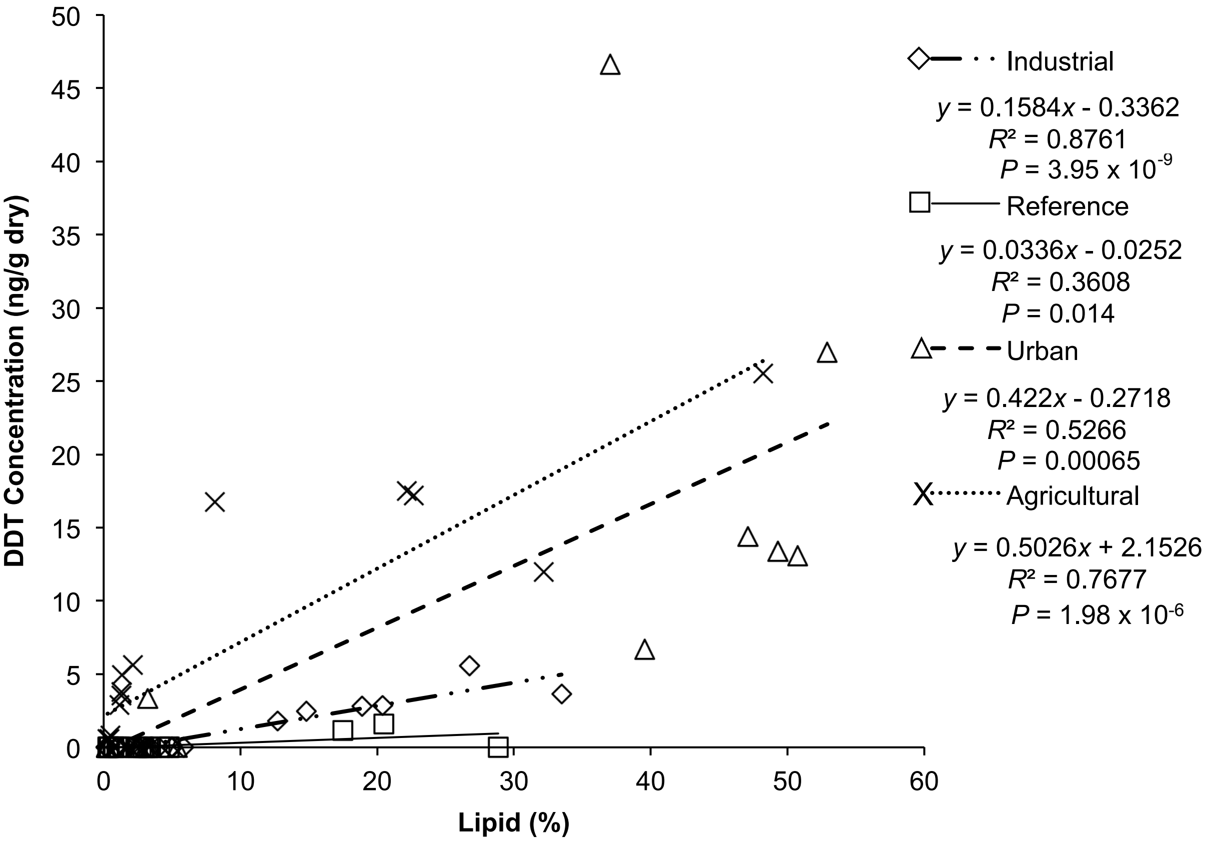

Figure 13. DDT concentration and lipid content of each sample with a regression line representing the relationship for each site.

CHAPTER 5

TROPICAL INSULAR FISH ASSEMBLAGES ARE RESILIENT TO FLOOD DISTURBANCE

(Job 4)

Abstract

A combination of deterministic and stochastic processes structures aquatic communities. Periods of stable environmental conditions, favoring development of communities regulated by deterministic processes, are interrupted by random periods of disturbance that may restructure communities. Disturbance may affect populations via habitat alteration, mortality, or displacement. We quantified fish habitat conditions, density, and movement before and after a major flood disturbance in a Caribbean island tropical river using habitat surveys, fish sampling and population estimates, radio telemetry, and passively monitored PIT tags. Native stream fish populations showed evidence of acute mortality and downstream displacement of surviving fish. All fish species were reduced in number at most life stages after the disturbance, but populations responded with recruitment and migration into presumably vacated upstream habitats. Changes in density were uneven among size classes for most species, indicating altered size structures. Rapid recovery processes at the population level appeared to dampen effects at the assemblage level, as fish assemblage parameters (species richness and diversity) changed minimally. The native fish assemblage appeared resilient to flood disturbance, rapidly compensating for mortality and displacement with increased recruitment and recolonization of upstream habitats. In tropical island streams, major flood disturbance may act as a community filter to resist invasion by exotic species with minimal net effect on natives, thereby maintaining relatively stable native stream fish assemblages.

Introduction

Ecological communities are structured by a combination of deterministic biotic and stochastic abiotic factors. Deterministic biotic interactions (e.g., competition and predation) are density dependent and develop as populations grow in number, and stochastic abiotic events (e.g., floods and droughts) periodically reset community structure and interrupt the outcome of biological interactions (Connell 1978). Severe, temporary abiotic conditions (i.e., disturbances) result in the mortality or displacement of individuals (Sousa 1984), redistribution of habitat and substrate (Pickett and White 1985), and altered nutrient cycling (Pringle 1997). Abiotic factors set the physical template upon which ecological communities develop; species traits such as dispersal rate and environmental tolerances set the pool of available colonists from which communities are derived, and the disturbance regime (frequency, intensity, and predictability) modifies community composition over time (Poff and Ward 1989, Waide and Willig 2012). Knowledge of physical and biotic regulatory processes and their interaction is not only of interest for ecological conceptual development, but can enhance understanding of the distribution and abundance of organisms with direct conservation applications (Ludwig et al. 2001, Agrawal et al. 2007).

The role of deterministic biotic versus stochastic abiotic factors in structuring aquatic communities was the subject of scientific debate in the 1980s (Grossman et al. 1982, Matthews 1982, Yant et al. 1984, Grossman et al. 1985). Several studies on this topic reported variable stream fish assemblage structure at small scales in patchy stream habitats (Grossman et al. 1982) and high assemblage stability across very large spatial scales (Matthews 1982). After years of empirical study, research findings conformed to a unifying paradigm that stream systems followed a predictable continuum from highly-disturbed, stochastically structured communities to rarely-disturbed, deterministically structured communities (Resh et al. 1988, Poff and Ward 1989, Strange et al. 1992). Recently, enhanced understanding of community structuring has recognized that the physical template and disturbance regime feed back on populations over time, fostering the evolution of traits that modulate future responses to disturbances (Lytle and Poff 2004, Death 2010).

Understanding the specific role of disturbance in structuring communities leads to enhanced development and prediction of resource management success under various strategies. For example, many reservoir, lake, and pond ecosystems are successfully managed using a community-based strategy originally developed by Swingle (1956). These lentic systems are infrequently disturbed, and fish populations tend to reach high biomasses where density-dependent, biotic interactions regulate populations (Poff and Ward 1989). Conversely, density-dependent fishery assessment approaches have not produced reliable results in marine ecosystems (e.g., stock-recruit relationships, Hilborn and Walters 1992), where disturbance is intense and stochastic environmental effects often take precedence in structuring communities (Dayton 1971, Connell 1978, Harmelin-Vivien and Laboute 1986). Fish life histories have evolved to cope with different biotic and abiotic challenges under conditions of low versus high disturbance, and those life histories respond differently to exploitation and management (Winemiller 2005).

Lentic ecosystems generally experience infrequent disturbance, but lotic ecosystems may be so frequently disturbed that equilibrium is rarely reached, and the resulting system is in perpetual recovery from the previous disturbance event (Resh et al. 1988). Stochastic disturbance regimes favor rapidly-maturing, short-lived opportunistic species, while low levels of predictable disturbance favor late-maturing, long-lived equilibrium species (Winemiller 1995). Cycles of intense disturbance and favorable environmental conditions foster the development of high fecundity and low per capita investment in offspring (i.e., periodic life history). Opportunistic, periodic, and equilibrium species are each expected to respond differently to common resource management strategies (Winemiller 2005).

Ecological theory predicting the effects of disturbance on stream communities has been developed primarily from examples in temperate regions (e.g., Grossman et al. 1982, Matthews 1982, Poff and Ward 1989, Death 1996, Townsend et al. 1997). Disturbance regimes in tropical regions are contrastingly frequent, intense, and stochastic and could exert major ecological influences on stream communities (Ramírez et al. 2009); however, the specific effects of disturbance on, and its role in structuring, tropical insular stream communities have not been fully examined. To supplement this information gap in disturbance ecology, we conducted research to quantify the effects of a major flood disturbance, Hurricane Irene (August 2011), on the native riverine fish assemblage of a tropical island river, Río Mameyes, Puerto Rico, USA. Through a combination of fish sampling and population estimates, two approaches to fish tagging, and instream habitat surveys before and after dramatic flooding, we characterize the role of stochastic processes in shaping the fish assemblage of an insular tropical river system to enhance the understanding and conservation of tropical stream ecosystems.

Methods

Study system

Río Mameyes is a fourth-order, free-flowing river draining the Luquillo Mountains of northeastern Puerto Rico, USA, the eastern-most island of the Greater Antilles in the Caribbean Sea (Fig. 1). The Luquillo Mountains are of volcanic origin and reach heights greater than 1,000 m within 20 km of the island coast (Pike et al. 2010). The steep topography interacts with northeasterly trade winds and frequent tropical storms to produce extremely high rainfall (averaging nearly 5 m annually, Lugo et al. 2012) and flood-dominated rivers, typical in hydrology to other Antillean rivers. Spates 50 times greater than base flow are common in the region, and river hydrographs are flashy, often peaking and returning to near base flows within 24 hours (USGS 2011).

Río Mameyes is one of only a few undammed rivers in Puerto Rico (Cooney and Kwak 2013) with continuous aquatic connectivity from the headwaters to the ocean. It has a steep, short drainage, with an origin at 850 m above sea level, flowing north just 12 km to the Atlantic Ocean. The upper watershed consists of protected secondary forest within the El Yunque National Forest, and the lower watershed contains primarily secondary forest, agricultural land, and small urbanized areas, including the villages of La Vega, Palmer, and Fortuna (Martinuzzi et al. 2007). Río Mameyes' unrestricted connection to the ocean, short total length, and generally undeveloped watershed render it an ideal system to study a tropical aquatic community with minimal anthropogenic influence.

Native amphidromous fauna dominates Puerto Rico freshwater streams with uninterrupted connectivity to the estuary, whereas exotic fauna dominates streams above larger obstructions to aquatic migration, such as waterfalls and large dams (Holmquist et al. 1998, Kwak et al. 2007, Cooney and Kwak 2013). Thus, native amphidromous fish and shrimp fauna are the dominant aquatic community members in the free-flowing Río Mameyes. Amphidromous larvae temporarily occupy estuaries and river mouths before migrating back into freshwater as metamorphosing juveniles (McDowall 1988). Amphidromous shrimp, *Xiphocaris elongata, Atya* spp., and *Macrobrachium* spp., and amphidromous fishes, mountain mullet (*Agonostomus monticola*), bigmouth sleeper (*Gobiomorus dormitor*), sirajo goby (*Sicydium* spp.), river goby (*Awaous banana*), and smallscaled spinycheek sleeper (*Eleotris perniger*) are the most common native macrofauna. A sixth diadromous fish, the American eel (*Anguilla rostrata*) follows a catadromous life history and is also common among Puerto Rico streams (Kwak et al. 2007). Globally, diadromous fish of the Gobiidae, Eleotridae, and Anguillidae families are dominant fish assemblage members on tropical volcanic islands and are in need of comprehensive conservation strategies (McDowall 1988, 1999). Thus, Río Mameyes can be considered representative of the undeveloped streams with native diadromous fish assemblages and intense flood regimes, found on tropical islands throughout the world, and our findings may be applied toward broad-scale conservation.

Riverine habitat

Before and after Hurricane Irene, standardized instream habitat surveys of a 200-m coastal plain river reach were conducted to describe the magnitude of physical effects from the storm. Water depth, mean-column velocity, and dominant substrate were measured at 10 evenly spaced points along cross-sectional transects that were evenly separated by a distance of two mean river widths. At points of less than 0.5-m depth, velocity was measured at 60% of total depth, and at points greater than 0.5-m depth, velocity was measured at 20% and 80% of total depth then averaged. The dominant substrate (areal coverage) within 1 m of each sampling point was classified according to a modified Wentworth particle size scale (Bovee and Milhous 1978). Habitat parameter data were tested for normality with a Shapiro-Wilk test. Non-normal data were tested for shifts in the median using Mann-Whitney tests, and normal data were tested for a change in location of the mean using two-sample t-tests (R Development Core Team 2012). All habitat statistical comparisons were interpreted at $\alpha = 0.05$.

Fish density

Fish density was estimated using a standardized three-pass removal procedure (Hayes et al. 2007, Kwak et al. 2007) in randomized 100-m river reaches from just upstream of the estuary (river km 2) to the highest accessible site (river km 10.7). Fish were sampled using two Smith-Root LR-24 backpack electrofishers (400 V, 30% pulse width, 0.2–0.3 A) and three sequential passes through the 100-m reach. Either block nets or natural barriers (e.g., steep riffles and cascades) were used to close each reach to fish movement during sampling. All fish were collected without replacement during each 30-minute pass and then measured and weighed before release subsequent to the final pass.

During the summers of 2009 and 2010 and the winter of 2010, 22 pre-disturbance removal estimates were completed, and during the two weeks following Hurricane Irene (August 26 – September 9, 2011), six post-disturbance estimates were completed (Fig. 2). Kwak et al. (2007) found seasonal differences in fish abundance throughout Puerto Rico, so only summer estimates were compared. We considered summer 2009 and 2010 samples to be representative of expected undisturbed summer fish densities. Winter samples were included in the model to improve estimates of capture probability, which was held constant across seasons in some candidate removal models. Each species was stratified into three size categories, juveniles, sub-adults, and adults, based on estimates of size at maturation (unpublished data). Mountain mullet males reach much smaller asymptotic sizes compared to females, and no external characters distinguish the sexes. Therefore, we considered the sub-adult size to be representative of sub-adult female and adult male mountain mullet. The presence of American eels in collecting tanks increased mortality among other fishes, which interfered with fish tagging, so American eels were only collected during the three-pass removal samples of summer 2009. In samples after the storm, American eels were enumerated only in the first pass, and a single-pass density estimate of density was generated.

Removal data were modeled in a Bayesian framework using OpenBUGS software (Lunn et al. 2009) and the multiple-pass Bayesian removal model described by Wyatt (2002). Removal models estimated two parameters, abundance and capture probability, representing a hierarchy of state and observation processes (Williams et al. 2002). Abundance estimates were converted to density (number per unit area), which in turn were considered draws from a median density for each size class and species. Median fish density after the storm was subtracted from the expected value (median density during previous summer samples), and the difference was divided by the expected value to yield an estimate of the proportional change in fish density associated with the storm. Pre- and post-disturbance estimates of fish density were summed among size classes to calculate community parameters, species richness, assemblage heterogeneity, and the rank order abundance of each species (Kwak and Peterson 2007). Species richness was estimated as the total number of species observed in each sampled reach, and t-tests identified changes in mean species richness after the disturbance. Change in heterogeneity was quantified as the difference of post- and pre-hurricane Shannon-Weaver index values (H', Shannon and Weaver 1949).

Derived parameters of the fish density model included proportional change in density and change in community parameters (pre- versus post-disturbance). Credible intervals for these population and community parameters that did not contain zero indicated significant departures from expected densities and assemblage heterogeneity.

Fish movement

Fish movement was monitored at two spatial scales, a broad, basin scale using large numbers of PIT-tagged fish, and a small, reach scale using a smaller number of fish tagged with radio transmitters. Fish were collected from two sequential passes of nine randomized 150-m reaches in both June and July 2011 as described above. Only mountain mullet and bigmouth sleeper were large enough for radio transmitter implantation, and only mountain mullet larger than 120 mm total length and bigmouth sleeper larger than 130 mm total length were PIT-tagged. No fish smaller than 200 mm total length received a radio transmitter.

Fish receiving only a PIT tag were lightly anesthetized in aerated river water containing 80 mg/L tricaine methane sulphonate (MS 222) for 2 minutes before transfer to a tank containing a maintenance level of anesthetic (30 mg/L MS 222). Total length, weight, sex, and capture location were recorded for each fish. Half-duplex PIT tags (23 mm, 0.6 g; Texas Instruments) were implanted intra-abdominally via a 4-mm incision posterior to the pelvic fin. Fish longer than 250 mm total length were implanted with larger 32-mm tags (0.8 g). Before each surgery, all surgical equipment and PIT tags were disinfected in a solution of Benzall surgical disinfectant. Each PIT tag implantation lasted approximately one minute and total sedation time was 7.5 minutes on average, after which fish recovered in a 30-L live well, with flowing river water, for 1–2 hours before release. Only fish in apparently good condition were released.

Fish tagged with radio transmitters were captured and allowed to recover following the same procedures for PIT tagging. Fish receiving a radio transmitter were anesthetized for 4 minutes in 80 mg/L MS 222. A small radio transmitter (Advanced Telemetry Systems, Model F1545, 0.9 g) and a 23-mm PIT tag were implanted intra-abdominally through a 12-mm incision that was closed with two sterile sutures. Trailing antennas were coiled and implanted intra-abdominally (not inserted through body wall). Each surgery lasted for approximately 10 minutes. Greater measures were required to ensure that each fish survived radio tag implantation in good condition, including closure of the incision wound and artificial circulation of water containing 30 mg/L MS 222 over the gills with a hand siphon.

To monitor the movement of PIT-tagged fish, pairs of PIT antennas (i.e., arrays) were installed in Río Mameyes across the entire river channel, one in the coastal plain and one in the foothills (Fig. 1). Each antenna was driven by an Oregon RFID multiplexed reader and constructed from a single loop of 8-gauge stranded copper cable in a horizontal, pass-over design. The use of paired antennas enabled a determination of the direction of fish movement; however, we elected to avoid assumptions regarding detectability of movement direction by summing all movement detections by day to quantify general fish activity levels (Aymes and Rives 2009). Radio-tagged fish were manually tracked weekly, and a hand-held Global Positioning System unit was used to record each fish location. Fish locations were later converted to river km using Google Earth (Google, Inc. 2011).

The location of radio-tagged fish in relation to the storm was quantified two ways, the change in location proximately after the river returned to base flow and the change in mean location during the weeks before and after the flood. Locations during the four weeks prior to the flood were averaged as an estimate of mean pre-flood location, and locations during the two weeks after the flood were averaged to generate an estimate of mean post-flood location. Change in location immediately following reduced flow and the mean change in location in the weeks following the storm were pooled by species and tested for before-after differences using a Mann-Whitney test (R Development Core Team 2012).

Results

Hurricane Irene struck Puerto Rico on August 22, 2011, and rain persisted for four days. The heaviest rainfall occurred during the first day of the storm and produced a peak daily discharge in Río Mameyes of 39 m^3/s (Fig. 2), representing the highest flow among the previous 7 years; the 30-year median August flow was 1.6 m^3/s. Discharges of this magnitude or greater have occurred in Río Mameyes on average every four years over the last 30 years that the streamflow gauge has operated. During Hurricane Irene, the hydrograph peak was brief, but elevated water levels persisted for six days.

Habitat parameter data revealed that water in the surveyed reach of Río Mameyes was significantly deeper and faster after the flood (Table 1), despite greater river discharge during the initial survey than during that after the flood (USGS 2011). A deep channel was scoured along the downstream portion of the reach, where water velocity ($P = 0.022$) and depth ($P < 0.01$) were greater. Further, a large 30 x 75 m gravel bar was deposited along the shore, resulting in a narrower channel ($n = 4$; $P = 0.07$) in the downstream portion of the reach.

Averaged among species and size classes, a 12% decline in fish density was observed after the flood disturbance (Fig. 3). Bayesian information criteria (Schwarz 1978) of three-pass removal models indicated that the best model included capture probabilities that varied by sample (Table 2). Most examined fish groups showed a reduction in density after the flood. Only sub-adult mountain mullet, juvenile sirajo goby, and adult smallscaled spinycheek sleeper were found at expected or higher than expected densities after the flood (Fig. 3). Excluding one outlier in fish density (subadult mountain mullet), the average change in fish assemblage density was a 49% decrease. Species richness did not appear to change during the disturbance, as all common species and size classes were present before and after the storm. The disturbance did not extirpate any common fish species from Río Mameyes; furthermore, the change in fish assemblage heterogeneity was minimal (Table 3). The rank order of species density was similar before and after the storm, but American eel declined from the most abundant species to the third most abundant species (Table 4).

Detections of PIT-tagged fish indicated peaks in activity following the flood disturbance, but no major downstream displacement of fish. Totals of 280 bigmouth sleeper and 179 mountain mullet were PIT-tagged and released during June and July 2011 and available for detection at automated PIT arrays. Including both the coastal plain and foothills PIT arrays, 8 PIT-tagged bigmouth sleeper and 14 mountain mullet were detected during the month prior to the flood, and 10 bigmouth sleeper and 28 mountain mullet were detected during the two weeks after the storm. PIT arrays were disabled during the flood and reinstalled 5-11 days later. After the flood, peaks in the activity of PIT-tagged fish occurred in both species (Fig. 4). Post-flood activity levels were especially high for bigmouth sleeper in the coastal plain and for mountain mullet in the foothills. Most bigmouth sleeper detected (70% before, 87% after) were tagged locally, within 2 km of the array where they were detected. A smaller fraction of PIT-tagged bigmouth sleeper detected appeared to be transient, having been tagged greater than 2 km away from the array. However, the proportion of these transient fish was greater before the storm than after it (30% before, 13% after). A small fraction of all fish appeared to be transient, and some individuals of both species were detected after moving long distances downstream to the coastal plain (>3 km; four bigmouth sleeper and six mountain mullet detected at the coastal plain array), demonstrating a low level of downstream displacement during the disturbance. The most notable dispersal observed in tagged fishes was a large number of mountain mullet initially captured and tagged 3-5 km downstream in the coastal plain that were observed in the foothills (22 PIT-tagged fish, 82% of all mountain mullet detections in the foothills), moving upstream following the flood. This pulse of migrating fish represented more than 10% of all PIT-tagged mountain mullet.

Radio-telemetered fish showed moderate mortality and movement associated with the flood disturbance. Nine bigmouth sleeper and 12 mountain mullet were tagged with radio transmitters in early June and survived until Hurricane Irene. During the storm, two mountain mullet (17% of total tagged fish) and one bigmouth sleeper (11% of total tagged fish) died. Mortality was confirmed by retrieving radio transmitters from the streambed. Among all radio-telemetered fish, neither immediate location nor average location was significantly different after the disturbance ($P > 0.05$). Movement direction was generally stochastic. The most notable change in location associated with the storm was one mountain mullet found in a large pool 1 km upstream from its pre-flood location on the first day that fish were relocated after the disturbance. This fish returned downstream to its area of core use within one week. A final exceptional observation of fish movement occurred outside of the study period during a brief spate, three weeks subsequent to Hurricane Irene. A mountain mullet previously located in the mountains near river km 9 (82 m above sea level) was detected 1.25 km downstream (36 m above sea level). Two days later, the same fish was relocated upstream in its core use area (rkm 9).

On August 28, six days after the hurricane's eye passed and on the first day of reduced discharge in Río Mameyes, a large recruitment migration of juvenile sirajo gobies was observed at the location of the coastal plain PIT array (river km 2). For approximately 8 hours, a 0.5-m wide column of postlarval sirajo gobies (22-mm mean total length) was observed moving upstream 1 m from the shore. This recruitment event was similar to those described by previous researchers studying sicydiine gobies and goby fry fisheries (Erdman 1961, Bell 1994).

Discussion

Hurricane Irene represented an intense disturbance event in a tropical insular river, and substantial biotic effects resulted at the population level. However, rapid recovery processes, recruitment, and recolonization of upstream habitats were evident within populations and reduced the effects of the disturbance at the assemblage level. Our results reveal fish mortality, displacement, and increased activity that occurred due to the disturbance. Reduced densities among species and size classes indicated that significant mortality or displacement of native fishes occurred, and the mortality of radio-telemetered fishes during the disturbance confirm mortality as a consequence. Anecdotally, a large recruitment migration of juvenile sirajo gobies was observed in the receding floodwaters, and high densities of juvenile sirajo gobies were present throughout the river during removal sampling. Episodic recruitment migrations into riverine habitats are a regular feature in large tropical insular river systems (Erdman 1961, Bell 1994), and our observation of recruitment migration after a major flood suggests that juveniles are induced to migrate upstream by flooding or receding floodwaters. The higher than expected densities of sub-adult mountain mullet in the coastal plain and foothills reaches surveyed after the flood were likely displaced from upper elevations, and the small number of PIT tagged fish moving downstream after the disturbance confirmed the potential for a low level of downstream displacement. Displacement and crowding of sub-adult diadromous fishes in downstream reaches following hurricanes has been associated with increased flows in other aquatic systems (Fitzsimons and Nishimoto 1995, Houde et al. 2005). High fish densities in lower reaches in our study were subsequently associated with upstream migration into the headwaters, observed in PIT-tagged mountain mullet moving through the foothills. Population parameters changed significantly after the storm, but community parameters, assemblage species richness, heterogeneity, and species rank order changed minimally. Thus, we conclude that native fish assemblages are resilient to the effects of flood disturbance in tropical insular rivers.

Hurricane Irene was a major flood disturbance that persisted over several days and significantly altered riverine habitat. Floods of equal or greater magnitude occur on average every four years in Río Mameyes, approximately equal to or slightly less than the maximum life span of fishes in the assemblage, based on growth and survival rates (Chapter 4). Thus, the native fish assemblage has adapted to this type and frequency of disturbance, and we should expect those adaptations to manifest in the native assemblage response to disturbance (Lytle and Poff 2004). Among fish populations, two general patterns were observed in fish density and movement – low densities in the foothills and coastal plain and little evidence of displacement (pattern 1) and high densities in lower reaches and greater evidence of displacement (pattern 2). All species were reduced in number, with the exception of sub-adult mountain mullet. Further, among tagged fishes, only mountain mullet were observed migrating after the disturbance. We hypothesize that all species suffered significant mortality due to catastrophic displacement. Most species followed pattern 1, but mountain mullet, consistent with pattern 2, suffered displacement as well but were able to survive the event and migrate into presumably vacated headwaters.

Previous studies of reproductive ecology in Caribbean amphidromous fishes have suggested that mountain mullet undergo a downstream migration to spawn, which would characterize them as a catadromous species (Anderson 1957, Phillip 1993). Although mountain mullet larvae have been captured from the ocean (Anderson 1957), spawning in lower river reaches or marine habitats has not been documented. Our results indicate that mountain mullet may periodically inhabit lower reaches of rivers; however, their presence in lower river reaches in this study was likely due to involuntary displacement from higher elevations, rather than directed, broad-scale downstream migration. The data presented here do not indicate catadromy and further confirm mountain mullet as amphidromous. Future studies of reproductive migrations in amphidromous species must separate the effects of displacement during disturbance from downstream migration and spawning to elucidate fish reproductive life history.

Our results support the conclusion that upstream movement of mountain mullet following disturbance is associated with atypically higher densities in lower reaches. Two hypotheses could explain this phenomenon. Mountain mullet may have become crowded in lower reaches and responded with density dependent emigration from the coastal plain, or these facultatively predatory fish may have followed migrating juvenile sirajo gobies that we observed at lower elevations. Studies of fish migration and movement associated with a flood event during a season when post-larvae do not migrate en masse (e.g., winter-spring) could separate these confounding influences during summer floods.

Movement analysis was enhanced by incorporating both broad-scale, basin-wide data from PIT-tagging and remote monitoring with fine-scale reach data from radio-telemetry. Both study approaches indicated that most fish movement was local (<2 rkm), with the exception of mountain mullet migrating into upper elevations following the disturbance. Approximately 10% of all PIT-tagged mountain mullet were observed migrating upstream. Only 10 of 12 radio-telemetered mountain mullet survived the disturbance, and only one moved a short-distance upstream (1 km). Chance alone could explain the lack of telemetered mountain mullet migrating at the scale observed in PIT tagged fishes (>3 km), and the incorporation of large numbers of PIT-tagged fish facilitated the quantification of a small fraction of migrating fish.

Mountain mullet may have experienced greater difficulty than other species in locating hydrodynamic refuge during the flood disturbance, resulting in their downstream displacement. Morphology and the potential for downstream displacement can limit the upper altitude distribution of native stream fishes in flashy tropical streams like Río Mameyes (Schoenfuss and Blob 2007). Among the six native freshwater fishes found in Río Mameyes, mountain mullet are the only water-column dwelling species; all others are demersal, making them less susceptible to involuntary displacement. The potential for extensive downstream displacement of mountain mullet was observed in one radio-telemetered individual during a brief but intense spate outside of the study period and six PIT-tagged mountain mullet after the disturbance. Furthermore, only mountain mullet were observed at high densities in the coastal plain and foothills reaches we surveyed after the disturbance. These findings suggest a higher probability of downstream displacement in mountain mullet than other fish species.

The flood disturbance effects on an amphidromous fish assemblage documented here are consistent with previous studies showing similar changes in the spatial distribution of amphidromous shrimp fauna in another northeastern Puerto Rico watershed following Hurricane Hugo in 1989 (Covich et al. 1996). At least one shrimp genus, *Atyid*, appeared to follow a similar pattern as that of mountain mullet, with a 20% reduction in abundance at high elevations followed by greater than a 100% increase in abundance at lower elevations. Further, at long, annual time scales another shrimp genus, *Macrobrachium*, was relatively unaffected by flood disturbances (Covich et al. 2006). We suggest that short-term alterations to the distribution of *Macrobrachium* may have occurred but were not observed because populations were monitored at a coarse time scale. Thus the conclusions of these studies on amphidromous shrimp (Covich et al. 1996, 2006) are consistent with ours. Amphidromous populations experience frequent, acute effects due to flood disturbance, but amphidromous populations and assemblages are resilient to flood disturbances in the long term.

The removal methods we employed to estimate fish densities are recognized to be biased in some populations by heterogeneity of capture probability associated with the number of removal passes, fish size or age, and environmental variation (Williams et al. 2002, Peterson et al. 2004, Dauwalter and Fisher 2007). Previous assessment determined that three removal passes were sufficient sampling effort to accurately assess fish densities in Puerto Rico streams (Kwak et al. 2007). We directly accounted for variability in capture probability related to size or age by estimating size-specific capture probability. Estimates of capture probability in the removal model were highly variable among samples, and some estimates were imprecise, indicated by wide credible intervals. Such variability in capture probability likely reflects habitat heterogeneity. Most fish did not rise to the water surface after being stunned by the electrofisher; they instead sank to the bottom, where they were more difficult to locate under cobble and boulders. Larger substrates exacerbated this heterogeneity. Imprecision in capture probability estimates resulted in imprecision in estimates of the proportional change in fish density; therefore, some of our estimates of fish density change were not significantly different from zero (i.e., the credible interval included zero). However, the overall trend in proportional change in fish density was consistent among species and size classes. When the one outlier group, sub-adult mountain mullet, was omitted from analysis, the mean net change in fish density was significantly negative. Although we did not have sufficient statistical power with our sample sizes and model to conclude that most density estimates changed significantly, aggregated data for the entire fish assemblage indicated that overall fish densities were reduced after the disturbance.

Ecological and management implications

We observed a stochastic mortality and dispersal event that demographically and spatially restructured tropical stream fish populations. Consistent with the predictions of Poff and Ward (1989), populations experiencing intense, stochastic disturbance at a moderate frequency (approximately once every four years in this case) were structured by abiotic events. Restructuring occurred only at the population level, manifesting as mortality and altered dispersal patterns. The disturbance, however, appeared to trigger rapid recovery processes that were related to deterministic, biotic effects, including recruitment that replaced diminished adult populations and crowding or predatory aggregation in lower reaches. Population recovery processes appeared to dampen the assemblage level effects of the disturbance, as assemblage parameters did not change significantly. To fully understand the forces structuring populations and communities in tropical streams, we rely on an interpretation of the traits that amphidromous species have evolved to cope with frequent and intense disturbance (Lytle and Poff 2004, Death 2010).

The amphidromous life history has been interpreted as an adaptation for colonizing distant island stream habitats then recolonizing those same habitats following catastrophic disturbance (McDowall 2010). The critical life stage in this colonization adaptation is the marine larval dispersal phase (Fitzsimmons and Nishimoto 1995). Larval dispersal can seed newly-formed volcanic islands across vast areas of ocean, and at the same time, marine larvae can act as a local pool of colonists that are relatively protected from the floods that occur in freshwater habitats. We add to this theory of amphidromous dispersal that adults of some species are also capable of recolonizing headwater habitats after downstream displacement.

We further conclude that the disturbance regime of tropical streams like Río Mameyes provides resistance to establishment of non-native species. An appropriate analogy from the terrestrial literature may be fire disturbance in pine ecosystems, where frequent and intense disturbances act as a community filter, preventing the establishment of exotic species that are not fire-adapted and enhancing the native ecosystem (Levin 2009). In contrast, anthropogenic alterations to stream hydrology (e.g., dams and resulting impoundments) and the extensive floodplains of larger rivers provide a lentic environment where exotics find hydrodynamic refuge during intense disturbance events (Johnson et al. 2008). Supporting this conclusion, exotic fish species proliferate in Puerto Rico streams with dams somewhere in the watershed, including more permeable low head dams that do not restrict native amphidromous fauna (Kwak et al. 2007, Cooney and Kwak 2013). Río Mameyes is one among only a few free-flowing, unregulated streams on the island and supports minimal levels of exotic species.

Results from our study may inform conservation and management of diadromous fish assemblages on tropical islands globally. Our findings indicate the importance of high flows in regulating fish assemblage structure and the resilience of native species. Managing water releases from dams on regulated rivers has long been applied as a conservation tool for diadromous species (Annear et al. 2004) and more recently as management tool for limiting the spread of exotic fish species (Fausch et al. 2001). Dam construction has facilitated the invasion of aquatic habitats by species that are not resilient to the natural disturbance regime (Poff et al. 1997), but regulation of water released from dams can be a valuable tool in the maintenance and restoration of native aquatic communities. We conclude that an ecological flow regime, providing minimum flows for continuous connectivity between fresh and marine waters and periodic high flow events to filter aquatic communities is among the most important management strategies for conserving native fish assemblages on tropical islands.

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Table 1. Results of Mann-Whitney tests for shifts in habitat characteristics in a coastal plain reach of Río Mameyes after Hurricane Irene.

Habitat characteristic	Shift in median	*P*
Dominant substrate (mm diameter)	0	0.52
Velocity (m/s)	0.040	0.022
Depth (m)	0.11	0.0003

Table 2. Bayesian information criterion (BIC) for all candidate removal models. The best model (i.e., lowest BIC) is indicated in bold.

Model	BIC
Capture probability fixed over time	8832
Capture probability varies seasonally	8787
Capture probability varies by sample	**7814**

Table 3. Change in Shannon-Weaver diversity index (H') for the Río Mameyes fish assemblage before and after the disturbance of Hurricane Irene.

	H'	95% credible interval
Pre-disturbance	0.23	0.22–0.24
Post-disturbance	0.27	0.24–0.30
Change	0.038	0.0083–0.076

Table 4. Rank order of fish density estimates in Río Mameyes before and after the disturbance of Hurricane Irene.

	Pre-disturbance		Post-disturbance	
Rank	Species	Density (number/ha)	Species	Density (number/ha)
1	American eel (*Anguilla rostrata*)	2021	Mountain mullet (*Agonostomus monticola*)	1384
2	Mountain mullet (*Agonostomus monticola*)	1360	Sirajo goby (*Sycidium* spp.)	497
3	Sirajo goby (*Sycidium* spp.)	1295	American eel (*Anguilla rostrata*)	307
4	Bigmouth sleeper (*Gobiomorus dormitor*)	577	Bigmouth sleeper (*Gobiomorus dormitor*)	299
5	Smallscaled spinycheek sleeper (*Eleotris perniger*)	258	Smallscaled spinycheek sleeper (*Eleotris perniger*)	141

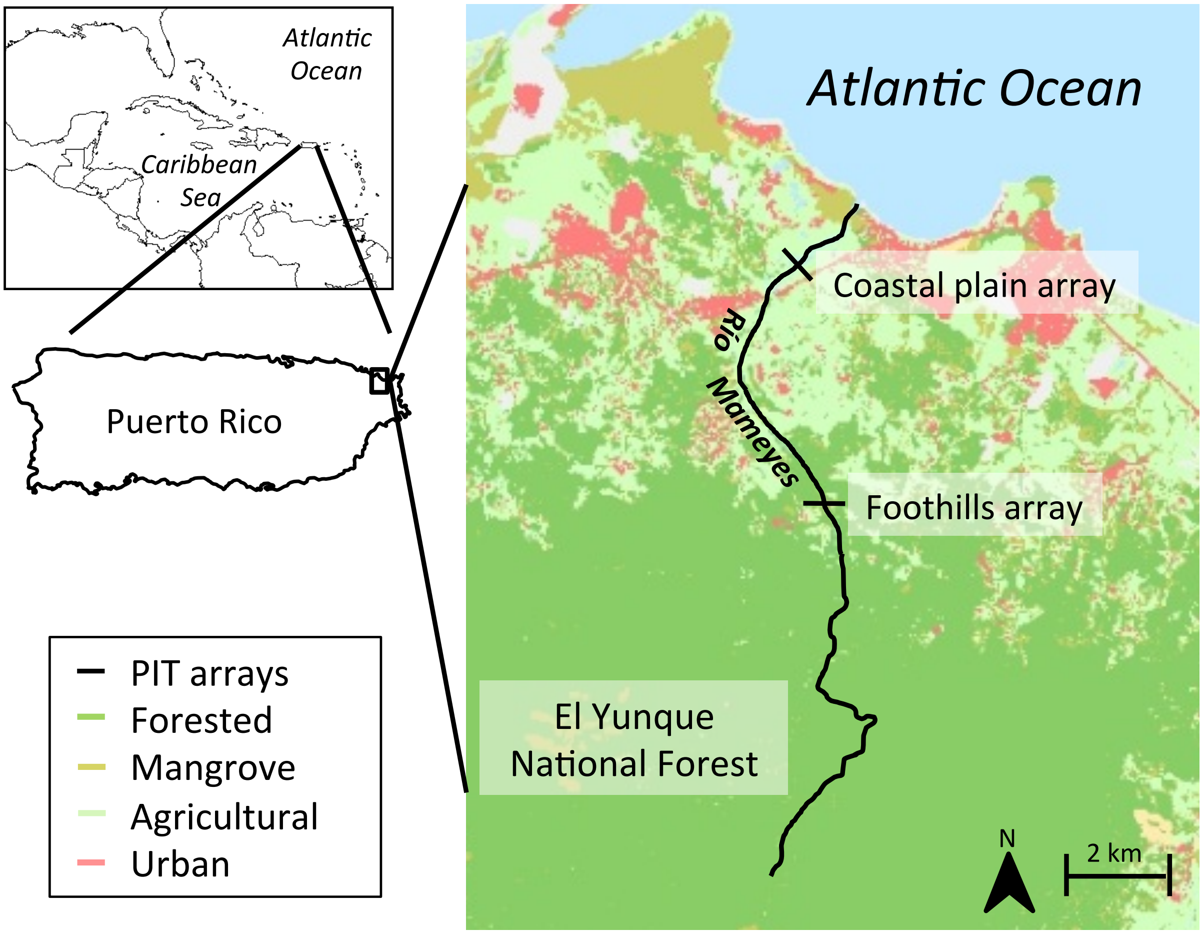

Figure 1. Land cover map of northeastern Puerto Rico, showing Río Mameyes and passive integrated transponder array and locations. The coastal plain array was located 2.2 km upstream of the ocean (rkm 2.2; 2 m above sea level), and the foothills array was located at rkm 6.4 (42 m above sea level).

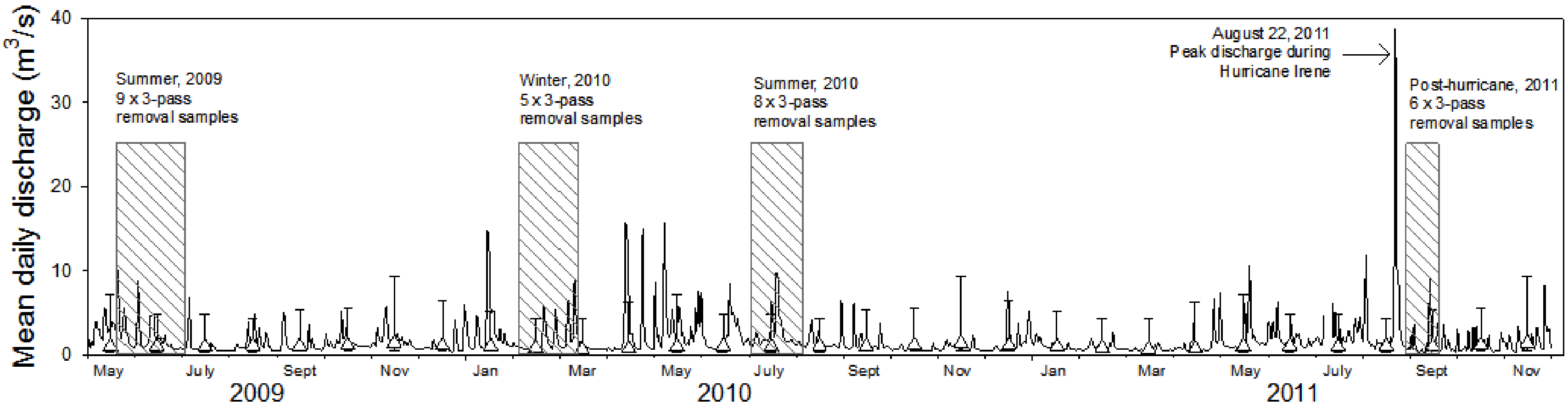

Figure 2. Flow over time in Río Mameyes in relation to fish sampling events and Hurricane Irene. The solid line represents daily discharge in Río Mameyes over 2.5 years. Hatched boxes indicate dates of 28 three-pass depletion samples. Triangles and bars indicate monthly median flows and standard deviations estimated from 30 years of streamflow data collected at the U.S. Geologic Survey gauge station on Río Mameyes (USGS 2011).

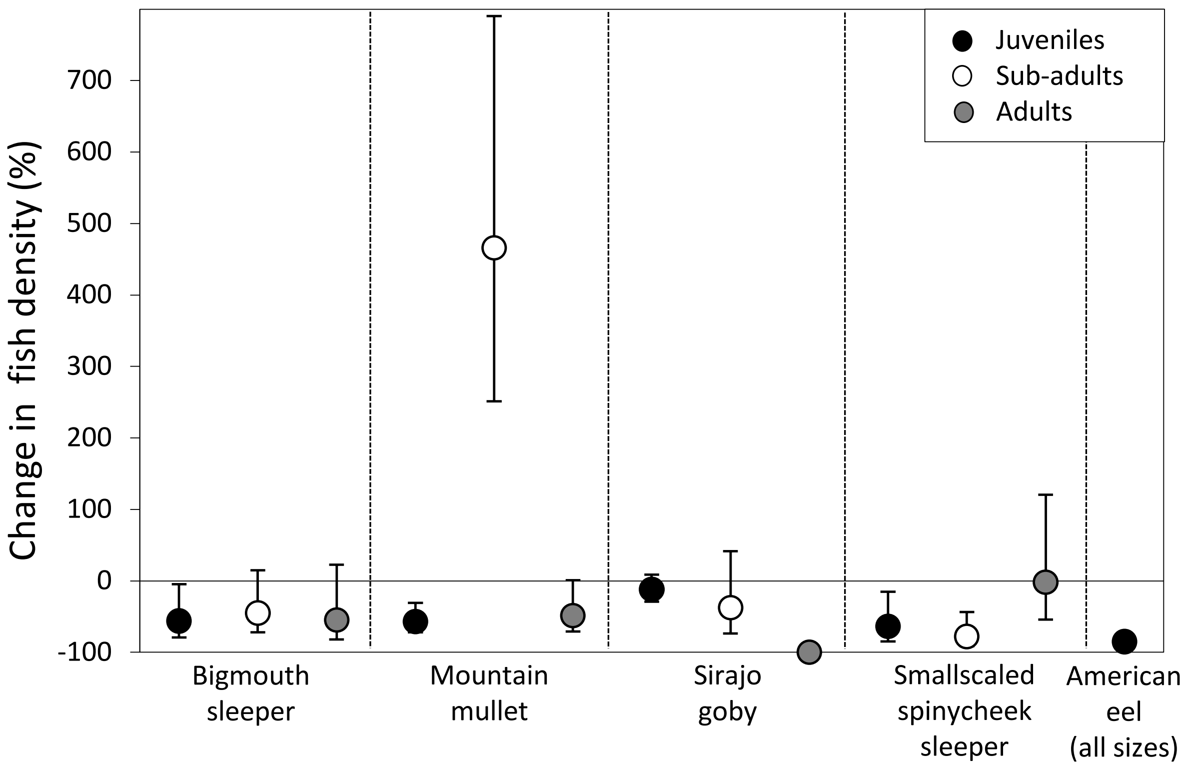

Figure 3. Percent change in fish population densities estimated from three-pass removal electrofishing procedures of bigmouth sleeper (*Gobiomorus dormitor*), mountain mullet (*Agonostomus monticola*), sirajo goby (*Sycidium* spp.), smallscaled spinycheek sleeper (*Eleotris perniger*), and American eel (*Anguilla rostrata*) in Río Mameyes following Hurricane Irene. Vertical bars indicate 95% credible intervals.

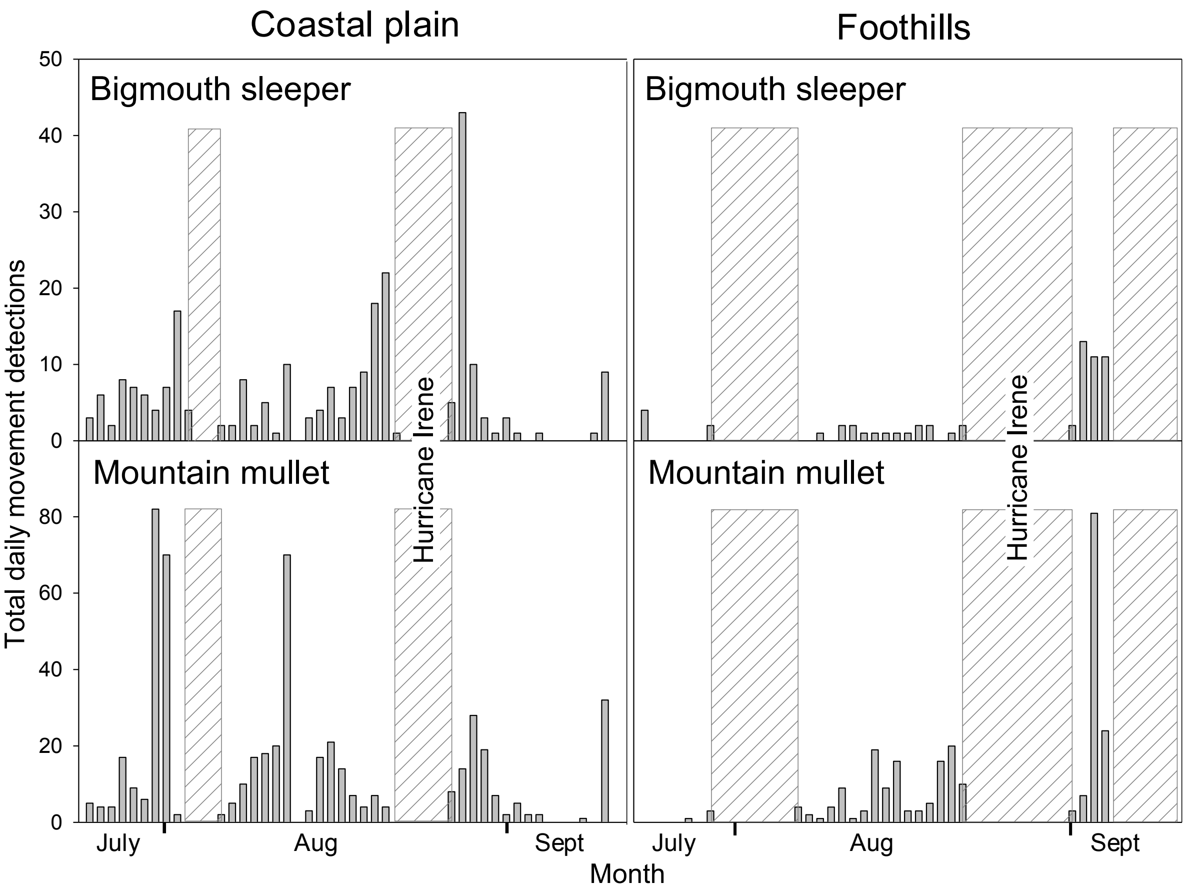

Figure 4. Activity indices, total number of movement detections, for two passive integrated transponder tagged species, bigmouth sleeper (*Gobiomorus dormitor*) and mountain mullet (*Agonostomus monticola*), before and after a major flood disturbance, Hurricane Irene. Hatched areas indicate periods when passive integrated transponder arrays were disabled.

CHAPTER 6
A CAPTURE-RECAPTURE MODEL OF AMPHIDROMOUS FISH DISPERSAL
(Job 4)

Abstract

Characterization of migratory scale is critical to the successful conservation and management of diadromous fishes. We quantified adult movement scale for two tropical diadromous fishes, bigmouth sleeper *Gobiomorus dormitor* and mountain mullet *Agonostomus monticola*, using passive integrated transponders and radio telemetry. Large numbers of fish were tagged in Río Mameyes, Puerto Rico, USA, with passive integrated transponders and monitored at three fixed locations over a 2.5-year period, generating information to estimate transition probabilities between upper and lower elevations and survival probabilities with a multistate Cormack-Jolly-Seber model. A subset of fish was tagged with radio transmitters and tracked at weekly intervals, generating fine-scale spatial information to estimate the scale of dispersal. Changes in spatial and temporal distributions of tagged fishes indicated that neither *G. dormitor* nor *A. monticola* moved into the lowest, estuarine reaches of Río Mameyes during two consecutive reproductive periods, thus demonstrating that both species follow an amphidromous, rather than catadromous, migratory strategy in this system. Further, both species were relatively sedentary, with restricted linear ranges. While substantial dispersal of these species occurs at the larval stage during recruitment to freshwater, our results indicate little dispersal in spawning adults. We conclude that successful conservation of diadromous fauna on tropical islands requires management at both broad basin and localized spatial scales.

Introduction

Diadromous fauna pose unique challenges for conservation because they migrate extensively between wide ranging habitats. As they migrate between marine and freshwaters, diadromous organisms cross jurisdictional borders, perform important ecological functions, and are often the target of valuable fisheries (McDowall, 1992). Characterizing the scale of migration is a critical component in defining the most effective management unit for diadromous fisheries (McDowell, 1999); however, migratory scale is poorly understood for many diadromous species.

Much of the native freshwater fauna found on tropical islands throughout the world follow a diadromous life cycle termed amphidromy (Myers, 1949; McDowell, 1988). Amphidromous organisms complete the majority of their life cycle in freshwater, and only larvae experience estuarine or marine conditions after hatching in freshwater and being passively transported downstream (McDowell, 1988; Keith, 2003). After a period of estuarine or marine development which may last up to six months in some species (Bell, 1994; Keith & Lord, 2011), metamorphosing larvae recruit to freshwater habitats, where they develop, grow, and remain as adults. Diadromous migrations may be important sources of longitudinal nutrient and energy transport (Flecker *et al.*, 2010), and amphidromous recruitment migrations are the target of locally important artisanal fisheries (Erdman, 1961; Bell, 1999; Castellanos-Galindo *et al.*, 2011). Amphidromous recruitment migration at the basin scale, ranging from the estuary to stream headwaters, has been documented across taxa (Keith, 2003; Kikkert *et al.*, 2009; Keith & Lord, 2011). Furthermore, interbasin and even interisland recruitment is known to occur (Cook *et al.*, 2010). In contrast, the scale of amphidromous fish migration after recruitment into freshwater, whether adults are sedentary, mobile, or migrate for spawning remains uncertain. Some evidence indicates that amphidromous fish in Hawaii make annual reproductive migrations to the estuary [*Awaous guamensis* (Valenciennes, 1837)] (Kido & Heacock, 1992), and otolith microchemistry evidence suggests that another group of Pacific freshwater gobies, *Sicyopterus* spp., may undergo adult migrations between upper and lower river reaches (Lord *et al.*, 2011). Little is otherwise known about amphidromous fish dispersal after recruitment into freshwaters or how migratory patterns are structured within amphidromous fish assemblages.

Native Caribbean stream fishes, bigmouth sleeper *Gobiomorus dormitor* (Lacepède 1800) and mountain mullet *Agonostomus monticola* (Bancroft 1834), have each been considered amphidromous and catadromous (i.e., migrating to the ocean for spawning; Myers, 1949) by different authors over the years (Anderson, 1957; Nordlie, 1981; McDowall, 1988; Phillip, 1993; Winemiller & Ponwith, 1998). *A. monticola* migratory patterns were an example of the ambiguity in the distinction between catadromy and amphidromy in Myers' (1949) description of diadromous migration and life histories. The critical distinction between catadromy and amphidromy lies in the scale of movement of adults during the spawning season; do they migrate to the estuary or beyond to the ocean? If they do, their life history is consistent with catadromy; if not, they should be categorized as amphidromous. Evidence to support reproductive migrations to lower river reaches has been anecdotal, indirect, and sparse (Anderson, 1957; Nordlie, 1981; McDowall, 1988; Phillip, 1993; Winemiller & Ponwith, 1998), and no investigator has documented the dispersal patterns of adults using a direct method.

We directly estimate the scale of dispersal of *G. dormitor* and *A. monticola* with a multistate capture-recapture model and fish tagging data from passive integrated transponder (PIT) and radio telemetry in a free-flowing Caribbean river. Tagged fish were monitored and resampled using a combination of passive PIT arrays and electrofishing recaptures over 2.5 years and weekly radio-telemetry relocations over a reduced period. The multistate model allows estimates of survival probabilities and transition probabilities between spatial strata to be calculated based on empirical field data. Transition probabilities indicated the likelihood of moving from upper to lower elevations (i.e. downstream migration), and estimates of linear ranges from fine-scale radio telemetry data indicated the scale of dispersal in adult *G. dormitor* and *A. monticola*.

Methods

Study system

Río Mameyes is a fourth-order, free-flowing river draining the Luquillo Mountains of northeastern Puerto Rico, USA, the smallest and eastern-most island of the Greater Antilles in the Caribbean Sea. The Luquillo Mountains are of volcanic origin and reach heights greater than 1,000 m within 20 km of the island coast (Pike *et al*., 2010). The steep topography interacts with northeasterly trade winds and frequent tropical storms to produce extremely high rainfall (averaging nearly 5 m/year; Lugo *et al*., 2012) and flood-dominated rivers, typical in hydrology to other Antillean rivers. Río Mameyes is one of only a few undammed rivers in Puerto Rico (Cooney & Kwak, 2013) with continuous aquatic connectivity from the headwaters to the ocean. It has a steep, short drainage, with an origin 850 m above sea level, flowing north just 12 km into the Atlantic Ocean. The upper watershed consists of protected secondary forest within the El Yunque National Forest, and the lower watershed contains primarily of secondary forest, agricultural land, and small urbanized areas, including the villages of La Vega, Palmer, and Fortuna (Martinuzzi *et al*., 2007; Fig. 1). Río Mameyes's unrestricted connection to the ocean, condensed length, and generally undeveloped watershed render it an ideal system to study tropical lotic fish populations with minimal anthropogenic influence.

Native diadromous fauna dominates Puerto Rico freshwater streams with uninterrupted connectivity to the estuary (Holmquist *et al*., 1998; Kwak *et al*., 2007; Cooney & Kwak, 2013). Thus, native diadromous fish and shrimp fauna are the primary aquatic community inhabitants in the free-flowing Río Mameyes (Kwak *et al*., 2007). Río Mameyes may be considered representative of the highly disturbed, undeveloped streams with native diadromous fish assemblages found on tropical islands throughout the Caribbean and the world, and our findings are applicable to broad-scale conservation, considering local variation.

Fish tagging and recapture

Fish were PIT tagged and recaptured during 13 sampling periods over 2.5 years (2009–2011; Fig. 2). Sampling periods were one month in duration and were selected to compare early (May–July) and late (August–September) fish spawning seasons to a non-spawning period (February–March). Data from an annual time series of gonadal development in *G. dormitor* and *A. monticola* in Puerto Rico indicated that spawning for *G. dormitor* and *A. monticola* occurred from late spring through early fall (Chapter 4). Populations of PIT-tagged fish were sampled using two different methods, backpack electrofishing at both random and fixed locations and PIT arrays at fixed locations in the coastal plain, foothills, and mountains (Fig. 1). Fish were tagged with passive integrated transponders during each sampling period until August 2011. PIT arrays were deployed and populations were resampled by backpack electrofisher during each sampling period after March 2009.

Fish populations were sampled by pulsed direct-current backpack electrofisher (Smith-Root Model LR-24; www.smith-root.com; 400 V, 30% pulse width, 0.2-0.3 A) from nine 100–150-m reaches during each sampling period, five randomized reaches in the coastal plain (rkm 2–6.5) and four upper elevation reaches (rkm 6.5–10.2). Upper elevation river access is limited, so we sampled among 10 fixed sites by backpack electrofisher in the foothills and mountains. Assuming some degree of fish capture and tagging stress, we did not resample reaches in consecutive sampling periods. Sampling tagged fishes occurred concurrently with sampling to estimate fish density (see Chapter 1). We sampled 100-m reaches in three passes to tag and recapture fish while estimating fish density. Otherwise, 150-m reaches were sampled in two passes to tag and recapture fish only.

Tagging protocols were designed to minimize fish stress by tagging only adult fish (Bateman & Gresswell, 2006), sterilizing all surgical equipment, briefly anaesthetizing fish during surgery, and minimizing the total handling time of each fish. Only *A. monticola* larger than 120 mm total length and *G. dormitor* larger than 130 mm total length were implanted with PIT tags or radio transmitters. Fish receiving a PIT tag were lightly anesthetized in aerated river water containing 80 mg/L tricaine methane sulphonate (MS 222) for two minutes before transfer to a tank containing a maintenance level of anesthetic (30 mg/L MS 222). Total length, weight, sex, and capture location were recorded for each fish. PIT tags (Texas Instruments; www.ti.com/rfid; 23-mm, half-duplex, 0.6 g) were implanted intra-abdominally via a 4-mm incision posterior to the pelvic fin (Baras *et al*., 1999). Fish larger than 250 mm total length were implanted with larger 32-mm tags (0.8 g). Before each implantation, all surgical equipment and PIT tags were disinfected in a solution of Benzall surgical disinfectant. Each PIT tag implantation lasted approximately one minute, and total sedation time was 7.5 minutes on average, after which fish recovered in a 30-L live well, with flowing river water for 1–2 hours before release. Only fish in apparently good condition were released.

During the final sampling periods (June–September 2011), a small sample of large *G. dormitor* and *A. monticola* were fitted with radio transmitters. Fish tagged with radio transmitters were captured and allowed to recover after surgery following the same procedures for PIT tagging. Fish receiving a radio transmitter were anesthetized for 4 minutes in 80 mg/L MS 222. Radio transmitters required a 12-mm incision, and each surgery lasted for approximately 10 minutes. Greater measures were required to ensure that each fish survived radio transmitter implantation in good condition, including artificial water circulation over the gills and closing of the incision wound. Water containing 30 mg/L MS 222 was circulated over the gills using a hand siphon while a small radio transmitter (Advanced Telemetry Systems; http://www.atstrack.com; Model F1545, 0.9 g) and a 23-mm PIT tag were implanted intra-abdominally, including the trailing antenna (not inserted through body wall). Incisions were closed with two sterile sutures.

Tagged fish detection

PIT-tagged fish were monitored continuously at three fixed points in Río Mameyes in the coastal plain, the foothills, and the mountains (Fig. 1), with pairs of automated PIT antennas (PIT arrays). PIT antennas spanned the entire river channel and recorded the unique tag identification number and time at which tagged fish moved past stationary points in the river. Two antennas were placed 2-3 m apart at each site to improve the probability that tagged fish would be detected when present at array sites. The use of paired arrays enabled a determination of the direction of fish movement; however, we elected to avoid assumptions regarding detectability at each antenna by combining data from the two antennas at each PIT array site. Each antenna was connected to a series of capacitors and a multiplex PIT reader and automated data logger (Oregon RFID; www.oregonrfid.com).

Antennas were firmly secured to the river bed with concrete anchors and mounds of small boulders in a horizontal pass-over design for protection against high velocity flood waters. Vertical pass-through antenna designs yield greater PIT detection efficiencies but are more vulnerable to damage during flooding compared to pass-over designs (Nunnallee *et al.*, 1998; Greenberg & Giller, 2000). Shorter antennas tend to have greater read ranges and less surface area to create drag during periods of high water velocity, and pass-over antenna designs are most effective when animal movement is restricted to a narrow range of depths. Therefore, PIT array sites were selected to maximize detection efficiency by reducing total array length (river width) and water depth. Antennas were each 1-m wide and 17-m, 11-m, and 18-m in length in the coastal plain, foothills, and mountains, with respective read ranges of 0.38-m, 0.50-m, and 0.25-m. Based on the depth profile at each site, these read ranges were sufficient to cover >90% of the river channel under base flow conditions.

Radio-telemetered fish were manually located at weekly intervals through September 2011. We recorded individual fish locations with a hand-held Global Positioning System (GPS) device and also described their position relative to previous locations and prominent stream features or landmarks. Fish locations were converted to linear position in the river (rkm) using Google Earth (www.google.com/earth). A curvilinear path was delineated along the Río Mameyes corridor, and fish locations were assigned to the closest point on the Río Mameyes path.

Multistate model

We used a multistate Cormack-Jolly-Seber model (Seber, 1982; Brownie et al., 1993; Kery & Schaub 2011) to estimate the time-specific probabilities of transitioning from upper elevations (foothills and mountains) to lower elevations (coastal plain) and the probabilities of transitioning from lower to upper elevations, while accounting for apparent survival and observation probabilities. Transition probabilities significantly greater than 0.5 indicated high probabilities of leaving longitudinal strata (i.e. emigrating), while transition probabilities significantly less than 0.5 indicated low probabilities of emigration (i.e. sedentary behavior). Statistical significance was inferred when 95% credible intervals did not include 0.5. Significant probabilities of transitioning from upper to lower elevations during the spawning season were interpreted as evidence of the potential for a downstream spawning migration.

Multistate model estimates of dispersal (transition probabilities) were compared to observed long-distance dispersal. Long-distance dispersal was quantified by calculating linear ranges. Long-distance dispersal was defined as any movement greater than 4 km, the approximate distance required to traverse between physiographic regions in Río Mameyes. Linear ranges were calculated as the maximum distance between any two encounter locations (Hayne, 1949), assuming that dispersal occurred at the time of the second encounter. Linear ranges were also calculated for all radio-telemetered fish, and mean linear range values for each species, based on radio telemetry, were assumed to represent the average scale of dispersal.

Results

Large numbers of fish were PIT-tagged, generating a great quantity of coarse information about movement and survival, and a small number of fish were located using radio telemetry, generating complementary, fine-scale movement data. From March 2009 through September 2011, 1455 *G. dormitor* and 784 *A. monticola* were PIT tagged. Two-hundred sixty-four PIT-tagged *G. dormitor* and 241 PIT-tagged *A. monticola* were observed at PIT arrays, and 162 PIT-tagged *G. dormitor* and 63 PIT-tagged *A. monticola* were recaptured in electrofishing samples. From June through August 2011, 15 *G. dormitor* and 13 *A. monticola* were tagged with radio transmitters and later relocated at least four times before death or battery expiration.

The multistate model estimated low annual rates of apparent survival for both *G. dormitor* and *A. monticola* (Table I); however, few PIT tagged fish died during short-term mortality trials. Of 102 *G. dormitor* tagged and held for 16 hrs, only four died, and of 55 tagged *A. monticola*, only one died. Transition probability estimates were generally low or not significant, indicating sedentary behavior of PIT-tagged *G. dormitor* and *A. monticola* and low rates of migration (Fig. 3; panels A, B, D, and E). Observed patterns of long distance dispersal (linear ranges) also indicated sedentary behavior (Fig. 3; panels C and F). A small number of *G. dormitor* dispersed downstream during the early spawning season of 2011 and the late spawning season of 2011; however, the majority of all observed dispersal was local. A significant exception to the local movement patterns of *A. monticola* occurred during the late spawning season of 2011, when a large number of fish were observed in upper elevations after emigrating from lower elevations. The multistate model estimated an emigration probability of 0.78 for lower elevation mountain mullet during this period, which followed a major flood disturbance event (Chapter 1). Sedentary behavior was also observed in radio-telemetered fishes. Linear ranges of radio-telemetered *G. dormitor* were variable, with a mean of 0.30 km (SD = 0.4; range = 0.03-1.39 km). Linear ranges of radio-telemetered *A. monticola* were also variable, with a mean of 0.53 km (SD = 0.3; range = 0.16-1.27 km). No radio-telemetered individuals of either species were observed moving between upper and lower elevation strata.

Discussion

Direct evidence that we present here demonstrates that neither *G. dormitor* nor *A. monticola* migrated downstream from upper to lower elevations of Río Mameyes during two spawning seasons, indicating that spawning occurs in freshwater riverine habitats. Furthermore, the probability of long-distance downstream dispersal (e.g., a spawning migration) was generally low, and the period of greatest long-distance dispersal for both species, September 2011, was subsequent to a major flood disturbance, the most likely cause of adult dispersal observed during our study. Thus, we conclude that neither *G. dormitor* nor *A. monticola* are catadromous in this system, resolving any ambiguity related to previous indirect, anecdotal speculation (Anderson, 1957; Nordlie, 1981; Phillip, 1993; Winemiller & Ponwith, 1998). The movement patterns we observed are consistent with an amphidromous life history with spawning completed within freshwaters, directly confirming this presumption by previous investigators (Cruz, 1987; McDowall, 1988).

Estimates of survival rates, transition probabilities, and observation probabilities could each be biased by violations of the Cormack-Jolly-Seber model assumptions (Seber, 1982). Therefore, we designed field sampling and modeling protocols to maximize adherence to model assumptions and accounted for heterogeneity in survival probabilities and time at large during the initial period after tagging. The physical capture and tagging of fish was the process most likely to result in systematic heterogeneity of survival probabilities (Arnason & Mills, 1987), so we took measures to enhance the condition of each fish after capture during tagging, sedation, and recovery. Furthermore, we accounted for short-term survival of the capture and tagging process by tagging and holding a subset of fish for a short time to empirically estimate tagging mortality, and tagging mortality estimates were used to reduce individual survival probabilities during the initial period after tagging. Just as mortality from capture creates a smaller pool of tagged fishes available for later recapture, so too can the loss of tags, generating negative bias in survival estimates (Seber, 1982). The surgical implantation of PIT tags in *G. dormitor* and *A. monticola* resulted in a secondary mark, a small scar from the incision. This scar was visible on all electrofishing recaptured fish, and no fish were observed with a scar and no PIT tag. We interpreted this finding as anecdotal evidence of low tag loss, similar to that measured in other PIT-tagging studies (Gries and Letcher, 2002; Bateman & Gresswell, 2006; Iserman & Carlson, 2008). We accounted for heterogeneity in the exact time at large during the initial period after tagging by reducing individual mortality rates during the initial period of tagging by the fraction of the initial sampling period that passed before the individual was tagged. Permanent emigration from the study area can bias survival estimates, and temporary emigration can bias estimates of observation probabilities (Seber 1982); however, the restricted linear ranges of radio-telemetered *G. dormitor* indicated that emigration was generally unlikely. The high probability of *A. monticola* migration estimated by the multistate model during the final sampling period suggested that survival probabilities for this species may have been biased low due to upstream migration into inaccessible regions of Río Mameyes; however, this migratory period was associated with unusual environmental conditions, the most significant flood disturbance event observed of the course of the study. Furthermore, it occurred at the end of the study, and emigration during this period would not have affected survival estimates.

Earlier studies concluding that *G. dormitor* (Nordlie, 1981; Winemiller & Ponwith, 1998) and *A. monticola* (Anderson, 1957; Phillip, 1993) migrate to the estuary or ocean for spawning and are thus catadromous were based entirely on indirect, anecdotal evidence generated from studies not designed to comprehensively assess movement patterns. Winemiller & Ponwith (1998) and Phillip (1993) found ripe females in the estuary but not in freshwater; however, freshwater sample sizes in both studies were small. It is not clear that Nordlie (1981) sampled throughout the spawning season or actually assessed reproductive condition. Anderson (1957) found *A. monticola* post-larvae at sea, a considerable distance from the nearest possible freshwater source but never observed adults or spawning activity. While he concludes that the patterns observed were consistent with a catadromous life history, they were also consistent with an amphidromous life history that may include a marine larval dispersal phase (McDowall, 1988; Keith, 2003), which was poorly understood at the time. Our findings represent the first direct evidence of movement patterns in adults of any amphidromous fish species. The direct tagging method allowed individual movements to be tracked through the spawning season, and tagging large numbers of fish allowed robust statistical comparisons among seasons and within the spawning season. Counter to the conclusions of previous studies, direct estimates from our research indicate that *G. dormitor* and *A. monticola* were extremely sedentary with relatively low probabilities of long-distance dispersal or dispersal between upper and lower elevations. One exception to this general rule was documented in a radio-telemetered *A. monticola* that moved 1.25-km downstream after a brief, intense spate but returned to its activity center within two days. Major flood disturbance events may result in some temporary, downstream displacement (Chapter 1) but not population-scale movements to lower river reaches.

Our findings also include the first estimates of survival rates for adults of any amphidromous fish. Apparent survival estimates indicated that only 30–35% of adult *G. dormitor* and *A. monticola* populations in Río Mameyes survive from one year to the next, rates that are low relative to other fish populations with similar body sizes (Pauly, 1980). While illegal harvest of *G. dormitor* is known to occur in Río Mameyes (personal observation), illegal fishing at the scale observed is unlikely to remove a large proportion of the adult population of this species, but this has not been investigated and is difficult to measure. We observed only minimal harvest of either *G. dormitor* or *A. monticola* in lower reaches of the river, and the upper river is closed to fishing within the El Yunque National Forest. Thus, we conclude that our estimates of total mortality approximate natural mortality rates in this system.

The predominant cause of high mortality rates in Río Mameyes is likely the intense disturbance regime that characterizes lotic ecosystems in this region. The El Yunque National Forest receives nearly 5 m of rainfall annually (Lugo *et al*., 2012), including brief periods of intense rainfall during tropical cyclones. One tropical cyclone, Hurricane Irene, struck Puerto Rico during this study and resulted in a 10% reduction in mean fish density in Río Mameyes (Chapter 1). Flood disturbances of Hurricane Irene's magnitude (>250 cm of rain in 24 hours;

US National Weather Service; water.weather.gov/precip) occur only approximately every four years, but chronic flood disturbances of lesser intensity are characteristic of Puerto Rico watersheds (Ramírez *et al*., 2009; Pike *et al*., 2010). Repeated exposure of fish populations to discrete, minor mortality events may have an additive effect, resulting in considerable total mortality at an annual scale.

Conservation implications

Evidence presented here suggests the following model of *G. dormitor* and *A. monticola* migratory life history. Adult fish spawn in freshwater lotic habitat, and after hatching in freshwater, larvae are passively transported downstream to marine or estuarine habitats (Chapter 3). After a period of development, post-larvae actively return to freshwater, often in large aggregate migrations, where they locate suitable habitat and remain for the duration of their lives and spawn as adults. Periodically, flood disturbances may spatially and demographically redistribute fishes, but even subsequent to major flooding, most adults remain sedentary (also see Chapter 1). The sedentary behavior of adult *G. dormitor* and *A. monticola* suggests that management of adult amphidromous fish populations at the local site or reach scale may be an effective approach, contrasted with broad, basin-scale management. In contrast, amphidromous post-larvae are known to migrate from the estuary to the headwaters (Keith, 2003; Kikkert *et al.*, 2009; Keith & Lord, 2011); thus, management of amphidromous fish recruitment requires consideration of the entire basin. This model of amphidromous life history suggests that basin characteristics, such as the geology, land cover, and number and spatial location of dams of a watershed, influence the recruitment of juveniles into local habitats. After recruitment, more sedentary amphidromous adults are influenced by localized factors, such as exploitation and instream habitat quality.

The low apparent survival rates estimated here suggest that adult *G. dormitor* and *A. monticola* populations may be able to withstand substantial harvest rates, given adequate recruitment. In general, adding low to moderate additional mortality (i.e., fishing) when natural mortality rates are very high has little effect on fish populations (i.e., compensatory, rather than additive effects; Allen et al., 1998; Winemiller, 2005). However, information about juvenile survival, growth rates, size at maturation, and fecundity are necessary to fully characterize the harvest potential and population dynamics of these species. Furthermore, the metapopulation dynamics of amphidromous species are not fully understood, and the proportion and abundance of larvae emigrating from and immigrating to other systems has not been quantified. Models of amphidromous populations that can explicitly test population sensitivity to harvest at different life stages are not feasible with currently available information; thus, future research focused on migration and mortality of early life stages is warranted. While additional adult mortality may have little influence on the sustainability of *G. dormitor* and *A. monticola* populations, the effects of additional juvenile mortality remains to be determined.

Our findings represent the first comprehensive, direct elucidation of movement and mortality in adult amphidromous fishes, with implications for the management of tropical aquatic resources. Two species, *G. dormitor* and *A. monticola*, were conclusively shown to follow an amphidromous spawning pattern, with residence in freshwater throughout the annual reproductive period. Mortality rates were high, likely associated with periodic disturbance experienced by tropical streams. The model of migratory life history we developed is based on direct quantitative evidence and suggests that the management of amphidromous fauna should be approached from two spatial scales, a broad, basin scale that influences recruitment and a local scale that influences adults and potential fishery resources. We look forward to future research to enhance this understanding for other amphidromous species and life stages to inform conservation and management of tropical insular lotic ecosystems.

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Table 1. Modeled population parameters estimated by multistate capture-recapture models for two diadromous tropical fish species.

Parameter	Estimate	SD	95% Credible interval
	Gobiomorus dormitor		
Annual mortality rate (*Z*)	1.1	0.08	0.9–1.2
Annual apparent survival rate (*S*)	0.35	0.03	0.29–0.40
	Agonostomus monticola		
Annual mortality rate (*Z*)	1.2	0.09	1.0–1.4
Annual apparent survival rate (*S*)	0.30	0.03	0.25–0.36

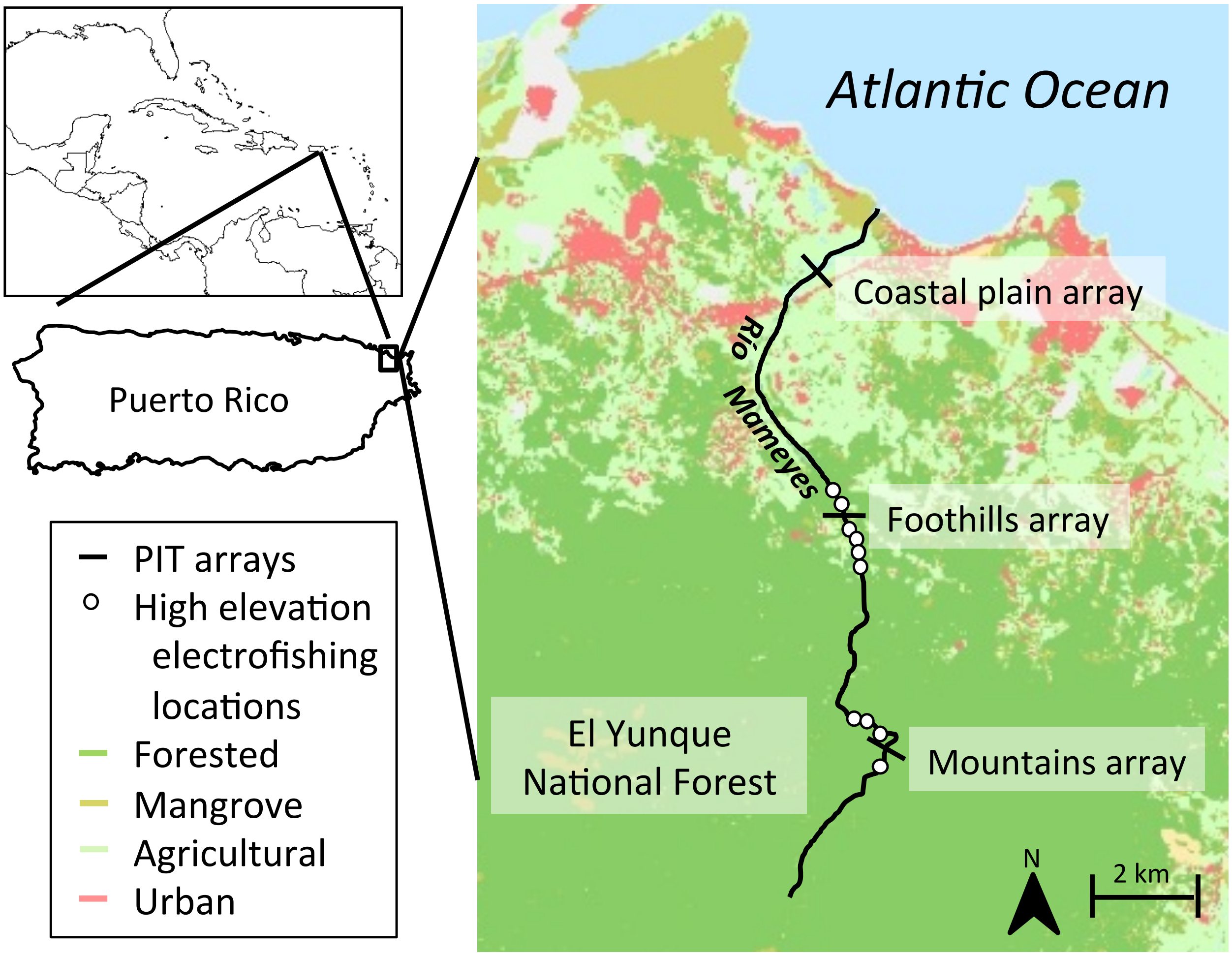

Figure 1. Land cover map of the Río Mameyes, Puerto Rico, watershed, showing locations of remote passive integrated transponder arrays and upper elevation electrofishing sites. The coastal plain array was located 2.2 km upstream of the ocean (rkm 2.2) [2 m above sea level (asl)]; the foothills array was located at rkm 6.4 (42 m asl); and the mountain array was located at rkm 9.9 (117 m asl).

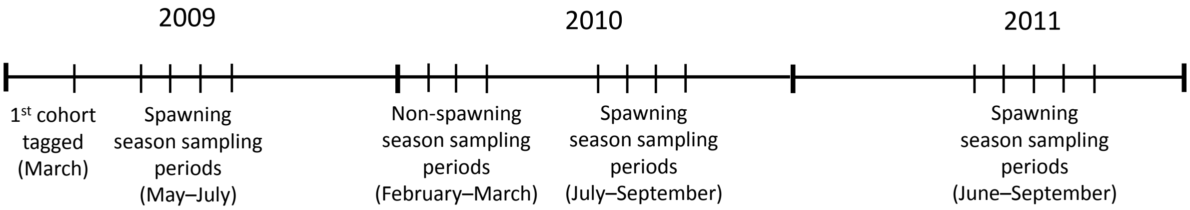

Figure 2. Sampling chronology showing fish capture-recapture periods. Tick marks indicate the beginning and end of each sampling period; bold tick marks delineate years.

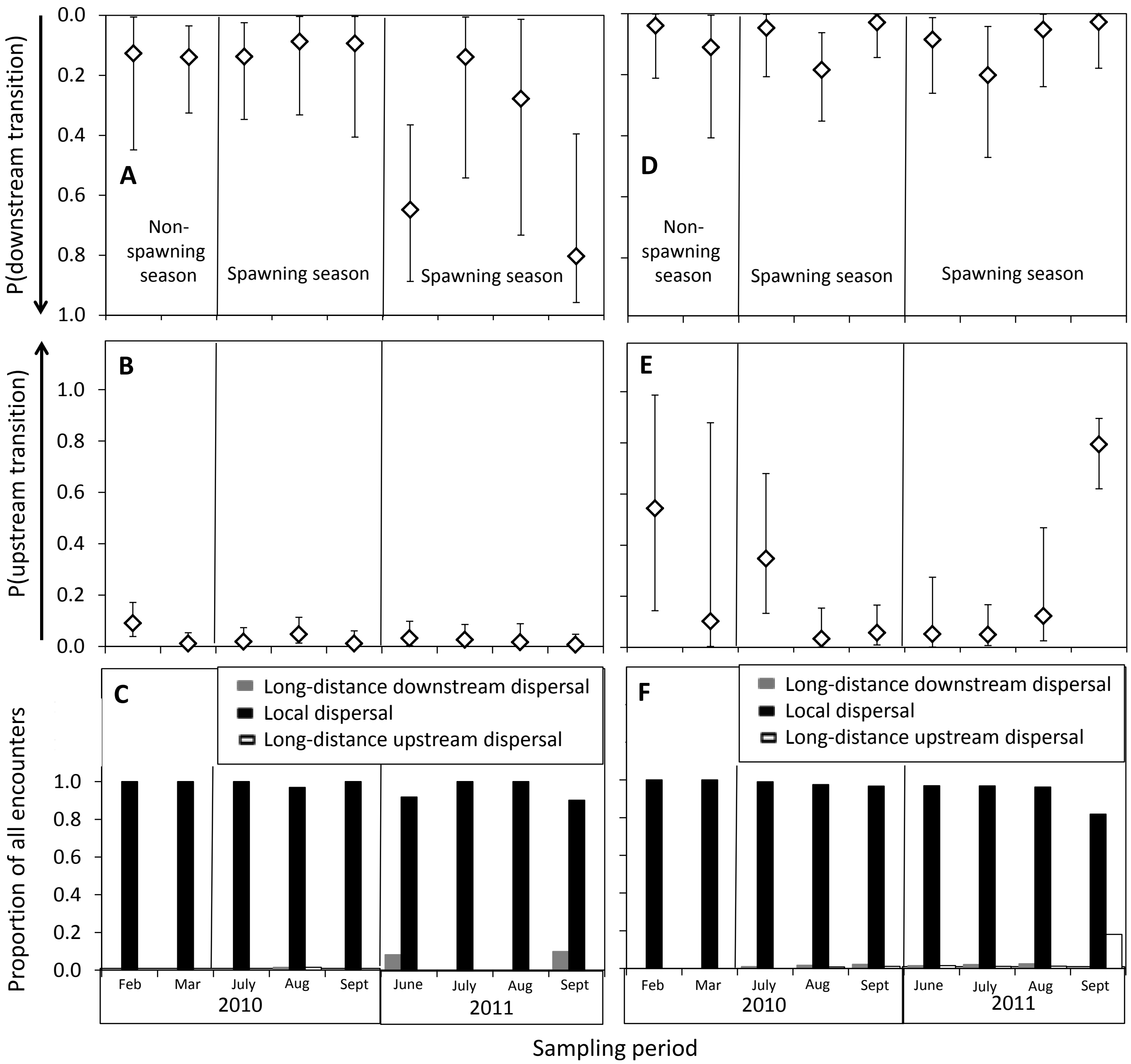

Figure 3. Transition probabilities estimated by the multistate model (A, B, D and E) and observed long-distance dispersal (C and F) for *Gobiomorus dormitor* (left panels) and *Agonostomus monticola* (right panels). P(upstream transition) (top panels) indicates the probability of moving from the foothills and mountains to the coastal plain, and P(downstream transition) (middle panels) indicates the probability of moving from the coastal plain to the foothills and mountains. Long-distance dispersal (bottom panels) is defined as any movement greater than 4 km, the approximate distance required to traverse between physiographic regions in Río Mameyes.

CHAPTER 7
OTOLITH MICROCHEMISTRY OF TROPICAL DIADROMOUS FISHES: SPATIAL AND MIGRATORY DYNAMICS
(Job 4)

Abstract

Classification of many tropical diadromous fishes as amphidromous or catadromous has not acknowledged that species or populations may follow a range of migratory patterns with full, partial, or no migration to the ocean. Otolith microchemistry is a useful technique to elucidate such migratory patterns and variation within and among species. We applied otolith microchemistry to quantify migratory variation and the proportion of native Caribbean stream fish that undergo full or partial marine migration. Strontium and barium water chemistry in four Puerto Rico U.S.A. rivers was clearly related to a salinity gradient; however, variation in water barium, and thus fish otoliths, was also dependent on river basin. Strontium was the most accurate index of longitudinal migration in tropical diadromous fish otoliths. Among four species examined, bigmouth sleeper *Gobiomorus dormitor*, mountain mullet *Agonostomus monticola*, sirajo goby *Sicydium* spp., and river goby *Awaous banana*, 9–12% of individual recruits were not amphidromous, with no evidence of marine elemental signatures in their otolith core. Populations of one species, *G. dormitor*, may have contained a small contingent of migratory, partially amphidromous adults that temporarily occupied marine habitat (4%); however, adult migratory elemental signatures may have been confounded with those related to diet and physiology. Our findings indicate the plasticity of migratory strategies of tropical diadromous fishes, which may be more variable than simple categorization might suggest.

Introduction

The larvae of many diadromous tropical fishes spend a period of time in marine habitats before returning to freshwater habitats as metamorphosizing post-larvae (Keith, 2003), consistent with an amphidromous or catadromous life history (Myers, 1949). Amphidromous fish spend the majority of their lives and spawn in freshwater, and larvae are passively transported to marine waters before migrating back to freshwater habitats as post-larvae. In contrast, catadromous fish undergo downstream migrations, leaving juvenile and adult freshwater habitats to spawn at sea, and larvae hatch in the marine environment before returning to streams.

The distinction between amphidromy and catadromy is based on the occurrence of adult migration to marine habitats (Myers, 1949) and is somewhat subjective and can render distinguishing these two migratory types difficult (Thibault *et al*., 2007). Diadromous reproductive migrations may not occur or could be completed entirely within freshwater (amphidromous), between fresh and estuarine waters (partially amphidromous), or between fresh and marine waters (catadromous). Further complicating the distinction between these reproductive strategies, diadromous populations may follow multiple migratory forms (Kerr *et al*., 2009). Reproductive adult migration to marine, estuarine, or downstream freshwater reaches, and recruitment from marine to freshwater or from downstream to upstream freshwater habitats could all occur within a single partially amphidromous fish population (Closs *et al*., 2003).

Analysis of otolith microchemistry is a relatively recent and useful technique for characterizing the dispersal patterns of diadromous fishes between aquatic habitats (Limburg, 1995; Secor & Rooker, 2000; Kerr *et al.*, 2009; Lord *et al.*, 2011; Tsunagawa & Arai, 2011). Fish otoliths record environmental chemistry information throughout an individual's life, and trace element signatures within the otolith have been used to determine natal origins (Thorrold *et al.*, 1998; Thorrold *et al.*, 2001; Rooker *et al.*, 2008), quantify metapopulation dynamics (Thorrold *et al.*, 2001; Chang *et al.*, 2008), and identify freshwater-marine migratory forms (Tsunagawa & Arai, 2008; Chino & Arai, 2009; Kerr *et al.*, 2009). The advantage of the indirect, otolith microchemistry approach compared to direct approaches to estimate fish dispersal rates, such as mark-recapture, is that otoliths store a record of the entire environmental history of a fish (Campana, 1999). Therefore, the otolith integrates a wealth of information relative to that possible by most direct approaches. Methods that integrate information over a long duration are especially advantageous when dispersal is temporary or episodic (Schilthuizen & Lombaerts, 1994; Wilson *et al.*, 2004). Furthermore, direct methods are often logistically limited to small spatial scales (Koenig *et al.*, 1996), whereas otolith samples can be easily obtained over broad spatial scales.

The migratory dispersal patterns of tropical diadromous stream fishes are largely unquantified, and evidence to support the categorization of Caribbean diadromous fishes as amphidromous or catadromous has been largely anecdotal (Anderson, 1957; Nordlie, 1981; Phillip, 1993; Winemiller & Ponwith, 1998). Here, we apply otolith microchemistry techniques to describe and quantify the recruitment and adult migratory forms that occur within and among Caribbean diadromous fish populations.

Methods

We sampled native stream fish populations in each of three river basins across the Caribbean island of Puerto Rico U.S.A., the Grande de Manatí, Sabana, and Cañas basins (Fig. 1). Two of the rivers (Grande de Manatí and Sabana) were located on the north side of the island and flow into the Atlantic Ocean, and the third was on the south side of the island (Cañas) and drains into the Caribbean Sea. These rivers were selected to represent a broad geographic distribution across Puerto Rico and because they each contained the full complement of native Puerto Rico stream fish species (Kwak *et al.*, 2007). Fishes at sites within at least two physiographic regions per basin were sampled by pulsed-DC backpack electrofisher (Smith-Root Model LR-24; www.smith-root.com) over a period of 1.5 years from July 2008 to August 2009 (Table I). Four of the most common native Puerto Rico stream fishes found were collected from each river, bigmouth sleeper *Gobiomorus dormitor*, mountain mullet *Agonostomus monticola*, sirajo goby *Sicydium* spp., and river goby *Awaous banana*. Additional samples of only *G. dormitor* were collected in Río Mameyes, and additional samples of only *A. banana* were collected from the Río Grande de Añasco basin (Fig. 1). Samples of only one species were collected in each of these two additional basins (Mameyes and Grande de Añasco) to supplement the more extensive sampling of native assemblages in the primary sampling locations (Grande de Manatí, Sabana, and Cañas). We chose to examine only the otoliths of the largest fish collected at each site, as these older fish possessed the longest records of environmental histories within their otoliths. Sagittal otoliths were extracted, cleaned with deionized water to remove all soft tissues, and dried. Cleaned otoliths were mounted in epoxy resin (Struers EpoFix; www.struers.com) and sectioned transversely with a diamond blade (Buehler series 15 HC diamond; www.buehler.com). Each sectioned otolith was mounted to a petrographic slide with thermoplastic glue (Crystalbond 509; www.crystalbond.com) and polished with 3-μm diamond slurry to reveal the core. Prepared otoliths were sonified for 15 minutes in ultrapure water to remove surface contamination.

The concentrations of Ba, Sr, and Ca in each otolith were analyzed using a laser ablation inductively coupled plasma mass spectrometer (LA-ICPMS) located at the GeoMed Analytical

Laboratory at University of Massachusetts, Boston, U.S.A. A linear raster scan ablation (width = 30 μm, rate = 5 μm/s) (Chang *et al.*, 2012) was made of each otolith from the core to the edge, measuring the concentrations of each element. Concentrations of Ba and Sr were expressed as ratios to Ca (Ba:Ca and Sr:Ca) to account for variation in the amount of material ablated. The calcium carbonate standard, MACS-3 (U.S. Geological Survey; crustal.usgs.gov) (Wolf & Wilson, 2007), was used to calibrate the LA-ICPMS. Calibrations were completed at the beginning and middle of each day to adjust for instrument drift. Based on 3 times the standard deviation of the blank Ar gas used by the LA-ICPMS, the detection limits for Ba:Ca and Sr:Ca were 0.016 mmol/mol and 0.19 mmol/mol, respectively.

Variation in otolith microchemistry may indicate transitions between marine and freshwater habitats, or simply a change in environmental conditions unrelated to fish movement (Elsdon & Gillanders 2002; Gillanders, 2002; Elsdon & Gillanders 2006). A difference between the variation in Ba:Ca and Sr:Ca profiles could indicate that environmental factors altered water chemistry (and thus otolith microchemistry) in addition to changes in ambient water salinity. To account for water chemistry and the potential for basin-specific effects in water chemistry, we analyzed water samples along a salinity gradient in each basin and tested for basin-specific patterns in otolith microchemistry variation. Triplicate water samples were collected along a salinity gradient from completely fresh (<0.5 ppt salinity) to at least half seawater (15–30 ppt salinity) at three points in each river basin sampled. Water samples were analyzed following procedures described by Dorval *et al.* (2005) and using a dynamic reaction cell ICPMS to quantify concentrations of Ba and Sr. The relationship between concentrations of Sr and Ba (dependent variables) and the corresponding salinity at each sampling location (independent variable) was analyzed with linear regression. Each river basin was modeled separately, and significant slope parameters ($P < 0.05$) indicated that Sr and Ba concentrations were related to salinity gradients and that otolith Sr and Ba could be applied as indices of a fish's salinity environment. Intercept parameters indicated regression model predictions in completely freshwater (i.e., 0 ppt salinity), so we compared the 95% confidence intervals of intercept parameters to evaluate the potential for basin-specific patterns in freshwater chemistry. Non-overlapping intercept parameter 95% confidence intervals were interpreted as significant differences.

Each individual fish's profile was stratified into a recruitment period and an adult period, and recruitment and adult patterns were analyzed separately. Amphidromous recruitment from marine or estuarine to freshwaters is characterized by a rapid decline in Sr:Ca near the otolith core (Tsunagawa & Arai, 2008; Lord *et al.*, 2011), so we assumed that the lowest Sr:Ca value near the core represented the point of recruitment to freshwater and used this point to stratify each individual profile into recruit and adult periods. Tropical diadromous larvae may transition between watersheds (Cook *et al.*, 2009; Cook *et al.*, 2010) and use marine habitats (Anderson, 1957; McDowall, 1988; Keith, 2003), where they are less affected by freshwater chemistry, so only the adult periods of otolith microchemistry profiles accurately represented environmental conditions experienced in riverine habitats. Thus we restricted analysis of the relationships between otolith microchemistry variation and river basin to adult periods.

Variation in otolith microchemistry profiles was quantified by calculating the range of Ba:Ca and Sr:Ca for each individual's adult period. Range was selected as an index of profile variability, because it is sensitive to outlying data, such as that produced in otolith microchemistry by episodic movement into varying salinity habitats. We tested the hypothesis that mean Ba:Ca and Sr:Ca ranges were different among basins using a one-way analysis of variance (ANOVA; $\alpha = 0.05$). The normality of each response variable was tested using the Shapiro-Wilk test, and non-normal variables were transformed with the Box-Cox power transformation. If ANOVA results revealed significant differences, mean Ba:Ca and Sr:Ca ranges were compared among basins using Tukey's test for multiple comparisons. All statistical test were performed in R (R Development Core Team, 2012). Agreement between tests on Ba:Ca and Sr:Ca variation was interpreted to support the conclusion that variability was a valid migratory signal, but disagreement between tests on Ba:Ca and Sr:Ca variability supported the conclusion that some unaccounted environmental factor influenced otolith microchemistry.

Recruitment periods were classified as amphidromous if a peak in Sr:Ca was found at the otolith core and Sr:Ca declined by at least 2 mmol/mol or non-amphidromous if the change in Sr:Ca was less than 2 mmol/mol. An absence of high Sr:Ca values could indicate that an individual completed its life cycle within freshwater or that the core was missed during otolith preparation. Therefore, for each individual classified as a non-amphidromous recruit, the polished and ablated otolith was microscopically reexamined to confirm that the core was sampled by the LA-ICPMS. Only individuals with a confirmed core sample were included in estimates of relative proportions of amphidromous and non-amphidromous recruits.

We also quantified the proportion of adults with microchemistry indicating a return to marine or estuarine waters (partial amphidromy or catadromy). For each species, we defined the threshold marine or estuarine signature as the minimum Sr:Ca value found in otolith cores of amphidromous recruits, and classified adult periods as partially amphidromous when two consecutive Sr:Ca values were greater than the threshold and amphidromous when fewer than two consecutive Sr:Ca values were greater than the threshold.

Results

A total of 279 diadromous fish otoliths were sampled and analyzed for microchemistry, and water samples were collected and analyzed from each basin sampled for otolith microchemistry, except Ro Grande de Añasco (36 water samples). Among all species, measured otolith Ba:Ca values ranged from below the detection limit during recruitment periods to 0.26 mmol/mol during adult periods, and Sr:Ca values ranged from 11.6 mmol/mol during recruitment to 0.41 mmol/mol during adulthood. Among all river basins sampled for water chemistry, freshwater Ba concentrations ranged from 8 x 10^{-4} to 9.6 x 10^{-3} mg/L, and freshwater Sr concentrations ranged from 0.03 to 0.27 mg/L. Estuarine Ba concentrations ranged from 6 x 10^{-4} to 5.6 x 10^{-3} mg/L, and estuarine Sr concentrations ranged from 0.55 to 1.84 mg/L.

Linear regression results confirmed significant positive Sr and negative Ba relationships between elemental concentration and salinity in water sampled from all rivers (Fig. 2). Therefore, otolith concentrations of these two elements may serve as suitable indices of a fish's transition through salinity gradients. No intercept parameters for Sr regression were significantly different among rivers, indicating similar Sr levels in the freshwater habitats of the rivers sampled. The regression intercept parameter fit to Río Cañas Ba values, however, was significantly lower than all other rivers, indicating that freshwater in Río Cañas was reduced in Ba compared to other sampled rivers. Water chemistry differences were reflected in otolith microchemistry signatures; ANOVA results indicated that variation in otolith Ba:Ca was associated with river basin, but variation in otolith Sr:Ca was not (Table II). Further, graphical comparison of otolith Ba:Ca and Sr:Ca temporal profiles showed that dramatic oscillations in Ba:Ca were not reflected in Sr:Ca, and conversely, changes in Sr:Ca did not correspond well with changes in Ba:Ca (Fig. 3). Mean Río Cañas otolith Ba:Ca values for adult periods were significantly lower than those of all other basins, and Río Sabana Ba:Ca values for adult periods were significantly higher than all other basins. This pattern was consistent among fish species, and indicated that some unknown environmental factor, specific to each river basin, produced variation in otolith Ba:Ca but not Sr:Ca. Given the extensive literature documenting the relationship between Sr:Ca and ambient water salinity across taxa (Radke, 1989; Farrell & Campana, 1996; Secor & Rooker, 2000; Walther & Limburg, 2012) and the conclusion of other researchers that Ba in water may cycle temporally, producing false marine Ba signatures in otoliths (Eldson & Gillanders, 2006), we elected to restrict all further otolith data analyses to Sr:Ca results to elucidate fish migratory patterns.

All species except *A. monticola* appeared to include a small proportion of individuals within populations that never experienced marine conditions during early life (Fig. 4). Seventy-five of 81 *G. dormitor*, 58 of 70 *Sicydium* spp., and 45 of 56 *A. banana* otoliths were confirmed to include microchemistry samples from the core, and of those, 9.3–12.1% had an entirely freshwater (non-amphidromous) Sr:Ca recruitment signature; 87.0–92.7% had an amphidromous recruitment signature (Table III; Fig. 4). All *A. monticola* otoliths ($n = 72$) had a marine or estuarine signature at the core, and core samples were confirmed for all *A. monticola*.

Applying the threshold marine Sr:Ca value estimated from otolith core samples (Table IV), only *G. dormitor* otoliths, among all species examined, contained evidence that adults experienced marine or estuarine conditions. The adult Sr:Ca periods of *G. dormitor* included a continuum of oscillatory to flat profiles, but all *A. monticola*, *Sicydium* spp., and *A. banana* adult periods were relatively flat with no evidence of a return to marine or estuarine conditions. Three of 81 (3.7%) adult *G. dormitor* periods contained marine or estuarine signatures (Fig. 5). Visual assessment of contingency tables revealed no obvious associations between river basins and the fraction of recruit and adult migratory forms among basins.

Discussion

Our findings are the first evidence of variation in migratory strategies within Caribbean amphidromous fish assemblages. To be considered amphidromous, fish must (a) occupy freshwater habitats during their adult life stage, and (b) inhabit marine or estuarine environments during their early life stages (Myers, 1949; McDowall, 1988). A proportion of *G. dormitor,* *Sicydium* spp., and *A. banana* (9–12%) did not show marine signatures in their otolith cores, indicating that they completed early life within freshwaters. All *A. monticola*, *Sicydium* spp., and *A. banana* otolith microchemistry adult periods were flat, with no evidence of a return to marine or estuarine waters; however, 4% of *G. dormitor* adults revealed oscillatory patterns, indicating periodic movement into higher salinity habitats. These results are evidence that the fish assemblages we examined are composed of a range of migratory contingents from amphidromous to partially amphidromous, and that varying degrees of plasticity exist in amphidromous fish migratory patterns. *A. monticola* is amphidromous, with all marine recruits and no adult return to the estuary or ocean. *Sicydium* spp. and *A. banana* populations contain contingents that recruited from both marine and fresh water habitats (amphidromous and non-amphidromous recruitment) but no contingent of migratory adults. *G. dormitor* populations consisted of both amphidromous and non-amphidromous recruit contingents and amphidromous and partially amphidromous adult contingents; however, marine signals in the migratory adult contingent may have been confounded with dietary or physiological signals. It is noteworthy that only small proportions of populations deviated from an amphidromous migratory pattern, and all populations examined in our study were predominantly amphidromous.

We found basin-specific patterns in water Ba but not Sr, validating the finding that Ba levels in otoliths were also associated with a fish's river basin. Low Ba in the freshwaters of Río Cañas produced false marine Ba signatures in the otoliths of Río Cañas fishes, and without an understanding Río Cañas water chemistry from spatially stratified water sampling, these patterns might have been erroneously interpreted as basin-specific migration patterns in diadromous fish assemblages. The large, basin-specific oscillations in otolith Ba:Ca that we observed have been noted by other investigators studying tropical diadromous fishes (Miles *et al*., 2009; Lord *et al.*, 2011). Previous researchers (Lord *et al.*, 2011), however, concluded that inconsistency in Ba:Ca within individual basins was evidence of migratory diversity within populations. In contrast, within species and among fish assemblages, we found high within-basin agreement in the magnitude of Ba:Ca ranges. Almost all fish captured in Río Cañas had low Ba:Ca ranges, and almost all fish captured in Río Sabana had high Ba:Ca ranges. The patterns in Ba:Ca we observed were clearly related to the basin in which a fish was captured and not necessarily longitudinal fish movements. Basin-specific patterns in otolith microchemistry are commonly used to identify natal source populations (Thorrold *et al.*, 1998; Thorrold *et al.*, 2001; Walther *et* *al.*, 2008) and are related to complex interactions of geological and environmental factors (Walther & Limburg, 2012). Puerto Rico is composed of many geological formations, including volcanic material in the central mountains and karst limestone near the coast (Kaye, 1957), each of which undergoes different weathering patterns and carries different trace element signatures. Further, seasonal weathering patterns on the hydric north side of the island differ from those of the xeric south side, which falls in the rain shadow of the Puerto Rico Cordillera Central (Hunter & Arbona, 1995). Thus, we conclude that Ba:Ca is not always a reliable indicator of freshwater-marine transitions in the otolith microchemistry of tropical insular fish assemblages. Validation by intensive spatial and temporal sampling of water chemistry, accounting for both seasonal and longitudinal variation in Ba would enhance interpretation of Ba:Ca microchemistry profiles in future research. An accurate model of water chemistry variation is central to the interpretation of otolith microchemistry (Rieman *et al.*, 1994; Campana, 1999; Elsdon *&* Gillanders 2006*;* Eldsdon *et al*., 2008).

Many *G. dormitor* adult microchemistry periods showed evidence of a return to a moderately higher salinity environment, but only 4% of *G. dormitor* adult periods contained values that might be interpreted as a return to a fully marine or estuarine environment. All *A. monticola* and gobiid adult periods were flat with no return to high Sr:Ca levels. Two general hypotheses may explain these patterns. (1) *G. dormitor* populations contain contingents that either remain in freshwater (dominant contingent) or periodically migrate to marine or estuarine habitats (marginal contingent). The function of such a migration may be related to feeding, reproduction, density-dependent mechanisms, or displacement from freshwater habitat by disturbance (e.g., drought or flood). *A. monticola, Sicydium* spp., and *A. banana* never experience higher salinity environments after recruitment to freshwater. Hypotheses 1 is consistent with a partially amphidromous adult life history for *G. dormitor* and a fully amphidromous adult life history for *A. monticola* and the gobiids. (2) No species in the fish assemblages we studied occupy marine or estuarine environments after recruitment into freshwater, but a contingent of *G. dormitor* populations feeds on a periodically available prey source that is enriched in marine elements, such as Sr. Hypotheses 2 is consistent with an amphidromous adult life history for all fish we sampled.

Several authors have concluded that *G. dormitor* and *A. monticola* are catadromous and make reproductive migrations to the mouths of rivers (Anderson, 1957; Phillip, 1993;

Winemiller & Ponwith, 1998), but very little direct information about individual fish movements has been published. In related research (Chapter 2), direct evidence from tagging fish in Río Mameyes indicated that *G. dormitor* and *A. monticola* were quite sedentary, and areas of highest fish density (aggregations) did not extend to estuarine waters. Even after a major flood disturbance during the spawning season, tagged fishes did not redistribute to lower reaches of the river (Chapter 1). This is in contrast to the finding presented here, that *G. dormitor* otoliths, including samples from Río Mameyes, contain marine or estuarine signals, indicating that a small proportion of populations may periodically occupy marine or estuarine habitats. However, otolith microchemistry reveals very little about the timing of these transitions, and it is likely that such a small proportion of migrants (4%) could be missed by monitoring tagged fish only during short, discrete periods (spawning seasons). The contradictory evidence between otolith microchemistry and tagging studies could be explained if transitions from fresh to estuarine waters occurred in a very small number of the tagged population, were episodic, or if a transition occurred when tagged fish were not monitored (e.g., during flooding events).

Sicydiine post-larval gobies are known to recruit to freshwater in large pulse migrations of several millions of individuals (Erdman, 1961; Castellanos-Galindo, 2011), and predatory fishes that aggregate with the post-larval migrations to feed attract local fishers in Puerto Rico (personal observation). *G. dormitor* is predatory, and the most common items in its diet are decapods and small fish (Winemiller & Ponwith, 1998; Bacheler *et al*., 2004). It is highly probable that *G. dormitor* opportunistically prey on recruiting diadromous post-larvae as they migrate up the river, transporting marine elements such as Sr. Fish otoliths incorporate diet information, in addition to a record of environmental and physiological conditions (Kennedy *et al.*, 2000; Buckel *et al.*, 2006; Walther & Thorrold, 2006; Sturrock *et al*., 2012); thus, *G. dormitor* could remain in freshwater and feed on a seasonally available prey enriched in marine elements, producing seasonal oscillations in otolith microchemistry that might otherwise be interpreted as a seasonal migration to a high salinity environment. Our finding of this phenomenon (i.e., marine signatures in adult *G. dormitor*) in a small proportion of the individuals examined, however, may indicate a size-selective or behavioral feeding mode that may limit the number of *G. dormitor* that utilize the seasonal food source (Bacheler *et al*., 2004), or it may further support an adult marine migratory hypothesis.

Based on direct evidence from tagging *G. dormitor* and *A. monticola* and the indirect otolith microchemistry patterns for the majority of the native stream fish assemblage, we conclude that native freshwater fishes in the Caribbean insular streams we examined are amphidromous, not catadromous, and adults generally do not return to natal habitats. No adult *A. monticola, Sicydium* spp., or *A. banana* otolith microchemistry periods showed marine or estuarine signatures, but a small fraction of *G. dormitor* adult periods did. The origins of the marine signatures in *G. dormitor* adult periods, whether migratory, dietary, or physiological, remains uncertain; however, if the marine signatures are the result of a migratory pattern, the effected proportion of adults is relatively small, and the modal migratory life history of *G. dormitor* is amphidromous.

Recent findings indicate that many diadromous populations are composed of a diversity of migratory contingents that conduct full, partial, or no migration between marine and fresh waters (Kerr *et al*., 2009). Migratory diversity within populations can confer resilience to frequent disturbance (Secor, 2007), such as the flood and drought regimes that commonly affect diadromous assemblages on tropical islands (Covich *et al*., 2006). Here, too, we documented plasticity in diadromous populations that follow multiple permutations of the typical migratory pattern. Larvae (except *A. monticola*) may be amphidromous or non-amphidromous, completing early life in either marine or freshwaters, and adult populations of *G. dormitor* may contain a small proportion of migratory, partially amphidromous adults. Future research on dispersal and migration in amphidromous fish assemblages focusing on the relationships between amphidromous and non-amphidromous recruitment and expatrial versus natal larval dispersal would elucidate the dynamics of the migratory plasticity that we revealed. River basin-specific factors, such as habitat quality and the degree of connectivity between fresh and marine waters, substantially influence larval dispersal patterns (Cook *et al*., 2009; Cooney & Kwak, 2013). Reduced streamflow from drought or water extraction might facilitate non-amphidromous recruitment (natal dispersal), and the seasonal formation of terminal estuaries may promote larval retention and the closure of populations. Metapopulation and source-sink dynamics are the likely primary forces structuring assemblages of native diadromous fishes on tropical islands (McRae, 2007; Ramírez *et al.*, 2012), and a greater understanding of the factors that determine metapopulation, source-sink, and migratory dynamics will inform enhanced conservation of tropical aquatic ecosystems and communities.

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Table 1. Number of each fish species sampled in Caribbean insular streams for otolith microchemistry according to physiographic region and river basin.

River basin	Coastal plain (0-20 m above sea level)	Foothills (21-70 m above sea level)	Mountains (>70 m above sea level)	Total sampled per basin
		Gobiomorus dormitor		
Cañas	10	2	10	22
Grande de Manatí	0	10	9	19
Mameyes	9	10	0	19
Sabana	10	11	0	21
		Agonostomus monticola		
Cañas	13	6	12	31
Grande de Manatí	0	8	10	18
Sabana	11	12	0	23
		Sicydium spp.		
Cañas	7	5	10	22
Grande de Manatí	0	8	15	23
Sabana	13	12	0	25
		Awaous banana		
Cañas	0	6	9	15
Grande de Manatí	0	0	16	16
Grande de Añasco	0	0	16	16

Table 2. Results of an analysis of variance of adult otolith Sr:Ca ranges, testing the association between otolith microchemistry and the basin in which fish were captured. Significant *P*-values are indicated in bold.

	P-value of the *F*-statistic	
Species	**Ba:Ca**	Sr:Ca
Gobiomorus dormitor	**<0.001**	0.35
Agonostomus monticola	**<0.001**	0.22
Sicydium spp.	**<0.001**	0.42
Awaous banana	**<0.001**	0.17

Table 3. Percentage of each species with otolith microchemistry (Sr:Ca) indicating amphidromous, non-amphidromous, or partially amphidromous recruitment and adult life histories. Amphidromous recruitment included a diadromous migration from marine or estuarine to freshwaters, but non-amphidromous recruitment did not include a marine larval phase (only a freshwater Sr:Ca signature). Amphidromous adults did not migrate to marine or estuarine waters after recruitment; partially amphidromous adults returned to marine or estuarine waters.

	Recruitment pattern	
Adult pattern	Non-amphidromy (%)	Amphidromy (%)
Partial amphidromy		
Gobiomorus dormitor	0	3.7
Agonostomus monticola	0	0
Sicydium spp.	0	0
Awaous banana	0	0
Amphidromy		
Gobiomorus dormitor	9.3	87.0
Agonostomus monticola	0	100.0
Sicydium spp.	12.1	87.9
Awaous banana	6.7	93.3

Table 4. Threshold marine or estuarine Sr:Ca signatures, estimated from the minimum otolith core values of fully amphidromous recruits. Greater values indicated that a fish experienced a higher salinity environment when the corresponding otolith section was formed.

Species	Threshold marine Sr:Ca value (mmol/mol)
Gobiomorus dormitor	3.0
Agonostomus monticola	3.3
Sicydium spp.	3.1
Awaous banana	2.8

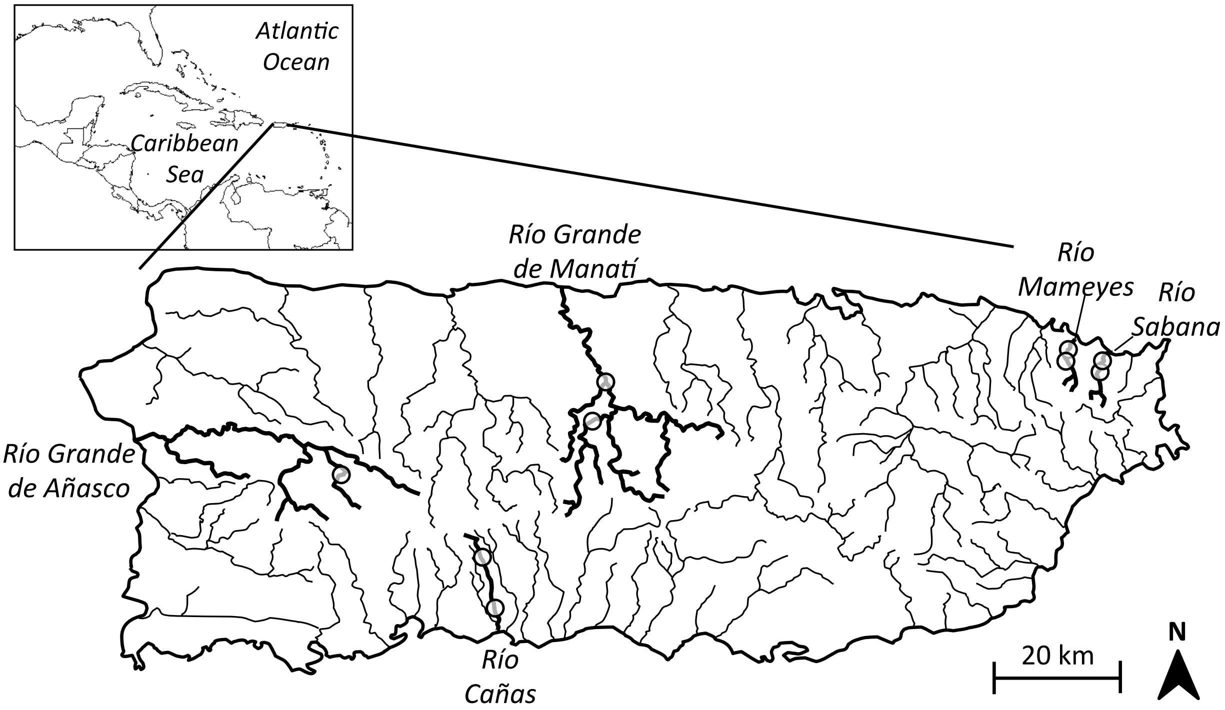

Figure 1. Map of Puerto Rico, U.S.A., showing river basins sampled for fish otolith microchemistry. Circles indicate exact locations of sampling.

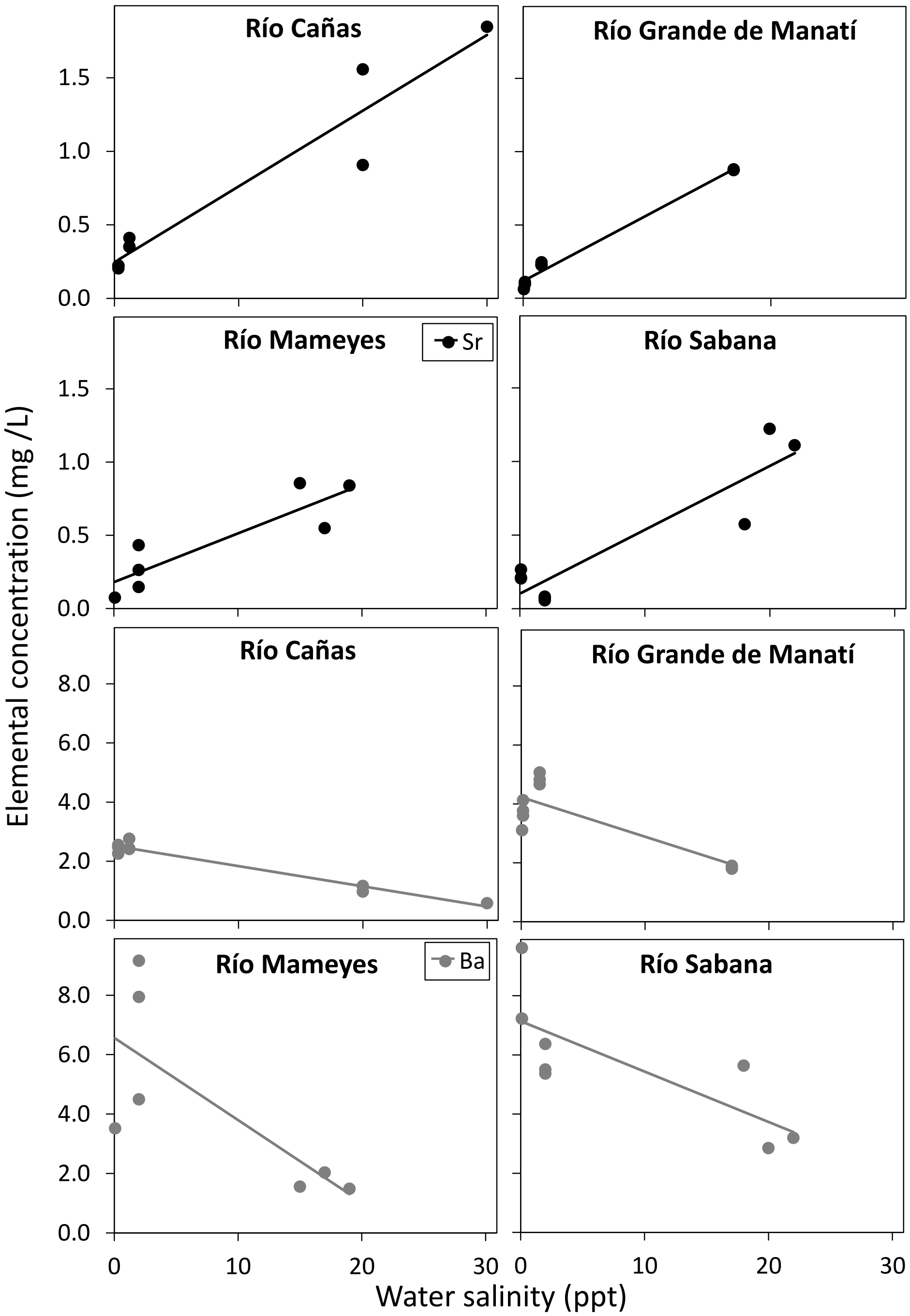

Figure 2. Water chemistry along a salinity gradient in four of the river basins sampled for fish otolith microchemistry.

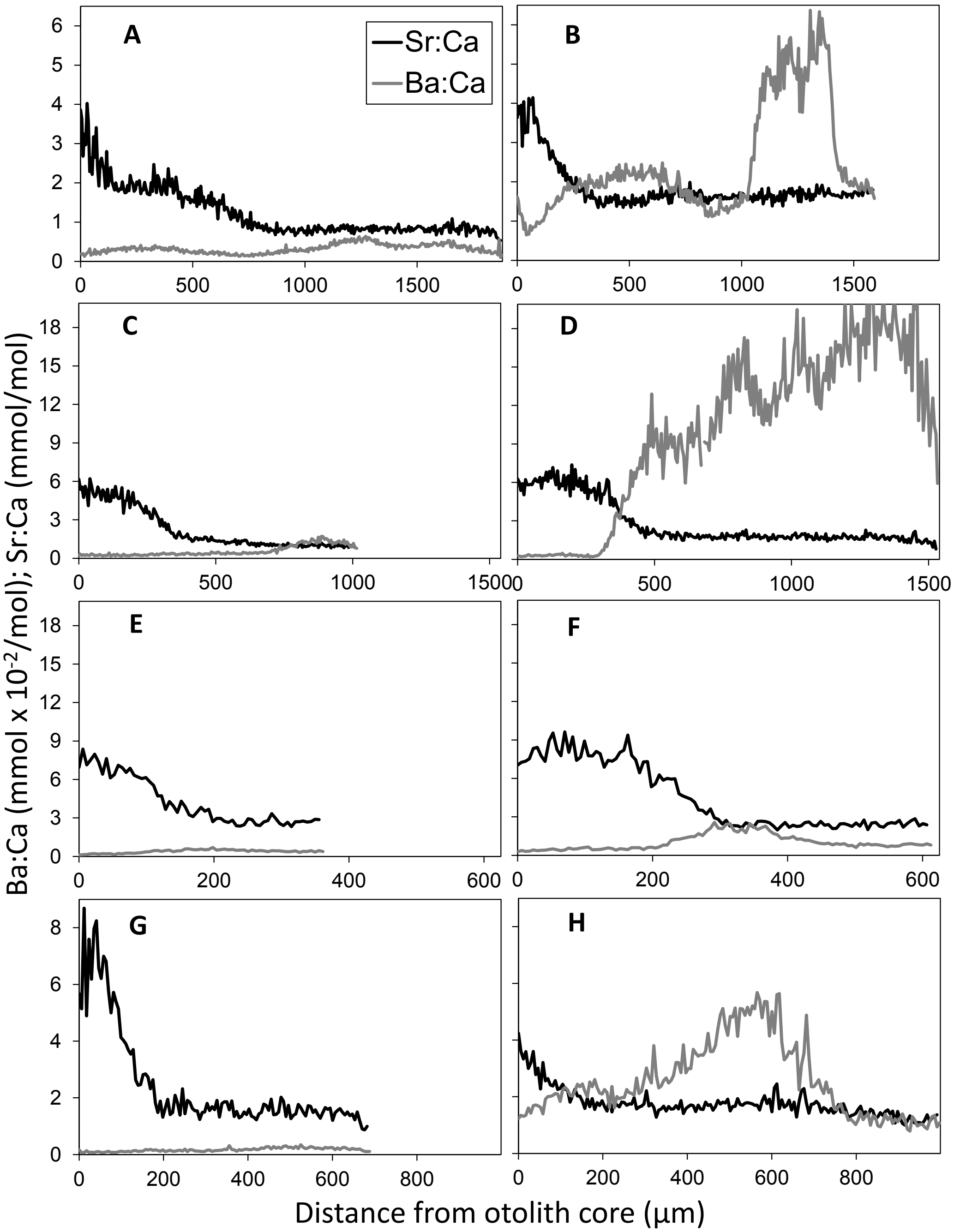

Figure 3. Characteristic otolith microchemistry profiles for bigmouth sleeper *Gobiomorus* dormitor (A-B), mountain mullet *Agonostomus monticola* (C-D), sirajo goby *Sicydium* spp. (E-F), and river goby *Awaous banana* (G-H), demonstrating discrepancies between Ba:Ca and Sr:Ca profiles. Panels on the left show instances of patterns in Sr:Ca that were not reflected in Ba:Ca, and panels on the right show instances of patterns in Ba:Ca that were not reflected in Sr:Ca.

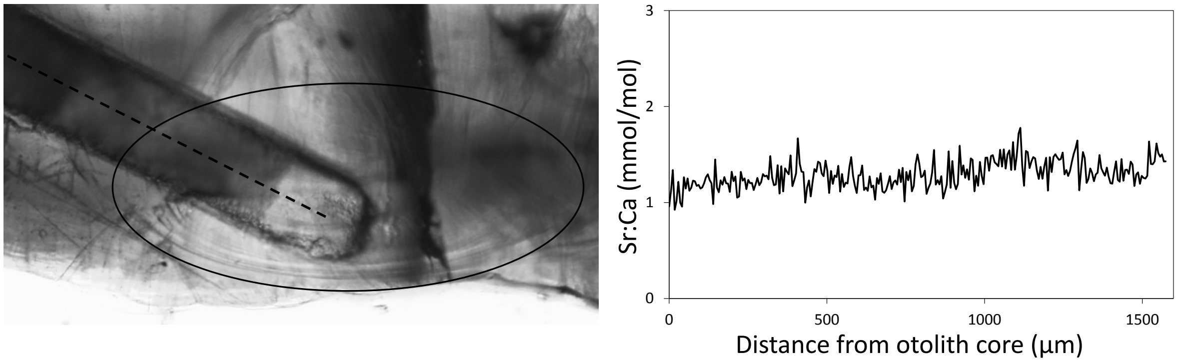

Figure 4. Example of a river goby *Awaous banana* otolith microchemistry profile with no marine or estuarine signature at the core (left margin of right panel), indicating that the life cycle was completed entirely in freshwater, and corresponding otolith image (left panel), showing the location of the otolith core that was sampled by laser ablation inductively coupled plasma mass spectrometer (LA-ICPMS). The oval indicates the area of the otolith core, and the dashed line indicates the path of the LA-ICPMS.

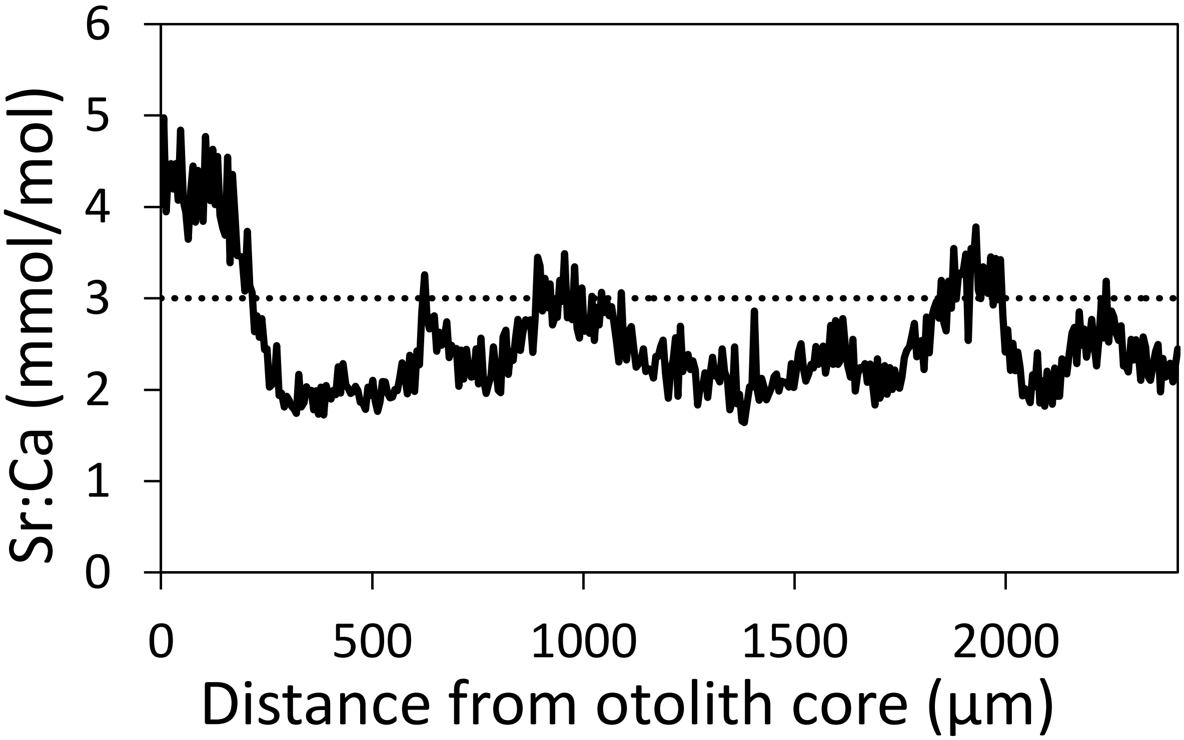

Figure 5. Bigmouth sleeper *Gobiomorus dormitor* otolith microchemistry profile indicating multiple returns to marine or estuarine water. The dotted reference line indicates the threshold marine or estuarine Sr:Ca value.

CHAPTER 8
LIFE HISTORY OF NATIVE CARIBBEAN STREAM FISHES: CONSERVATION AND MANAGEMENT IMPLICATIONS
(Job 4)

Abstract

Sound natural resource conservation and management rely on quantitative predictions of population response to exploitation and management, but predictive models are frequently limited by a lack of quantitative information on population dynamics. The management of data-limited species can be informed by a general understanding of life history patterns and dynamics and the suitability of common management strategies to particular life history traits. We quantified a suite of life history parameters for native Caribbean amphidromous fishes and compared those to life history parameters of other fish species to define the life history traits of the native fish assemblage. The amphidromous fishes examined follow an intermediate, periodic-opportunistic life history strategy. Deterministic and density-dependent management models are less likely to be effective for periodic and opportunistic populations, relative to models that account for environmental variability. We conclude that the amphidromous fish assemblages examined are robust to low to moderate exploitation of adults, and conservation measures, such as maintenance of stream habitat quality, environmental flows, and ecosystem connectivity may be the optimal approach to conserving native community structure and sustainable amphidromous fisheries.

Introduction

Native Caribbean freshwater fishes perform important ecological functions and are exploited in small-scale fisheries throughout their ranges (Bell 1999; Castellanos-Galindo et al. 2011; Inda-Diaz 2011). In many Latin American communities, freshwater fish are a primary source of protein (Cerdeira et al. 2000), and rapidly-growing human populations threaten these fish and fisheries with overexploitation and habitat degradation (Allen et al. 2005). Anthropogenic pressures can become especially intense on tropical islands (Neal et al. 2009), where many native freshwater fishes follow an amphidromous life cycle, migrating between marine and freshwater habitats as juveniles, complicating conservation and management strategies (Myers 1949; McDowall 1988). There is a clear need to ascertain and develop life history information about these fishes to successfully conserve and manage fish populations as the exploitation and habitat alteration by humans continue to expand.

Amphidromous fish populations in tropical regions throughout the world are exploited by humans at post-larval and adult stages. Certain species of amphidromous gobies recruit from marine or estuarine habitats to freshwater in pulse migrations of several millions of individuals (Erdman 1961), exposing recruits to a unique post-larval fishery (Manacop 1953; Bell 1999). Catch data indicate that a large fraction of the post-larval fishes harvested by this fishery are Sicydiine gobies, and other recruiting amphidromous species may also be captured (Castellanos-Galindo 2011). Recruiting post-larval fish are very small in size (approximately 20 mm SL; Bell 1994), but catches can be as large as 44 kg by a small group of fishers during a single trip (Castellanos-Galindo 2011). Post-larvae appear to be the primary target of fisheries for amphidromous fishes in lower river reaches near river mouths, and fisheries for amphidromous adult fishes are of greater importance inland, where larger species, such as bigmouth sleeper (*Gobiomorus dormitor*) and mountain mullet (*Agonostomus monitcola*), are harvested in recreational and subsistence fisheries (Cruz 1987; Corujo 1989; Inda-Diaz 2009).

Despite the value of amphidromous fishes in the tropics, little is known about their life history traits, such as spawning characteristics or demographic parameters that would inform the conservation and management of these species. The annual reproductive period has been documented in several specific locations for some individual species (Phillip 1993; Bell 1994; Bacheler et al. 2004), but no study has documented spawning patterns at the scale of management action (i.e., region- or island-wide) or for a full assemblage of native amphidromous fishes. Maturation, growth, and mortality are likewise poorly understood, but important demographics to guide fishery harvest. Quantification of spawning time and certain population rates allows fishery managers to define critical periods in a species' life history and adopt conservation measures that account for these sensitive periods (Poff et al. 1997; Winemiller 2005).

In this research, we identify critical gaps in the ecological understanding of Caribbean amphidromous fish assemblages, estimate critical population parameters, and identify management strategies that are applicable to these life history traits to improve the conservation and management of amphidromous fish resources. We develop a model of annual spawning chronology for amphidromous fishes in Puerto Rico, USA, to quantify periods when they are more sensitive to riverine connectivity to the estuary and ocean and quantify the life history parameters, maturation, growth, and survival, of two common Caribbean amphidromous fishes to explore their harvest potential and conservation.

Methods

Data collection consisted of two components, examination of fish reproductive tissues and a fish mark-recapture study. Data collected from reproductive tissues were used to characterize the annual timing of reproduction and size at maturation, and those from mark-recapture sampling were used to estimate growth rates. Reproductive tissues were sampled from stream fish assemblages in three broadly distributed basins on the Caribbean island, Puerto Rico, USA (Fig. 1). Samples were pooled among basins to make inferences at the metapopulation-island scale. Two river basins (Grande de Manatí and Sabana) were sampled on the north side of the island that flow into the Atlantic Ocean, and a third basin was sampled on the south side of the island (Río Cañas) that drains into the Caribbean Sea. These basins were selected to represent a broad geographic distribution across Puerto Rico and because they contained the full complement of native Puerto Rico stream fish species (Kwak et al., 2007). Beginning in September of 2008, fishes of each basin were sampled by pulsed-DC backpack electrofisher (Smith-Root Model LR-24) every two months until March 2009, and every month from May to August 2009. To obtain a representative sample, two sites per basin were sampled during each time period, one reach near the lowest elevation that the sampling gear was effective and one reach near the highest elevation that we expected to find the study species.

Four native Caribbean stream fishes, representing two thirds of the common native stream fish species, bigmouth sleeper, mountain mullet, sirajo goby (*Sicydium* spp.), and river goby (*Awaous banana*) were collected during each sampling period. All study species are amphidromous (McDowall 1988). A maximum of 15 specimens of each target species were collected per site during each sampling period. Total weight, total length (TL), sex, macroscopic gonad stage (Table 1), gonad weight, and eviscerated weight were measured for each specimen. Gonads from each individual were fixed in a 10% buffered formalin solution and preserved in 40% isopropyl alcohol. From each macroscopic stage of each species and sex, a random subsample was selected for histological validation (Tables 2 and 3). Paraffin-embedded sections of gonadal tissue were mounted on slides, stained with hematoxalin and eosin during histological preparation, and staged based on the most advanced gametes observed (Tables 4 and 5). Histological staging of gonadal tissues is considered the most accurate method to determine maturity, while macroscopic staging can be associated with considerable observation error (West 1990). We assumed there was no observation error in histological maturity classification and used histological subsampling to correct for macroscopic staging errors.

Gonadosomatic index (GSI) is a common metric in fish biology used to quantify annual spawning periods (Snyder 1983). GSI is the proportion of an individual's mass invested in reproductive tissue (gonads) and can indicate annual periods of high reproductive investment or spawning. GSI was calculated as gonad weight divided by eviscerated weight. Using eviscerated weight eliminated variability in total weight associated with gut fullness or recent feeding intensity. Mountain mullet were generally indistinguishable between sexes without histological examination of gonadal tissues. Mean male and female GSI were not significantly different for mountain mullet that were sexed histologically (t-test; $P = 0.2$); thus, male and female mountain mullet GSI data were combined. Sexes were modeled separately for all other species. The annual cycle of GSI was modeled with a periodic regression in which day of capture (day of the year, converted to radians $[2\pi\,(day)/365]$) was a predictor and GSI was a response, $GSI = b_0 + b_1[\sin(day)] + b_2[\cos(day)]$ (deBruyn and Meeuwig 2001), where b_0, b_1, and b_2 were regression coefficients. The day of peak GSI was estimated as $day_{\text{peak}} = \tan(b_1/b_2) + \pi$. Plots of observed GSI indicated the initiation and termination of the spawning season among species, and periodic regression predictions of GSI indicated the period of peak spawning.

Size-at-maturation ogives were fit to maturity data using hierarchical logistic regression models that separated the state process, maturation, from the observation process, macroscopic staging. Maturity status (1 or 0) and TL were the response and predictor in the model. Individual states of maturity, the first hierarchical level were considered random outcomes of a Bernoulli random variable with likelihood equal to the logistic model, $p(maturity_i) = 1/(1 + e^{-(a+b(TL))})$, where a and b were regression coefficients. Total length, where the predicted probability of maturation equaled 0.5, L_{50}, was estimated as $-a/b$. Observations of maturity status from macroscopic gonadal staging, the second hierarchical level, were also considered outcomes of a Bernoulli random variable, with separate likelihood,

$$p(observing\ maturity_i | stage_i) = \begin{cases} (1 - \lambda_{\text{stage}}) & \text{if } stage_i = 1\ (immature) \\ \lambda_{\text{stage}} & \text{if } stage_i = 2{:}\,6\ (mature) \end{cases},$$

where λ_{stage} is an estimate of the classification success for each sex and macroscopic gonadal stage (inverse of observation error). λ accounted for uncertainty in the model related to observational error and was estimated by comparing histological maturity classification to macroscopic maturity classification in a second, independent sample of gonads. λ was set equal to the number of each species, sex, and macroscopic stage with correct macroscopic maturity classification, divided by the total number examined histologically. As mountain mullet could not be reliably sexed, all mountain mullet used to estimate size at maturation were histologically staged, which reliably separated males and females. Thus, observational error was deemed minimal for mountain mullet data, and all λ were set to 1.0, eliminating the second hierarchical level of the size at maturation model. To minimize the bias related to gonadal abatement after spawning (Hunter and Macewicz 2003), only data collected during early through peak reproductive periods were used to estimate size at maturation.

Over a 2.5-year period beginning in March 2009, samples of bigmouth sleeper and mountain mullet populations were tagged and resampled in Río Mameyes, Puerto Rico, USA (Fig. 1) to estimate dispersal, survival, and growth rates. Fish were tagged with passive integrated transponders and detected or recaptured using a combination of passive integrated transponder arrays (Chapter 2) and randomized backpack electrofishing using the sampling gear described above. Of the four study species, only bigmouth sleeper and mountain mullet were large enough to hold a surgically implanted passive integrated transponder and thus provide information to estimate growth rates. Further details of site characteristics, sampling design, and survival estimation may be found in Chapter 6.

The physical recapture of previously tagged fishes during backpack electrofishing generated growth information (i.e., change in TL) over a known time duration. Von Bertalanffy growth model parameters, TL_∞ (asymptotic maximum total length) and k (growth coefficient), were estimated using the Fabens (1965) non-linear regression model for mark-recapture data. Although t_0, the hypothetical age when TL equals zero, cannot be estimated using the Fabens (1965) model, t_0 can be calculated using a known TL at an early age and the other parameters of the von Bertalanffy model, TL_∞ and k (Atason et al. 1999). We used the larval size at age estimated for bigmouth sleeper by Harris et al. (2011; 1.25 mm TL at hatch). No estimates of larval size at age have been published for mountain mullet, so we used the size at hatch for another mugilid species with similar egg diameter, assuming that similar egg diameters would produce similarly-sized larvae in two closely related species. Marin and Dodson (2000) estimated mean oocyte diameter for white mullet, *Mugil curema*, to be 426 μm, slightly larger than the mean oocycte diameter of mountain mullet, 362 μm (Eljaiek and Vesga 2011). Thus, we assumed that the larval size at age estimated for white mullet by Houde et al. (1976; 2.1 mm TL at hatch), was an adequate proxy for mountain mullet.

Bigmouth sleeper and mountain mullet are sexually dimorphic (Aiken 1998; Bacheler 2002). We addressed the potential for sexual differences in growth and maturation patterns by comparing combined and separate sex models using Akaike's Information Criterion (AIC, Akaike 1974; Burnham and Anderson 2002). We compared a model estimating separate TL_∞ for male bigmouth sleeper and female bigmouth sleeper to a model estimating a single TL_∞ for both sexes. We also compared separate and combined sex maturation models, with different logistic regression parameters. The smallest tagged mountain mullet (>130 mm TL) were either large males or small females. As males and females show clear differentiation in growth pattern and we had no non-lethal alternatives to determine the sex of tagged mountain mullet, we only used mark-recapture data from mountain mullet that were greater than the average TL of males sampled for maturation and spawning analyses plus one standard deviation. Thus, we restricted our analysis of mountain mullet growth to larger mountain mullet that were most likely female. The most parsimonious models of bigmouth sleeper and mountain mullet growth were graphically examined for model fit by plotting residuals versus time at large, change in TL versus initial TL, and change in TL versus time at large.

All models to estimate life history parameters were implemented in a Bayesian framework using the OpenBUGS software (Lunn et al. 2009). We assumed that the change in length data used in the non-linear regression growth model were normally distributed, with expected value equal to the Fabens (1965) model. All logistic regression and growth model parameters were given uninformative normal priors ($\mu = 0$, $\tau = 1 \times 10^{-6}$) ($\tau = 1/\sigma^2$), linear and nonlinear regression errors were given uninformative gamma priors ($\alpha = 0.001$, $\beta = 0.001$), and λ_{stage} in the size-at-maturation model was given an uninformative beta prior ($\alpha = 1$, $\beta = 1$).

Estimates of growth and natural mortality rates (M) from our research and fecundity estimates from previous studies were used to characterize the general life history traits and strategy (periodic, opportunistic, or equilibrium) (Winemiller and Rose 1992) of bigmouth sleeper and mountain mullet. Growth, asymptotic size, and natural mortality (estimated in Chapter 2) were compared to a list of estimates for 175 fish species compiled by Pauly (1980). All growth and asymptotic size parameters were natural log transformed, and natural mortality was converted to survival (e^{-M}) in order to facilitate graphical comparisons by using a linear scale. The rank of bigmouth sleeper and mountain mullet estimates among these comprehensive lists was applied to indicated harvest potential relative to other fish species with implications for potential management strategies.

Results

In general and among species, high GSI values were measured from late spring through fall (May–November; Fig. 2). Bigmouth sleeper GSI declined by September and was reduced to baseline values by November. Mountain mullet, female sirajo goby and female river goby GSI were elevated through November and were reduced by January; however, a small number of ripening female sirajo gobies and bigmouth sleeper were observed in early March, indicating that some minimal spawning may occur throughout the year. Periodic regression model predictions estimated peak GSI from late June through late August among the fishes we sampled. Inactive male goby reproductive structures were difficult to extract due to their extremely small mass. Samples representing the minima of the GSI distribution were generally absent in male river goby and sirajo goby, so we were unable to complete spawning chronology analyses for males of those species.

Sexual differences in bigmouth sleeper size at maturation were not significant. Model selection criteria indicated that the best model of bigmouth sleeper size at maturation did not separate sexes (Table 6); furthermore, the candidate model estimating separate sizes at maturation indicated that bigmouth sleeper TL_{50} was only 20 mm different between males and females. TL_{50} estimates indicated that bigmouth sleeper greater than 144 mm TL (95% CI: 137–154 mm TL) were likely to be mature (Fig. 3). Histological validation of the macroscopic gonadal staging process indicated variable classification errors between sexes and for most macroscopic stages (Table 3). Lack of fit (observed versus predicted values) of the bigmouth sleeper logistic regression model indicated that the hierarchical model accounted for gonad classification errors by adjusting the probability of maturation higher for resting macroscopic stages, where observation errors were most prevalent and most individuals were greater than TL_{50}, thus shifting the predicted size-at-maturation ogive.

Mountain mullet observed data fit closely to the logistic regression predictions, because all mountain mullet used in size-at-maturation analyses were staged histologically and did not require correction for observation errors. The TL_{50} estimated for female mountain mullet indicated that individuals greater than 113 mm TL (95% CI: 99–122 mm TL) were likely to be mature (Fig. 3). Inadequate data were collected to fit a logistic regression model of male mountain mullet, sirajo goby (male and female), and river goby (male and female) sizes at maturation, so we elected to note the maximum size of immature individuals and minimum size of mature individuals observed. This range of sizes at maturation served as an approximate characterization of maturation patterns for these groups (Table 7).

Data to fit growth model parameters were generated from the recapture of 79 previously PIT-tagged female bigmouth sleeper, 99 recaptures of male bigmouth sleeper, and 41 recaptures of mountain mullet greater than 160 mm TL. Model selection criteria indicated that the most parsimonious model of bigmouth sleeper growth estimated separate TL_{∞} for males and females (Table 6). Male TL_{∞} was greater than female TL_{∞} (Fig. 4), consistent with our observations that the largest bigmouth sleeper encountered during this study were males. Compared to female bigmouth sleeper growth model predictions, female mountain mullet reached similar asymptotic sizes but grew at a faster rate during early life. Diagnostic plots showed no consistent patterns, indicating that growth rate estimates were relatively unbiased (Fig. 5).

Graphical comparisons with life history parameters compiled by Pauly (1980), indicated that bigmouth sleeper and mountain mullet grew slowly compared to similarly sized fishes and fishes with similar survival rates (Fig. 6). Bigmouth sleeper and mountain mullet survival rates fell below the median value for other fishes, indicating low annual survival relative to other fish species.

Discussion

Our extensive investigation of the reproductive traits of amphidromous fishes from throughout Puerto Rico and an intensive study of the growth of amphidromous fishes in a single river generated data to quantitatively estimate the annual spawning chronology, size at maturation, and growth rates of two native Caribbean amphidromous fishes. Spawning chronology indicated that these native fishes spawned seasonally, over a protracted period from late spring through late fall. Bigmouth sleeper reproductive activity declined earlier in the year compared to other species. Based on the maturation and growth models presented here, most bigmouth sleeper and mountain mullet mature moderately early, by ages of 2 or 3 years, but another amphidromous species, sirajo goby, appeared capable of rapid maturation at small sizes. Growth rates of bigmouth sleeper and mountain mullet were slower than average compared to similarly-sized fishes.

The annual chronology of gonadal development for Puerto Rico amphidromous fishes quantified in our findings shows that spawning peaks from June through August and may continue through December. Assuming that the upstream migration of amphidromous recruits occurs two to three months subsequent to spawning (Keith and Lord 2011), our results indicate August through November as the peak period of amphidromous recruitment to freshwater in Puerto Rico. Collectively, this reproductive chronology suggests that June through November is an annual period when riverine connectivity between fresh and marine waters is critical for the persistence of the native stream fish assemblages of Puerto Rico. The flow regime, patterns in stream discharge volume and fluctuations, during this critical period will influence passive transport of recently hatched larvae to estuarine and marine waters and facilitate upstream migration of post-larval recruits to the river system. Therefore, flow regulation in dammed streams and rivers to meet these life history needs of tropical amphidromous fishes is most critical during the June–November fish reproductive and rearing period, and this information can guide river managers in developing effective environmental flow regimes.

Estimates of growth rates and size at maturation from this study and survival rates from related research (Chapter 2) indicate that the amphidromous fishes, bigmouth sleeper and mountain mullet, follow an intermediate periodic-opportunistic life history (Fig. 6). Their annual survival and growth rates are relatively low, and they are capable of early maturation. Most bigmouth sleeper and mountain mullet mature during their second or third year of life; however, mature female bigmouth sleeper were observed at sizes as small as 90 mm TL (age 1, based on growth model), as well as extremely small mature sirajo gobies (36–40 mm TL). This pattern is consistent with a life history that optimizes reproductive output under high mortality risk and stochastic resource and environmental dynamics, characterized as an opportunistic life history strategy (Fig. 6) (Winemiller and Rose 1992). Estimates of fecundity by other investigators (Aiken 1998; Bacheler 2002) indicate that bigmouth sleeper and mountain mullet have moderately high fecundity, a characteristic of the periodic life history strategy. We propose that amphidromous fishes maximize fecundity and mature early by limiting per capita investment in offspring (e.g., no parental care) and producing very small eggs (McDowall 2009). Amphidromous fishes inhabit an environment that may be considered intermediate between the patchy, stochastic environment associated with opportunistic life histories and the broadly cyclic environments associated with periodic life histories. Caribbean streams are chronically disturbed by floods of lesser intensity acting at small, watershed scales (Ramírez et al., 2009; Pike et al., 2010) and periodically disturbed by major flood events from tropical cyclonic activity acting at large, regional scales (Chapter 1).

The estimates of growth rate presented here represent the first published for bigmouth sleeper or mountain mullet. Growth model parameters did not appear to be biased for the adult fishes that were tagged, as residual plots showed only random scatter; however, the growth models should be cautiously interpreted for younger ages and smaller sizes, because only larger adult fish were included in the analysis. In general, fish growth patterns are not consistent between larval and juvenile stages (Fuiman 1983). This bias can be avoided by anchoring the growth model with a known size at age after the larval period; however, size-at-age information for *G. dormitor* and *A. monticola* were only available for recently hatched larvae.

Our size at maturation estimates for bigmouth sleeper and mountain mullet were lower than those of previous studies (Bacheler 2002; Eljaiek and Vesga 2011); however, previous investigators did not account for observation error using a robust statistical approach. The hierarchical logistic regression model of maturation we applied incorporated independent estimates of macroscopic staging error, generating robust estimates of the probability of maturation. Classification error rates indicated that macroscopic staging of resting females, which tended to be larger, was prone to error. Similar errors may have resulted in an overestimation of size at maturation in other studies, as larger resting females were more likely to be incorrectly classified as immature, positively biasing estimates of size at maturation. Environmental or genetic interpopulational variation may also have contributed to the differences in life history parameter estimates among studies. The bigmouth sleeper population studied by Bacheler (2002) was a landlocked population that follows a life history pattern that is an exception to the typical amphidromy found in the species. The South American mountain mullet populations studied by Eljaiek and Vesga (2011) are located at a more tropical latitude with different environmental conditions than the insular Caribbean populations we studied. Thus, computational, geographic, or life history variation may account for differences in life history parameter estimates among populations of the same fish species.

Life history implications for conservation and management

Fish life histories can be described in terms of the stochasticity and severity of environmental conditions and an associated optimization of survival rates, maturation patterns, and fecundity (Fig. 6). Furthermore, the characterization of life history traits leads to predictions of demographic resilience that are highly relevant to resource management and conservation (Winemiller 2005). The life history parameters quantified here indicate that amphidromous fishes in tropical stream assemblages follow a periodic-opportunistic strategy, with relatively low annual survival rates, rapid maturation, and high fecundity in a stochastic environment (Chapter 1). Predictions of periodic and opportunistic population dynamics suggest that abundance will vary stochastically at short time scales, but over longer time scales, periods of higher abundance and strong recruitment will cycle with periods of favorable environmental conditions. Populations will most commonly fall well below carrying capacity and be structured by abiotic events, but occasionally, density-dependent effects may influence assemblages during periods of high abundance

Winemiller (2005) identified several potential pitfalls in resource management associated with periodic and opportunistic life history traits. Populations following an opportunistic life history often have poor stock-recruitment relationships and relaxed density dependence. The environment regulates recruitment patterns, rather than the number of spawning adults in opportunistic and periodic populations; therefore, deterministic population projection models will be less effective than models that account for environmental variation. One common aquatic conservation approach, instream flow assessment, may be particularly applicable for managing amphidromous species, as it explicitly accounts for environmental variation (Annear et al. 2004). Yet biological deterministic components of instream flow assessment, such as habitat suitability criteria, may not be reliable indices for the management of opportunistic species, because stochastic disturbances frequently restructure habitat and populations (Chapter 1; Poff and Ward 1989; Winemiller and Rose 1992; Winemiller 2005). Opportunistic and periodic populations are particularly sensitive to juvenile survival, but can be sensitive to adult survival at extremely low spawner biomass. Amphidromous stream fish assemblages may become sensitive to adult biomass when fish stocks become severely depleted, and based on the moderate growth rates estimated here, a delayed age at entry into the fishery (i.e., a size limit) could be effective for maximizing fish abundance and fishery yield. However, resource managers should generally expect resilience to harvest and sustainable fishing under moderate to low fishing pressure. In the context of aquatic natural resource conservation and management in Puerto Rico and other tropical systems, these findings in aggregate indicate that an environmental flow regime, providing habitat connectivity, flows that enhance reproductive and migratory functions, and community filtering through periodic flood disturbance (Chapter 1), is among the most potentially effective management tools for maintaining native aquatic ecosystems in these areas.

Although the harvest of adults is unlikely to result in deleterious effects on populations of the amphidromous fishes examined here, some species are also harvested as post-larvae before recruitment to freshwater has been completed (Erdman 1961; Bell 1999; Castellanos-Galindo et al. 2011). The harvest and natural mortality rates of young amphidromous fishes remains uncertain, but large catches in terms of biomass and small sizes of individual fish in the catch suggest that post-larval fishing mortality may be substantial (Castellanos-Galindo et al. 2011), but at what level it may become detrimental is unknown. Furthermore, the fraction of recruitment lost to expatrial dispersal to other rivers or islands and conversely, the fraction of recruitment immigrating to local rivers from other areas is uncertain for all amphidromous species. As periodic and opportunistic populations are sensitive to juvenile mortality, a more detailed understanding of the early life history and metapopulation dynamics of amphidromous fishes is required to fully assess the vulnerability of these fishes to overharvest at various life stages.

The information gained by our study may contribute to development of a comprehensive conservation and management strategy for the amphidromous fish assemblages found in many tropical streams and rivers throughout the world. Annual spawning chronologies define periods when instream flow regulation will be most effective for the enhancement of amphidromous recruitment, and the quantification of life history parameters and traits indicates the potential for successful conservation under various resource management strategies. The fish populations with life history traits explored in our study can become sensitive to adult mortality when spawner biomass is low and juvenile mortality persists over large temporal and spatial scales (Winemiller 2005). However, the nature of life history and exploitation patterns of amphidromous fishes suggests that the maintenance of stream habitat quality, environmental flows, and ecosystem connectivity may be the optimal approach to conserving native community structure and sustainable amphidromous fisheries.

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Table 1 Macroscopic gonad classification criteria used for all species in this study (modified from Hjort 1910 and Pollard 1972). Stages III-VI were considered mature. Stage I-III mountain mullet males and females were not distinguishable, as males begin to show milky coloration very late in gonad maturation, and took a more turgid shape similar to an ovary.

Macroscopic stage	Description of gonads
I. – Immature	Gonads are very small and thin; transparent or little color. Sexes indistinguishable.
II. – Developing	Still small, but ovaries beginning to show yellow-orange color and testes beginning to show milky color. Ovaries turgid; testes strap-like.
III. – Resting	Gonads larger and colored but show no sign of impending reproduction.
IV. – Fully developed	Gonads very large. Ovaries yellow-orange in color; often highly vascularized with oocytes visible. Testes milky-white and vascularized.
V. – Running ripe	Ovaries are large and soft with many large, free-flowing (with slight pressure) hydrated oocytes. Milt flows freely from testes.
VI. – Spent	Gonads are large and highly vascularized but have a flaccid appearance. Ovaries are pale yellow to red, and testes are grey.

Table 2 Numbers of macroscopically staged gonads for each species.

Macroscopic stage	Bigmouth sleeper		Mountain mullet		Sirajo goby		River goby	
	Female	Male	Female	Male	Female	Male	Female	Male
Immature	42	45	46	23	25	19	3	14
Developing	30	37	113	9	37	50	14	9
Fully developed	97	80	5	0	139	27	29	12
Resting	16	23	37	5	12	36	18	12
Spent	0	0	0	1	0	0	0	0
Total	185	185	201	38	213	132	47	64

Table 3 Numbers of histologically staged bigmouth sleeper gonads and classification success rate of each macroscopic stage (λ, in parentheses).

Macroscopic stage	Female	Male
Immature	18 (0.61)	14 (0.71)
Developing	21 (0.71)	19 (0.58)
Fully developed	4 (1.0)	5 (1.0)
Resting	4 (0.5)	4 (0.5)
Spent	0	0
Total	43	47

Table 4 Microscopic ovarian stage criteria used for all fish species in this study (based on West 1990). Stages III-VII were considered mature (Murua and Saborido-Rey 2003).

Histological stage	Description of ovaries
I. – Early perinucleolar	One or few large nucleoli, basophilic ooplasm, no cytoplasmic inclusions
II. – Late perinucleolar	Less basophilic ooplasm, a cortical alveolus and/or a lipid droplet
III. – Cortical alveolar	Many small lipid droplets and cortical alveoli dispersed in cytoplasm
IV. – Late lipidogenic	Lipid droplets fill ooplasm and coalesce centrally, cortical alveoli at follicular envelope
V. – Vitellogenic	Yolk protein globules present
VI. – Final maturation	Germinal vesicle breakdown, lipid and yolk coalesced, may be hydrating
VII. – Postspawning	Postovulatory follicles, atretic oocytes

Table 5 Microscopic testicular stage criteria used for all fish species in this study (based on Pollard 1972). Stages III-IV were considered mature.

Histological stage	Description of testes
I. – Primary germ cell	Triangular lobules filled with dense connective tissue. Primary germ cells contain nuclei with peripheral chromatin and a single central nucleolus.
II. – Spermatagonial formation	Elongated lumina are lined by a single layer of spermatogonia.
III. – Spermatagonial proliferation and spermiogenesis	Cysts of dividing spermatogonia. Spermatozoa may be present and proliferating in most advanced testes.
IV. – Residual sperm	Lobule walls are loosely contracted and primary germ cells are present along with residual sperm.

Table 6 Akaike information criterion (AIC) model selection results indicating the most parsimonious models (lowest AIC) of bigmouth sleeper growth and maturation in bold.

	Maturation model			Growth model		
Model	k	AIC	AIC weight	k	AIC	AIC weight
Combined sexes	**10**	**894**	**0.88**	3	1541	0
Separate sexes	12	898	0.12	**4**	**1525**	**1.0**

Table 7 Observed size ranges of maturation and logistic regression prediction of length at 50% maturation (TL_{50}; 95% credible interval in parentheses) for Puerto Rico native stream fishes.

	Smallest mature (mm TL)	Largest immature (mm TL)	TL_{50}
Bigmouth sleeper male	115	157	144 (137–154)
Bigmouth sleeper female	90	167	144 (137–154)
Mountain mullet male	96	—	—
Mountain mullet female	98	168	113 (99–122)
Sirajo goby male	40	99	—
Sirajo goby female	36	92	—
River goby male	74	—	—
River goby female	73	104	—

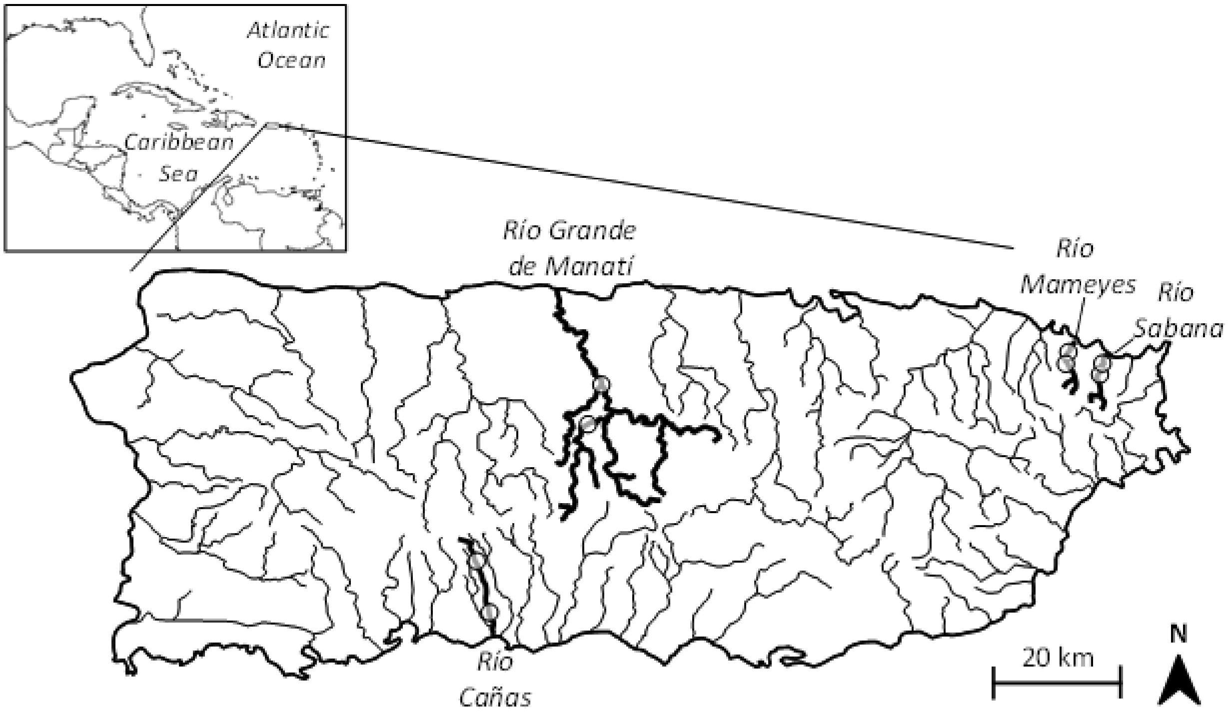

Figure 1. Map of Puerto Rico showing sampling locations in Ríos Cañas, Grande de Manatí, Mameyes, and Sabana. Ríos Cañas, Grande de Manatí, and Sabana were sampled for reproductive analyses and fish from Río Mameyes were tagged for growth analyses.

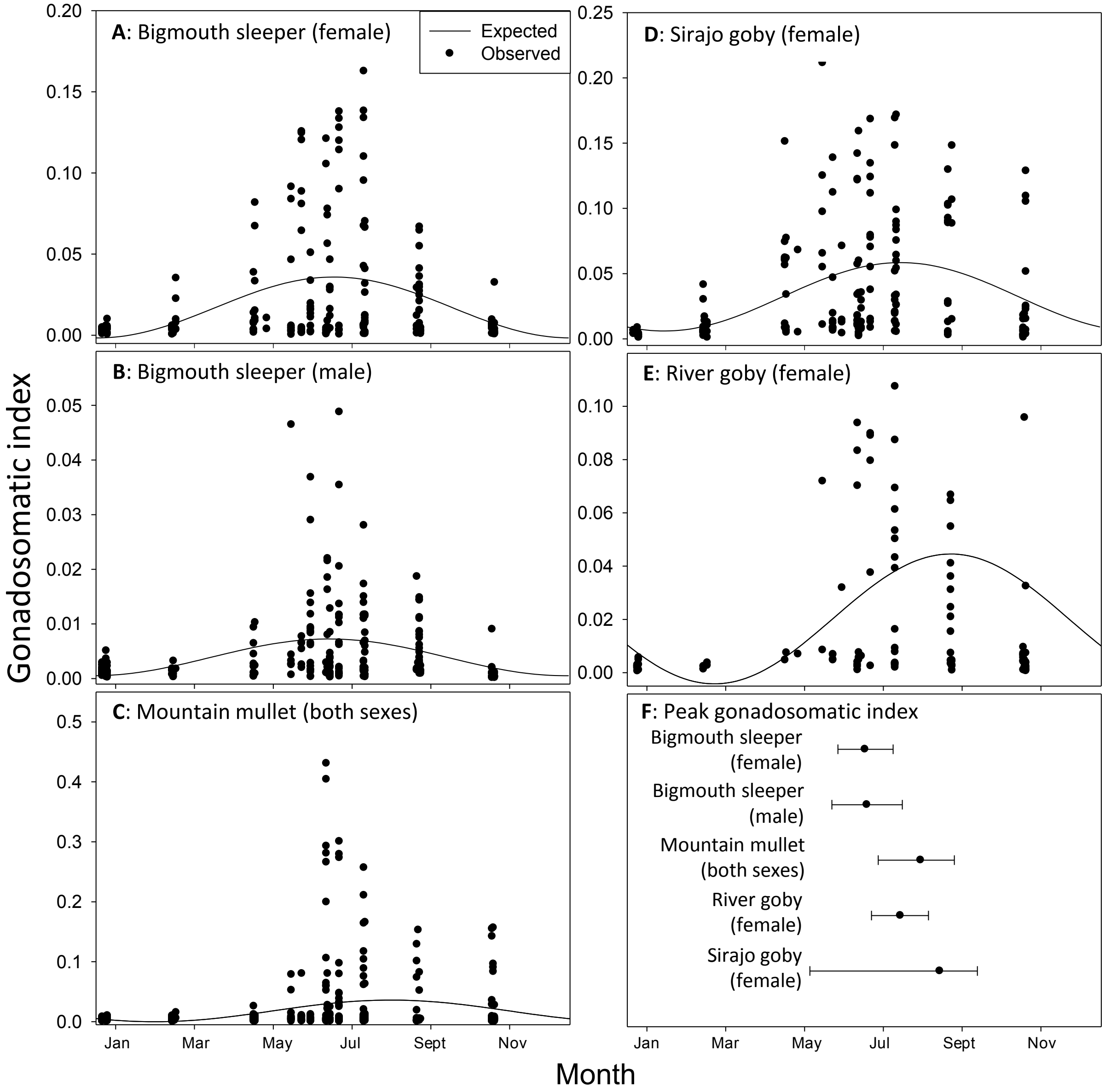

Figure 2. Annual time series of gonadosomatic index (GSI) and periodic regression predictions for four common native Caribbean stream fishes collected from July 2008 through August 2009 (A–E). Periodic regression estimates and 95% credible intervals (brackets) for the time of peak GSI (F).

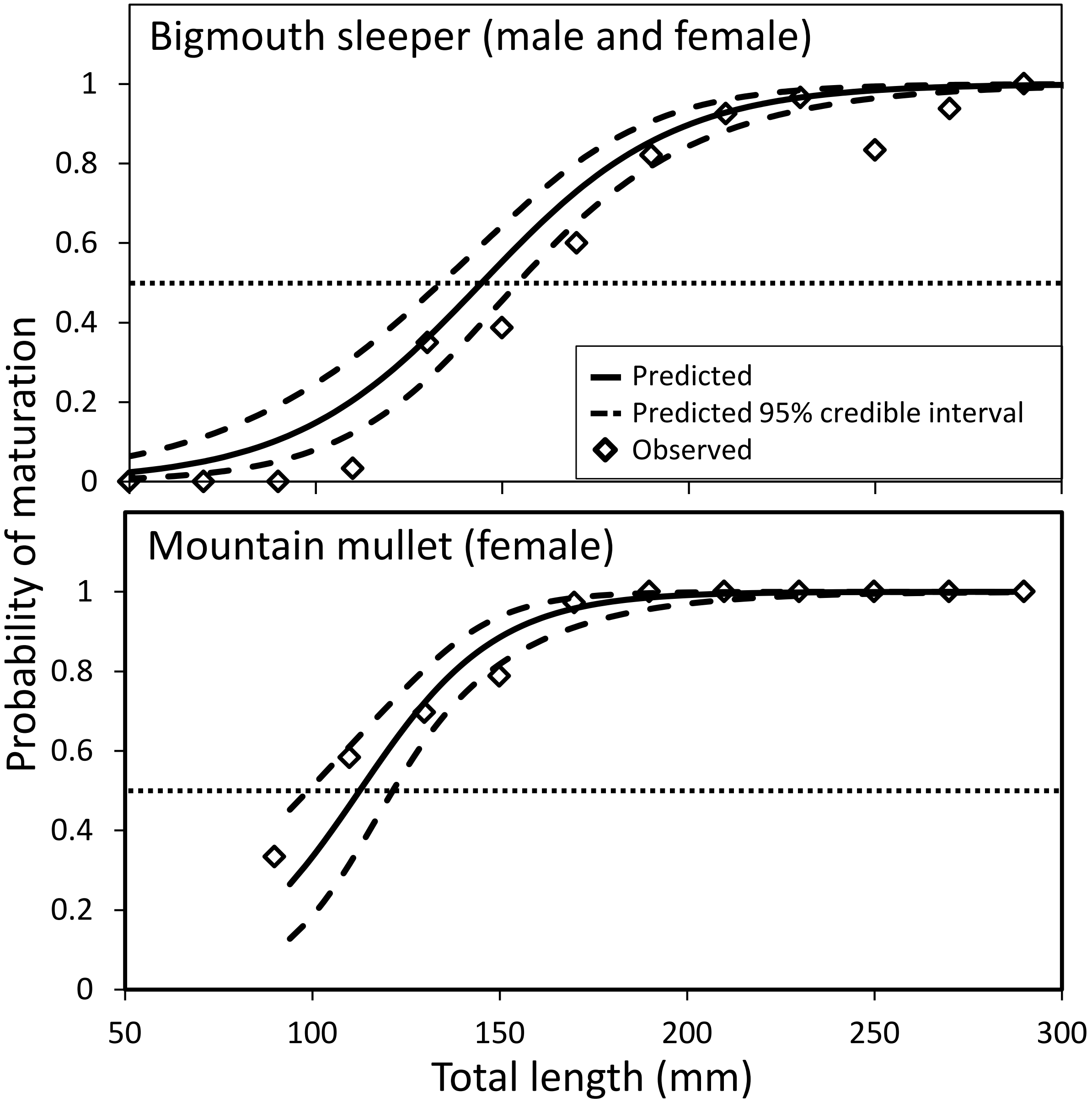

Figure 3. Predicted probability of maturation and observed proportion mature for bigmouth sleeper and mountain mullet. Solid lines indicate predicted probability of maturation, and dashed lines indicate 95% credible intervals for the prediction. Dotted horizontal lines indicate the 50% predicted probability of maturation (*TL*$_{50}$). Median *TL*$_{50}$ predictions were 144 mm TL for bigmouth sleeper and 113 mm TL for mountain mullet.

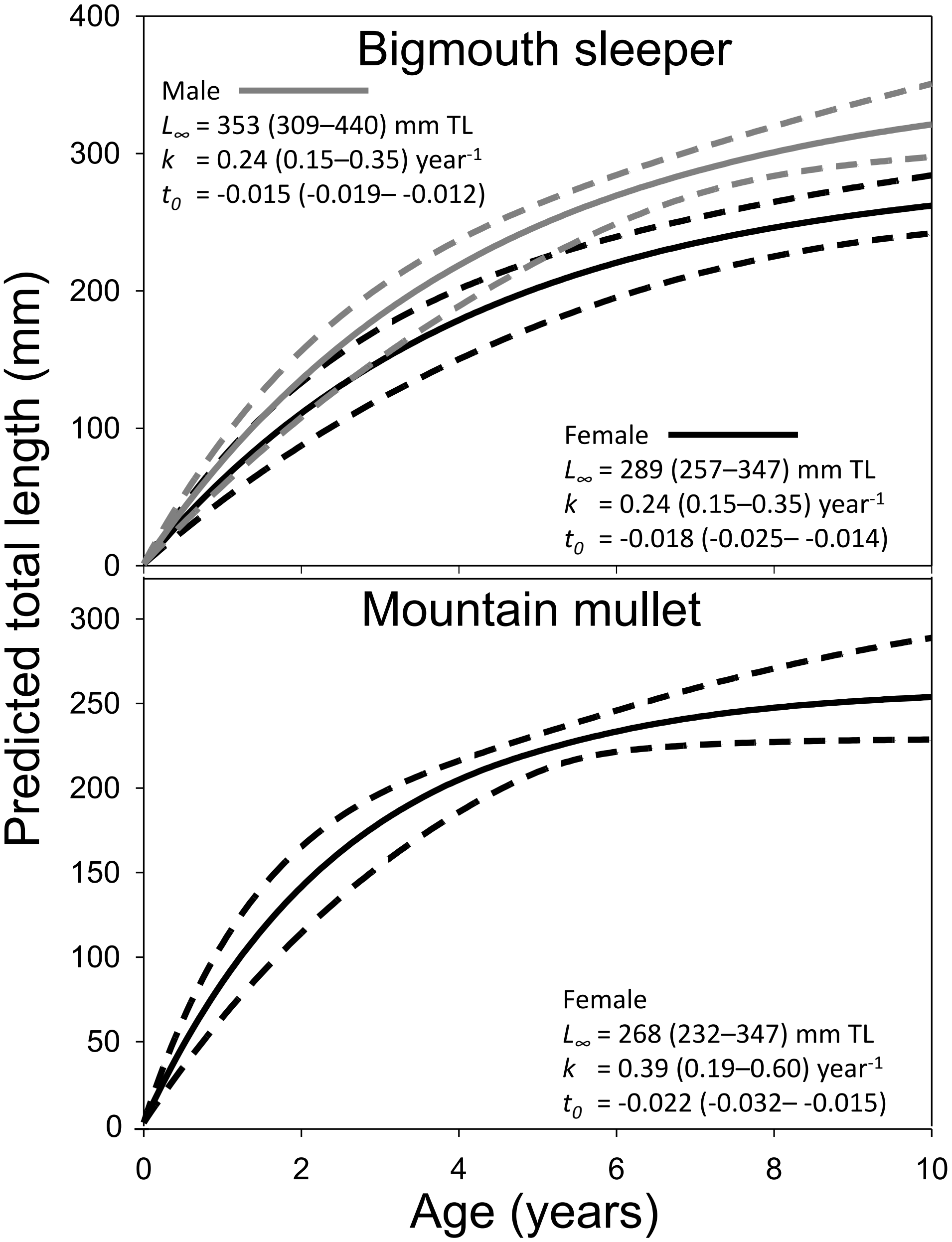

Figure 4. Predicted size at age (solid line) and 95% credible intervals (dashed lines) for bigmouth sleeper and mountain mullet estimated by a von Bertalanffy growth model, including growth model parameters and 95% credible intervals.

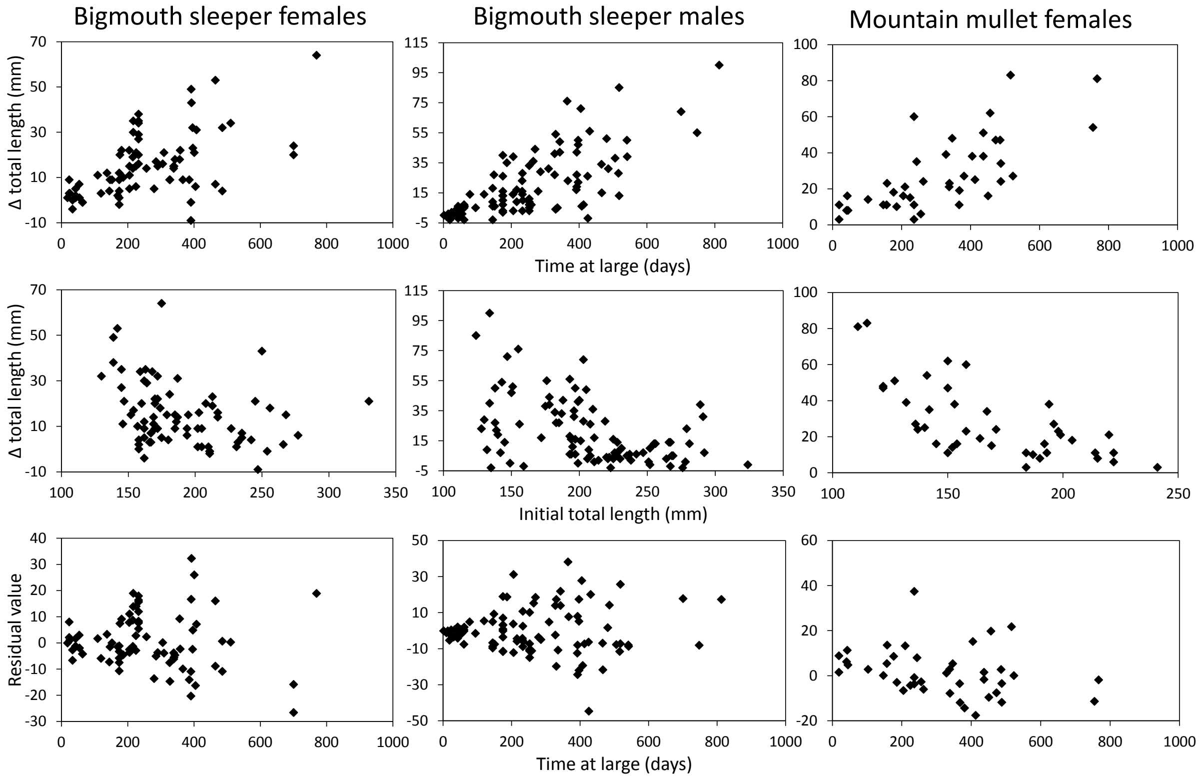

Figure 5. Diagnostic plots for all growth models.

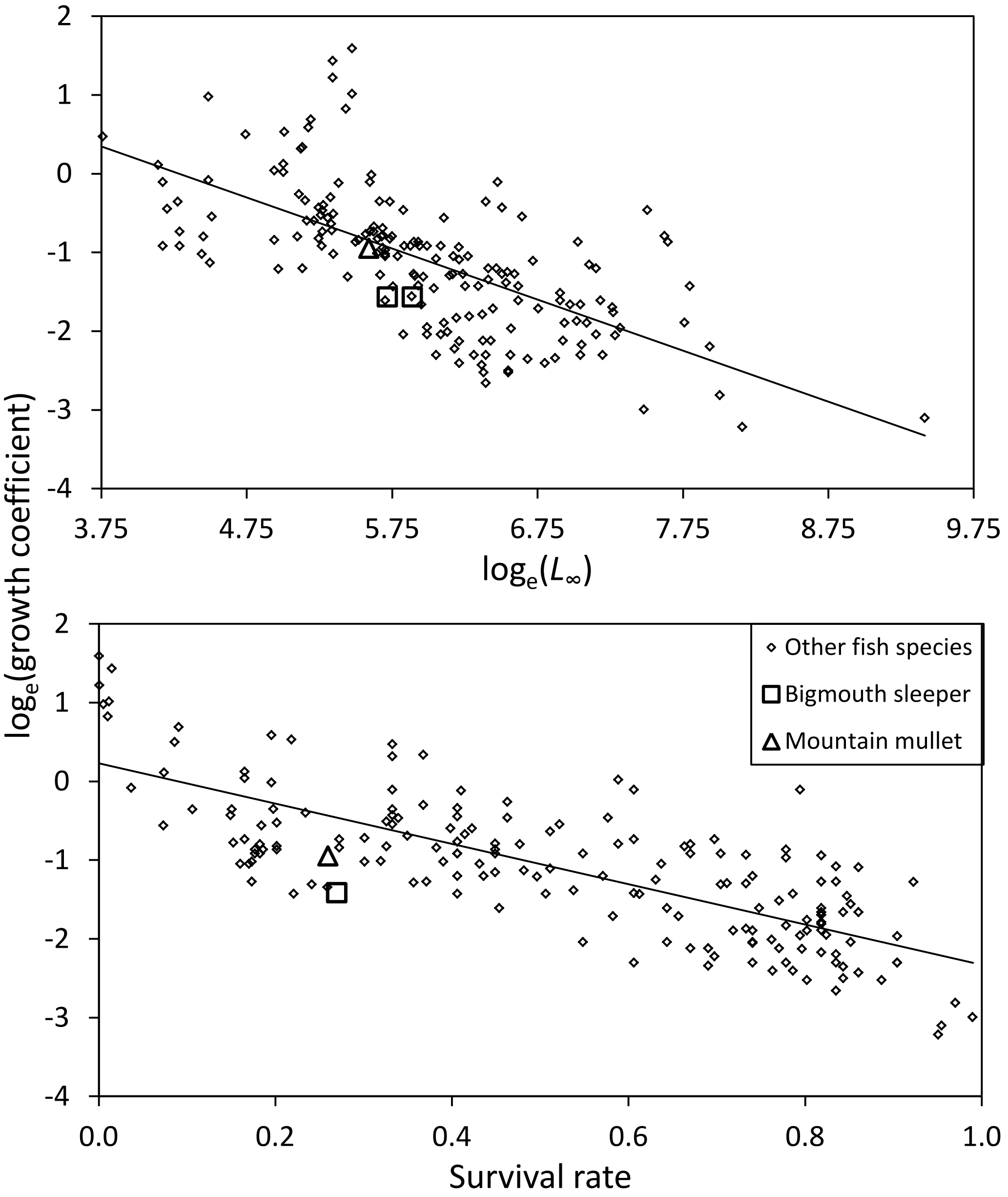

Figure 6. Growth rate, mortality rate, and asymptotic maximum size for bigmouth sleeper and mountain mullet compared to those for 175 fish species listed in Pauly (1980). Lines indicate mean fish growth rates at a given asymptotic total length (TL_∞) and survival rate.

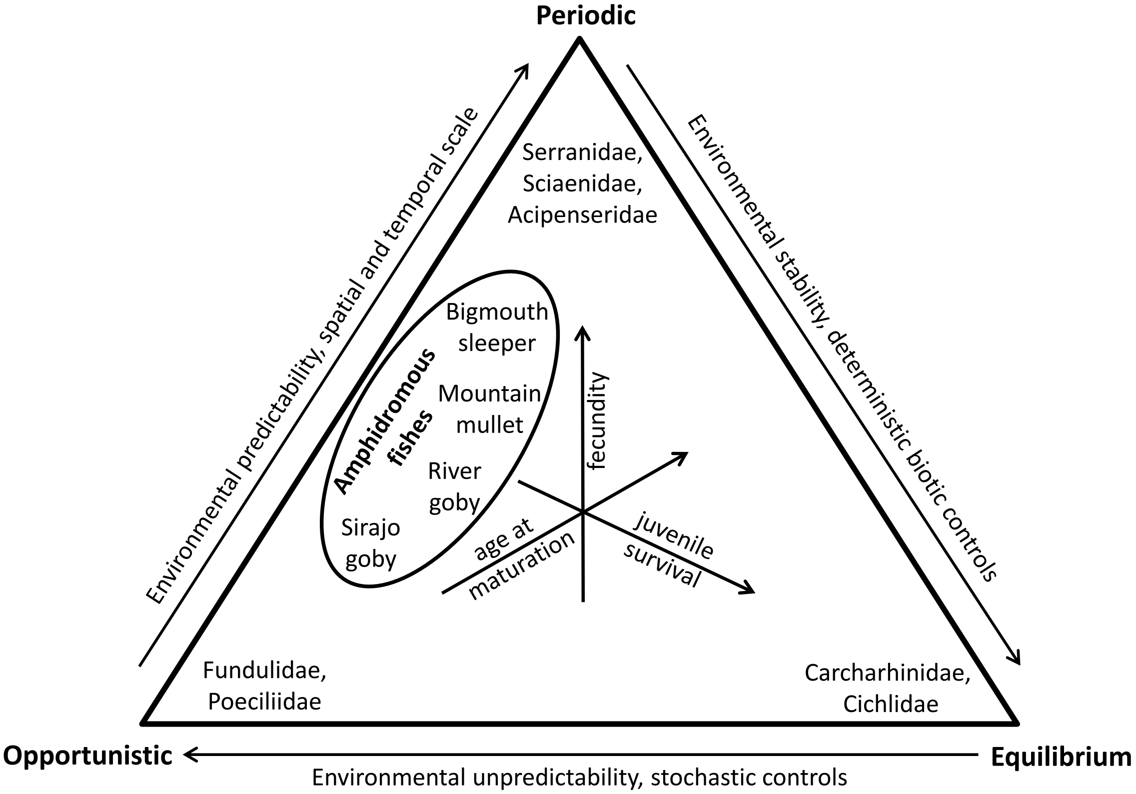

Figure 6. Conceptual life history model (after Winemiller 1995) illustrating environmental gradients, associated life history endpoints, and example fish taxa, including the amphidromous species examined in our study. Equilibrium populations are structured by deterministic biotic interactions, whereas opportunistic populations are structured by stochastic abiotic factors acting at local scales. Periodic populations inhabit cyclically variable environments that vary over very large temporal and spatial scales.