ABSTRACT

BROWN, CHRISTIN HAMBRICK. Sampling Bias, Selectivity, and Environmental Influences of Puerto Rico Stream Fishes. (Under the direction of Dr. Thomas J. Kwak.)

Puerto Rico, an island in the Caribbean Sea, is known for its marine sport and commercial fisheries, but the freshwater habitats of the island also support a substantial number of fishes, which provide recreational and subsistence fishery values. There are about 80 fish species that inhabit Puerto Rico freshwaters. Of those, there are fewer than 10 native fish species that reside within the rivers, and they are of primary management concern. Management of these stream fish resources would be enhanced by an understanding of gear catchability, a standardized sampling method, and accurate population estimates. My primary objectives for this study were to (1) quantitatively describe gear efficiency and selectivity relationships to estimate stream fish populations in Puerto Rico; (2) evaluate population models among species using electrofishing catch results analyzed with mark-recapture and removal methods to identify the most suitable parameter-estimating model; (3) use these findings to develop a standardized stream fish sampling protocol to be applied island-wide; and (4) develop empirical, hierarchical models that describe relationships between fish catchability and instream habitat and water quality parameters for each native fish species.

In my first research component, I compared two fish sampling gear types (electrofishing and seining) and four models for estimating fish population parameters (Petersen mark-recapture and removal estimators of 2–4 sampling passes) to provide the quantitative basis for development of a standardized sampling protocol for Puerto Rico stream fish. I found electrofishing more efficient and logistically feasible than seining for collecting fish in these environments. I determined that three- and four-pass removal models were more accurate than the Petersen mark-recapture model or a two-pass removal model, and that accuracy was similar between three- and four-pass removal models. I investigated variations of models that account for assumption violations and found model M_b, that adjusts for fish behavioral effects, to provide the overall best and most parsimonious fit for estimating population parameters.

Based on these findings, I propose a standard fish sampling protocol for Puerto Rico wadeable streams that includes sampling stream reaches from 100 m to 200 m long, using the appropriate electrofishing gear (backpack or barge electrofishers) and conducting three sampling passes of equal effort. A Zippin-type, maximum-likelihood estimator will then be used to calculate estimates of fish population densities.

I sampled fish in 81 wadeable stream reaches island-wide, totaling 105 sampling occasions, using the standardized sampling protocol with backpack or barge electrofishers. I estimated fish catchability using the standard maximum-likelihood removal estimator for 2-5 pass removals. At each sampling location, I measured seven instream habitat and 13 water quality parameters. I employed a correlation matrix to reduce 20 environmental parameters to seven, then developed hierarchical regression models and used AIC model selection to quantify the most parsimonious relationships between catchability and environmental variables.

Mean catchability among six fish species ranged from 0.30 to 0.55. I found no trend relating environmental parameters to variation in catchability among benthic and water-column species. The most influential environmental parameters on fish catchability were mean water column velocity, mean stream width, and percent cover. Catchability was negatively correlated to mean column water velocity and mean stream width and positively to percent cover. Turbidity was not closely associated with electrofishing catchability within the range of my sampling. The regression models that I developed can be used to better understand environmental variables that influence electrofishing catchability and may be applied to more efficiently estimate fish populations. As these models correct for bias associated with varying sampling conditions, they can be utilized with single-pass electrofishing data to estimate stream fish populations. These models will enable fisheries researchers and managers in Puerto Rico to obtain fish population estimates with a single field sample, saving time and expense, with minimal bias. More complete, quantitative estimates of the fish community may then form the basis for improved stream fish and ecosystem management.

Sampling Bias, Selectivity, and Environmental Influences
of Puerto Rico Stream Fishes

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Fisheries and Wildlife Sciences

Raleigh, North Carolina

2008

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BIOGRAPHY

Christin Hambrick Brown was born in Greensboro, North Carolina, on June 24, 1979. She is the daughter of Rebecca Reckard and William Brown. Christin was blessed with a younger brother, Fields Brown, and sister, Elaina Brown, from her father and stepmother, Joy Brown. At five years of age, she moved from Greensboro to Raleigh, North Carolina, where she attended Millbrook Elementary, East Millbrook Middle, and Millbrook High School, where she graduated in 1997.

After high school, Christin was not quite ready to attend college, so she spent some time traveling around the United States, visiting national parks and various music festivals. She soon realized that the vagabond lifestyle was only a temporary career and that she would need to continue her education if she wanted to be successful in life. She attended Wake Technical Community College for two years, with hopes to transfer to a four-year university. She was undecided on what field to focus, but she knew she loved science and decided to transfer into the College of Agriculture and Life Sciences at North Carolina State University. Christin focused on zoology, but was interested in the marine ecosystem. After taking a marine biology class at NCSU, she decided to study abroad for a semester in Wollongong, Australia, were she became a certified scuba diver. While diving in Australia, Christin realized she was interested in the dynamics of the marine ecosystem. With her new found love of fish, she enrolled in Dr. John Miller's Fish Biology class. This class was a great experience. She learned about several techniques used in fisheries and gained hands-on field experience. At the end of the semester, she became a summer intern at the North Carolina Aquarium at Fort Fisher, where she taught all age levels about the world of fishes.

In the winter of 2004, Christin graduated from North Carolina State University with a Bachelor of Science in Zoology. She then moved to the coast and worked as a waitress, while applying to graduate school. During summer 2005, Christin accepted a position as a Master's student studying Puerto Rico native stream fish populations. About two weeks later, she was packed-up and moving to Boquerón, Puerto Rico, for her first field season. The following pages describe her activities over the past three years in detail, enabling her to accomplish her goal of receiving a Master's degree.

ACKNOWLEDGMENTS

I would like to gratefully acknowledge my graduate committee members, whose advice and expertise have guided me and helped me throughout the entire journey of completing my thesis. Dr. Tom Kwak, chair of the advisory committee, has been instrumental in developing my skills as a biologist, through his patience, guidance, and support. I would also like to thank Dr. Ken Pollock and Dr. Jim Gilliam for their suggestions and logistic help during this research. I gratefully acknowledge Patrick Cooney, biologist on the Puerto Rico project, who assisted in site selection, field work, data entry, helped with revisions on my thesis, and was always open to discuss and answer questions related to the project. I am also grateful to Michael Fisk, field technician; his assistance, good attitude, and patience in the field made even the most challenging conditions enjoyable.

The Puerto Rico Department of Natural and Environmental Resources (PRDNER) provided funding for this research through Federal Aid in Sport Fish Restoration Funds (Project F-50). I am grateful for the assistance of many biologists and administrators of the PRDNER, who assisted not only in the field but provided administrative and logistical support. Craig Lilyestrom assisted in study design, site selection, logistic and practical discussion, and field sampling. José Berríos Diaz and Aitza Pabon Valentin also assisted with field sampling. Aida Rosario, Daniel Matos Caraballo, Miguel Figuerola Fernandez, and the staff at the PRDNER Joyuda Fisheries Lab provided lab, office, and field staging facilities that greatly facilitated this research. Darien Lopez of the Lucchetti Reservoir Field Management Station assisted with stream site selection and access.

Throughout the six seasons of field work many people provided field assistance under challenging conditions. I would like to thank all those who helped in the field, including Michael Fisk, Keith Gibbs, Aaron Bevill, Kristian Bonilla Santana, Julie Harris, Wilfredo A. Matamovos, Wilfredo M. Matamovos, Nate Harris, Dave Behler, Chris Mace, Paula Rosado, Andres Rosado, Christopher Cheleuitte, Sergio Mandina, Benjamin Fuentes, and Anastacio Gomez. Wayne Starnes of the North Carolina Museum of Natural Sciences identified fish voucher specimens and clarified their taxonomy. Lucy Bunkley-Williams and Bert Williams of the University of Puerto Rico granted permission to modify their Puerto Rico base map to display sampling sites, supplemented field supplies, and shared their knowledge of parasites known to impact freshwater stream fish of Puerto Rico. Jim Peterson of the U.S. Geological Survey, Georgia Cooperative Fish and Wildlife Research Unit assisted with hierarchical modeling and provided SAS macros that facilitated model development. Ramon Littell of the University of Florida assisted with help in the use of SAS Proc MIXED. Wendy Moore of the North Carolina Cooperative Fish and Wildlife Research Unit facilitated grant administration, purchases, housing arrangements, and other logistic necessities. Wendy was also always available to help with logistical questions, making my life less stressful, for which I am extremely grateful.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

LIST OF FIGURES

CHAPTER I

CHAPTER 1
INTEGRATING GEAR BIAS AND SELECTIVITY INTO DEVELOPMENT OF A STANDARDIZED FISH SAMPLING PROTOCOL FOR PUERTO RICO STREAMS

Introduction

Puerto Rico is a 8,959-km^2 island in the Caribbean Sea with diverse geology and habitats, including tropical rainforest, mountain, karst, and coastal plain regions. A mountain range transects the island longitudinally that averts the Northeast Trade Winds creating a rainshadowing effect, with northern areas receiving more rainfall than those in the south (Hunter and Arbona 1995). These factors contribute to the high diversity of fresh waters in Puerto Rico, and the 1,200 streams in Puerto Rico are a vital part of the ecological and human environment (Erdman 1972). Puerto Rico streams function to provide habitat to aquatic animals and for recreation, irrigation, hydroelectric power, and human drinking water. They also transport excess water off land and connect the coastal and mountain regions (March et al. 2003).

The human history of Puerto Rico has greatly impacted its streams. The early 1900s was a period of rapid industrialization, increasing the need for energy production (Hunter and Arbona 1995). In response to this need, the Puerto Rican government dammed the first stream in 1907 for hydroelectric power. The results of this and subsequent dam construction were positive for industry, but a hindrance for migrating fish species that rely on access between upper and lower stream reaches to complete their life cycle (Erdman 1984; Holmquist et al. 1998). The key to stream migration is unimpeded access to and from the estuarine environment for larvae dispersal (Brasher 2003). Further, the industrial boom was coupled with a large human population expansion that increased water pollution and water withdrawal (Hunter and Arbona 1995).

Puerto Rico is isolated with no access to large amounts of freshwater, creating a challenge when supplying drinking water to a growing human population (Hunter and Arbona 1995; March et al. 2003). Streams provide the primary supply of drinking water on the island, so protecting them from pollution is crucial (Hunter and Arbona 1995). The maintenance of freshwater fish populations is also dependent upon pollution control and adequate flow (Erdman 1984). Stream diversion results in a reduction of water flow and depth that directly affects habitat availability (Brasher 2003). A greater understanding of Puerto Rico streams is needed for proper management to sustain fish communities, other aquatic life, and the streams where they reside.

A vast number of organisms live within Puerto Rico stream systems, including fishes, crustaceans, mollusks, and other freshwater vertebrates. There are about 80 fish species that inhabit the freshwaters of Puerto Rico, and many of these have commercial or sport fish value. Some of these fishes are also a vital food source for important recreational and subsistence fisheries. Many of the riverine fish are amphidromous, spending their adult life in streams, and larvae migrate to the estuaries, while others are catadromous, living in freshwater and spawning in the ocean (March 2003). Native species that utilize both upper and lower stream reaches include gobies (Gobiidae), sleepers (Eleotridae), mountain mullets (Mugilidae), and eels (Anguillidae) (Holmquist et al. 1998). Upstream reaches are dominated by sirajo goby *Sicydium plumieri,* whereas, lower stream reaches are dominated by mountain mullet *Agonostomus monticola*, American eel *Anguilla rostrata*, bigmouth sleeper *Gobiomorus dormitor*, and river goby *Awaous banana* (Holmquist et al. 1998). Bigmouth sleeper is the only one of these species that is known to be able to complete its entire life cycle in a riverine environment (Bacheler et al. 2004). Mountain mullet is a recreationally important amphidromous fish, spawning in early summer and returning to upper stream reaches as an adult (Corujo Flores 1980; Erdman 1984). Sirajo goby and river goby have a modified ventral sucker disc that allows them to climb waterfalls or dams with any flow or leakage and return to upper stream reaches after spawning. The larvae of these fish are a local delicacy (Keith 2003). American eels are catadromous and found in lowland stream reaches (Erdman 1972). The smallscaled spinycheek sleeper *Eleotris perniger* and fat sleeper *Dormitator maculatus* are two native stream fishes found restricted to lower reaches or brackish water (Corujo Flores 1980). Understanding the occurrence and relative abundance of each species in a community will serve as the foundation for management of this valuable resource.

Few studies have been conducted on fishes in the streams of Puerto Rico, making them difficult to manage. Quantitative knowledge of stream fish can be used to assess the well being of fish communities and their habitats. Fishes can be used as a direct measurement of biological conditions in a stream and are reliable organisms used to indicate environmental quality (Simon 1999). Fish are desirable indicator organisms because they generally remain in the same area seasonally, recover well from natural disturbance, have long life spans, are highly visible, and their life history and taxonomy are well documented (Simon 1999).

Human impacts on streams, such as water quality or habitat degradation, can be assessed by biological monitors in a stream habitat. A fish's relationship with its environment and relative species abundance can be used as biological monitors to characterize stream health and integrity of a stream (Maret 1999). An Index of Biotic Integrity (IBI) was designed to assess biological integrity of aquatic ecosystems by incorporating fish assemblage and population attributes, relative abundance of a species, and condition of individuals within a sample (Karr 1990; Kwak and Peterson 2007). The IBI was first developed in midwestern U.S. warmwater streams by Karr et al. (1986) and would be a useful concept to characterize stream health in Puerto Rico streams if quantitative fish data were available.

Gear selection is an integral part of planning for sampling fish populations, as well as selection of region, amount of effort required within a region, personnel, and data analysis (Willis and Murphy 1996). When sampling fish, use of the appropriate gear is important because all fish sampling gears are variably selective. Types of gear selectivity that can affect sampling are those associated with fish species, size, and sex. All of these factors can lead to an over- or under-representation of the fish present in the region.

Two common gears used in stream fish sampling are seine nets and electrofishing. Seines are inexpensive, light weight, not restricted by turbidity, and have low fish mortality (Onorato et al. 1998). Seines are typically deployed in areas of low flow and relatively flat bottoms because they are not as effective as electrofishing in streams with high flow and large substrate (Hayes et al. 1996). Compared to seining, electrofishing gear is more expensive, heavier, and restricted by turbidity, but it is more effective for measuring stream fish abundance and biomass (Bohlin et al. 1989; Kruse et al. 1998). Relative to seining, electrofishing allows for more standardization of sampling effort, is less selective, and requires fewer personnel (Anderson 1995).

Gear efficiency, the amount of effort expended and the ability of a gear to capture the target organism, is affected by gear selectivity (Hubert 1996). Electrofishing efficiency is influenced by biological, environmental, and technical factors (Hubert 1996; Fievet et al. 1999; Peterson et al. 2004) and is especially important to consider when sampling fish communities (Kwak and Peterson 2007). Influential biological factors include fish morphology, physiology, and behavior. Capture efficiency of electrofishing is affected by fish size and favors capture of larger individuals and species (Bohlin 1982; Anderson 1995; Peterson et al. 2004). Influential environmental factors may be water conductivity, depth, and turbidity. Electrofishing efficiency is inversely related to water depth. Turbidity exhibits a bell-shaped curve with gear efficiency, because in clear waters fish can detect sampling personnel, but as water becomes more turbid, fish detectability decreases (Hubert 1996). Technical factors related to personnel, procedures, and equipment can be controlled to minimize the misrepresentation of a population in a sample and to most accurately represent a fish community (Kwak and Peterson 2007). Catchability is the proportion of fish captured in a standardized unit of effort, and any changes in fishing effort expended by the gear or shifts in spatial distribution of the fish can change the catchability (Fabrizio and Richards 1996). Failure to account for differences in selectivity, efficiency, and catchability can significantly misrepresent population estimates (Peterson et al. 2004).

Estimates of fish population parameters can be obtained by mark-recapture or removal methods (Seber 1982; Pine et al. 2003; Hayes et al. 2007). Mark-recapture methods can be applied to both open and closed populations (no births, deaths, or emigration), whereas the removal method is applied only to closed populations (Pine et al. 2003). In the simple Petersen mark-recapture method, applied to closed populations, a sample of fish is collected, marked, and returned to the population. Fish are allowed time to return to their original location and resume normal behavior, and a second sample is collected. Marked and unmarked individuals are recorded and compared to the original number of individuals marked to estimate actual population size with associated estimates of sampling error (Ricker 1975; Seber 1982). When applying mark-recapture methods to a closed population, certain assumptions must be met to attain accurate results. These include that all animals have the same probability of being caught, marking does not affect probability of capture, animals do not lose their marks, and all marks are recorded (Otis et al. 1978; Seber 1982). Mark-recapture methods can yield biased estimates, because handling may affect fish behavior (Rodgers et al. 1992; Peterson et al. 2004), but in general, marked fish are assumed to be released in good condition and are as likely to be captured as unmarked fish (Pine et al. 2003). In addition to handling effects, mark-recapture population estimates will be biased if the fish exhibit a behavioral response to the gear. The most common fish behavioral response to gear is a "trap shy" response, where subsequent recapture probability is lower than that for initial capture, and the population estimate will be biased high, or overestimated.

In the removal method, a portion of the population is removed in each of multiple successive sampling passes, and the total population is estimated by the rate of decline over repeated fishing efforts (Seber 1982). The removal method assumes a closed population and the probability of capture remains constant (Zippin 1958). In stream fish sampling, the assumption of a closed population can be reasonably met by setting blocknets at both ends of the reach or utilizing natural barriers to fish movement (Thompson and Rahel 1996; Heimbuch et al. 1997; Peterson et al. 2004). The removal method is preferred if fish exhibit a behavioral response to the sampling gear; however, this method will generally underestimate or overestimate fish populations if capture probability varies over time.

Evaluation of gear efficiency and catchability requires an unbiased estimate of the true population of fishes within a site, and there are several approaches used to estimate sampling bias or correct for such bias when it occurs (Fievet et al. 1999; Peterson et al. 2004). Fievet et al. (1999) utilized a three-pass removal method, and corrected for bias by estimating fish populations considering only the last two passes and then adding the catch from the first pass as a total population estimate. They did not estimate fish from the first pass because in the first pass, there was no preliminary disturbance that would affect catchability, and thus, they considered subsequent passes to have equal catchability. Peterson et al. (2004) stratified fish into three size classes for analysis and used two different removal estimators, the Zippin model (M_b) and model M_{bh} (Otis et al. 1978; Pollock et al. 1990). The latter model accounts for size related bias by including heterogeneity in capture probability among individuals. They then used a linear regression analysis to examine the relationship among estimate bias, site characteristics, fish body size, and number of removal passes. Rosenberger and Dunham (2005) estimated bias by comparing a known number of observed fish to estimates from removal and mark-recapture methods.

Population model assumptions that are violated related to variable capture probability can be corrected by using several alternative models available in the program MARK, a software application for estimating population size and capture probability (White and Burnham 1999; Pine et al. 2003). Population dynamicists have developed a series of models to account for variation in capture probability. Model M_h allows for variance in capture probability due to heterogeneity (most commonly due to fish size); heterogeneity, or the size, gender, and social status of a fish, among and within species, can lead to violations of the equal catchability assumption for estimating population size (Pollock 1982; Pine et al. 2003). Model M_b was designed to allow for trap responses after initial capture. Behavioral responses of a fish to a selected gear may vary after capture; therefore, an animal may be more or less likely to be recaptured (Pine et al. 2003). Behavioral responses include a "trap happy" fish that is easily caught each pass or fish that avoid capture and are never caught, that is "trap shy" fish. Model M_{bh} adjusts for both heterogeneity and behavioral responses. Models M_b and M_{bh} are the only models that can be applied to removal data; however, every model can be tested with mark-recapture data (Otis et al. 1978). Capture probability can also vary over time or subsequent passes; thus, a population can be overestimated or underestimated to varying degrees. Model M_t allows capture probability to vary over time and corrects for bias associated with varying capture probability. Multiple models may be applied to a single sampling occasion or data set, and Akaike's Information Criterion (AIC; Akaike 1973) model selection approach can be employed to determine which of the considered models is the most parsimonious and yields the least biased population estimates for a particular population (Burnham and Anderson 2002).

There are many scientific and practical reasons to standardize fish sampling procedures within specific habitats and regions (Bonar and Hubert 2002), and knowledge of gear efficiency and catchability for potential sampling gears is critical for protocol development. Ideally, biologists should compile knowledge and information on the sampling attributes of all potential gears, including practical considerations as well as their ability to represent actual population parameters, before standardized protocols are developed. Unfortunately, reliable information on those attributes may not be readily available for specific gears, habitats, and regions, and investigators may be required to attain applicable information empirically.

Objectives

The primary focus of this first research component was to quantitatively describe gear efficiency and selectivity relationships to estimate fish populations in two river drainages in Puerto Rico, and to use these results to develop standardized sampling techniques that can be applied island-wide. I also intended to evaluate population models among species using electrofishing catch results analyzed with both mark-recapture and removal methods to identify the most suitable parameter-estimating model.

I developed procedures to quantify fish populations and communities in Puerto Rico streams and better understand sampling dynamics by intensively sampling multiple sites repeatedly during three seasons (spring, summer, and fall). Toward the development of a standardized sampling protocol, I used fish catchability estimates to estimate gear efficiency and selectivity of electrofishing gears among species and sizes within and among species. A better understanding of gear bias will increase accuracy in population estimates and provides ecological information on population density, biomass, and community structure. By estimating bias and accuracy of both mark-recapture and removal methods, I could determine the most efficient and accurate stream fish sampling method, and then I applied the most efficient, accurate, and practical methods to a standardized sampling protocol.

Methods

Site Description

I conducted this research on two watersheds in western Puerto Rico that receive varying annual rainfalls. Río Cañas (Figure 1) is a xeric watershed, characterized by lower annual rainfall, dry periods, and reduced flow. Río Guanajibo (Figure 2) is a mesic watershed, characterized by relativity high annual rainfall and flow. Within each watershed, a number of representative sampling reaches were selected spanning varying longitudinal gradients, allowing comparison of fish communities based on flow, depth, and longitudinal position in the watershed. The mountain stream headwaters tend to have steep gradients with short pools, well defined riffles, and larger substrates, creating high velocities (Erdman 1972). The coastal regions are mostly comprised of floodplains with low-gradient stream reaches that flow slowly over clay and sand substrates (Erdman 1972; Bass 2003). Within watersheds, I selected sampling sites above and below dams and natural barriers (i.e., waterfalls) that impede fish migration (March et al. 2003; Fievet et al. 1999).

The sampling site closest to the headwaters of the Río Cañas is located at latitude 18°05'10.25"N and longitude 66°39'22.61"W at 220.8 m elevation and is about 5.6 km north-northwest of Ponce (site C1, Table 1, Figure 1). The farthest downstream sampling site is located at latitude 18°01'29.14"N and longitude 66°38'24.54"W (Site C4). The Río Cañas drainage area is approximately 16.8 km^2 and is a major tributary of Río Matilde (U.S. Geological Survey 2006).

The Río Guanajibo watershed (89.6 km^2) is over five times larger than that of Río Cañas, with peak stream flows in September and October (U.S. Army Corps of Engineers 1998). The most upstream sampling site is located at latitude 18°10'36.44"N and longitude 66°58'46.78"W and is located about 0.3 km south of the Maricao (Site G1, Table 1, Figure 2). The highest elevation was at this site in the headwater region and was 426.2 m. The mouth of the stream is located at latitude 18°09'32"N and longitude 47°10'29"W (U.S. Geological Survey 1991-2002).

I sampled 12 stream sites for instream habitat, water quality, and fish populations during each of three seasons including, spring (March-April 2006), summer (June-July 2005), and fall (November-December 2005). Four of the 12 sites sampled were located in the Río Cañas watershed in the Río Cañas proper (Figure 1). The remaining eight stations were located in the Río Guanajibo watershed from five tributaries of Río Guanajibo, including Río Duey, Río Maricao, Río Rosario, Río Nueve Pasos, and Río Hoconuco (Figure 2). The lengths of the 12 sampling reaches ranged from 108 to 144 m (Table 3).

Fish Sampling Procedures

I sampled stream fish using electrofishing techniques during three seasons, spring (2006), summer (2005), and fall (2005). Sampling among seasons allowed for a representation of a broad range of habitat types and sampling conditions. Two types of electrofishing gear were employed to capture fish, a backpack electrofisher and a barge electrofisher. The Smith-Root model 12-B, pulsed-DC backpack electrofisher consists of a battery, hand-held anode, and a trailing cathode cable. At each site selected for backpack electrofishing, two backpacks were employed simultaneously operating at about 0.25 A. The Smith-Root SR-6 electrofishing tote barge is a small boat that holds a generator and is pushed by an operator. The barge electrofisher was powered by a Smith-Root GPP 2.5 power source and converter (2.5 kW) that typically operated at about 3 A. It can power up to three anode probes, and the boat has an attached cathode plate. A minimum of four people operated the barge fisher, and a minimum of three people sampled when using the backpacks. All personnel operating anodes also netted fish, and any additional crew assisted with additional dip nets. The type of gear used at each site was based upon stream width, depth, and substrate composition. All sites selected were shallow enough to effectively sample by wading. Backpacks were most suitable in reaches with large substrate materials (large cobble or boulders) or in reaches of shallow depths and narrow widths. The barge electrofisher was used at all other sites, especially those with few instream impediments (e.g., boulders or physical structure), deep enough draft, and suitable stream width.

I selected sites based on accessibility, stream habitat, and position in the watershed. Sites consisted of at least one pool–riffle sequence (Lyons and Kanehl 1993; Thompson and Rahel 1996; Thompson 2003). A pool was defined as a deep area of sluggish current that flowed over silt, gravel, cobble, or boulder. A riffle was a shallow area with swift current and surface turbulence that flowed over sand, gravel, or cobble substrates. At each site, 21.3-m by 1.8-m blocknets, with 7-mm mesh knotless nylon, surface floats, and a bottom lead-line, were used to close off both upstream and downstream ends of the sampling site. I assumed that blocknets formed a closed system for sampling purposes by preventing fish movement (Heimbuch et al. 1997). Sites with natural barriers, such as a waterfall or a low-head dam, eliminated the need for a blocknet at that barrier.

Once a site was closed and the proper gear was selected, three to five upstream electrofishing passes of equal effort (by time) were conducted, and fish of all species and sizes were collected. Following the first pass, fish were weighed (g), measured (total length, mm), and marked with a partial upper caudal fin clip. Each fish was then released in the middle of the reach and allowed at least one hour to recover and return to a suitable location before the next successive pass. One hour has been shown to be sufficient for a fish to recover from the effects of electricity and handling (Rodgers et al. 1992). Following the second pass, each fish collected was weighed, measured, checked for an upper caudal fin clip, received a partial lower caudal fin clip, and was released in good condition. Following the third pass, fish collected were weighed, measured, and checked for upper and lower caudal fin clips.

I conducted a five-pass removal procedure at a subset of locations (C2, C3, C4, G4, G5, and G6) in both watersheds during fall (2005) sampling and at every location during spring (2006) sampling to further evaluate accuracy of the removal method. Fish captured on passes four or five were temporarily removed from the stream and not marked, but marked fish were recorded. Fish that were removed from the stream were temporarily held in a mesh basket that I located in the stream.

I also performed a five-pass mark-recapture procedure at a subset of locations (C1, G1, G3, G7, and G1) during the spring (2006) sampling season on both the Río Guanajibo and Río Cañas watersheds to further evaluate the accuracy of the mark recapture method. Fish collected on the third pass received a partial right pectoral fin clip, and fish collected on the fourth pass received a partial left pectoral fin clip. All fish collected were weighed, measured, and all marks were recorded according to the sampling pass.

Previous accounts of freshwater Puerto Rico fishes (Hildebrand 1935; Erdman 1961, 1986) reported the presence of only one species of *Sicydium,* the sirajo goby, *Sicydium plumieri*; however, Watson (2000) recently examined fish holdings of a number of museums and other collections from Puerto Rico and determined that four species of *Sicydium* occur in the streams of Puerto Rico (*S. buski*, *S. gilberti*, *S. plumieri* and *S. punctatum*). Due to the minute physical distinctions between species that are difficult to distinguish in the field, I considered all four species one taxon, the sirajo goby *Sicydium plumieri*, for this study, as I presumed that the capture probability and sampling attributes would be similar among the four species.

Testing Assumption Violations

Upon completion of removal and recapture sampling, I deployed an electrofisher outside of the blocknets at a subset of four sites (G1, G2, G4, and G7) to assess if the assumption of a closed system was violated. I sampled 30-m reaches upstream and downstream of the sampling reach, at an effort sufficient to collect all of the fish within the given area. Fish collected were identified, weighed (g), measured (total length, mm), and any marks were recorded. Any fish captured outside of the reach that was marked would represent a violation of the assumption that the population was closed.

Instream and Riparian Habitat Surveys

I characterized habitat by a cross-sectional transect survey at each sampling site within the two study drainages (McMahon et al. 1996). Ten cross-sectional transects within each sampling reach were measured and spaced at a distance apart that equals one stream width. Placement of the first transect was within the downstream 1/10 of the sampling reach with the exact point chosen randomly. I measured at least 10 equally-spaced points for microhabitat parameters on each transect. Habitat characteristics measured were bank angle, riparian land cover, instream physical cover, substrate composition, water depth, mean column velocity, and stream width (Simonson et al. 1994; McMahon et al. 1996).

I used a clinometer to measure bank angle on both banks, if the bank was undercut the width of the undercut bank was also measured. I visually estimated riparian land cover, instream physical cover, and substrate composition. Riparian land cover was estimated on each bank of each transect in a zone 50 m from the bank and was classified as residential, forested, agricultural, or road. Instream physical cover type was visually classified and listed as one of the following: course woody debris, fine woody debris, rootwad, leaf litter, undercut bank, emersed plant, submersed plant, terrestrial plant, boulder, cobble, or trash. Substrate composition was visually classified as the most dominant size class according to particle diameter (mm) following a modified Wentworth scale (Bovee and Milhous 1978).

Substrate particle size was classified as one of the following: silt/clay (>0-0.06 mm), sand (0.06-1.00 mm), very course sand (1-2 mm), pea gravel (2-4 mm), fine gravel (4-8 mm), medium gravel (8-16 mm), course gravel (16-32 mm), very course gravel (50-64 mm), small cobble (64-130 mm), large cobble (130-250 mm), small boulder (250-500 mm), medium boulder (500-1,000 mm), large boulder (1,000-2,000 mm), very large boulder (2,000-4,000 mm), and mammoth boulder (>4,000 mm).

I measured stream water depth to the nearest centimeter using a Scientific Instruments, 1.5-m top-setting wading rod, and water velocity was measured using a Marsh-McBirney Flo-Mate Model 2000 digital meter. Mean column velocity was measured at a point 60% of the depth below the surface (McMahon et al. 1996). When depth exceeded 1.0 m, velocity was recorded at 20% and 80% depth below surface, and those rates were averaged for the column mean. Upon completion of the cross-sectional habitat survey, geographic coordinates for the site were recorded using a Garmin Model V Global Positioning System.

I calculated stream discharge volume using the width between points along the cross-sectional transect, depth, and mean column velocity from a transect of laminar flow (McMahon et al. 1996). Total discharge (Q, m^3/s) for that transect was calculated by multiplying for each cell on the transect cell width (w_n), depth (d_n), and velocity (v_n) and then summing the resulting volumes for each cell as below.

$$Q = w_1 d_1 v_1 + w_2 d_2 v_2 + \ldots\ldots + w_n d_n v_n.$$

Water Quality Analyses

I measured selected water quality parameters at each sampling site. Water temperature (ºC), total dissolved solids (g/L), conductivity (µS/cm), dissolved oxygen (mg/L), and salinity (ppt) were measured with a Yellow Springs Instrument (YSI) model 556 Multiprobe Instrument. These measurements were taken by lowering the YSI probe into an area of the stream of laminar flow. At each site, a water sample was also collected and placed on ice for subsequent analyses in the lab. A Hach CEL/850 Aquaculture Laboratory was used to measure alkalinity, hardness, turbidity, pH, and concentrations of nitrate, nitrite, ammonia, and phosphorus. Alkalinity was measured by titrating a sample with phenolthaline as an indicator with sulfuric acid, measuring levels from 10 to 400 mg/L as $CaCO_3$ using a digital titrator. Hardness was measured by a digital titration method using EDTA as an indicator to measure levels from 10 to 400 mg/L as $CaCO_3$. Turbidity was measured in FAU using a DR/850 colorimeter and comparing a deionized water blank to the water sample. Measurements of pH were conducted using a sension 1 pH meter and was measured to an accuracy of 0.01. Nitrate concentration was measured by a cadmium reduction method measuring levels from 0.3 to 30.0 mg/L NO_3^- using a DR/850 colorimeter. Nitrite concentration was measured by a diazotization method measuring levels from 0.002 to 0.300 mg/L NO_2^- using the same colorimeter. Ammonia as nitrogen was measured by a salicylate method that measures levels from 0.01 to 0.50 mg/L NH_3 using the same colorimeter. Phosphorous was measured by the orthophosphate ascorbic acid method that measure levels from 0.02 to 2.50 mg/L PO_4^- using the same colorimeter.

Bias Assessment

I used mark-recapture and removal methods to calculate population estimates of each fish species based on electrofishing catch among samples. I developed and calculated a bias estimator for both mark-recapture and removal methods to indicate relative accuracy and how confident I can be in interpreting the population estimates. The bias estimator analyses on the mark-recapture method was developed using fish that were caught in the first pass and released as a subpopulation of known size. Fish recaptured in the second pass that had been marked in the first pass (upper caudal fin clip) then represented the sample of marked fish (m) from a typical first pass sample in the bias estimator. Fish recaptured in the third pass that had been captured and marked in the first two passes (both upper and lower caudal clips) represented recaptured fish (r). All fish caught that were previously marked in either first or second pass (any clip) represented the total catch for the second mark-recapture sample (c). A simple Petersen population estimate (N) was calculated using the data from the second and third passes ($N = mc/r$) and compared to the known population from the first pass total catch. This procedure yielded information on the directional bias and percent accuracy of the mark-recapture method, and demonstrated the level of confidence in the estimating procedure.

The removal method that I evaluated was a maximum-likelihood estimator (model

M_b) and was estimated in program MARK. Similar to mark-recapture bias estimating, the removal estimate based only on recaptured fish (upper caudal clip) from the second and third passes was compared to the known population from the first pass. At sampling occasions where a five-pass removal was conducted, maximum-likelihood estimates were calculated on two-, three-, and four-pass removals and compared to the known first-pass population. This allowed for comparison of directional bias and percent accuracy among three removal procedures. Accuracy was considered the absolute difference between the known population size and the estimate, and bias was considered a systematic trend among the differences between the known population and the estimate.

Model Selection

I conducted both mark-recapture and removal method procedures concurrently at all sampling occasions. With these methods, a suite of models can be used to estimate fish capture probability and population sizes. To determine the most efficient model for sampling the entire fish assemblage, I analyzed three models available in program MARK, the null model (M_o), the time variation model (M_t), and the behavioral model (M_b). I then calculated an AIC weight, a probability that allows for model comparison to identify the best fit and most parsimonious model. Each sampling occasion was analyzed separately resulting in a separate AIC weight among each site and species sampled at that site; the best overall model was determined by the percent of times AIC weights selected the model and the mean AIC weight.

I then analyzed model M_b further to determine if fish displayed a behavioral response to the gear. Using model M_b results, I plotted capture probability (p) against recapture probability (c) to indicate any trap response. If recapture probability is greater than initial capture probability, this indicates a "trap-happy" response, and if recapture probability is less than initial capture probability, this indicates a "trap-shy" response by the fish to the gear. Based upon results from AIC model selection and these additional analyses on model M_b, I selected the most efficient model for sampling and estimating population sizes for the entire fish community in Puerto Rico streams.

Catchability and Population Sizes

I estimated fish catchability, density (fish/ha), and biomass (kg/ha) of each species sampled using Pop/Pro Modular Statistical Software, a program designed for electrofishing field data that utilizes single-census mark-recapture or removal methods (Kwak 1992). I incorporated length of individual fish to calculate catchability and population density estimates, and both fish length and weight to estimate biomass. I stratified all parameter estimates according to fish size to reduce electrofishing bias related to size selectivity.

Three-pass removal data were used to calculate all of these estimates, but if any population in the community was not depleted in three passes (i.e., fish caught on the last pass exceeded the number of fish caught on the first pass), catchability was not estimated, and population density and biomass were calculated as a minimum estimate with no variance by summing the catch of all passes. For all other samples, the entire fish community was estimated by species that were stratified by size. I stratified all estimates into 5-cm size groups, but if sample size was low in any size group, successive groups were combined. Species mean and site mean catchability were then determined for each species and site. Population density and biomass estimates for each species were converted to standard units (fish/ha, kg/ha) using the area of the respective sampling reach. Population density, biomass, and associated variances were calculated according to Newman and Martin (1983). Variance associated with each parameter estimate (sampling error) was calculated and presented as standard error (square-root of variance).

Results

A total of 12 sites were sampled in two Puerto Rico drainages over three seasons (spring, summer, and fall) to yield a total of 36 sampling occasions. Backpack electrofishers were deployed on 19 sampling occasions and a barge electrofisher on 17 sampling occasions. I collected data sufficient to study three-sample mark-recapture estimates for 32 sampling occasions, five-sample mark-recapture for four sampling occasions, three-pass removal for 19 sampling occasions, and five-pass removal for 17 sampling occasions (five-pass removal sampling includes data sufficient for three- or four-pass estimates). A total of 12 fish species were collected in spring samples, 11 in the summer, and 12 in the fall; six of the seven native riverine species were found among all three seasons. Of the seven native riverine species, the fat sleeper *Dormitator maculatus* was the only one not collected.

The six native riverine species were sympatrically located among sites downstream of significant migration barriers, and only goby species were sampled upstream of barriers. American eel were located at eight sites consistently among seasons, with the addition of being sampled at site G4 during the summer. Smallscaled spinycheek sleeper were only found at downstream sample locations during fall and summer (C4, G5, and G8); however, they were sampled farther upstream during the spring (G6 and G7). Bigmouth sleeper were collected at all downstream sample locations among seasons, as well as an upstream location (G4, 26.4 km from the river mouth); however, their absence at other upstream sampling sites was probably related to the presence of barriers that impede fish migration. Among seasons, river goby were sampled at both up and downstream sample locations, but highest densities were found at downstream sites (C3, C4, and G5). Sirajo goby were detected at both up and downstream locations among seasons, and were the dominant fish species collected at site C1, located above a waterfall. Mountain mullet were overall the most abundant fish species collected among seasons, but they were not collected at the most upstream sampling sites (C1, G1, and G3).

Sampling to assess the assumption of a closed system associated with my methods indicated good compliance with that assumption. I electrofished outside of the sampling reach at 4 sites during spring 2006. I collected five native species within 30 m of the block nets, American eel, bigmouth sleeper, river goby, sirajo goby, and mountain mullet. Overall, I sampled a total of 92 fish outside the nets on the four sampling occasions (Table 2). Of these fish, only two were marked (2.2%), and they were both mountain mullet (2 of 53, 3.8% for the species).

Habitat Characteristics

Instream habitat characteristics varied among seasons and between drainages, but riparian habitat was similar between drainages. The Río Cañas and Río Guanajibo mean bank angles ranged from 96.3º to 163.3º (Table 3), and both included sites with undercut banks and vegetation, offering additional cover for fish and invertebrate species. Generally, substrate composition and the presence of rocky cover followed a trend with an increase in substrate size as occurrence of large cobble and boulders with elevation, with sampling reaches following a typical riffle, run, and pool sequence of macrohabitats. Average water velocities and depths varied within and among the stations. Among seasons, average water velocities were lower in the Río Cañas drainage than the Río Guanajibo, and the lowest mean velocities were measured during spring (overall range 0.026-0.236 m/s, Table 3). In the Río Cañas and the Río Guanajibo watersheds, average mean stream width was generally lower in headwater reaches (overall range 3.7-5.6 m) and mostly decreased at every site in the spring (overall range 2.43-10.75 m, Table 3). Discharge peaked in the fall and summer (overall range = 0.087-1.813 m^3/s). Peak discharge occurred in the fall at sample location G2 (Table 4). The Río Cañas watershed had lower discharge values than the Río Guanajibo for all seasons (overall range = 0.041-0.703 m^3/s, 0.010-1.813 m^3/s, respectively Table 4).

Slight differences in average water quality parameter measurements were apparent between the two river drainages. Within each sampling season, the mean temperature varied and was about 0.5 ºC higher in Río Cañas sites, than in those of Río Guanajibo during summer and fall, perhaps explaining the slightly higher dissolved oxygen concentrations measured in Río Guanajibo sites. However, during the spring sampling season, average temperature was lower in the Río Cañas sites by about 2.0 ºC, but dissolved oxygen concentrations did not increase (Table 5). Mean turbidity and conductivity levels on average were higher in Río Cañas samples among seasons, although mean turbidity was slightly higher in Río Guanajibo during spring, mostly owing to substantially higher turbidity at sites G2 and G8. Among seasons, mean phosphorus and mean nitrate concentrations were higher in Río Guanajibo. Average pH (8.42, Table 5) did not vary greatly among seasons and ranged from 7.71-9.21.

Bias Assessment

I estimated bias for two-sample Petersen mark-recapture population estimates, and two-pass, three-pass, and four-pass removal estimates for four native fish species at 25 sampling occasions. I developed bivariate plots of the estimated population size of each estimate versus the known population size (i.e., the sample marked in initial sampling) and included a 100%-accuracy line, where the estimated population size was equal to that of the known population (Figure 3). The direction of any bias and accuracy of each method can be derived from these plots; points located above the 100%-accuracy line indicate an underestimation in the population, and points clustered below the line would indicate an overestimation, with proximity to the line representing accuracy. Figure 3 shows points that are distributed equivalently above and below the line for each removal method, thus indicating no systematic bias for any of the three removal methods evaluated. However, for the Petersen mark-recapture method nearly all points fell below the line, suggesting that the method is negatively biased.

Both the three-pass and four-pass removal methods resulted in relatively concentrated groupings around the 100%-accuracy line, indicating these methods were more accurate than the Petersen mark-recapture or two-pass removal methods (Figure 3). Overall, the three-pass removal mean accuracy was 87.9% (95% CI ± 3.3) and four-pass removal was 89.5% (95% CI ± 4.5; Figure 4). Ninety-five percent confidence intervals suggest that these accuracies were significantly greater than those for the Petersen mark-recapture method (82.6%, 95% CI ± 5.6), but not significantly different than those for the two-pass removal method (85.1%, 95% CI ± 7.2; Figure 4).

Population Model Selection

To determine the best model to estimate fish populations in Puerto Rico, I analyzed the performance of three models for four native species with sufficient sample sizes, bigmouth sleeper, river goby, sirajo goby, and mountain mullet. I based model selection on AIC weights (w_i) and found that the best model varied among species. For the bigmouth sleeper, there were 10 sampling occasions used to select the best model; according to w_i probabilities, the percent frequency each model was selected was 30% for M_o and 35% each for M_t and M_b (Table 6). The best overall model was M_b for river goby (10 sampling occasions) and sirajo goby (four sampling occasions) with it selected 70-75% of sampling occasions. The model selected most frequently for mountain mullet was model M_o at 42% among 24 sampling occasions.

In further analysis of model M_b results, I found variation among species in their behavioral response to electrofishing. Plots of capture probability (*p*) versus recapture probability (*c*) demonstrated a clear behavioral response ("trap shyness") to the electrofishing gear for bigmouth sleeper, river goby, and sirajo goby (Figure 5b-d). Recapture probability was lower than initial capture probability for every sampling occasion for bigmouth sleeper, nine of 10 for the river goby, and four of five for the sirajo goby. Mountain mullet comparisons suggest no substantial behavioral response in that species, further explaining why the M_o model was most frequently selected for this species (Table 6, Figure 5a).

Population Size Structure

American eel abundance and size ranges were similar among seasons and sites. Abundance ranged from one to 16 fish at a given location, and size ranged from 132 to 885 mm (Figure 6). The largest American eel was located at site C4 during the summer sampling season. At this location, a total of 15 American eels were captured ranging from 203 to 885 mm. This site made up 28% of the total catch of American eel among all sites and seasons.

Bigmouth sleeper abundance varied among sites and seasons; however, the general size range remained similar among seasons (overall range = 47-441 mm, Figure 7). Size groups greater than 200 mm did not vary greatly in number among seasons. However, there was a peak in the number of 100-200 mm fish during the spring, but this peak coincided with a lower relative biomass. Bigmouth sleeper density was similar between spring and summer (Tables 10 and 11), but biomass was 35% lower during the spring, suggesting a high density of juvenile fish during spring (Figure 7, Table 13). During spring, the 100-200 mm size classes made up 70% of the total catch at downstream reaches on Río Cañas (sites C3 and C4) and 72% in the fall. Overall, Río Cañas contributed 65% of total bigmouth sleeper catch of the 100-200 mm size classes.

I found minimal variation in smallscaled spinycheek sleeper abundance and size classes among seasons (overall range = 51-179 mm, Figure 8). The most abundant size class was 100-150 mm fish, and their numbers increased slightly in the summer and peaked in the fall. Overall they were the least abundant native species.

River goby abundance varied greatly among seasons, but the size range remained similar (overall range = 32-303 mm, Figure 9). Peak abundance occurred during the spring with a large mode at 75-100 mm. The lower reaches of Río Cañas (sites C3 and C4) yielded 88% of the total catch of the 25-150 mm size classes for the Río Cañas watershed, and the lower reaches of Río Guanajibo (sites G2, G5, G6, G7, G8) contributed 94% of the total catch of the 25-150 mm size classes for that watershed. This suggests that spawning occurs in late winter or early spring and that juvenile river gobies are utilizing downstream locations.

Sirajo goby abundance varied greatly among seasons, with a similar size range of 12 to 176 mm fish (Figure 10). Abundance peaked in spring, owing to the high occurrence of juveniles (25-50 mm). The lower reach of Río Cañas (site C4), 4.9 km from the river mouth, contributed 50% of the total catch of the 25-50 mm size class, not including the Río Guanajibo catch. Juveniles were collected at both upstream and downstream locations in Río Cañas and were observed ascending the nearly vertical waterfall located at the downstream edge of site C1.

Mountain mullet abundance was the highest of the six native species sampled. It varied widely among seasons with peak abundance occurring in the 50-100 mm size class of approximately 1,600 fish (Figure 11). Size range remained relatively consistent among seasons (overall range = 25-347 mm). The abundance of individuals greater than 100 mm remained similar among seasons and was approximately 5 to 200 fish per size class. The lower reaches of the Río Cañas watershed (sites C3, C4) contributed 95% of the total catch of 25-100 mm fish, not including the Río Guanajibo watershed. The lower reaches of the Río Guanajibo (sites G2, G4, G5, and G6) yielded 85% of the total catch of those size classes.

Catchability, Density, and Biomass

Fish catchability means and ranges among sites and species were generally similar among seasons. In the spring sampling season, catchability was estimated for nine of the 13 species from within both watersheds (overall range = 0.223-0.620, mean 0.457, Table 7). Summer sampling results were similar (overall range = 0.172-0.516, mean 0.409, Table 8) and were estimated for nine of the 13 species. I estimated catchability for eight of 13 species for the fall sampling season (overall range = 0.285-0.560, mean 0.450, Table 9).

I estimated species mean catchability for all of the native species encountered among all seasons, and on average estimates were high but varied by species, site, and season. American eel catchability was highest during spring (mean 0.481, Table 7) and ranged from 0.200-0.650 among all seasons (Table 7-9). Catchability estimates for bigmouth sleeper did not vary greatly by site or by season and ranged from 0.112-0.654 among seasons. There were only two catchability estimates less than 0.20 and these were associated with sparse populations (catches less than 20 fish). Smallscaled spinycheek sleeper estimates were highest during fall (mean 0.469, Table 9) and ranged from 0.159 to 0.566; 50% of the total catch of smallscaled spinycheek sleepers among seasons was during fall sampling. Overall catchability for river gobies was high with a range from 0.122 to 0.709, the only estimate less than 0.20 occurred at site G8 where only four river gobies were collected (Tables 7-9). Sirajo goby catchability was highest (0.729) at site C3 during spring, where over 100 sirajo gobies were collected; catchability was generally high at downstream sample reaches on Río Cañas. On average, mountain mullet catchability was high (0.095-0.916, Tables 7-9). I found that the greatest probability of capture occurred at site G7 during spring, where I collected 123 mountain mullet and recaptured 101 fish on the second pass. This site was unique among the 12 sampling sites in being very narrow, shallow, with low flow volume (mean stream width = 2.43 m, mean depth = 7.9 cm, mean column velocity = 0.079 m/s; see Table 3).

Fish density estimates peaked during the spring sampling season and ranged among sites from 301.0 to 27,492.8 fish/ha (Table 10). The summer range was 648.7-8,078.4 fish/ha (Table 11) and that for fall was 209.4-4,609.3 fish/ha (Table 12). Native fish were found at every sampling site. Densities of American eel and smallscaled spinycheek sleeper were similarly low among sites (range = 9.5-462.0, range = 7.5-212.1, Tables 10-12). Bigmouth sleeper density peaked in the summer at 2,681 fish/ha, and river goby, sirajo goby, and mountain mullet densities peaked during spring (1,544, 11,475, and 17,087 fish/ha, respectively; Table 10). The highest density of non-native species I encountered was at site G7 during spring, which was dominated by green swordtails *Xiphophorus hellerii* (18,018 fish/ha, Table 10). Green swordtails were the most abundant non-native species sampled and were located at one site on Río Cañas and seven sites on Río Guanajibo (Tables 10-12).

Total fish biomass estimates varied widely among sites with a range of 1.6-621.9

kg/ha. The highest biomass estimate (621.9 kg/ha) was associated with site C4 during summer sampling with substantial biomass of American eel, bigmouth sleeper, and mountain mullet (Table 14). This high biomass estimate did not coincide seasonally with the greatest density estimate among sites and seasons associated with this site (C4) during spring (Table 10).

Discussion

My research objectives were to examine the sampling attributes of fishing gears and deployment methods and applicability of population models to resulting catch data. My ultimate goal in setting those objectives was to incorporate those findings into development of a standard fish sampling protocol for Puerto Rico stream fishes. Criteria that I considered in protocol development were to prescribe a set of procedures that would be as accurate as possible among options and logistically feasible and efficient in the field.

Ichthyologists routinely sample streams and other shoreline habitats using small seines with the intent of collecting as many fishes as possible to describe species occurrences. Such sampling is important to define geographic distributions of fish species, but is not intended to estimate fish population parameters or community structure for ecological relevance. Such objectives require intensive sampling and the application of parameter-estimating methods that I examined here, such as mark-recapture or removal models (Ricker 1975; Seber 1982; Pine et al. 2003).

I attempted to sample stream fish using two types of sampling techniques, seining and electrofishing. Initial pilot sampling using seines found the gear to be ineffective, owing to fish behavior, instream channel morphology, and associated cover. Thus, I sampled fish using the two electrofishing techniques described in Methods above, backpack electrofishers and a barge electrofisher, and I evaluated their sampling attributes and compared population models to estimate fish catchability and population size among species. The conductivity of Puerto Rico stream water is moderate (100-1,000 μS/cm, with most waters 200-500 μS/cm; Díaz et al. 2005), which is optimal for sampling with typical electrofishing gears (Reynolds 1996). My water quality sampling confirmed optimal conductivity for electrofishing among 81 stream sampling sites with a mean of 321.6 μS/cm (SD = 131.8 μS/cm; range = 59-780 μS/cm; see Chapter 2). Thus, I expected and demonstrated relatively high catchability in stream habitats using electrofishing gear (seasonal means among sites and species ranged from 0.41 to 0.46; Tables 7-9), and I confidently recommend its application over netting techniques in wadeable Puerto Rico streams.

A Standardized Fish Sampling Protocol

I compared two fish sampling gear types (electrofishing and seining) and four population models for estimating fish population parameters (Petersen mark-recapture and removal estimators of 2-4 sampling passes) to provide the quantitative basis for development of a standardized sampling protocol for Puerto Rico stream fish. I found electrofishing substantially more efficient and logistically feasible for collecting fish in these environments. I also determined that the three- and four-pass removal models were more accurate than the Petersen mark-recapture model or the two-pass removal model, and that accuracy was similar between the three- and four-pass removal models (Figures 3 and 4). I further investigated variations of models that account for assumption violations among models and found model M_b to have the overall best and most parsimonious fit for estimating population parameters (Table 6).

Thus, based on my empirical findings, I propose a standard fish sampling protocol for Puerto Rico wadeable streams that includes sampling stream reaches from 100 m to 200 m and using the appropriate electrofishing gear (backpack or barge electrofishers) depending on stream morphology and instream habitat conditions. Three sampling passes of equal effort (by time) will be conducted with sufficient time between passes for fish to reorient to their environment after the disturbance of sampling (ca. 1 h). Fish will be held in suitable containers separately for each pass until they can be measured for length and weight, and all fish, except those retained as voucher specimens, will be returned to the stream. A Zippin-removal-type, maximum-likelihood estimator (Seber 1982) will then be used to calculate population size estimates for the reach, and then fish catch among passes, fish weight data, and site dimension measurements (length and mean width) will be used to calculate estimates of fish catchability, density, and biomass and associated variances in standard units for each species in the community (Kwak 1992; Hayes et al. 2007). Ancillary habitat and water quality parameters may be measured in association with fish sampling following the procedures described here as a guide, but specific variables to be measured may vary with study objectives.

Implications of the Sampling Protocol and its Development

My findings that support the use of the three-pass removal method and model (M_b) with electrofishing data as a robust estimator of population parameters of Puerto Rico stream fish are contrary to those of several other studies evaluating multipass removal models for stream-dwelling salmonids. In related research in Rocky Mountain (USA) coldwater streams, other investigators found removal estimators for salmonid populations (species) to be systematically biased, yielding inflated catchability estimates and underestimates of actual population size (Riley and Fausch 1992; Peterson et al. 2004; Rosenberger and Dunham 2005). Those researchers cited low sampling efficiency that decreased among successive sampling passes as the likely explanation for the bias. They also found bias related to stream habitat, fish species, and fish size. My findings that the three-pass removal estimator was 87.9% accurate on average and showed no systematic bias suggest that sampling conditions in Puerto Rico streams and the response by native and introduced fishes in those habitats are conducive to the sampling gear and removal methods. It may not be surprising that results would differ between field studies conducted in Puerto Rico tropical island streams and those in coldwater mountain streams of the western U.S., given the dramatic differences in environments and fish faunas.

In situations where a three-pass fish sampling protocol is not feasible or where data precision for density and biomass is not critical, the estimates of catchability that I developed can be used to approximate fish density and biomass from a single electrofishing pass. The catch from a single electrofishing sample may be divided by catchability (as a proportion, not a percent) to yield an estimate of population number in the sampling reach. The catchability used in such a calculation should be as specific as possible for the fish species, habitat, and sampling conditions. For example, the catchability results that I present in Tables 7-9 are stratified by fish species, site, and season, and applying the specific catchability estimate for a species and season would result in the most accurate population estimate. Other investigators have proposed this approach as an efficient means to index fish population sizes with a single electrofishing sample (Lobón-Cerviá and Utrilla 1993; Kruse et al. 1998). The precision of population estimates by this means can be improved by incorporating environmental covariates (e.g., stream size or water conditions) into regression models, and I developed and present such models in Chapter 2.

The scientific and practical benefits of standardizing fish sampling procedures within specific habitats and regions are numerous (Bonar and Hubert 2002). The advantages to using the standard sampling protocol that I present here are many and include the ability to describe the fish communities of Puerto Rico streams in a quantitative manner that allows confident comparison among populations and communities, stream sites and reaches, and over time. This is possible because all parameter estimates account for variation in gear efficiency and selectivity and are presented in standard comparable units. Further, fish population and community data from Puerto Rico streams may be compared and placed in perspective relative to stream ecosystems in other regions. Another benefit of understanding gear efficiency and bias in stream fish sampling is that historical fish collections can be interpreted with greater relevance.

There are also potential drawbacks related to standardized sampling. A standardized sampling protocol is generally specific to the region where the protocol was developed and may not be reliable in other regions with dissimilar fish taxa, instream habitat, and water quality variables. Further, within a region if sampling conditions are highly variable among stream networks, standardized sampling may not be as reliable. The standardized sampling protocol that I developed for Puerto Rico stream fish is a regional protocol related to collecting fish abundance data within Puerto Rico streams. Since the protocol is rather specific other caution should be exercised when considering applicability for other regions.

An important consideration when applying any sampling method is the objectives and goals of the study. Depending on the objective of sampling, a biologist may need to sample with more or less effort, rather than a standardized unit of effort. The effort required to meet objective needs depends on required accuracy and precision of data, attributes of the stream, taxa of interest, and efficiency of the protocol (Angermeier and Smoger 1995). For example, less sampling effort may be required when the objective need is fish abundance or relative abundance data, but more effort would be required to collect the total number of species present (Angermeier and Smoger 1995). If rare species are of interest, the detection probability of these species is lower in a shorter sampling distance. Lyons (1992) recommended sampling stream reaches 35 times mean stream width, when species richness data is of interest. If a biologist is interested in utilizing an index to assess a stream related to presence and absence of species within a reach, there is a strong correlation between reach length and richness (Angermeier and Karr 1986). Finally, attributes of any standardized sampling protocol should be considered with regard to bias, selectivity, and the resulting parameters and indices at the fish community level (Kwak and Peterson 2007).

The development of this effective and efficient fish sampling protocol is an important step toward providing the components of information required to further develop management plans for Puerto Rico freshwater streams and fisheries. The first step in management planning is to develop effective sampling protocols for fishery resources, including the fishes and their habitats, and this objective is now complete. This protocol will be useful to improve the resolution, quality, and relevance of fish population and community data and can facilitate the establishment of monitoring programs to identify unique fish resources, document physical and biotic changes in stream fish communities over time, guide the ongoing development of stream fisheries, and evaluate future fishery or habitat management actions.

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Table 1. Geographic descriptions of 12 fish, water quality, and instream habitat sampling sites in the Río Cañas and Río Guanajibo drainages in Puerto Rico.

Site	Drainage basin	River	Municipality	Location	Elevation (m)
C1	Cañas	Cañas	Ponce	5.6 km NNW of Ponce	220.8
C2	Cañas	Cañas	Ponce	5.0 km NNW of Ponce	164.2
C3	Cañas	Cañas	Ponce	3.1 km NW of Ponce	57.7
C4	Cañas	Cañas	Ponce	2.0 km NW of Ponce	30.0
G1	Guanajibo	Maricao	Maricao	0.3 km S of Maricao	426.2
G2	Guanajibo	Rosario	San Germán/ Mayagüez	4.5 km SW of Rosario	48.8
G3	Guanajibo	Nueve Pasos	San Germán	2.9 km ESE of Rosario	199.3
G4	Guanajibo	Nueve Pasos	San Germán	1.3 km SE of Rosario	61.4
G5	Guanajibo	Duey	San Germán	1.5 km SE of Rosario	47.7
G6	Guanajibo	Duey	San Germán	2.0 km SSE of Rosario	39.2
G7	Guanajibo	Hoconuco	San Germán	2.6 km SSE of Rosario	41.6
G8	Guanajibo	Rosario	Hormigueros	1.5 km SE of Hormigueros	10.2

Table 2. Number and percent of total catch of fish species sampled outside of the closed sampling reach within 30 m of blocknets at four sampling sites during spring 2006 to assess compliance with the closed-population assumption.

Species	Total catch	Number marked (%)
American eel	8	0
Largemouth bass	5	0
Bigmouth sleeper	10	0
River goby	13	0
Sirajo goby[a]	3	0
Mountain mullet	53	2 (3.8)
Total	92	2 (2.2)

[a] Four species of *Sicydium* occur in Puerto Rico, combined here.

Table 3. Instream habitat and sampling reach characteristics from sampling sites located within the Río Cañas and Río Guanajibo drainages.

Site number	Season	Reach length (m)	Mean width (m)	Area (m^2)	Mean depth (cm)	Mean velocity (m/s)	Dominant substrate	Mean bank angle (˚)	% Cover
C1	Summer	112	4.35	487	14.4	0.452	Medium boulder	117.1	95
C1	Fall	112	5.16	578	18.9	0.501	Medium boulder	131.5	86
C1	Spring	112	3.84	430	14.9	0.081	Medium boulder	136.8	59
C2	Summer	118	4.97	586	17.1	0.143	Medium boulder	110.5	98
C2	Fall	118	6.53	771	17.7	0.105	Medium boulder	126.0	80
C2	Spring	118	4.64	548	12.2	0.048	Medium boulder	136.3	67
C3	Summer	108	5.01	541	26.0	0.217	Very coarse gravel	137.5	56
C3	Fall	108	6.03	651	29.2	0.106	Very coarse gravel	138.3	51
C3	Spring	108	4.61	498	30.9	0.026	Very coarse gravel	141.5	54
C4	Summer	118	5.12	604	21.7	0.450	Sand	128.3	65
C4	Fall	118	8.16	963	14.3	0.217	Sand	114.0	34
C4	Spring	118	8.17	964	14.5	0.202	Sand	131.3	54
G1	Summer	118	3.70	437	15.0	0.115	Small cobble	133.3	60
G1	Fall	118	5.63	664	12.9	0.199	Small cobble	135.3	57
G1	Spring	118	3.75	442	9.6	0.055	Small cobble	147.4	75
G2	Summer	130	10.30	1,339	26.7	0.362	Small cobble	117.8	38
G2	Fall	130	11.14	1,448	25.3	0.379	Small cobble	113.3	40
G2	Spring	130	10.75	1,397	18.4	0.236	Small cobble	118.8	51
G3	Summer	134	3.94	527	12.1	0.720	Very coarse gravel	131.8	84
G3	Fall	134	4.02	538	12.4	0.232	Very coarse gravel	116.8	35
G3	Spring	134	2.94	394	6.7	0.057	Very coarse gravel	145.8	66
G4	Summer	124	5.26	652	15.0	0.619	Very coarse gravel	138.5	71
G4	Fall	124	6.13	760	12.8	0.273	Very coarse gravel	148.3	61
G4	Spring	124	3.98	493	10.5	0.083	Very coarse gravel	156.5	75
G5	Summer	144	4.99	718	19.6	0.377	Small cobble	124.0	46
G5	Fall	144	7.31	1,053	19.2	0.939	Small cobble	137.3	53
G5	Spring	144	4.54	654	17.8	0.073	Small cobble	149.3	78
G6	Summer	144	7.50	1,080	11.7	0.435	Very coarse sand	119.1	38
G6	Fall	144	7.71	1,110	16.8	1.031	Very coarse sand	114.5	23
G6	Spring	144	6.90	994	9.4	0.041	Very coarse sand	125.3	56
G7	Summer	144	4.71	678	10.1	0.592	Small cobble	145.8	66
G7	Fall	144	5.16	742	18.5	0.259	Small cobble	145.8	37
G7	Spring	144	2.43	350	7.9	0.079	Small cobble	163.3	60
G8	Summer	114	7.64	871	39.6	0.405	Clay	108.3	27
G8	Fall	114	7.11	811	47.6	0.341	Clay	96.3	24
G8	Spring	114	6.71	764	35.9	0.189	Clay	116.0	44

Table 4. Discharge measurements for 12 sampling sites during 2005-2006 in the Río Cañas and Río Guanajibo drainages, calculated from instream measurements (water depth and velocity) taken in association with fish sampling.

	Discharge volume (m^3/s)			
Site	Spring	Summer	Fall	Site mean
C1	0.061	0.361	0.465	0.296
C2	0.041	0.204	0.703	0.316
C3	0.063	0.317	0.155	0.178
C4	0.264	0.508	0.365	0.379
G1	0.019	0.087	0.235	0.114
G2	0.520	1.227	1.813	1.187
G3	0.010	0.322	0.160	0.164
G4	0.036	0.772	0.329	0.379
G5	0.048	0.403	1.661	0.704
G6	0.035	0.319	1.811	0.722
G7	0.024	0.367	0.318	0.236
G8	0.585	1.778	1.657	1.346
Season mean	0.142	0.555	0.806	0.501

Table 5. Mean water quality parameters from the Río Cañas (4 sites) and the Río Guanajibo drainages (8 sites) during 2005-2006.

Drainage	Season	Water temperature (°C)	Total dissolved solids (g/L)	Conductivity (µS/cm)	Salinity (ppt)	Nitrate (mg/L as NO_3^-)	Nitrite (mg/L as NO_2^-)	Ammonia (NH_3)	Phosphorous (mg/L as PO_4^-)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	Turbidity (FAU)	pH	Dissolved oxygen (mg/L)
Cañas	Spring	23.31	0.26	394.00	0.19	2.28	0.07	0.04	0.30	164.25	177.00	6.50	8.63	8.38
Guanajibo	Spring	25.27	0.22	335.25	0.16	5.21	0.05	0.06	1.09	147.88	169.38	8.88	8.76	8.84
Cañas	Summer	26.26	0.23	357.00	0.17	1.25	0.01	0.02	0.14	144.50	157.25	8.00	8.17	7.91
Guanajibo	Summer	25.52	0.21	317.50	0.15	1.28	0.02	0.03	0.54	151.75	160.88	4.75	8.15	8.43
Cañas	Fall	24.07	0.29	445.50	0.22	2.10	0.03	0.08	0.14	156.75	170.00	12.00	8.22	8.57
Guanajibo	Fall	23.48	0.20	308.25	0.15	2.59	0.02	0.01	0.71	155.75	157.38	4.13	8.46	9.16

Table 6. Percent frequency and the mean probability (AIC weight, w_i) that a model was selected as the most parsimonious according to AIC among a suite of models developed for specific sampling occasions. The number of sampling occasions appears in parentheses.

	Bigmouth sleeper (10)		River goby (10)		Sirajo goby[a] (4)		Mountain mullet (24)	
Model	% Selected	Mean w_i	% Selected	Mean w_i	% Selected	Mean w_i	% Selected	Mean w_i
M_o	30	0.20	10	0.03	0	0	42	0.29
M_t	35	0.45	20	0.27	25	0.27	27	0.41
M_b	35	0.35	70	0.70	75	0.73	31	0.30

[a] Four species of *Sicydium* occur in Puerto Rico, combined here.

Table 7. Spring electrofishing catchability estimates for Puerto Rico stream fishes at 12 sampling sites during 2005-2006 in the Río Cañas and Río Guanajibo drainages. Standard error estimates appear in parentheses.

Site	American eel	Bluegill	Largemouth bass	Fat snook	Mozambique tilapia	Bigmouth sleeper	Smallscaled spinycheek sleeper	River goby	Sirajo goby[a]	Burro grunt	Mountain mullet	Green swordtail	Guppy	Site mean
C1								0.436	0.329					0.383
								(0.342)	(0.043)					(0.172)
C2						0.552		0.453	0.476		0.753			0.558
						(0.133)		(0.136)	(0.096)		(0.037)			(0.054)
C3	0.650					0.517		0.375	0.729		0.733			0.619
	(0.152)					(0.073)		(0.028)	(0.097)		(0.006)			(0.039)
C4	0.641					0.608		0.371						0.540
	(0.051)					(0.066)		(0.112)						(0.047)
G1			0.504						0.334					0.419
			(0.103)						(0.184)					(0.105)
G2	0.404					0.534		0.464			0.503			0.476
	(0.212)					(0.149)		(0.410)			(0.044)			(0.122)
G3												0.339	0.337	0.338
												(0.070)	(0.018)	(0.036)
G4						0.559		0.709	0.580		0.631			0.620
						(0.266)		(0.127)	(0.254)		(0.037)			(0.098)
G5						0.523		0.601	0.436		0.577	0.425		0.512
						(0.039)		(0.055)	(0.281)		(0.008)	(0.055)		(0.059)
G6	0.230					0.310	0.159	0.377			0.606			0.337
	(0.402)					(0.055)	(0.350)	(0.130)			(0.012)			(0.110)
G7						0.374		0.580	0.394		0.916	0.021		0.457
						(0.113)		(0.103)	(0.153)		(0.024)	(0.088)		(0.047)
G8						0.112		0.122			0.436			0.223
						(0.314)		(0.294)			(0.342)			(0.183)
Species	0.481		0.504			0.464	0.159	0.449	0.468		0.644	0.261	0.337	0.457
Mean	(0.120)		(0.103)			(0.054)	(0.350)	(0.067)	(0.067)		(0.044)	(0.042)	(0.018)	(0.029)

[a] Four species of *Sicydium* occur in Puerto Rico, combined here.

Table 8. Summer electrofishing catchability estimates for Puerto Rico stream fishes at 12 sampling sites during 2005-2006 in the Río Cañas and Río Guanajibo drainages. Standard error estimates appear in parentheses.

Site	American eel	Bluegill	Largemouth bass	Fat snook	Mozambique tilapia	Bigmouth sleeper	Smallscaled spinycheek sleeper	River goby	Sirajo goby[a]	Burro grunt	Mountain mullet	Green swordtail	Guppy	Site mean
C1									0.396				0.407	0.401
									(0.285)				(0.448)	(0.266)
C2	0.407					0.486					0.358			0.417
	(0.448)					(0.273)					(0.079)			(0.177)
C3	0.200					0.195		0.435			0.733			0.391
	(0.537)					(0.262)		(0.179)			(0.032)			(0.156)
C4	0.288					0.654		0.566	0.660		0.414			0.516
	(0.295)					(0.141)		(0.255)	(0.091)		(0.073)			(0.086)
G1			0.452											0.452
			(0.223)											(0.223)
G2	0.333					0.530		0.486			0.617			0.492
	(0.609)					(0.255)		(0.258)			(0.078)			(0.178)
G3												0.172		0.172
												(0.215)		(0.215)
G4											0.362			0.362
											(0.067)			(0.067)
G5	0.407				0.500	0.315			0.566		0.401			0.438
	(0.448)				(0.612)	(0.221)			(0.442)		(0.099)			(0.182)
G6	0.500					0.486					0.409			0.465
	(0.597)					(0.273)					(0.086)			(0.221)
G7						0.263		0.682	0.382		0.538			0.466
						(0.413)		(0.158)	(0.437)		(0.084)			(0.157)
G8						0.566					0.095			0.331
						(0.442)					(0.287)			(0.264)
Species	0.356		0.452		0.500	0.437		0.542	0.501		0.436	0.172	0.407	0.409
Mean	(0.204)		(0.223)		(0.612)	(0.106)		(0.109)	(0.173)		(0.040)	(0.215)	(0.448)	(0.055)

[a] Four species of *Sicydium* occur in Puerto Rico, combined here.

Table 9. Fall electrofishing catchability estimates for Puerto Rico stream fishes at 12 sampling sites during 2005-2006 in the Río Cañas and Río Guanajibo drainages. Standard error estimates appear in parentheses.

Site	American eel	Bluegill	Largemouth bass	Fat snook	Mozambique tilapia	Bigmouth sleeper	Smallscaled spinycheek sleeper	River goby	Sirajo goby[a]	Burro grunt	Mountain mullet	Green swordtail	Guppy	Site mean
C1									0.432					0.432
									(0.104)					(0.104)
C2	0.567					0.629		0.326	0.183		0.750			0.491
	(0.497)					(0.133)		(0.262)	(0.140)		(0.037)			(0.119)
C3	0.399					0.543		0.417			0.706			0.516
	(0.150)					(0.077)		(0.130)			(0.047)			(0.055)
C4	0.272					0.564	0.474	0.490	0.348		0.579			0.455
	(0.245)					(0.073)	(0.157)	(0.060)	(0.079)		(0.032)			(0.053)
G1			0.542						0.381					0.461
			(0.149)						(0.222)					(0.134)
G2	0.515					0.329		0.486			0.351			0.420
	(0.224)					(0.164)		(0.248)			(0.119)			(0.098)
G3									0.500					0.500
									(0.612)					(0.612)
G4						0.571		0.347	0.357		0.402			0.419
						(0.110)		(0.231)	(0.155)		(0.078)			(0.077)
G5					0.230	0.366		0.219	0.259		0.350			0.285
					(0.402)	(0.137)		(0.132)	(0.360)		(0.049)			(0.115)
G6	0.297				0.558	0.348	0.368				0.368			0.388
	(0.191)				(0.169)	(0.141)	(0.147)				(0.081)			(0.067)
G7						0.648	0.566	0.501	0.389		0.696			0.560
						(0.213)	(0.442)	(0.224)	(0.193)		(0.051)			(0.115)
G8						0.500					0.557			0.528
						(0.259)					(0.313)			(0.203)
Species	0.410		0.542		0.394	0.500	0.496	0.398	0.356		0.529			0.455
Mean	(0.129)		(0.149)		(0.218)	(0.052)	(0.164)	(0.075)	(0.101)		(0.041)			(0.060)

[a] Four species of *Sicydium* occur in Puerto Rico, combined here.

Table 10. Spring density (fish/ha) estimates for Puerto Rico stream fishes at 12 sampling sites during 2005-2006 in the Río Cañas and Río Guanajibo drainages. Standard error estimates appear in parentheses.

Site	American eel	Bluegill	Largemouth bass	Fat snook	Mozambique tilapia	Bigmouth sleeper	Smallscaled spinycheek sleeper	River goby	Sirajo goby[a]	Burro grunt	Mountain mullet	Green swordtail	Guppy	Total
C1								56.8	11,475.0			116.5	69.9	11,718.2
								(9.6)	(374.5)			(0)	(0)	(374.6)
C2	55.2					294.5		295.0	3,029.0		3,212.0			6,885.7
	(0)					(145.7)		(132.7)	(234.5)		(15.3)			(306.7)
C3	246.4					1,710.0		787.9	3,844.0		5,083.8			11,672.1
	(17.4)					(46.5)		(103.0)	(35.5)		(22.1)			(121.8)
C4	53.5					466.6	212.1	592.7	9,080.9		17,087.0			27,492.8
	(5.1)					(33.3)	(257.2)	(28.8)	(55.2)		(255.4)			(369.3)
G1			807.0		22.6			90.5	208.0			22.6	22.6	1,173.4
			(26.9)		(0)			(0)	(51.1)			(0)	(0)	(57.8)
G2	169.0				6.8	347.0		974.0	20.4	6.8	1,947.9	6.8		3,478.7
	(119.4)				(0)	(25.1)		(111.8)	(0)	(0)	(88.0)	(0)		(187.4)
G3								25.4	76.1			1,599.0	484.0	2,184.5
								(0)	(0)			(144.5)	(102.6)	(177.2)
G4						139.4		265.6	82.4		2,807.0	20.5		3,314.9
						(50.8)		(3.1)	(4.8)		(23.5)	(0)		(56.2)
G5	45.9				76.5	287.9	15.3	1,544.0	94.6		10,544.0	775.1	76.5	13,459.7
	(0)				(0)	(25.0)	(0)	(17.6)	(10.0)		(48.4)	(34.5)	0	(67.5)
G6	37.8					305.5	104.9	197.4	20.2		2,117.9	90.0	10.1	2,883.8
	(28.7)					(57.3)	(162.9)	(22.3)			(51.7)	(0)	(0)	(183.9)
G7	57.5					734.1	28.7	544.5	453.8		3,537.0	18,018.0	86.2	23,459.8
	(0)					(332.2)	(0)	(83.4)	(205.6)		(22.5)	(73,885.2)	(0)	(73,886.2)
G8	11.6			13.1		86.9	91.7	59.9		13.1	24.6			301.0
	(0)			(0)		(172.5)	(0)	(93.1)		(0)	(6.1)			(196.1)

[a] Four species of *Sicydium* occur in Puerto Rico, combined here.

Table 11. Summer density (fish/ha) estimates for Puerto Rico stream fishes at 12 sampling sites during 2005-2006 in the Río Cañas and Río Guanajibo drainages. Standard error estimates appear in parentheses.

Site	American eel	Bluegill	Largemouth bass	Fat snook	Mozambique tilapia	Bigmouth sleeper	Smallscaled spinycheek sleeper	River goby	Sirajo goby[a]	Burro grunt	Mountain mullet	Green swordtail	Guppy	Total
C1								20.5	3,011.7				63.0	3,095.2
								(0)	(336.0)					(336.1)
C2	79.4					134.4		329.0	395.0		4,015.0			4,952.8
	(40.8)					(44.3)		(0)	(0)		(676.0)			(678.7)
C3	462.0					759.0		498.0	18.5		3,159.0			4,896.5
	(1,110.0)					(1,346.5)		(324.8)	(0)		(477.9)			(1,838.2)
C4	388.9					2,681.0	49.7	307.6	624.7		4,026.5			8,078.4
	(236.1)					(3,580.5)	(0)	(21.3)	(32.3)		(488.6)			(3,621.6)
G1			452.8					15.3	180.6					648.7
			(395.0)					(0)	(10.4)					(395.2)
G2	157.0	7.5				90.6		82.6			758.5			1,096.2
	(142.3)	(0)				(19.2)		(18.7)			(25.9)			(147.1)
G3									37.5			1,044.0	56.3	1,137.8
									(0)			(1,050.7)	(0)	(1,050.7)
G4	15.3					107.0		122.3	76.5		4,761.7	18.8		5,101.6
	(0)					(0)		(0)	(0)		(827.3)	(0)		(827.3)
G5	63.8				37.6	324.6		224.9	13.9		4,289.9			4,954.7
	(23.3)				(32.6)	(174.2)		(0)	(4.7)		(1,027.2)			(1,042.6)
G6	55.6				41.7	141.1	166.8	69.5	13.9		2,314.4			2,803.0
	(48.2)				(0)	(37.4)	(0)	(0)	(0)		(247.8)			(255.2)
G7	13.9					137.4		167.1	195.8		2,074.3			2,588.5
	(0)					(142.4)		(12.4)	(86.5)		(121.9)			(206.8)
G8	11.6					25.3	34.8			11.6	670.4			753.7
	(0)					(8.6)	(0)			(0)	(1,825.0)			(1,825.0)

[a] Four species of *Sicydium* occur in Puerto Rico, combined here.

Table 12. Fall density (fish/ha) estimates for Puerto Rico stream fishes at 12 sampling sites during 2005-2006 in the Río Cañas and Río Guanajibo drainages. Standard error estimates appear in parentheses.

	American eel	Bluegill	Largemouth bass	Fat snook	Mozambique tilapia	Bigmouth sleeper	Smallscaled spinycheek sleeper	River goby	Sirajo goby[a]	Burro grunt	Mountain mullet	Green swordtail	Guppy	Total
C1								23.5	2,166.0					2,189.5
								(0)	(674.9)					(674.9)
C2	95.4					233.0		62.3	378.0		2,231.0			2,999.7
	(40.8)					(10.0)		(22.8)	(192.6)		(11.3)			(198.7)
C3	188.3					736.0		758.0	181.0		2,746.0			4,609.3
	(50.8)					(63.3)		(158.1)	0		(56.3)			(186.4)
C4	60.9					962.0	90.9	705.0	770.0		1,968.0			4,556.8
	(29.1)					(19.6)	(8.3)	(22.2)	(110.1)		(20.4)			(119.7)
G1			397.9					50.8	308.9					757.6
			(104.9)					(0)	(174.8)					(203.8)
G2	70.1				6.9	287.0	7.5	90.1		6.9	474.6	13.8		956.9
	(14.8)				(0)	(141.9)	(0)	(37.5)		(0)	(103.3)	(0)		(180.1)
G3									74.8			117.8	16.8	209.4
									(64.8)			(0)	(0)	(64.8)
G4						261.0		86.8	189.6		1,565.7			2,103.1
						(19.0)		(24.6)	(34.7)		(84.7)			(96.6)
G5	9.5				26.1	186.2		248.9	57.4		2,896.0	75.9		3,500.0
	(0)				(27.0)	(38.8)		(91.2)	(74.6)		(296.0)	(0)		(322.1)
G6	220.8				108.2	339.0	185.6	153.4	13.9		2,379.0			3,399.9
	(83.9)				(39.6)	(72.1)	(29.6)	(0)	(0)		(355.1)			(375.2)
G7	59.7					347.0	25.7	180.9	149.7		1,612.0	106.0	11.8	2,492.8
	(0)					(87.6)	(8.7)	(182.9)	(36.5)		(182.9)	(0)	(0)	(275.7)
G8	47.6			11.9		95.2	154.7	47.6			57.0			414.0
	(0)			(0)		(24.5)	(0)	(0)			(29.5)			(38.3)

[a] Four species of *Sicydium* occur in Puerto Rico, combined here.

Table 13. Spring biomass (kg/ha) estimates for Puerto Rico stream fishes at 12 sampling sites during 2005-2006 in the Río Cañas and Río Guanajibo drainages. Standard error estimates appear in parentheses.

Site	American eel	Bluegill	Largemouth bass	Fat snook	Mozambique tilapia	Bigmouth sleeper	Smallscaled spinycheek sleeper	River goby	Sirajo goby[a]	Burro grunt	Mountain mullet	Green swordtail	Guppy	Total
C1								2.3	14.8			0.1	0.001	17.2
								(0.5)	(1.4)			(0)	(0)	(1.5)
C2	6.8					30.6		7.0	2.1		95.6			142.1
	(0.4)					(30.2)		(3.2)	(0.2)		(2.6)			(30.5)
C3	48.4					100.2		19.3	1.4		286.0			455.3
	(4.5)					(13.7)		(4.6)	(0.1)		(8.6)			(17.4)
C4	4.0					28.4	7.0	3.8	6.9		118.4			168.5
	(0.8)					(2.5)	(8.5)	(0.3)	(0.2)		(3.2)			(9.5)
G1			83.9		3.7			10.0	4.2			0.005	0.001	101.8
			(3.9)		(0)			(0)	(1.7)			(0)	(0)	(4.3)
G2	32.0				1.1	26.6		15.3	0.1	10.1	48.4	0.01		133.6
	(28.6)				(0)	(2.9)		(5.0)	(0)	(0)	(2.4)	(0)		(29.3)
G3								5.8	2.5			0.6	0.1	8.9
								(0)	(0)			(0.2)	(0)	(0.2)
G4						37.5		4.1	1.6		44.5	0.020		87.8
						(24.3)		(0.3)	(0.4)		(3.6)	(0)		(24.6)
G5	11.1				3.9	30.0	1.1	37.0	0.8		183.0	1.1	0.007	267.9
	(0.7)				(0)	(4.8)	(0)	(2.1)	(0.1)		(4.3)	(0.1)	(0)	(6.8)
G6	4.5					17.6	3.8	4.4	0.5		28.8	0.2	0.001	59.8
	(2.2)					(4.9)	(6.0)	(0.9)	(0.03)		(1.3)	(0)	(0)	(8.2)
G7	6.3					26.7	1.5	14.4	7.6		38.9	19.5	0.008	114.9
	(0)					(25.0)	(0)	(7.6)	(2.6)		(1.8)	(79.8)	(0)	(84.0)
G8	0.3			0.2		7.7	1.2	1.0		0.3	0.2			10.9
	(0)			(0)		(16.4)	(0)	(1.7)		(0)	(0.1)			(16.5)

[a] Four species of *Sicydium* occur in Puerto Rico, combined here.

Table 14. Summer biomass (kg/ha) estimates for Puerto Rico stream fishes at 12 sampling sites during 2005- 2006 in the Río Cañas and Río Guanajibo drainages. Standard error estimates appear in parentheses.

Site	American eel	Bluegill	Largemouth bass	Fat snook	Mozambique tilapia	Bigmouth sleeper	Smallscaled spinycheek sleeper	River goby	Sirajo goby[a]	Burro grunt	Mountain mullet	Green swordtail	Guppy	Total
C1								0.9	12.1				0.030	13.0
								(0)	(1.2)				(0)	(1.2)
C2	12.5					9.3		3.3	2.8		94.0			121.9
	(5.2)					(2.6)		(0)	(0)		(17.9)			(18.8)
C3	151.1					113.0		31.1	0.3		225.5			521.0
	(365.5)					(116.4)		(10.0)	(0)		(30.7)			(384.9)
C4	182.9					250.7	2.2	6.9	1.6		177.6			621.9
	(139.8)					(369.9)	(0)	(0.6)	(0.2)		(28.7)			(396.5)
G1			20.8					1.0	3.1					24.9
			(19.7)					(0)	(0.2)					(19.7)
G2	44.1	1.0				8.6		2.1			20.1			75.9
	(43.4)	(0)				(1.1)		(0.8)			(2.4)			(43.5)
G3									1.5			0.644	0.005	2.1
									(0.3)			(0.7)	(0)	(0.7)
G4	1.9					13.1		3.1	1.8		158.4	0.002		178.3
	(0)					(0)		(0)	(0)		(82.6)	(0)		(82.6)
G5	16.3				10.6	41.4		62.5	0.1		172.7			303.6
	(2.6)				(10.4)	(18.8)		(0)	(0.1)		(34.4)			(40.7)
G6	10.0				3.2	14.8	5.7	1.2	0.2		82.6			117.7
	(8.7)				(0)	(2.2)	(0)	(0)	(0)		(18.2)			(20.3)
G7	1.1					15.9		1.8	2.7		34.6			56.1
	(0)					(20.2)		(0.2)	(1.3)		(2.5)			(20.4)
G8	0.5					4.1	0.7			17.4	7.5			30.1
	(0)					(1.7)	(0)			(0)	(20.8)			(20.9)

[a] Four species of *Sicydium* occur in Puerto Rico, combined here.

Table 15. Fall biomass (kg/ha) estimates for Puerto Rico stream fishes at 12 sampling sites during 2005-2006 in the Río Cañas and Río Guanajibo drainages. Standard error estimates appear in parentheses.

Site	American eel	Bluegill	Largemouth bass	Fat snook	Mozambique tilapia	Bigmouth sleeper	Smallscaled spinycheek sleeper	River goby	Sirajo goby[a]	Burro grunt	Mountain mullet	Green swordtail	Guppy	Total
C1								1.2	12.1					13.3
								(0)	(1.1)					(1.1)
C2	22.2					20.5		3.5	2.0		70.4			118.6
	(12.1)					(2.1)		(2.3)	(1.2)		(1.5)			(12.6)
C3	21.7					62.3		1.4	1.2		142.6			229.2
	(4.4)					(4.2)		(2.6)	(0)		(3.7)			(7.6)
C4	12.7					38.5	4.2	5.9	3.3		38.6			103.2
	(6.8)					(1.3)	(0.7)	(0.4)	(0.5)		(1.3)			(7.1)
G1			24.1					2.7	4.3					31.1
			(4.0)					(1.7)	(0.9)					(6.1)
G2	10.3				2.0	29.2	0.3	1.9		10.3	18.3	0.023		72.3
	(3.4)				(0)	(19.5)	(0)	(0.2)		(0)	(4.9)	(0)		(20.4)
G3									1.5			0.1	0.003	1.6
									(1.5)			(0)	(0)	(1.5)
G4						27.7		1.9	2.3		46.5			78.4
						(1.6)		(0.8)	(0.6)		(5.0)			(5.3)
G5	5.4				0.7	34.1		6.6	1.2		66.3	0.1		114.4
	(0)				(0.9)	(13.7)		(2.9)	(1.7)		(4.7)	(0)		(14.9)
G6	26.1				8.3	33.1	6.8	4.9	0.3		54.5			134.0
	(15.4)				(2.6)	(9.8)	(1.3)	(0)	(0)		(5.0)			(19.1)
G7	13.3					17.4	2.2	9.4	1.9		22.1	0.1	0.001	66.4
	(2.6)					(1.5)	(0.8)	(11.7)	(0.6)		(1.1)	(0)	(0)	(12.2)
G8	2.5			1.9		5.4	2.7	0.3			1.2			14.0
	(0.7)			(0)		(2.5)	(0)	(0)			(0.4)			(2.6)

[a] Four species of *Sicydium* occur in Puerto Rico, combined here.

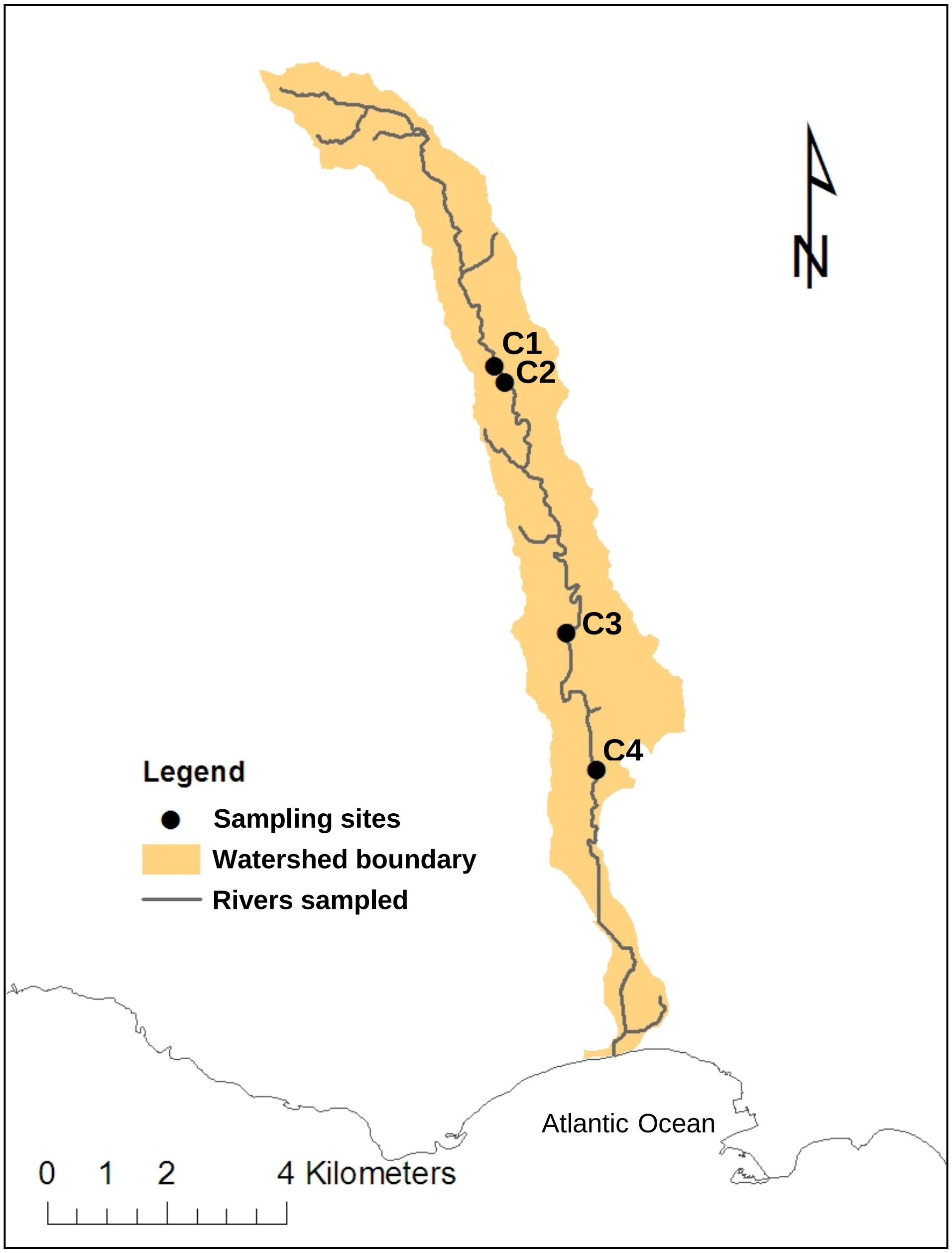

Figure 1. Four fish, water quality, and instream habitat sampling sites (C1-C4) within the Río Cañas watershed, a major tributary of Río Matilde near Ponce, Puerto Rico.

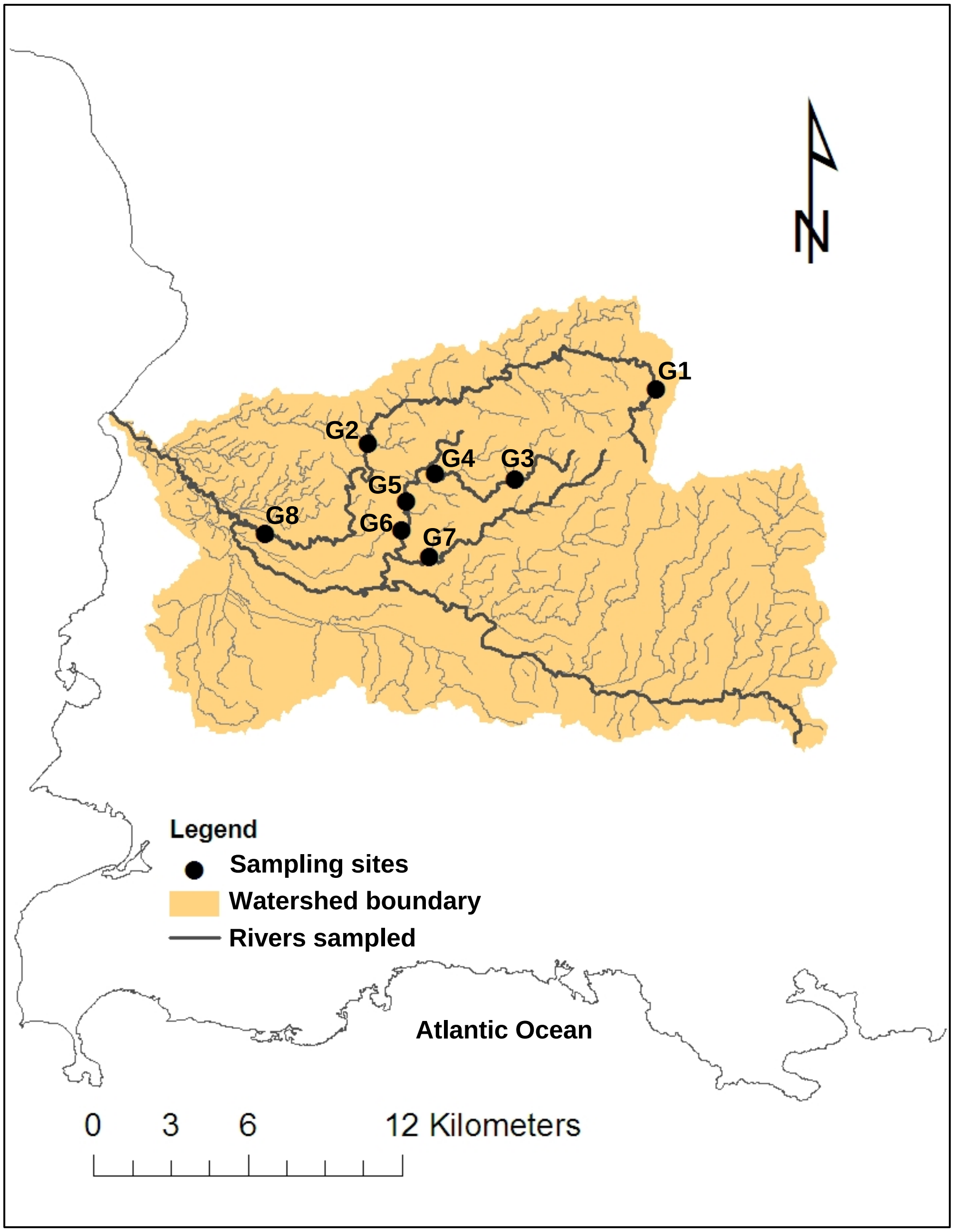

Figure 2. Eight fish, water quality, and instream habitat sampling sites (G1-G8) within the Río Guanajibo watershed near Mayagüez, Puerto Rico.

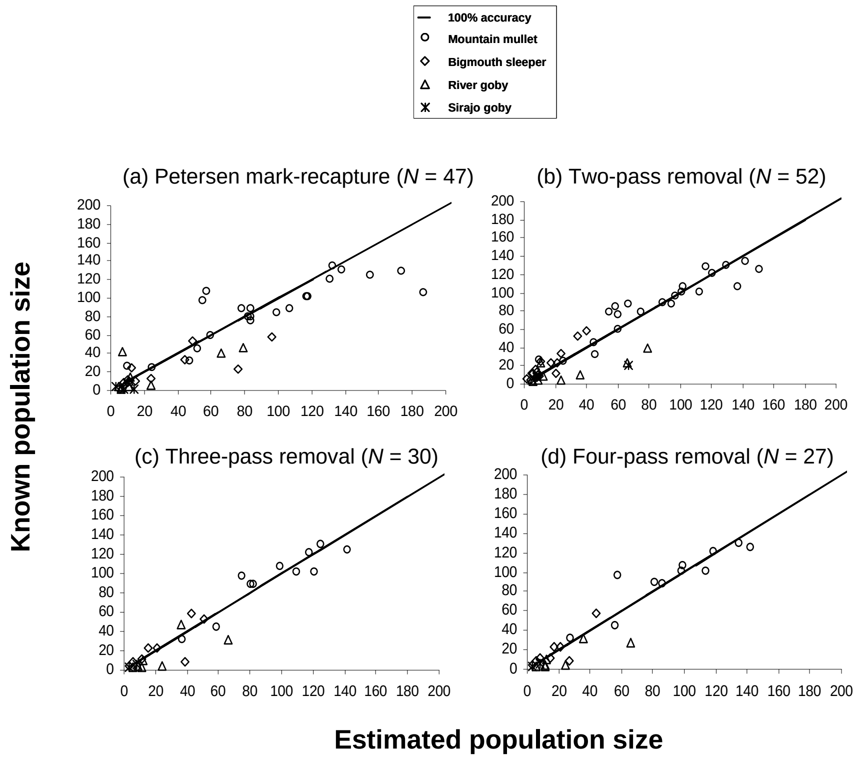

Figure 3. Accuracy assessment of four population models for estimating population size of Puerto Rico stream fishes. Points falling on the diagonal line represents high accuracy. Those above the line indicate underestimation, and those below the line are overestimates of population size.

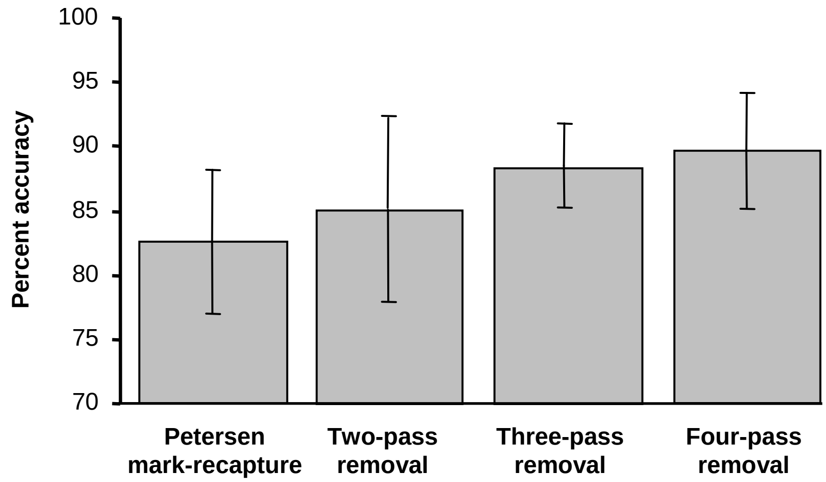

Figure 4. Mean percent accuracy of Petersen mark-recapture (N = 47), two-pass (N = 52), three-pass (N = 30), and four-pass removal (N = 27) models to estimate population size of Puerto Rico stream fishes. Error bars represent 95% confidence intervals.

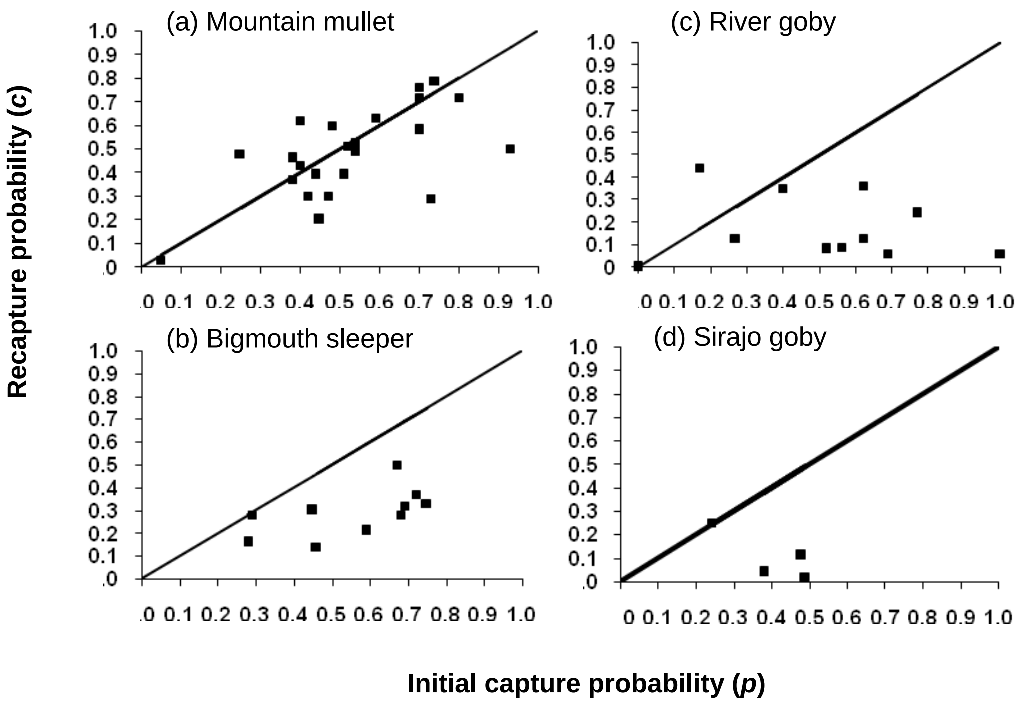

Figure 5. Plots of initial capture probability versus recapture probability to assess behavioral response of four Puerto Rico stream fishes to electrofishing gear. Points above the diagonal line of equal capture probability indicate a "trap-happy" response, and those below indicate "trap-shy" behavior.

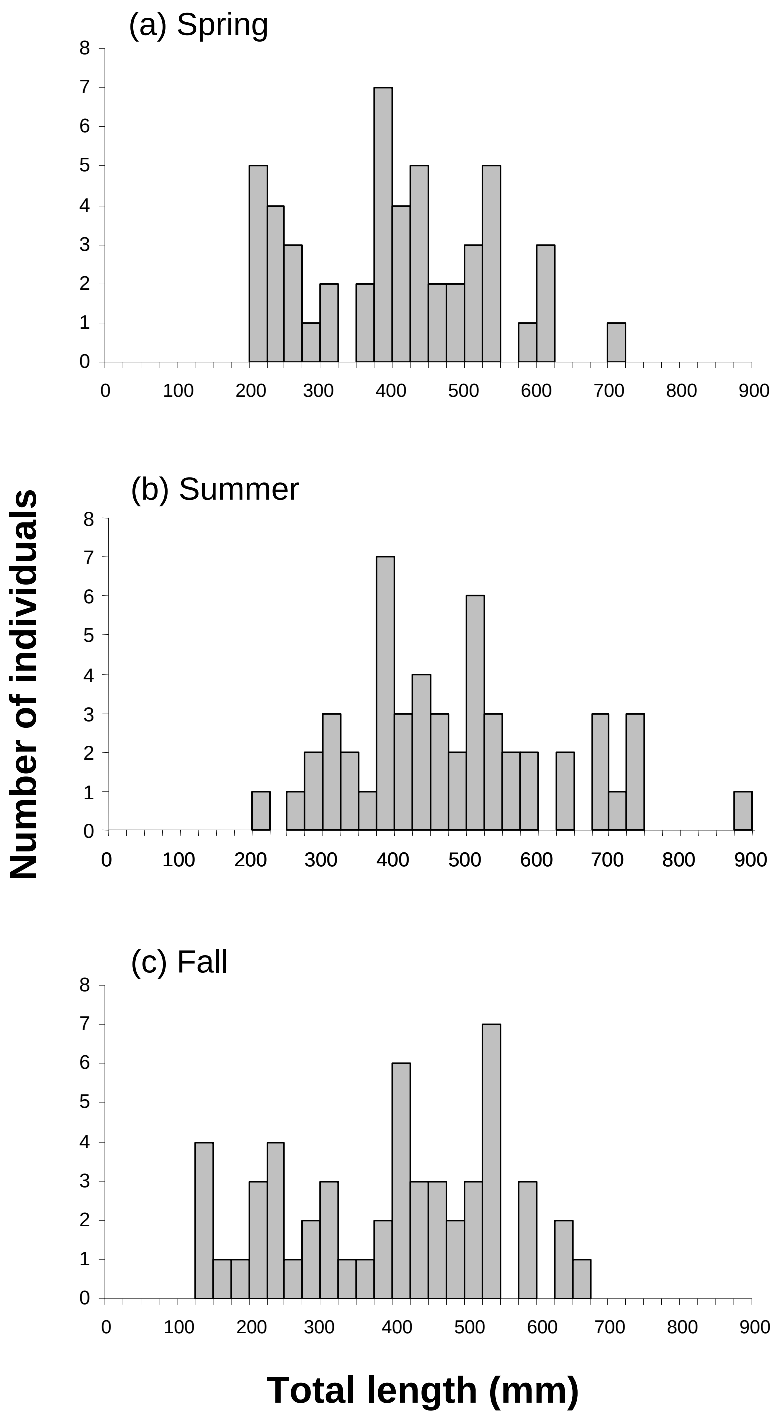

Figure 6. Length-frequency histograms of American eel combined for populations from nine sampling sites in Río Cañas (three sites) and Río Guanajibo (six sites) among three seasons during 2005-2006.

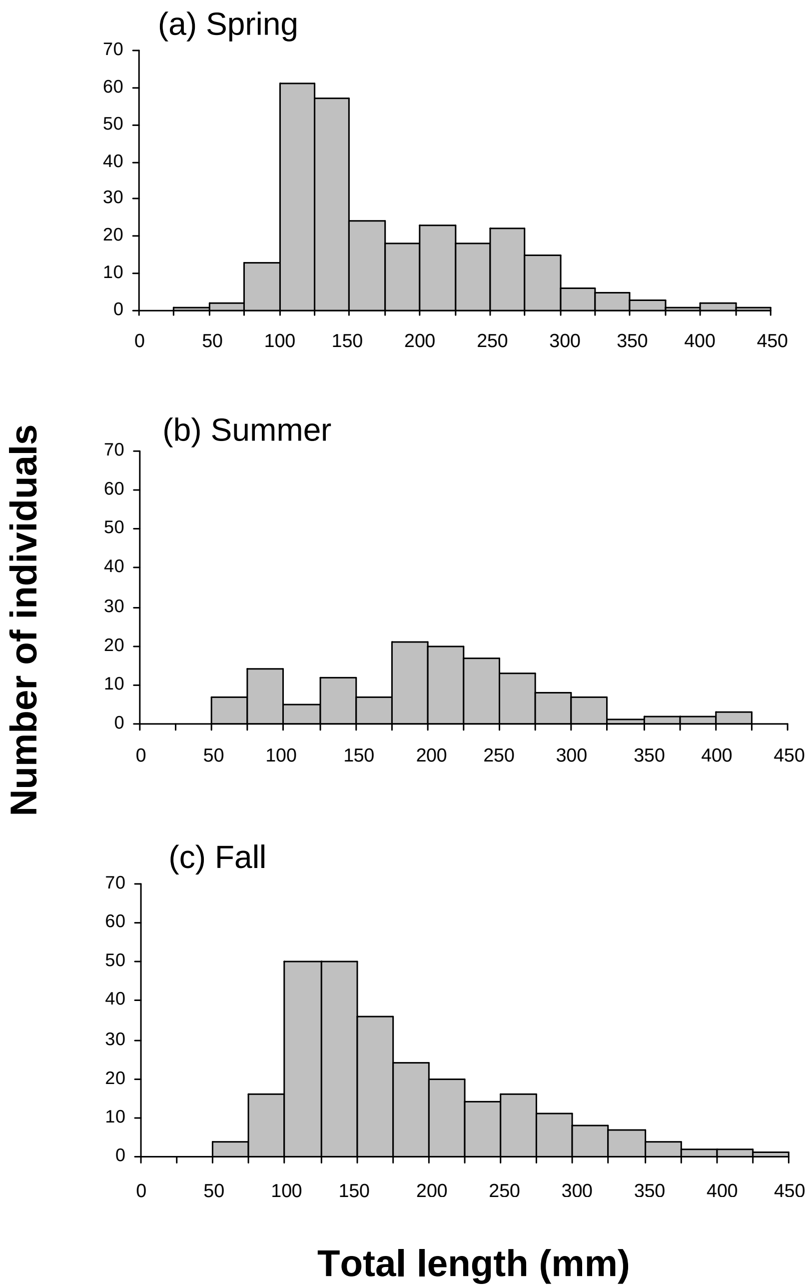

Figure 7. Length-frequency histograms of bigmouth sleeper combined for populations from nine sampling sites in Río Cañas (three sites) and Río Guanajibo (six sites) among three seasons during 2005-2006.

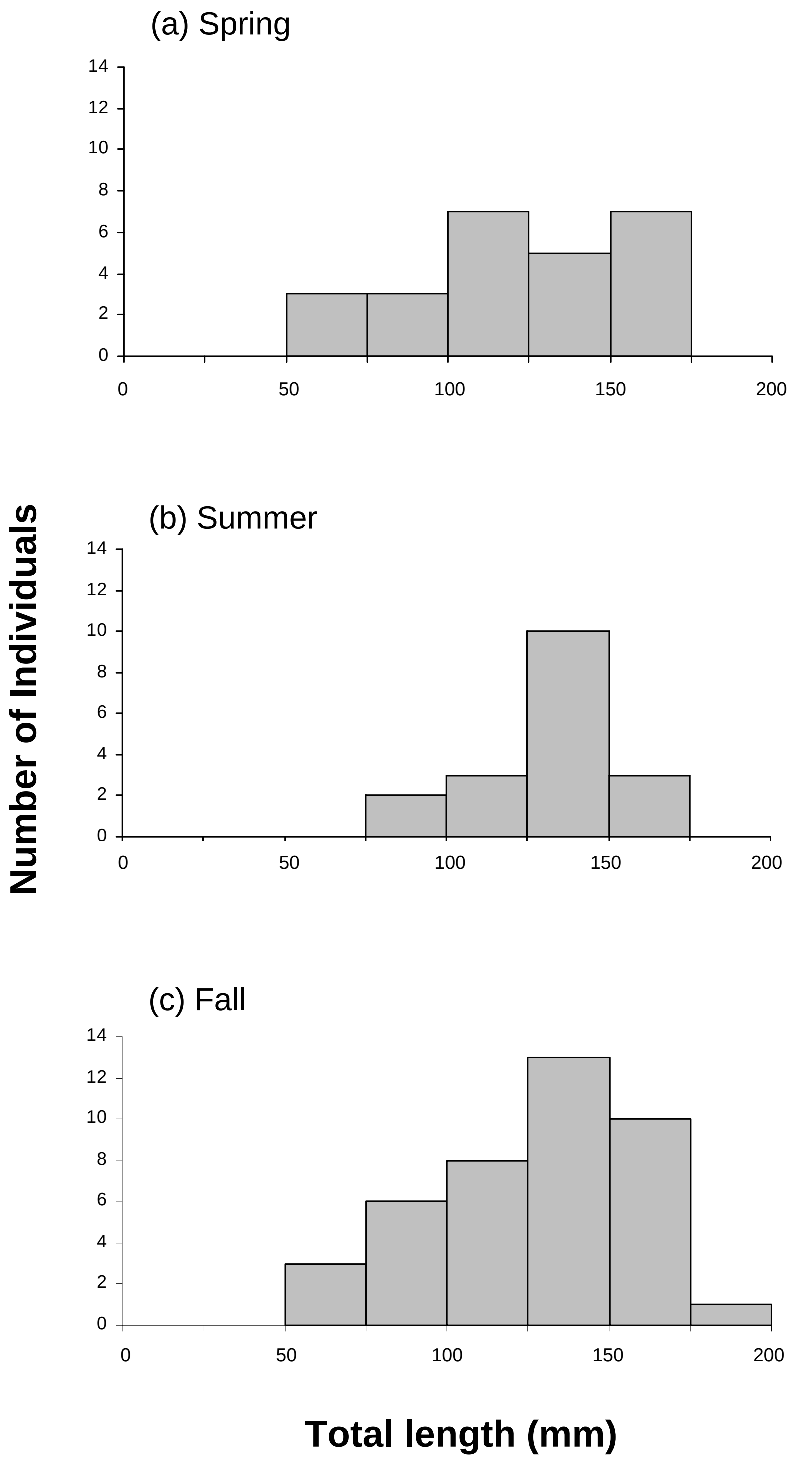

Figure 8. Length frequency-histograms of smallscaled spinycheek sleeper combined for populations from six sampling sites in Río Cañas (one site) and Río Guanajibo (five sites) among three seasons during 2005-2006.

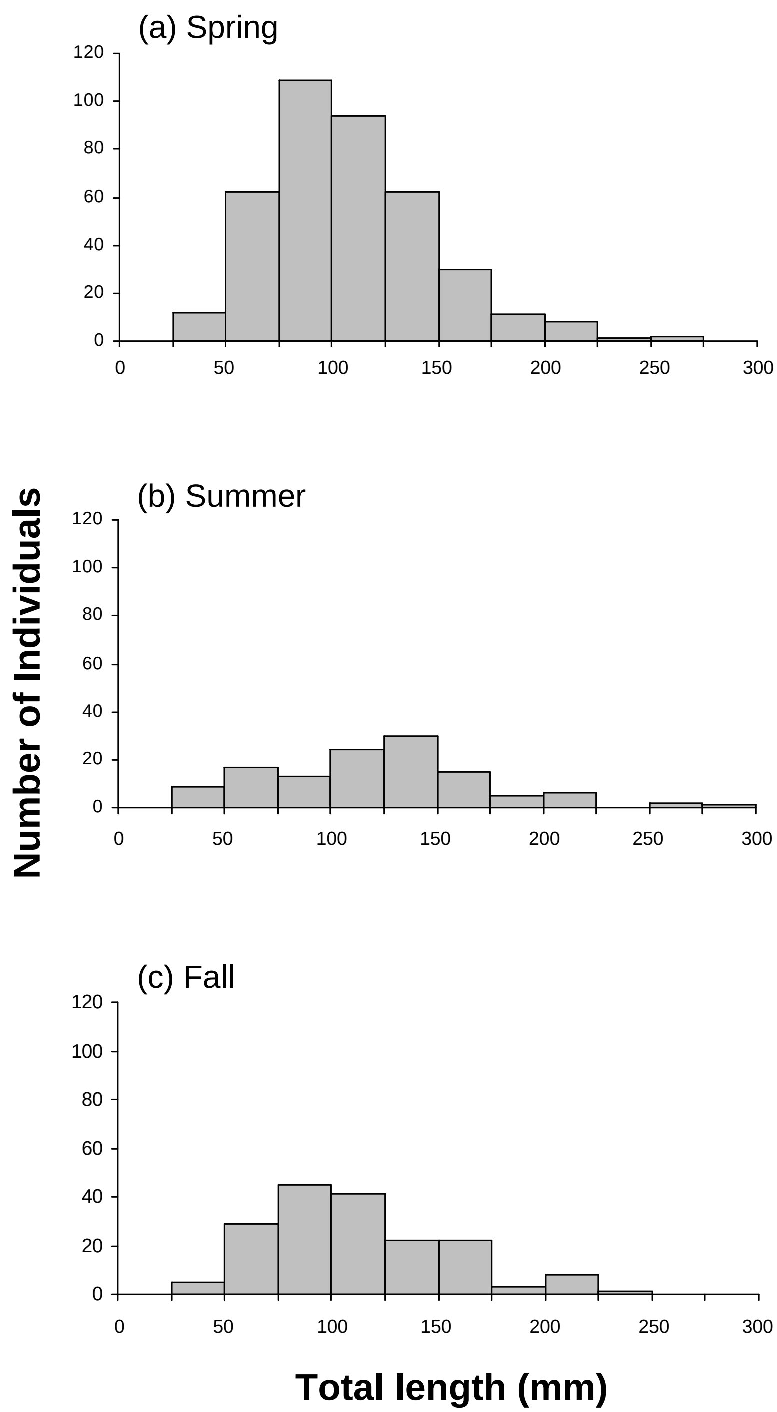

Figure 9. Length-frequency histograms of river goby combined for populations from 12 sampling sites in Río Cañas (four sites) and Río Guanajibo (eight sites) among three seasons during 2005-2006.

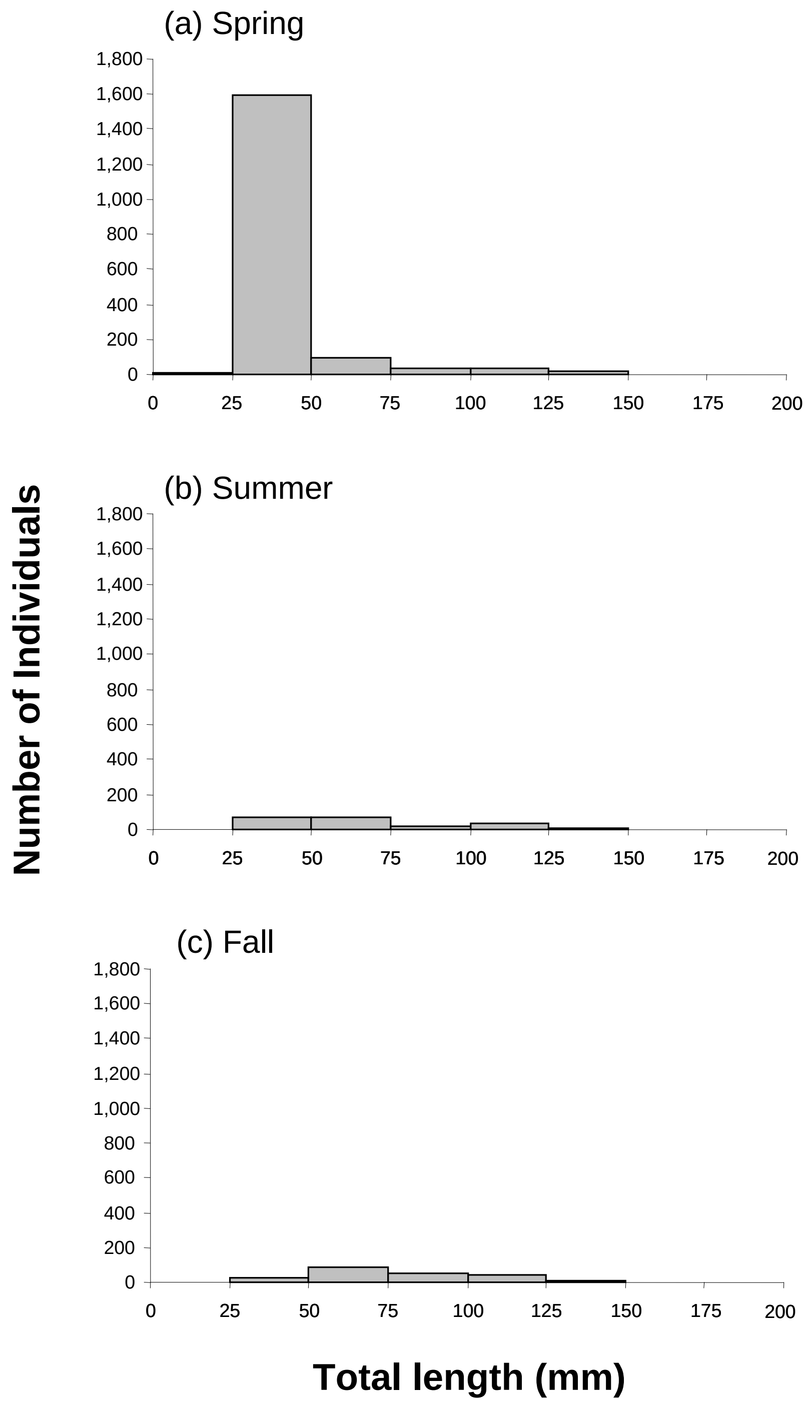

Figure 10. Length-frequency histograms of sirajo goby combined for populations from 11 sampling sites in Río Cañas (four sites) and Río Guanajibo (seven sites) among three seasons during 2005-2006.

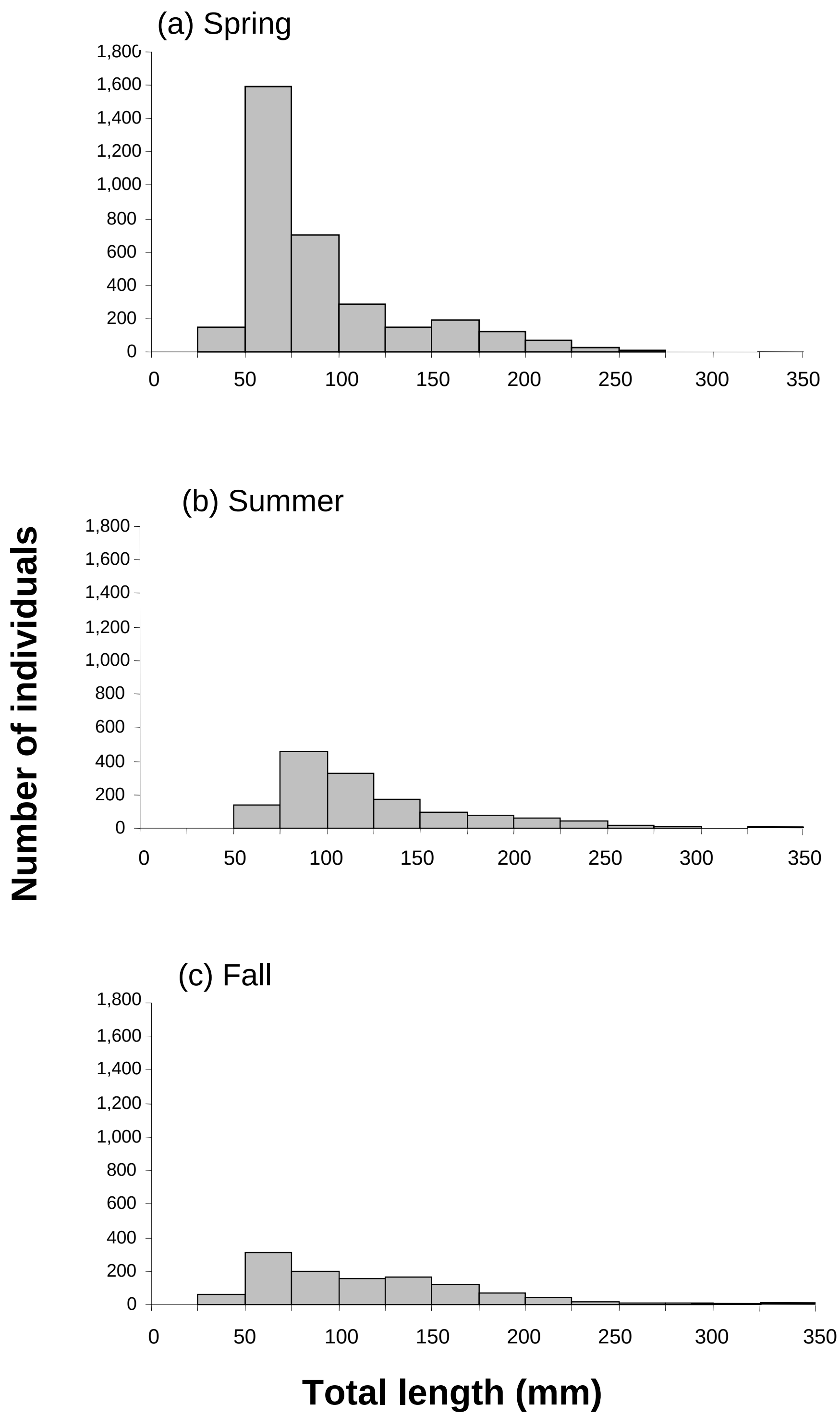

Figure 11. Length-frequency histograms of mountain mullet combined for populations from nine sampling sites in Río Cañas (three sites) and Río Guanajibo (six sites) among three seasons during 2005-2006.

CHAPTER 2
INFLUENCES OF INSTREAM HABITAT AND WATER QUALITY PARAMETERS ON ELECTROFISHING CATCHABILITY OF PUERTO RICO STREAM FISHES

Introduction

Accurate estimates are necessary to describe and understand stream fish communities and guide management decisions. This is especially true for warmwater streams that typically receive less attention from fisheries biologists and management agencies (Fisher et al.1998; Rabeni and Jacobson 1999). Warmwater streams are susceptible to anthropogenic impacts including sedimentation, erosion, pollution, and altered flow regimes, yet these waters are utilized for recreational fishing, subsistence fishing, swimming, and drinking water (Rabeni and Jacobson 1999). Unfortunately, warmwater stream management agencies generally receive a limited budget that is disproportionate to the economic value of those fisheries (Fisher et al. 1998), so sampling techniques must be both cost efficient and effective.

A vast number of organisms occur within the tropical streams of Puerto Rico, including fishes, crustaceans, mollusks, and other freshwater organisms. There are about 80 fish species that inhabit the freshwaters of Puerto Rico, and many of these have commercial or sport fish value. Some of these fishes also serve as a food source in important recreational and subsistence fisheries. However, few studies have been conducted on fishes in the streams of Puerto Rico, making it difficult to manage them in these systems. Quantitative estimates of stream fish populations can be used to assess the well being of fish communities and their habitats.

There are fewer than 10 native diadromous fish species that reside within Puerto Rico rivers, and they are of primary management concern and provide sport fishing opportunities and cultural value to the people of Puerto Rico. Most of the native riverine fishes are amphidromous, spending their adult life in streams, and larvae migrate to the estuaries, while one, the American eel *Anguilla rostrata* is catadromous, living in freshwater and spawning in the ocean (March 2003). Native species that utilize both upper and lower stream reaches include gobies (Gobiidae), sleepers (Eleotridae), mountain mullets (Mugilidae), and eels (Anguillidae) (Holmquist et al. 1998). Upstream reaches are dominated by sirajo goby *Sicydium plumieri,* whereas lower stream reaches are dominated by mountain mullet *Agonostomus monticola*, American eel, bigmouth sleeper *Gobiomorus dormitor*, and river goby *Awaous banana* (Holmquist et al. 1998). Bigmouth sleeper is the only one of these species that is known to be able to complete its entire life cycle in a riverine and reservoir environment (Bacheler et al. 2004). Mountain mullet is a recreationally important amphidromous fish, spawning in early summer and returning to upper stream reaches as an adult (Corujo Flores 1980; Erdman 1984). Sirajo goby and river goby have a modified ventral sucker disc that allows them to climb waterfalls or dams with any flow or leakage and return to upper stream reaches. The larvae of these fishes are a local delicacy (Keith 2003). American eels are catadromous and found in lowland stream reaches (Erdman 1972). The smallscaled spinycheek sleeper *Eleotris perniger* and fat sleeper *Dormitator maculatus* are two native stream fishes restricted to lower reaches or brackish waters (Corujo Flores 1980). To guide management of these valuable resources, the proper sampling gears and techniques must be selected to accurately estimate abundance and occurrence of native fish populations.

Electrofishing is a common gear used to collect and assess fish abundance through catch per unit effort indices or by either mark-recapture or removal population estimating methods. However, electrofishing efficiency is influenced by biological, environmental, and technical factors (Hubert 1996; Fievet et al. 1999; Peterson et al. 2004), and this is especially important to consider when sampling fish communities (Kwak and Peterson 2007). Influential biological factors include fish morphology, physiology, and behavior. Capture efficiency of electrofishing is affected by fish size and favors capture of larger individuals and species (Bohlin 1982; Anderson 1995; Peterson et al. 2004). Environmental factors that influence electrofishing may be related to both instream habitat and water quality parameters. Electrofishing efficiency is known to be inversely related to water depth. Turbidity may exhibit a bell-shaped curve with gear efficiency, because in clear waters fish may detect sampling personnel, but as water becomes more turbid, fish detectability decreases (Reynolds 1996). The morphology of the stream, water quality, and the species being sampled all effect the efficiency of the electrofishing gear, and this affect directly impacts the catchability of the fish.

Capture probability refers to the probability of an individual fish being captured by a gear in a particular sample (Williams et al. 2002; Pine et al. 2003). Capture probability can be estimated by either mark-recapture or from successive removal passes. In a two-sample mark-recapture study using the Lincoln-Petersen estimator, capture probability in the first sample ($\hat{p}_1$) is calculated as the quotient of the number of fish captured and marked in the first sample (n_1) and the population estimate from the mark-recapture event ($\hat{N}$) as

$$\hat{p}_1 = n_1 / \hat{N}, \text{ whereas}$$

$$\hat{p}_2 = n_2 / \hat{N}.$$

In cases of more than two samples, there is a suite of models available to estimate capture probability (Otis et al. 1978). Using a removal procedure based on successive sampling without replacement, capture probability is estimated using a maximum-likelihood approach. Although both of these methods estimate capture probability, it is important to consider that these estimates are influenced by the dynamics of the habitat and the stream fish being sampled, and failure to account for such differences can significantly misrepresent population abundances (Peterson et al. 2004).

If scientists can conduct intensive mark-recapture or removal studies to estimate capture probability, then they may utilize single-pass electrofishing to estimate fish abundance, which will reduce the cost of sampling and time spent in the field (Kruse et al. 1998; Dauwalter and Fisher 2007). Whereas, a single mark-recapture event can be used to estimate populations, it requires more effort than single-pass electrofishing and assumes equal capture probability among samples (Dauwalter and Fisher 2007). In cases where capture probability does not vary greatly among sites, successive removal samples can be used to estimate capture probability and then be applied to single-pass electrofishing at a larger subset of sites (Jones and Stockwell 1995). A simple model using single-pass catch (C) can be applied to estimate a fish population ($\hat{N}$) by dividing the catch by the capture probability ($\hat{p}$) of that fish as

$$\hat{N} = C / \hat{p}.$$

Kruse et al. (1998) utilized this method to estimate fish populations in trout streams that had sparse habitat and low densities. However, they cautioned against using this method in complex habitats. Stream habitat, width, and depth have been shown to affect stream fish capture probability, and this effect may vary among species. Dauwalter and Fisher (2007) found that capture probability of stream fish varied related to substrate composition and channel geomorphology and that the effects of those parameters varied among species. Single-pass electrofishing data can be used to predict stream fish abundance in complex habitats, when variation in capture probability related to environmental parameters is considered and incorporated into a developed regression model (Lobón-Cerviá and Utrilla 1993; Reid et al. 2008).

In this study, I followed the standardized sampling protocol developed in Chapter 1 at 81 Puerto Rico stream sampling sites to estimate fish catchability within a broad range of habitats and sampling conditions. From these data, I developed empirical, hierarchical models that describe relationships between fish catchability and instream habitat and water quality parameters. For each native fish species, I sought to determine the most parsimonious overall model that explained variation in catchability. The application of these models is two-fold. First, a better understanding of fish catchability and factors that affect it will define the quality of stream fish population data as applied to management decisions. And second, these developed models more accurately quantify variation in fish catchability among streams and sites and may be utilized to estimate fish populations based on single-pass electrofishing data, thus reducing time spent in the field and financial cost of fish sampling. It is my overall goal that these findings improve our understanding and conservation of Puerto Rico stream fishes.

Methods

Sampling Sites

Sampling sites included 81 Puerto Rico stream reaches located in 34 of 46 river drainages (Figure 1). Sampling occurred among six seasons from summer 2005 to spring 2007. Twelve of the sampling sites were sampled during three seasons (summer 2005, fall 2005, and spring 2006); eight of these were located in the Río Guanajibo drainage basin (sites 35A-H, Figure 1), and four sites were in the Río Cañas river drainage (sites 28A-D, Figure 1). This intensive sampling was in conjunction with research described in Chapter 1. The remaining 69 sites were sampled once among three sampling seasons (summer 2006, fall 2006, and spring 2007). Thus my total effort was 105 sampling occasions from June 2005 to April 2007. Each sampling site was a wadeable reach that incorporated multiple microhabitats habitats (run, pool, and riffle); sites varied in elevation and geomorphology to represent the diverse stream habitat located within the island (Figure 1).

Fish Sampling Procedures

I sampled stream fish using electrofishing techniques following a three-pass removal protocol during six seasons, spring (2006 and 2007), summer (2005 and 2006), and fall (2005 and 2006). Sampling among seasons allowed for a representation of a broad range of habitat types and sampling conditions. Two types of electrofishing gear were deployed to capture fish, a backpack electrofisher and a barge electrofisher. The Smith-Root model 12-B, pulsed-DC backpack electrofisher consists of a battery, hand-held anode, and a trailing cathode cable. At each site selected for backpack electrofishing, two backpack units were employed simultaneously operating at about 0.25 A. The Smith-Root SR-6 electrofishing tote barge is a small boat that holds a generator and is pushed by an operator. The barge electrofisher was powered by a Smith-Root GPP 2.5 power source (2.5 kW) and converter that was typically operated at about 3 A. It can power up to three anode probes, and the boat has an attached cathode plate. A minimum of four people operated the barge fisher, and a minimum of three people sampled when using the backpacks. All personnel operating anodes also netted fish, and any additional crew assisted with additional dip nets. The type of gear used at each site was based upon stream width, depth, and substrate composition. All sites selected were shallow enough to effectively sample by wading. Backpacks were most suitable in reaches with large substrate materials (large cobble or boulders) or in reaches of shallow depths and narrow widths. The barge electrofisher was used at all other sites, especially those with few instream impediments (e.g., boulders or physical structure), deep enough draft, and suitable stream width.

I selected sites based on accessibility, stream habitat, and position in the watershed. Sites consisted of at least one pool–riffle sequence and ranged in length from 100 m to 155 m (Lyons and Kanehl 1993; Thompson and Rahel 1996; Thompson 2003). A pool was defined as a deep area of sluggish current that flowed over silt, gravel, cobble, or boulder. A riffle was a shallow area with swift current and surface turbulence that flowed over sand, gravel, or cobble substrates. At each site, 21.3-m by 1.8-m blocknets, with 7-mm mesh knotless nylon, surface floats, and a bottom lead-line, were used to close off both upstream and downstream ends of the sampling site. I assumed that blocknets formed a closed system for sampling purposes by preventing fish movement (Heimbuch et al. 1997). Sites with natural barriers, such as a waterfall or a low-head dam, eliminated the need for a blocknet at that barrier.

Once a site was closed and the proper gear was selected, three upstream electrofishing passes of equal effort (by time) were conducted, and fish of all species and sizes were collected. Following the first and second pass, fish were weighed (g), measured (total length, mm), and held. Following the third pass, fish collected were weighed, and measured, and then all fish were released into the sampling reach.

Instream Habitat Surveys

I characterized habitat by a cross-sectional transect survey at each sampling site (McMahon et al. 1996). Ten cross-sectional transects within each sampling reach were measured and spaced at a distance apart equal to one stream width. Placement of the first transect was within the downstream 1/10 of the sampling reach with the exact point chosen randomly. I measured microhabitat parameters at least 10 equally-spaced points on each transect.

Habitat characteristics measured were bank angle, instream physical cover, substrate composition, water depth, mean column velocity, and stream width (Simonson et al. 1994; McMahon et al. 1996). I used a clinometer to measure bank angle on both banks; if the bank was undercut, the width of the undercut bank was also measured. I visually estimated instream physical cover and substrate composition. Instream physical cover type was visually classified and listed as one of the following: course woody debris, fine woody debris, rootwad, leaf litter, undercut bank, emersed plant, submersed plant, terrestrial plant, boulder, cobble, or trash. Substrate composition was visually classified as the most dominant size class according to particle diameter (mm) following a modified Wentworth scale (Bovee and Milhous 1978). Substrate particle size was classified as one of the following: silt/clay (>0-0.06 mm), sand (0.06-1.00 mm), very course sand (1-2 mm), pea gravel (2-4 mm), fine gravel (4-8 mm), medium gravel (8-16 mm), course gravel (16-32 mm), very course gravel (50-64 mm), small cobble (64-130 mm), large cobble (130-250 mm), small boulder (250-500 mm), medium boulder (500-1,000 mm), large boulder (1,000-2,000 mm), very large boulder (2,000-4,000 mm), and mammoth boulder (>4,000 mm).

I measured stream water depth to the nearest centimeter using a Scientific Instruments, 1.5-m top-setting wading rod, and water velocity was measured using a Marsh-McBirney Flo-Mate Model 2000 digital meter. Mean column velocity was measured at a point 60% of the depth below the surface (McMahon et al. 1996). When depth exceeded 1.0 m, velocity was recorded at 20% and 80% depth below surface, and those rates were averaged for the column mean. Upon completion of the cross-sectional habitat survey, geographic coordinates for the site were recorded using a Garmin Model V Global Positioning System.

Water Quality Analyses

I measured selected water quality parameters at each sampling site. Water temperature (ºC), total dissolved solids (TDS; g/L), conductivity (µS/cm), dissolved oxygen concentration (mg/L), and salinity (ppt) were measured with a Yellow Springs Instrument (YSI) model 556 Multiprobe Instrument. These measurements were taken by lowering the YSI probe into an area of the stream of laminar flow. At each site, a water sample was also collected and placed on ice for subsequent analyses in the lab. A Hach CEL/850 Aquaculture Laboratory was used to measure alkalinity, hardness, turbidity, pH, and concentrations of nitrate, nitrite, nitrogen, and reactive phosphorus. Alkalinity was measured by titrating a sample with phenolthaline as an indicator with sulfuric acid, measuring levels from 10 to 400 mg/L as $CaCO_3$ using a digital titrator. Hardness was measured by a digital titration method using EDTA as an indicator to measure levels from 10 to 400 mg/L as $CaCO_3$. Turbidity was measured in FAU using a DR/850 colorimeter and comparing a deionized water blank to the water sample. Measurements of pH were conducted using a Sension 1 pH meter and were measured to an accuracy of 0.01. Nitrate concentration was measured by a cadmium reduction method detecting concentrations from 0.3 to 30.0 mg/L NO_3^- using a DR/850 colorimeter. Nitrite concentration was measured by a diazotization method to detect concentrations from 0.002 to 0.300 mg/L NO_2^- using the same colorimeter. Ammonia as nitrogen was measured by a salicylate method that detects concentrations from 0.01 to 0.50 mg/L NH_3 using the same colorimeter. The reactive phosphorous method was an orthophosphate ascorbic acid method that measures levels from 0.02 to 2.50 mg/L PO_4^- using the same colorimeter.

Correlation Among Instream Habitat and Water Quality Parameters

For each sampling occasion, instream habitat and water quality parameters were measured, totaling 20 environmental parameters. Many of these parameters were correlated and redundant in their description of stream habitat. To eliminate redundant variables and reduce the number of variables for application to model development, I used a simple linear correlation matrix that included all 20 variables. The matrix examined the relationship among the variables and identified variables that were significantly correlated (correlation coefficient, r). By comparing all correlated variables within the matrix, I was able to reduce 20 variables to seven. Among subsets of correlated variables, I selected the variable that is the most common or simplest to measure, and this was the parameter that was incorporated into model development.

Catchability Estimates

I estimated fish capture probability for each species sampled using a maximum-likelihood estimator for three-pass removal sampling with estimates and variance computed using Pop/Pro Modular Statistical Software (Seber 1982; Kwak 1992). If any population in the community at any site was not depleted in three passes (i.e., the number of fish caught on the last pass exceeded that of the first pass), capture probability was not estimated. If sample size was low (i.e., total number of fish caught was less than 10), capture probability was not estimated. Species mean capture probability was then determined for each species. Initially, catch for each species was stratified among sites into 50-mm size classes to assess a size bias in electrofishing. However, I found no trend in catchability related to fish size, and thus, all size classes were combined, increasing sample size.

Hierarchical Models

For each of the six native riverine fish species sampled, I developed hierarchical regression models using Proc Mixed within SAS 9.1 software (SAS 1996; Singer 1998) to investigate the relationships between fish catchability and instream habitat (velocity, width, substrate, and cover) and water quality (temperature, conductivity, and turbidity) parameters. I examined residuals and found no evidence of heteroscedasticity, and thus used untransformed data for model development. Twelve of the sampling sites were each sampled for three seasons, and 14 of 34 drainage basins contained multiple sampling sites within the drainage. Sampling sites located within the same drainage or among the three seasons created a dependency at those locations. Thus, a nested all-subsets regression procedure within SAS Proc Mixed was used to investigate and quantify dependence among sampling sites and season for the selected variables. To account for dependence of location within drainage and season, hierarchical models were constructed with the subject option as location, nested within drainage for all models. For those where seasonal effects created dependence, season was used as a group option with a repeated measures statement. Influential environmental variables were selected by Akaike's Information Criterion (AIC; Akaike 1973), which selects the model that explains variation in catchability with the fewest parameters. For each species, the most parsimonious models were selected based on AIC_c (AIC value with a second-order bias correction term) and Akaike weights (w_i) (a given w_i is the weight of evidence in favor of a model being the best model in a set of models) (Burnham and Anderson 2002). Using

AIC_c and w_i , I was able to evaluate the relative fit of each model and identify and rank a subset of the most parsimonious models for catchability.

The relative importance of variables among all possible models was estimated to quantify the influence of each variable in the model set and improve confidence in the most parsimonious models that may be used for prediction. The importance of each variable was estimated by summing the w_i over all models that included the particular variable. The larger the overall sum of w_i , the more important the variable is in the model set, relative to other variables (Burnham and Anderson 2002).

Results

Instream Habitat and Water Quality

Habitat measurements among the 105 sampling occasions varied, reflecting differences in topography and geomorphology of the sampling site. Among the 105 sampling occasions, average sampling reach length was 141 m, ranging from 100 m to 155 m (Table 1). Instream habitat measurements varied widely throughout the island, with no trend related to sample reaches in the headwater region versus lower altitude reaches in the coastal plain. Site 38E, about 12.8 km from the river mouth, represented the most narrow reach (1.58 m) and was a channelized tributary with bottom substrate of sand and cement (Figure 1). The widest reach (15.08 m) was located in the Culebrinas drainage basin about 15.0 km from the river mouth. Site 23A had the lowest mean depth of 2.43 cm, the site consisted of mostly gravel substrate, a mean velocity of 0.056 m/s, and at this location the only native species collected were the river goby and sirajo goby (Figure 1). Sample reaches with mean depths greater than 30 cm were downstream locations that averaged about 11.05 km from the river mouth. Mean column velocity varied among sampling occasions with the highest velocity reach located at site 35F, a site characterized by both width and depths that were average among sampling occasions (7.71 m , and 16.8 cm, respectively) and a substrate that was dominated by very coarse sand. Site 32B had the lowest mean column velocity (0.014 m/s), the width at this location was similar to the average width among sampling occasions; however, the mean depth at this location was 21.8 cm, about 6 cm greater than the average mean depth for all sampling occasions.

Among the 105 sampling occasions, water quality parameters varied with no trend related to location on the island or within watershed. Water temperature varied throughout the island but not substantially by season (Table 2). Site 3A, sampled in the spring, located in northeastern Puerto Rico, had the lowest water temperature (20.27 ˚C), and site 28D, located in the southern portion of the island, was also a relatively cool temperature reach (20.51 ˚C; Figure 1). Site 35G had the highest water temperature (30.20 ˚C; Figure 1) and was also sampled in the spring. Mean conductivity was moderate (321.6 μS/cm), and the overall range was suitable for sampling using electrofishing gears, ranging from 59-780 μS/cm. Site 23A had the highest total dissolved solids concentration (0.507 g/L); this location was also the most conductive (780.6 μ/cm), with the highest salinity (0.38 ppt). Turbidity levels at 81% of the sampling sites were less than 10 FAU and at the most turbid location, 43B (52 FAU), no native fish were collected, the reach was dominated by guppies *Poecilia reticulata*.

The degree of correlation among 20 instream habitat and water quality variables that I measured was significant for those variables that were of similar ecological function (Table 3). I was able to reduce the number of environmental variables from 20 to seven, and those variables where incorporated into the hierarchical modeling analysis. Four variables were selected to describe instream habitat (mean width, mean column velocity, mean substrate diameter, and percent cover) and three variables where selected to describe water quality (water temperature, conductivity and turbidity; Table 3).

Fish Catchability

I estimated capture probability at 70 of 105 sampling occasions for the American eel (21 occasions), bigmouth sleeper (37 occasions), smallscaled spinycheek sleeper (9 occasions), river goby (35 occasions), sirajo goby (35 occasions), and mountain mullet (51 occasions) (Table 4). Overall, capture probability ranged widely from 0.066 to 0.916. Mean capture probability among species was lowest for the smallscaled spinycheek sleeper (0.299, ranging from 0.090 to 0.522). Bigmouth sleeper, river goby, and mountain mullet species had similarly high mean (0.547-0.554) and maximum (0.907-

0.916) capture probabilities. Thus, at these locations, we collected approximately 55% (up to 92%) of the total population in the first electrofishing sample. Mean capture probability for American eel (0.496) and sirajo goby (0.458) was lower; with maximum capture probabilities of only 0.792 and 0.815, respectively (Table 4).

Of the 105 sampling occasions, two sites had the highest mean capture probability for native species detected (0.796 and 0.786), sites 7A and 7B (Figure 1). These relatively low-velocity (0.060 m/s and 0.057 m/s), narrow (3.66 m and 2.61 m), and shallow (12.6 cm and 11.5 cm) reaches included rocky substrate consisting of course gravel and small cobble with prevalent cover averaging 62.5 % of the reaches. At both locations, the water was clear (0 FAU turbidity), average water temperature was 23.0˚ C, and average conductivity was 141 µS/cm.

Sites resulting in low capture probability (<0.30) varied among species. For American eel, site 5A had the lowest capture probability (0.211). The reach had good visibility (0 FAU), moderate conductivity (177 µS/cm), and flowed over rocky substrate (medium gravel) with 49% cover. The mean reach width was 7.64 m, with a mean depth of 8.9 cm, and a mean water column velocity of 0.073 m/s. The low capture probability at this site may be related to a high abundance of small-bodied juvenile eels that were not found in high numbers at other sampling sites. Site 28C during the summer had the lowest capture probability for bigmouth sleeper, at this location width averaged 5.01 m, mean depth was 26.0 cm, and mean column velocity was 0.217 m/s. The site was characterized by gravel substrate, 56% cover, moderate conductivity (376 µS/cm), and moderately turbid water conditions (13 FAU). Independent of sampling conditions, the low capture probability may also be associated with a sparse bigmouth sleeper population at that site (catch of less than 20 fish). The lowest capture probability of mountain mullet was at site 35H and was also associated with a low-density population (15 fish caught) and a deep (mean 39.6 cm), moderately wide reach (mean 7.64) with an average velocity of 0.405 m/s, over clay substrate, with slight cover (27%), and moderate water clarity (8 FAU). At site 15A, I was only able to estimate capture probability for the river goby, and it was lower than all other estimates for river goby (0.163). The site was a low-velocity (0.154 m/s), narrow reach (4.20 m), with fine substrate (very coarse sand). The lowest capture probability of sirajo goby was associated with a sparse population (catch of less than 25) and a slightly turbid (12 FAU), moderately wide reach (average 8.04 m) with fine substrate (very coarse sand) and moderate water ionic content (149 µS/cm). In general, capture probability of the smallscaled spinycheek sleeper was low, relative to other species; the lowest capture probability was at site 5A, where only 17 smallscaled spinycheek sleepers where collected.

Hierarchical Models

The most influential of the seven independent environmental variables that explained variation in fish catchability were percent cover, mean stream width, and mean column velocity (Tables 5 and 6). Other variables associated with catchability, but for only a single species, were water conductivity, mean substrate diameter, and water temperature; turbidity did not explain variation in catchability for any species.

In general, the most parsimonious model explaining variation in catchability included those variables with the highest relative importance (Tables 5 and 6). For both American eel and mountain mullet, the most parsimonious models explained variation in catchability as positively correlated to percent cover and negatively to mean column velocity (Table 5). Thus, for both of these species, catchability was higher in streams with cover and low velocity. Mean column velocity was also negatively correlated to catchability for the river goby; however, variation in catchability was also explained by a negative relationship with mean stream width, reflecting that the highest river goby catch was in narrow streams with low velocity. The primary variable explaining catchability of the smallscaled spinycheek sleeper was a negative correlation to mean stream width (Table 5). Catchability of the sirajo goby was positively correlated to water conductivity and temperature and negatively to mean substrate diameter. For the bigmouth sleeper, each of the seven environmental variables had similar AIC weights and relative importance weights, suggesting that there was no relationship between catchability and environmental variables or that each of these physical parameters influence catchability equally (Tables 6 and 7). In this case where there is no clear relationship with environmental variables, catchability may be estimated by a simple model that does not include environmental variables.

Model precision for the six most parsimonious models was reasonable, with an average 95% confidence interval of 18.1% of the estimate for catchability values for the mean of measured environmental variables (Table 5). The most precise model among all best models was the top model for mountain mullet (7.7% of estimate). The model with the lowest precision (33.2% of estimate) explaining variation in catchability was the best model for smallscaled spinycheek sleeper. For the six most parsimonious models there was no model in which the capture probability estimates, at the mean of environmental variables, had a 95% confidence interval that overlapped zero. Precision of the models for environmental variables at the 10th and 90th percentiles varied by species as well as by upper and lower ranges of the environmental variables (Table 6). For the six most parsimonious models, the average precision at the lower range was 26.2% of the estimate and at the upper range was 60.4% of the estimate. The low precision at the upper range, among the average of all models, can be explained by very low precision of the top model for smallscaled spinycheek sleeper (222.8% of estimate). The most precise model at both upper and lower ranges, among all best models was the top model for the bigmouth sleeper (11.1% at the lower range of the estimate and 20.1% at the upper range of the estimate).

Discussion

My research objective was to identify influential factors and develop species-specific models that explain variation in catchability of electrofishing related to instream habitat and water quality parameters. I successfully developed regression models for six native Puerto Rico stream fish species. Environmental parameters that explained variation in electrofishing catchability were mean stream width, mean substrate diameter, percent cover, mean column velocity, water conductivity, and water temperature (Table 5). Stream width, cover prevalence, and water velocity were the most important predictors of fish capture probability (Table 5). Turbidity was not closely associated with electrofishing catchability in my sampling. These developed models can be utilized to understand and account for electrofishing bias for Puerto Rico stream fish.

Electrofishing catchability is known to be influenced by a number of physical and biotic factors; for example, the species being sampled, behavior of the fish, fish morphology, experience of the sampling personnel, instream water quality, and stream geomorphology can all affect catchability (Larimore 1961; Bohlin et al. 1989; Reynolds 1996; Bayley and Dowling 1993). When the objective of sampling is to collect accurate and quantitative population data, it is important to understand these factors and how they influence electrofishing catch rates.

Species composition may influence the efficiency of electrofishing, and my results showed mean capture probability to vary only moderately from 0.30 to 0.55 among species (Table 4). Further, the physical variables that explained catchability varied among species. Other investigators have found varying electrofishing catchability according to species in warmwater and coldwater streams (Larimore 1961; Speas et al. 2004). The body shape, size, and behavior of a fish alter the vulnerability of a fish to electrical current and capture. In general, fish that reside in shallow waters are more readily captured than fish found in deeper pools (Zalewski and Cowx 1990). Benthic fish that occupy microhabitats between boulders and undercut banks tend to be negatively buoyant, lowering sampling efficiency. Many water column species may avoid capture behaviorally, but these fish tend to be lightly colored, and personnel can chase them up to riffles or blocknets that enhance their capture (Zalewski and Cowx 1990). Nonetheless, I observed no trend in catchability or influential factors related to fish species habitat affinity (e.g., benthic versus water column).

Size selective bias may also influence efficiency of electrofishing, with efficiency increasing with fish size, but I found no evidence of such a trend within species of Puerto Rico stream fish. Considering potential for size bias within a species, I initially estimated capture probability by stratifying the catch of each species into 50-mm length groups. I detected no evidence that capture probability was influenced by fish size, so I combined groups to increase the sample size. Researchers in other stream ecosystems, however, have demonstrated size selectivity in electrofishing within and among species. Peterson et al. (2004) determined that electrofishing of stream-dwelling salmonids was size selective, and that the probability of capture was lower for smaller species. They concluded that failure to account for this factor will result in biased population estimates. Other researchers determined that the size selectivity also varied with the taxon that was sampled, where certain taxa were less vulnerable to size effects (Bayley and Dowling 1993; Bayley and Austen 2002).

Conductivity can have an effect on electrofishing catchability, where at low levels the electrofishing gear is not as efficient because the electrical field is reduced, and at high levels the fish is less conductive and the electrical current becomes distorted. Optimal sampling for electrofishing ranges at intermediate levels from 90 μS/cm to 1,500 μS/cm (Zalewski and Cowx 1990). During sampling of Puerto Rico streams, conductivity ranged from 59 μS/cm to 780 μS/cm (Table 2), with the majority of sites greater than 200 μS/cm. Water conductivity explained variance in capture probability in the best model of only the sirajo goby, and the relationship was positive (Table 5). Because the range of water conductivity was optimal at a majority of locations in my study, it may not have exerted a substantial influence on the catchability of most species among sites. Similarly, others have found that when sampling waters at intermediate levels of conductivity, efficiency of electrofishing tends to be more related to environmental variables, other than conductivity (Zalewski and Cowx 1990). Bayley and Austen (2002) examined efficiency of boat electrofishing related to conductivity in warmwater lakes and found that conductivity did not influence catchability for any of the fish groups sampled. However, their samples also included intermediate ranges of conductivity, resulting in findings and conclusions similar to mine for Puerto Rico stream fish sampling.

The water temperature of Puerto Rico streams sampled ranged from 20.3˚C to 30.2˚C and was not included in any of the best models for any species, however was in the most supported models of sirajo goby (Tables 5 and 7). Warm water temperatures can negatively impact electrofishing catchability by increasing fish metabolism that enables fish to escape current once detected (Zalewski and Cowx 1990). However, the relationship between catchability and water temperature for sirajo goby and mountain mullet was positive, suggesting that catchability increases with increasing water temperature, contrary to the expectation associated with a fish metabolic mechanism. Borkholder and Parsons (2001) concluded that catch rates of age-0 walleye demonstrated a curvilinear relationship with declining catch at low and high temperatures, and highest catchability was at an intermediate temperature from 10 to 20˚C. In general, efficiency of electrofishing related to water temperature is normally distributed with varying optimum ranges depending upon the species being sampled (Zalewski and Cowx 1990; Reynolds 1996). Considering that these fish are tropical species, their optimum temperature for catchability will vary from that of temperate fishes, and sampling conditions in Puerto Rico streams may not reflect temperature ranges warm enough to negatively impact their catchability. The possibility also exists that temperature is confounded with another influential variable (e.g., conductivity) that is the mechanistic influence on catchability.

In Puerto Rico streams, varying levels of turbidity did not explain variance in electrofishing catchability for any of the six native fish species examined. This finding is contrary to most research on the effects of turbidity on electrofishing efficiency. The influence of turbidity typically exhibits a bell shaped curve, where in low turbidity waters the fish can detect personnel and escape, and in highly turbid water personnel cannot visually detect fish; in both extreme scenarios catchability declines (Zalewski and Cowx 1990; Reynolds 1996). In Puerto Rico, over half of the sampling sites had turbidity levels less than 5 FAU, where you would expect reduced catchability (Zalewski and Cowx 1990; Reynolds 1996); however, sites with low turbidity (0 FAU) had catchabilities higher than the mean for all species collected. This could be explained by fish movement away from personnel into cover, riffles, and blocknets that enabled the sampling crew to readily capture the fish. While I observed this phenomenon in these relatively small wadeable streams in Puerto Rico, catchability may be reduced in larger substrate streams with very low turbidity, where fish may be able to avoid electrofishing gear and operators. In contrast, highly turbid waters may negatively influence electrofishing catchability of Puerto Rico stream fish, by decreasing the netters ability to detect fish that are shocked, especially demersal species that are usually camouflaged and sink to the bottom. My sampling was generally under favorable conditions for electrofishing in Puerto Rico, and the water was relatively clear (mean turbidity of 6.6

FAU, Table 2). Certainly, had I sampled fish in these systems after rain events that cause flooding and elevated turbidity, catchability would be reduced substantially, but that approach is not practical or recommended for deployment of electrofishing gear (i.e., sampling under clearly suboptimal conditions).

Substrate and instream cover can exert varying influences on electrofishing catchability, and this was revealed in best models of several Puerto Rico stream fishes. Substrate size can negatively affect efficiency both at fine and coarse substrate sizes. Fine substrates such as clay and silt are more conductive than gravel/cobble bottoms, causing a reduction in current density of an electrical field (Zalewski and Cowx 1990). Larger substrates can reduce catchability, creating difficult electrofishing conditions by reducing the operator's ability to net fish. When evaluating the efficacy of using removal population estimates for salmonids, Peterson et al. (2004) found that electrofishing efficiency decreased over larger substrates and along undercut banks, because fish were utilizing both the substrate and overhead cover to hide and avoid capture. Speas et al. (2004) determined that catchability of rainbow trout *Oncorhynchus mykiss* was greater over sand/silt substrate than over rocky substrates. For Puerto Rico stream fish, I found that catchability was related to substrate size for only the sirajo goby, and the relationship was negative (Table 5). The highest catchability of the sirajo goby occurred with dominant substrates consisting of gravel, whereas the lowest catchability estimate was in a reach dominated by boulder substrate. Cover, such as rootwad, woody debris, and submersed vegetation, can increase catchability, concentrating fish into an area where they can then easily be collected. Conversely, in some cases excessive cover can impede fish capture, creating an environment where it is difficult to see and collect fish. Cover was positively correlated to catchability in the best models of American eel and mountain mullet in my research (Table 5). Areas with more prevalent cover made it easier to concentrate and hold fish that avoided the gear and were escaping the electric current. Once fish were concentrated in cover, they appeared less likely to flee, and we could then collect stunned fish with favorable capture efficiency.

Increasing velocity can decrease electrofishing catchability, and I found that catchability of the best models for mountain mullet and river goby were explained by negative relationships with velocity (Table 5). As velocity increases so does the chance of the fish moving past the personnel undetected once stunned (Zalewski and Cowx 1990). Typically, demersal species, that are dark colored, may be harder to detect at higher velocities (Zalewski and Cowx 1990); however, the mountain mullet, a light colored, water column species, showed a negative relationship with velocity, as did, the demersal, dark colored river goby, suggesting that for Puerto Rico stream fishes the catchability relationship with velocity is not associated with habitat affinity or fish color (Table 5).

Stream width may affect electrofishing catchability, and my results showed this for two Puerto Rico fish species (Table 5). Typically as stream width increases, electrofishing catchability declines (Bayley and Dowling 1993). This decline is related to the inability of the gear's electrical field to cover the entire reach width, allowing fish to escape past sampling personnel (Zalewski and Cowx 1990). Kennedy and Strange (1981) found an inverse relationship between river width and efficiency of electrofishing for salmonids. Peterson et al. (2004) found that salmonid capture efficiency was inversely proportional to mean area of sampled streams. These findings are consistent with the relationship I found in the best models of river goby and smallscaled spinycheek sleeper in Puerto Rico streams (Table 5). For the smallscaled spinycheek sleeper, there was a strong negative correlation between stream width and catchability. Stream width was the only environmental variable that explained variance in catchability for this species. The smallscaled spinycheek sleeper is a demersal species that was primarily netted along banks and in cover, such as large woody debris. Stream width was also negatively correlated to catchability of the river goby. To improve capture efficiency of these species, as stream width increases, both gear (i.e., number of anodes or backpack units) and personnel should be increased to reduce the fish's ability to escape.

Sampling Implications

The regression models that I developed (Table 5) can be used to better understand and determine environmental variables that influence electrofishing catchability. They also quantify the directional bias of each influential variable and allow correction for bias related to these variables. Each model is species-specific and includes the most influential environmental parameters on electrofishing catch (Tables 5 and 7). Because these models correct for bias associated with varying sampling conditions, they can be utilized with single-pass electrofishing data to estimate stream fish populations. These models can reduce time spent in the field, allowing fisheries biologists to collect one fish sample and measure a few environmental variable and reasonably quantify and assess stream fish populations within a sample reach.

If you select the top model for an individual species and collect data on the influential environmental variables, you can predict capture probability from single-pass catch data. In general, these estimates of capture probability will be similar to those from a three-pass removal procedure conducted at the same site for that species. For example, I selected the top model for river goby and applied habitat and single- pass catch data collected from a site on the Río Guanajibo drainage (35E, Figure 1) to further show the utility of these models. At this sampling location, average mean column velocity (0.073 m/s) and mean stream width (4.54 m) measurements were collected and applied to the top model

for river goby as

$$\hat{p} = -0.2484(\text{velocity})-0.0297(\text{width})+0.8050, \text{ and}$$

$$\hat{p} = 0.652.$$

Once capture probability at this sampling location was estimated it could then be applied to single-pass catch data (66 river goby) from this location to estimate the river goby population:

<u>Single-Pass Catch Estimate</u>

$$N = 66/0.652$$

$$N = 101.2 \pm 11.0$$

The three-pass removal estimate for river goby at this site on that day was 92 fish ± 4.1. In this example, the model estimate is 9.0% higher than that derived using a three-pass removal procedure, and the 95% CI of the model estimate overlaps the removal estimate.

Researchers have suggested the use of single-pass electrofishing data to estimate fish populations (Lobón-Cerviá and Utrilla 1993; Jones and Stockwell 1995; Kruse et al.

1998). By developing a regression model related to capture probability, at similar sampling locations, single-pass data combined with a simple model can predict fish abundance. However, researchers caution against using this method if the exact sampling protocol is not followed or at locations that have varying habitat and sampling conditions. Research and logic suggest that fish catchability can be influenced by instream habitat and electrofishing protocols that can also change with the fish species being sampled (Dauwalter and Fisher 2007). However, such bias can be corrected and variables that influence catchability can be used to predict capture probability and estimate fish abundance. Taking into account that catchability is also influenced by the species that is sampled, such predictive models should be species specific.

For Puerto Rico native stream fish, I developed regression models for each species that correct for bias related to instream habitat and water quality. These models can be utilized with single-pass electrofishing data to estimate stream fish abundance with reasonable precision. An important factor to consider is standard electrofishing sampling protocols, since following a different protocol can bias predicted capture probability estimates. When sampling Puerto Rico streams using single-pass data to predict abundance based upon the developed regression models, biologists should follow the sampling methods and gears used in this study. Further, sampling for utility of these models should only occur during optimal conditions, and not following a rain event that can displace fish in the stream and create a turbid, high-velocity environment that may negatively bias capture probability and lead to environmental conditions beyond the range of those used for model development. Sampling outside of the range of the environmental variables collected in this study may lead to bias and unreliable estimates. For example, the model for smallscaled spinycheek sleeper is not very reliable at the widest sampling reaches (i.e., >10.34 m), where estimates of capture probability are exceptionally low 0.092 and 95% confidence intervals overlap zero (Table 6). Since the regression model is not precise for smallscaled spinycheek sleeper at wide reaches, it would be advisable to conduct a three-pass removal procedure at such locations.

Considering these factors, biologists will be able to more efficiently and cost effectively sample stream reaches employing these predictive models that estimate capture probability. By collecting a few habitat measurements and conducting a single electrofishing sample, fish abundance can be estimated by dividing the number of fish caught on the first pass by the predicted capture probability. These models will enable fisheries researchers and managers in Puerto Rico to conduct fish population estimates with a single field sample, saving time and expense, with minimal bias. More complete, quantitative estimates of the fish community may then form the basis for improved stream fish and ecosystem management.

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Table 1. Mean sampling site and instream habitat characteristics from 105 sampling occasions at 81 sites in Puerto Rico during 2005-2007 surveys.

Parameter	Mean	SE	Min-max
Mean stream width (m)	5.92	0.27	1.58-15.08
Reach length (m)	140.8	1.4	100-155
Area (m^2)	836.6	39.9	237-2,262
Mean depth (cm)	15.09	0.73	2.4-47.6
Mean column velocity (m/s)	0.178	0.017	0.014-1.031
Cover (%)	54.4	1.8	16-98
Mean bank angle (˚)	135.4	1.4	96.3-171.3
Mean substrate diameter (mm)	345.2	113.5	0.06-6,000.0

Table 2. Mean water quality measurements from 105 sampling occasions at 81 sites in Puerto Rico during 2005-2007 surveys.

Parameter	Mean	SE	Min-max
Water temperature (°C)	24.32	0.18	20.3-30.2
Dissolved oxygen (mg/L)	8.19	0.12	4.12-11.11
Total dissolved solids (g/L)	0.209	0.008	0.038-0.507
Conductivity (µS/cm)	321.6	12.9	59-780
Salinity (ppt)	0.15	0.01	0.03-0.38
Nitrate (mg/L as NO_3^-)	3.65	0.39	0-25.8
Nitrite (mg/L as NO_2^-)	0.076	0.015	0-0.91
Ammonia (mg/L as NH_3)	0.08	0.01	0-0.60
Phosphorus (mg/L as PO_4^-)	0.65	0.07	0-2.75
pH	8.29	0.04	7.05-9.21
Alkalinity (mg/L as $CaCO_3$)	129.9	5.4	17-277
Hardness (mg/L as $CaCO_3$)	135.1	5.7	14-280
Turbidity (FAU)	6.6	0.7	0-52

Table 3. Instream habitat and water quality variables for 105 Puerto Rico stream sampling occasions. Twenty variables (7 instream habitat and 13 water quality) were reduced to seven primary variables for hierarchical modeling based on correlation coefficient (*r*) and related ecological function. Bold *r*-values denote significant correlation between primary and secondary variables ($P<0.05$). Critical absolute *r*-values are 0.190 for all correlations.

Primary representative variable	*r*	Correlated secondary variable
		Instream Habitat
Mean stream width (m)		
	0.974	Sampling reach area (m^2)
	0.418	Mean stream depth (cm)
	-0.184	Mean bank angle (˚)
Mean column velocity (m/s)		
Mean substrate diameter (mm)		
Percent cover		
		Water Quality
Water temperature (˚C)		
	-0.056	Dissolved oxygen concentration (mg/L)
Conductivity (μS/cm)		
	0.999	Total dissolved solids (g/L)
	0.987	Salinity (ppt)
	0.792	Alkalinity (mg/L $CaCO_3$)
	0.804	Hardness (mg/L $CaCO_3$)
	0.088	pH
	-0.220	Nitrate concentration (mg/L NO_3^-)
	0.098	Nitrite concentration (mg/L NO_2^-)
	-0.143	Ammonia concentration (mg/L NH_3)
	0.059	Phosphorus concentration (mg/L PO_4^-)
Turbidity (FAU)		

Table 4. Mean electrofishing catchability for six native Puerto Rico stream fishes among 105 sampling occasions at 81 sites during 2005-2007 surveys.

Species	*N*	Mean catchability	SE	Min-max
American eel	21	0.4963	0.0393	0.211-0.792
Bigmouth sleeper	37	0.5542	0.0273	0.172-0.907
Smallscaled spinycheek sleeper	9	0.2986	0.0569	0.090-0.522
River goby	35	0.5504	0.0293	0.163-0.907
Sirajo goby	35	0.4583	0.0289	0.066-0.815
Mountain mullet	51	0.5467	0.0271	0.095-0.916

Table 5. The most parsimonious models explaining variance in capture probability for six native riverine fishes among 81 Puerto Rico stream sampling reaches. K is the number of model parameters; ΔAIC_c, Akaike's Information Criterion, values corrected for bias, is the difference between the most parsimonious model and other candidate models; and w_i is the Akaike weight, or the weight of evidence in favor of the model being the best model. $\overline{p}$ is the mean catchability for each set of candidate models ± the variance associated at a 95% confidence interval.

Species and model	K	ΔAIC_c	w_i	$\overline{p}$ ± 95% CI
American eel				
0.2945(cover)+0.3353	1	0	0.1168	0.497 ± 0.154
-0.2665(velocity)+0.5487	1	0.0049	0.1165	0.500 ± 0.152
0.2089(cover)-0.1888(velocity)+0.4192	2	1.9608	0.0438	0.497 ± 0.436
Smallscaled spinycheek sleeper				
-0.0510(width)+0.6191	1	0	0.6028	0.286 ± 0.095
-0.0304(turbidity)+0.3629	1	3.6082	0.0992	0.185 ± 0.229
-0.3431(cover)+0.4810	1	3.7774	0.0912	0.290 ± 0.112
Bigmouth sleeper				
-0.0101(width)+0.5996	1	0	0.0532	0.552 ± 0.053
-0.1317(cover)+0.6057	1	0.0759	0.0512	0.548 ± 0.055
-0.0002(conductivity)+0.6098	1	0.0982	0.0506	0.542 ± 0.060
-0.1208(velocity)+0.5568	1	0.1369	0.0496	0.550 ± 0.052
-0.0078(temperature)+0.7274	1	0.3805	0.044	0.545 ± 0.055
0.00006(substrate)+0.5287	1	0.5555	0.0403	0.565 ± 0.060
-0.0011(turbidity)+0.5404	1	0.6606	0.0382	0.545 ± 0.047
River goby				
-0.2484(velocity)-0.0297(width)+0.8050	2	0	0.1337	0.580 ± 0.077
-0.0295(width)+0.7603	1	1.1226	0.0763	0.588 ± 0.075
0.1536(cover)-0.2294(velocity)-0.0290(width)+0.7143	2	1.6054	0.0599	0.581 ± 0.080
Sirajo goby				
0.0005(conductivity)-0.00007(substrate)+0.3007	2	0	0.1615	0.461 ± 0.068
0.0004(conductivity)-0.00007(substrate)+0.0159(temperature)-0.0519	3	1.3947	0.0804	0.457 ± 0.072
0.0245(temperature)-0.00007(substrate)-0.1343	2	1.5312	0.0751	0.448 ± 0.068
0.0006(conductivity)+0.2627	1	2.5944	0.0441	0.459 ± 0.074

Table 5 continued.

Species and model	K	ΔAIC_c	w_i	$\overline{p} \pm$ 95% CI
Mountain mullet				
0.2776(cover)-0.4454(velocity)+0.5054	2	0	0.1383	0.570 ± 0.044
0.2813(cover)-0.3965(velocity)-0.0114(width)+0.5658	3	0.9953	0.0841	0.575 ± 0.043
0.2932(cover)-0.4461(velocity)-0.0056(turbidity)+0.5248	3	1.1142	0.0792	0.570 ± 0.043
0.3225(cover)-0.4723(velocity)+0.0116(temperature)+0.2014	3	1.3014	0.0721	0.574 ± 0.044

Table 6. The most parsimonious models explaining variance in capture probability for six native riverine fishes among 81 Puerto Rico stream sampling reaches. p_{10} and p_{90} represent capture probability estimates at the 10 and 90 percentiles of the measured habitat variables, from all sampling occasions ± the variance associated at a 95% confidence interval.

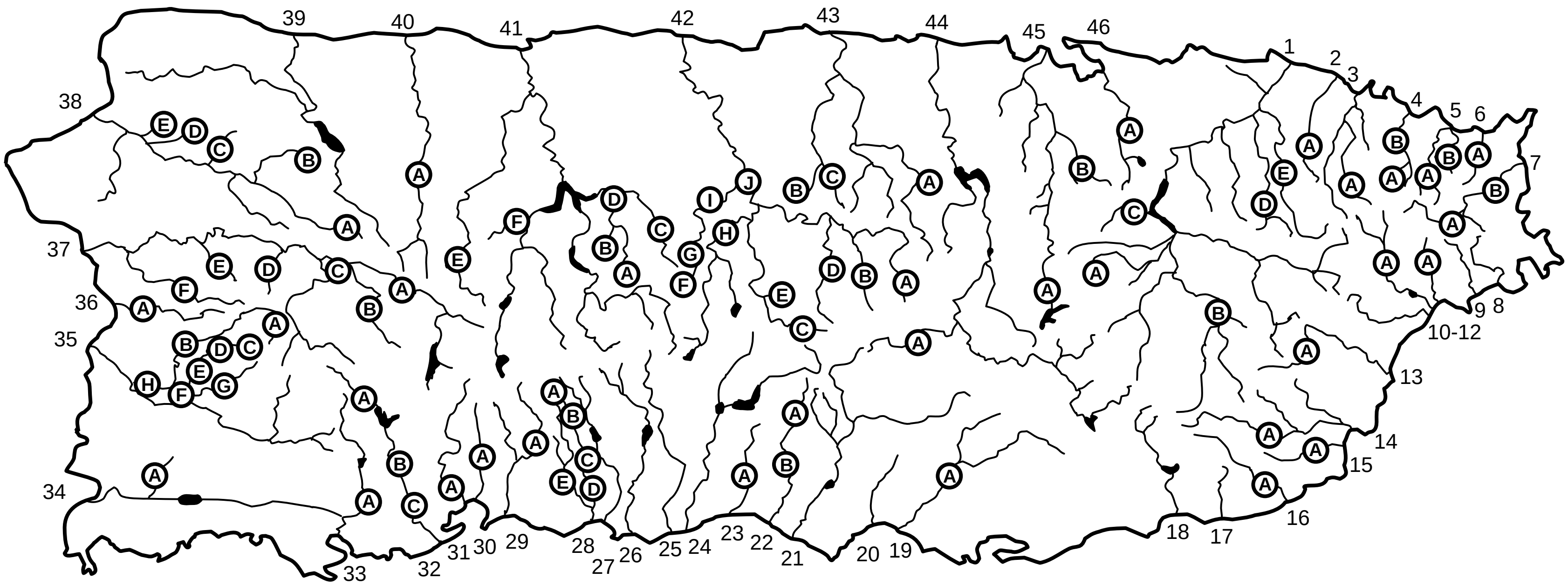
Species and model	p_{10} ± 95% CI	p_{90} ± 95% CI
American eel		
0.2945(cover)+0.3353	0.431 ± 0.233	0.564 ± 0.247
-0.2665(velocity)+0.5487	0.536 ± 0.190	0.441 ± 0.247
0.2089(cover)-0.1888(velocity)+0.4192	0.478 ± 0.917	0.493 ± 0.978
Smallscaled spinycheek sleeper		
-0.0510(width)+0.6191	0.439 ± 0.156	0.092 ± 0.205
-0.0304(turbidity)+0.3629	0.363 ± 0.161	0.005 ± 0.531
-0.3431(cover)+0.4810	0.372 ± 0.178	0.208 ± 0.204
Bigmouth sleeper		
-0.0101(width)+0.5996	0.597 ± 0.066	0.492 ± 0.099
-0.1317(cover)+0.6057	0.535 ± 0.078	0.562 ± 0.098
-0.0002(conductivity)+0.6098	0.530 ± 0.130	0.553 ± 0.079
-0.1208(velocity)+0.5568	0.573 ± 0.066	0.514 ± 0.072
-0.0078(temperature)+0.7274	0.544 ± 0.078	0.546 ± 0.079
0.00006(substrate)+0.5287	0.528 ± 0.059	0.649 ± 0.177
-0.0011(turbidity)+0.5404	0.607 ± 0.073	0.486 ± 0.077
River goby		
-0.2484(velocity)-0.0297(width)+0.8050	0.767 ± 0.100	0.321 ± 0.099
-0.0295(width)+0.7603	0.750 ± 0.099	0.372 ± 0.096
0.1536(cover)-0.2294(velocity)-0.0290(width)+0.7143	0.713 ± 0.134	0.384 ± 0.142
Sirajo goby		
0.0005(conductivity)-0.00007(substrate)+0.3007	0.387 ± 0.108	0.507 ± 0.119
0.0004(conductivity)-0.00007(substrate)+0.0159(temperature)-0.0519	0.374 ± 0.116	0.520 ± 0.131
0.0245(temperature)-0.00007(substrate)-0.1343	0.421 ± 0.087	0.464 ± 0.100
0.0006(conductivity)+0.2627	0.359 ± 0.107	0.549 ± 0.124

Table 6. continued.

Species and model	p_{10} ± 95% CI	p_{90} ± 95% CI
Mountain mullet		
0.2776(cover)-0.4454(velocity)+0.5054	0.575 ± 0.089	0.532 ± 0.115
0.2813(cover)-0.3965(velocity)-0.0114(width)+0.5658	0.601 ± 0.092	0.502 ± 0.128
0.2932(cover)-0.4461(velocity)-0.0056(turbidity)+0.5248	0.600 ± 0.094	0.496 ± 0.125
0.3225(cover)-0.4723(velocity)+0.0116(temperature)+0.2014	0.549 ± 0.101	0.562 ± 0.129

Table 7. Relative importance of predictor variables among 127 models and seven parameters for six native fish species. Akaike weights (w_i) were summed over all models that included a given variable to provide evidence of the importance of that variable.

	American eel	Smallscaled spinycheek sleeper	Bigmouth sleeper	River goby	Sirajo goby	Mountain mullet	Mean
Mean substrate diameter	0.203	0.053	0.257	0.189	0.781	0.199	0.280
Mean stream width	0.196	0.618	0.318	0.872	0.181	0.328	0.419
Percent cover	0.408	0.096	0.371	0.293	0.171	0.837	0.363
Mean column velocity	0.394	0.055	0.317	0.576	0.188	0.970	0.417
Water temperature	0.194	0.046	0.275	0.246	0.429	0.318	0.251
Conductivity	0.214	0.047	0.310	0.254	0.676	0.222	0.287
Turbidity	0.188	0.102	0.242	0.238	0.174	0.325	0.212



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. 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 = 81) spanning 34 of 46 drainage basins in Puerto Rico.