ABSTRACT

ENGMAN, AUGUSTIN CLARK Amphidromous Fish Recruitment and its Ecological Role in Caribbean Freshwater–Marine Ecotones. (Under the direction of Dr. Thomas J. Kwak).

Amphidromous fishes are ubiquitous on oceanic tropical islands where they typically dominate native stream fish richness. They are also a major component of stream fish assemblages in some continental tropical islands, temperate island chains, and in tropical coastal continental areas. In amphidromy, eggs are spawned in freshwater streams, and larvae drift to the ocean. After an oceanic pelagic larval phase, they transition to postlarvae, and migrate back into freshwater where they grow to adults. Despite the fact that amphidromous fishes are threatened, ecologically important, and culturally significant components of many stream communities, their early life history stages are understudied, especially in the Caribbean region.

My research quantifies the postlarval recruit abundance, recruitment phenology, and pelagic larval duration of several amphidromous fish species in multiple rivers in Puerto Rico and explores the effects of environmental variables on recruit abundances. I use stable isotope and contaminant analyses to infer the larval habitats of amphidromous fishes and the role of postlarvae as nutrient and contaminant biotransport vectors. I also determine the role of postlarval amphidromous fish recruitment in the fish assemblage structure and trophic ecology of a Caribbean freshwater–marine ecotone. Finally, I compare postlarval densities in multiple rivers, explore how watershed-scale habitat characteristics are related to recruit abundance in two goby taxa, and describe the riverine microhabitats that are utilized by amphidromous gobioid postlarvae during recruitment and upstream migration.

My study of postlarval recruit abundance, recruitment phenology, and pelagic larval durations revealed that two taxa—River Goby *Awaous banana* and Sirajo Goby *Sicydium*
spp.—are exceptionally abundant as postlarvae in Puerto Rico rivers. They recruit in pulses that are periodic at annual and lunar scales. Peak recruitment of River Goby and Sirajo Goby occurred during the last-quarter moon phase from June through January in the Caribbean. River Goby and Sirajo Goby recruit abundances varied between rivers, were greater at dawn than dusk, and are positively related to river discharge. The pelagic larval durations of four amphidromous fish taxa spanned a minimum of 28 to a maximum of 103 days with means between 43.1 and 65.2 days.

Stable isotope analyses indicated that recruiting postlarval amphidromous fishes are derived from marine organic matter sources, rather than riverine. Contaminant analyses revealed that Sirajo Goby postlarvae do not contain high loads of anthropogenic pollutants. Thus, amphidromous fish recruitment is a periodically available marine subsidy to tropical estuaries and streams that is not of ecological or human health concern. The study of the trophic-ecology effects of amphidromous fish recruitment revealed major changes in the diets of several freshwater–marine ecotone predator fish species, which are related to the consumption of amphidromous gobioids. The fish assemblage structure and distribution of individual predator species in this ecotone remained stable throughout a mass-recruitment event.

My study of postlarval habitat use indicated that recruit abundances of gobies are distinct among Puerto Rico rivers, and mean recruit densities of Sirajo Goby are correlated with river mouth location. In general, gobioid postlarvae at river mouths occupy low velocity waters with downstream flow direction and low salinities, and were found over sand substrates. River Goby at upstream locations occupied low velocity freshwaters, with upstream flow direction over sand substrates.
The findings and conclusions presented here quantitatively describe the ecological function of amphidromous fish recruitment and can be used to identify critical times to maintain river-ocean connectivity and stream flow for the benefit of the amphidromous fish migration and population dynamics and provide novel information on Caribbean gobioid recruitment habitat requirements. These results may guide Caribbean stream ecosystem conservation and management to ensure the viability of amphidromous fish populations and enhance the sustainability of the ecosystem functions and services that they provide.
Amphidromous Fish Recruitment and its Ecological Role in Caribbean Freshwater–Marine Ecotones

by
Augustin Clark Engman

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APPROVED BY:

Dr. Thomas J. Kwak
Committee Chair

Dr. W. Gregory Cope

Dr. James F. Gilliam

Dr. Brian Reich
BIOGRAPHY

I was born in Albuquerque, New Mexico, to two University of New Mexico graduate students. My mother, Sue, was an English major, reporter for The Daily Lobo and belly dance-enthusiast; my father, Marty, was the hardest-rocking and most anti-establishment person to ever teach a calculus course at UNM. Even though my family lived in the “student ghetto” and sometimes depended on student loans and government assistance to make ends meet, my early childhood was one of great privilege. I began my life in a world where the pursuit of knowledge, self-expression, love, and the celebration of diverse cultures all held high value, and my family and our friends were rich in this sense. My parents continued to surround me with these values throughout my life, and for that reason, I dedicate this dissertation to them.

The privileged life that my parents provided, allowed me to realize my career and life goals at a very young age. By the time I was eight years old, I knew that I would be a marine biologist. My career-choice stemmed from the excitement that I experienced during exploration and discovery in nature and a particular fascination (some might call it an obsession) with the aquatic world. Some of my earliest memories are of being mesmerized by the Koi in the UNM duck pond, driving to Sandia Peak in the middle of the night to watch the sunrise, and watching “Marty Stouffer’s Wild America” on the television while my dad grilled hamburgers. I picked up fishing—as a means for me to indulge my obsession—when my parents divorced and my mom and I moved to upstate New York. My parents—neither of whom actually like fishing—were always patient and supportive of my fishing habit. My dad spent a large portion of our limited time together during my childhood taking me fishing
everywhere from an urban stream in Plattsburg, New York, to the San Juan River, New Mexico, to Blue Marlin alley over the Puerto Rico trench. My mom—a part-time vegetarian with deep empathy for animals—semi-willingly gutted, cleaned, and cooked trout and bass for me when I was too young to handle a knife. She also spent way more money on a fly-fishing setup than any 11-year-old deserves. When my dad moved to San Juan, Puerto Rico, I had the opportunity to expand my indulgence in my aquatic-exploration-obsession through snorkeling and diving. My dad and I were SCUBA certified when I was 13, and I spent many hours of my teenage years exploring Caribbean reefs, seagrass beds, and mangroves.

One more very important part of my young life and beyond has been the presence of my siblings and extended family. When I was eight years old, my father had my sister, Sonia, and when I was eleven my Mom had my brother, Lex. Despite the fact that I have spent more time separated by distance from my brother and sister than is typical of most siblings, I have built great bonds and friendships with both of them. Watching them grow into the mature and amazingly talented people that they are today has been one of the great pleasures of my life and fills me with a tremendous sense of pride and purpose. The time that my siblings and I have spent with the various branches of our extended families has been fun, hilarious, tumultuous at times, but was always filled with love and support. All of our grandparents/aunts/uncles/cousins share in any career or personal successes any of us may have.

When I was a freshman in high school my parents reunited after a ten-year period of being divorced, which meant that my mom and I would move to Puerto Rico. Living in Puerto Rico as an adolescent and beyond has had a huge influence on who I am today. Although I moved around a lot growing up, surprisingly the easiest transition for me was not
a switch of school districts in upstate New York, but the move to another country, with a
different language and culture. This transition was made so agreeable by the warm and
inviting nature of the people of Puerto Rico. I often tell people that I consider myself a
‘Boricua adoptao’ because my friends, neighbors, teachers, and coaches in Puerto Rico truly
adopted me into their culture. They taught me many things, including how to speak Spanish,
dance (not saying I do it well—they just taught me), and most importantly how to live with
joy, passion, and warmth.

After high school I finally reached the long-awaited initiation into the education and
training for my career as a marine biologist. I could not have asked for a better undergraduate
experience than the one afforded to me by the University of Miami. The most important
aspect of my experience at UM was working in Dr. Alexandra Worden’s marine microbial
ecology lab. Alex hired me in the middle of my junior year and sent me to sea the very next
day. Even though I spent most of my first research cruise throwing up while failing to
properly learn to use a pipette on a rocking ship, Alex kept me in the lab and sent me out on
multiple research expeditions over the next two years. In Alex’s lab, I learned a lot of the
fundamentals of conducting field and laboratory research. I moved with Alex’s lab to
California for a brief period then chose to take a hiatus from science and returned to Puerto
Rico.

I spent a year working as a waiter in various restaurants in San Juan, then reinitiated
my career in science. I started a master’s program in biology at the University of Puerto Rico,
Río Piedras, in 2011 under the guidance of Dr. Alonso Ramírez. Alonso initiated me into the
world of stream ecology and gave me the opportunity to be the TA and a mentor in the
Luquillo research experience for undergraduates (REU) program. As the REU TA, I spent the summer of 2009—the first of many—at the El Verde field station in the El Yunque National Forest. There, I found my place among a dynamic community of field biologists and ecologists. The most important subset of this community for me was the DWTFS-bunch of aquatic ecologists with whom I have called the field station and stream house a shared, intermittent home for the last seven years.

Among this bunch were members of the Kwak lab who would eventually introduce me to Dr. Thomas Kwak and the idea of attending NC State University. In 2011, I was afforded the opportunity by Tom to work on this PhD, which has allowed me to conduct research on the very questions that I am most interested in studying and for that, I am very grateful. Tom’s guidance through the PhD process has been all that I could ever ask from an advisor, but his advice on life and the camaraderie that he has provided over the years have been the most valuable things that he has given me. Tom, and the rest of the ‘geezer guide service’ also initiated me into the world of hunting, which has been an extremely rewarding and necessary distraction from my doctoral work.

Although meeting Tom and many of my current colleagues at El Verde was pivotal for my career, the most important person that I met in that steamy, tropical forest was Ana Castillo. She has been the true love of my life and my best friend for over six years now. She has afforded me strength and has made me a better person and citizen during each day that I have been with her.
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First, I would like to thank Will Smith and Patrick Cooney, who taught me a lot of the basics of the biology of Puerto Rico’s stream fishes when I was a master’s student. The primordia of many of the ideas and questions addressed in this volume emerged from early conversations with these two over cold Medallas. Will was also essential for the transfer of logistical knowledge needed to run a research program in Puerto Rico while living in Raleigh; he aided in the development of my postlarvae sampling techniques, sampling design and provided great ideas and feedback related to the early stages of analysis. Will and Patrick have also been great friends and helped me get my feet on the ground when I showed up in Raleigh with a single checked bag full of all of my worldly possessions.

Next, I would like to thank my advisor Tom Kwak for his patient efforts to make me a better scientist and academic professional. I am also very grateful to the rest of my doctoral committee members, Greg Cope, Brian Reich, and Jim Gilliam for their thoughts and contributions to this body of work.

Jesse Fischer contributed tremendously to improving the quality of this project in almost every way possible. In short, he acted analogously to an additional committee member, was essential in the field, and was the only one who matched my enthusiasm for staying up for days-on-end during our most intensive sampling. Specifically, Jesse aided in project design, logistics, taught me a variety of fisheries techniques, including everything I needed to know about otoliths, field sampling, and aided in data analyses and interpretation of results. One of Jesse’s crowning achievements was converting our Zodiac into a boat electrofisher, which was absolutely essential to the results of chapter 3. Thank you, Jesse, for
continuing to come down to Puerto Rico even after I gave you walking pneumonia on your first trip.

There is no way that this project could have happened without the amazing help that so many provided in the field, laboratory, and office. Casey Grieshaber deserves special recognition for her long hours in the field, especially during my first major field season where she was essential for keeping me organized and for continuing field sampling when I had to be in Raleigh for classes. Then, after returning to Raleigh, Casey spent long hours in the laboratory and was essential in organizing our fish identification, measurement, and data management protocols. Mike Walter also deserves recognition for his perseverance at the microscope and talent with the otoliths. Andrew Maurer was always a tremendously helpful and independent worker during my second field season. I would also like to specifically thank Denny Padilla, Rafael Benítez Joubert, Edna Marie Díaz Negrón, and Edgardo Padilla for their efforts in the field and their friendship. Next, I want to recognize the NC State group of lab and field hands especially Emilee Wooster, James Wehbie, Spencer Gardner, and all of the Doris Duke scholars, especially Wilson Xiong and Mallory Gyovai. Adam Terando provided useful discussion on analyses, great moral support, and assisted in providing access to the precipitation dataset used in chapter one. Dr. Jared Flowers, my long-time office mate also provided great moral support and discussion on study design and analyses.

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CHAPTER 1: Recruitment phenology and pelagic larval duration in Puerto Rico

amphidromous fishes

Summary

1. Amphidromous fishes are major components of oceanic tropical island stream ecosystems, such as those of the Caribbean island, Puerto Rico. Fishes with this life history face threats related to the requirement of connectivity between freshwaters and the marine environment during early life stages.

2. Pelagic larval duration and recruitment phenology are two early life history processes that are crucial for the biology, ecology, conservation, and management of amphidromous fishes. However, these processes are understudied in the Caribbean in general and have never been quantified in Puerto Rico.

3. In this article, the recruit abundance, recruitment phenology, and pelagic larval duration of several Caribbean amphidromous fish species are quantified in multiple rivers in Puerto Rico. Furthermore, the effects of environmental variables on recruit abundances are explored.

4. Two taxa—River Goby *Awaous banana* and Sirajo Goby *Sicydium* spp.—were found to be exceptionally abundant as postlarvae and to recruit to Caribbean rivers in pulsed episodes that are periodic at annual and lunar scales. Sirajo Goby and River Goby recruit abundances varied between rivers, were greater at dawn than dusk and were positively related to river discharge.

5. The pelagic larval durations of four taxa ranged from a minimum of 28 to a maximum of 103 days with means between 43.14 and 65.17 days.
6. This study identifies the last-quarter moon phase during the months from June through January as periods of maximum amphidromous stream fish recruitment. The results and conclusions of this article can be used to identify critical times to maintain river-ocean connectivity and stream flow for the benefit of the amphidromous fish population dynamics, stream ecology, and natural resources of the Caribbean.
Introduction

Amphidromous fishes face multiple threats related to a life history that requires freshwater–marine connectivity during early life stages (Kwak et al., 2016). Fishes with this life history spawn in freshwater streams, and their larvae drift to the ocean immediately after hatching. After an oceanic pelagic larval phase, they transition to postlarvae prior to river ingress. Once postlarvae migrate into a river, they typically undergo a rapid metamorphosis to early juvenile stages and continue migration upstream to adult habitats (McDowall, 2007; Keith et al., 2008). Pertinent anthropogenic threats to amphidromous fishes include instream barriers to migration (Holmquist et al., 1998; Cooney & Kwak, 2013), stream flow alterations from water abstraction or hydroelectric operations (Brasher, 2003; Smith & Kwak, 2015; Kwak et al., 2016), and habitat alteration by stream channelization (Engman & Ramírez, 2012; Ramírez et al., 2012). Threats to amphidromous fish conservation are of special concern, because these fishes are globally distributed, ecologically important, and culturally significant components of stream communities (Fitzsimons et al., 1996; Keith, 2003; Feutry et al., 2012).

Amphidromous species are ubiquitous on oceanic tropical islands where the Gobiidae and Eleotridae families (suborder Gobioidei) typically dominate stream fish richness (Fitzsimons, Nishimoto & Devick, 1996; McDowall, 2004; Keith & Lord, 2011; Feutry et al., 2012). They are also a major component of stream fish assemblages on some continental tropical islands, temperate island chains, and in tropical coastal continental areas (McDowall, 2004, 2010; Thuesen et al., 2011). Amphidromous fishes play key ecological roles in the systems that they inhabit; they occupy multiple levels of stream food webs, from
primary consumer algivores (Schoenfuss & Blob, 2007) to top predators that influence the
distribution of other stream fauna (Hein & Crowl, 2010). Moreover, amphidromous
recruitment (i.e. river ingress by postlarvae) constitutes the upstream movement of biomass
from marine to freshwater systems, which may have important ramifications for riverine and
estuarine trophic ecology and production (Jenkins et al., 2010). Amphidromous fishes also
deliver valuable ecosystem services, including recreational and subsistence fisheries that
target multiple life-stages (Kwak et al., 2016). Artisanal fisheries that target postlarvae
during river ingress are of particular interest from a research and conservation standpoint
because these fisheries are widely practiced, culturally significant, and can be economically
valuable. However, their sustainability is unknown (Bell, 1999; Castellanos-Galindo et al.,
2011; Lefrançois et al., 2011).

In addition to the role of postlarval recruitment (i.e., river ingress) in ecosystem
functions and services, it is essential to the viability of amphidromous populations since
recruitment determines local and metapopulation dynamics in species with marine larval
phases (Caley et al., 1996). To understand this key life history process, the recruitment
phenology, size- and age-at-recruitment, and recruit abundances of some amphidromous fish
species have been examined (Bell, 1997; Benbow, 2004; Maeda, Yamasaki & Tachihara,
2007; Yamasaki, Maeda & Tachihara, 2007; Iida et al. 2008; Shen & Tzeng, 2008). The
results of these studies have revealed interesting commonalities, including lunar and annual
periodicity in the size, age, and abundance of recruiting postlarvae.

Despite the stressors faced by amphidromous fishes and their significance to streams
and people, this life history remains less studied than other types of diadromy (Omori et al.,
2012). Most prior studies on the early life history of amphidromous fishes have been conducted in the Indo-Pacific region, while published research on post-larval recruitment of Caribbean amphidromous fishes is scarce despite the fact that the majority of native stream fish richness in this region is composed of amphidromous gobioids (Keith, 2003). In fact—with the exception of two descriptive notes (Clark, 1905; Erdman, 1961)—one study on the island of Dominica, represents the only research on amphidromous fish recruitment in Caribbean islands (Bell, Pepin & Brown, 1995; Bell, 1997). The lack of basic knowledge (i.e., the abundance and phenology) about a key stage in the life history of a major component of stream ecosystems is a barrier to effective stream resource management in the Caribbean.

In this article, the recruit abundance and recruitment phenology of several Caribbean amphidromous fish species are quantified in multiple rivers in the Caribbean island of Puerto Rico. Specifically, the annual and lunar periodicity of postlarval recruit abundances is resolved. Additionally, interriver variability in the abundance of ingressing recruits and the effects of environmental variables on recruit abundances are explored. Finally, the length- and age-at-recruitment and hatching dates of Puerto Rico’s amphidromous fishes is quantified. The results and conclusions of this research are useful for stream and fisheries managers of Puerto Rico and the wider Caribbean region, and they improve the state of knowledge of tropical island stream ecosystem ecology.
Methods

Study systems

Puerto Rico is the smallest of the Greater Antilles and is centrally located in the Caribbean archipelago. Its streams harbor a typical oceanic tropical island stream fish assemblage; native species richness is low and—with the exception of the American Eel—all of the native stream fish are amphidromous (Neal, Lilyestrom & Kwak, 2009; Cooney & Kwak, 2013). The freshwater amphidromous fish taxa of Puerto Rico include the River Goby *Awaous banana*, Sirajo Goby *Sicydium* spp., Bigmouth Sleeper *Gobiomorus dormitor*, Smallscaled Spinycheek Sleeper *Eleotris perniger*, Fat Sleeper *Dormitator maculatus*, and Mountain Mullet *Agonostomus monticola* (Cooney and Kwak, 2013). In Puerto Rico the Sirajo Goby is a four-species-complex, which includes *Sicydium buski*, *Sicydium gilberti*, *Sicydium plumieri*, and *Sicydium punctatum*. These species are only morphologically distinguishable at the adult stage by microscopic examination of upper jaw dentition (Watson, 2000). In addition to being ubiquitous in Puerto Rico streams all of Puerto Rico’s amphidromous fish species commonly occur in streams throughout the Caribbean region (Lord et al., 2010; Cooney & Kwak, 2010; Nordlie, 2011).

Four rivers that flow to the north coast of Puerto Rico were selected as study systems. Two of the study rivers, the Río Grande de Arecibo and Río Grande de Manatí support artisanal amphidromous postlarvae fisheries. Fisher anecdotes suggest that postlarvae ingress to these rivers during mass recruitment episodes on the last-quarter of the moon phase from June through January (personal communication; Erdman, 1961). The Río Grande de Manatí and the Río Grande de Arecibo are adjacent drainages located in north-central Puerto Rico.
with headwaters in the volcanic Cordillera Central Mountain Range. They are two of the largest rivers in Puerto Rico and both flow through its Karst geology region in their middle and lower reaches (Zamora, Corujo Flores & Cham, 1986). The other two study systems were the Mameyes River and Sabana River. These adjacent drainages are located in northeastern Puerto Rico, have headwaters in the volcanic Luquillo Mountain Range, and are largely located in protected areas, including the El Yunque National Forest and the Northeast Ecological Reserve (Gould et al., 2008). These two river systems are of special conservation concern as they are considered among the least ecologically compromised systems on the island. For example, the Mameyes River is the only free-flowing river on the island with no migration barrier in Puerto Rico (Cooney and Kwak, 2013). Although the Mameyes and Sabana rivers are known to harbor abundant and native-dominated fish assemblages (Kwak, Cooney & Brown, 2007; Hein et al., 2011; Kwak et al., 2013), they do not support post-larval fisheries.

**Sampling protocol**

Postlarval amphidromous fishes were captured with a 1x1 m benthic kick-net with 1-mm mesh, fitted with wooden brails and a bottom lead line. To sample fishes, a two-person team swept the net directly towards the bank with the lead line lightly scraping the substrate. A large tub was placed on shore to collect the catch at the end of the net-haul. Captured fishes were separated from excess sediment and organic material, and preserved in a 10% buffered formalin solution. The depth of the starting point of each net-haul and its distance from shore was recorded.
All sampling occurred at the river mouth of each study system—defined as the point where the river-channel ends and ocean habitat begins. For feasibility, and because recruiting post-larvae have been observed migrating in columns along the shallow banks of rivers (Clark, 1905; Erdman, 1961), sampling was conducted near shore on the low-gradient bank side of the mouth. Each sampling occasion was composed of five net-hauls conducted over a 10-20 m section of the river mouth. The first net-haul was conducted at the down-stream end of the sampling area, and the location of subsequent net-hauls was moved incrementally upstream. No area of the mouth was ever swept twice on any sampling occasion. At times it was necessary to conduct less than five net-hauls on a sampling occasion due to inclement weather or logistical constraints, but no less than three net-hauls ever constituted a sampling occasion.

The postlarvae of all seven amphidromous fish taxa that are native to Puerto Rico were first sorted from other juvenile, postlarval, and larval fishes and crustaceans, which were sometimes present in preserved samples. Amphidromous fish postlarvae were identified and enumerated with an Olympus SZX7 optical microscope (Olympus VMZ, Germany). The total number of fishes captured in abundant samples (a total of 22 samples) was estimated by subsampling the catch. The first step of the subsampling procedure was to weigh the entire sample (± 0.01g). Then the entire sample was placed into a bowl and a tablespoon was used to mix the catch to ensure that individual fishes were evenly distributed. Next, three subsamples were taken from the whole sample by filling a tablespoon once for each subsample. Each subsample was weighed individually, all fish were counted, and the number of fish-per-gram-subsample of each species of interest was calculated. Then the mean
number fish-per-gram of each species from the three subsamples was calculated and multiplied by the total weight of the sample, which yielded an estimate of the total number of fish captured in the sample. The swept volume of each sample was calculated by approximating the area of a triangle with the depth and distance to shore measurements, and multiplying that area by the width of the kick-net. The catch-per-unit effort (CPUE) according to taxon of each sample was calculated by dividing the total number of fish captured in the sample by the swept volume.

**Temporal sampling design**

The sampling schedule was designed to reveal patterns of postlarval ingress to river mouths at two temporal scales, the annual scale, which is defined by the 365-day year cycle, and the lunar scale, which is defined by the 29.5-day cycle of the moon. All sampling was conducted during crepuscular hours, i.e. within one hour of sunrise (dawn) or sunset (dusk).

Lunar-scale sampling was conducted on all four rivers. In 2013, the Sabana River and Río Grande de Manatí were sampled during the June-July lunar cycle only, and the Río Grande de Arecibo and Mameyes River were sampled from June 18 to October 8 (i.e., four full lunar cycles). In 2014, all four rivers were sampled from May 29th through October 17th (i.e., five full lunar cycles). In 2013, postlarvae were sampled twice per lunar-quarter for a total of eight sampling events per lunar-month per river. In 2014, lunar-scale sampling was generally conducted three times per lunar-quarter for a total of 12 sampling events per lunar-month. Additional sampling was conducted during the July 2014 lunar cycle and is included in data presented here.
Annual-scale sampling occurred on two rivers, the Río Grande de Arecibo and the Mameyes River, over a 23-month period (June 2013 to April 2015). Postlarvae were sampled on two days per month during days of the last-quarter phase of the lunar cycle. Samples were collected during all months of the sampling period except for December 2013, February 2014, and April 2014. The last-quarter moon phase was selected based on evidence presented by Erdman (1961), fisher anecdotes, and because our initial lunar-scale sampling indicated that it was the period of maximum recruitment of River Goby and Sirajo Goby.

**Determination of length- and age-at-recruitment**

In addition to quantitative samples of postlarval density, recruiting postlarvae of all four native gobid stream fish taxa were examined to determine their length (total length, ± 0.01 mm) and age (days) at the time of recruitment. Samples of postlarvae for length and age determination were taken concurrently with both annual- and lunar-scale samples and typically occurred at times when high densities of postlarvae were observed. Samples of postlarvae for length and age measurements were retained on two dates at the Sabana River, eight dates at the Mameyes River, four dates at the Río Grande de Manatí, and 31 dates at the Río Grande de Arecibo. In total, 499 individuals from the four taxa were sampled for length and age analysis. Fishes were captured and identified as described above, but were preserved in 95% ethanol, rather than 10% formalin. Each individual fish was photographed with a Nikon Infinity digital camera (Nikon Inc. Tokyo, Japan) mounted to an Olympus SZX7 optical microscope (Olympus VMZ, Germany) and the total length (0.01 mm) was measured from the digital photograph with Image-Pro Insight 9 software (Media Cybernetics Inc. Rockville, MD, USA).
The age in days of individual postlarvae was determined by enumeration of sagittal otolith increments. Daily increment formation on otoliths is widely accepted for age estimation in larval fishes under most growth conditions (Brothers, Mathews & Lasker, 1976; Jones 1992) and has been validated in tropical amphidromous gobiods (Hoareau et al., 2007). Procedures described by Bell et al. (1995) were generally followed, where otoliths were extracted with forceps and a scalpel, cleaned of any adhered tissue and moved to a glass slide with the concave side of the otolith facing down. Our procedures differed from Bell et al. (1995) in that we used 95% ethanol to rinse the otoliths and did not mount the otoliths to the slides. The extracted otoliths were always sufficiently translucent to view daily increments without sanding or polishing, but a drop of immersion oil on each otolith was used to further improve clarity (Sponaugle & Cowen, 1994). Each otolith was photographed at 400X magnification using the with a Nikon Infinity digital camera (Nikon Inc. Tokyo, Japan) mounted to an Olympus SZX7 optical microscope (Olympus VMZ, Germany) and daily increments were enumerated from the digital photograph using Image-Pro Insight 9 software (Media Cybernetics Inc. Rockville, MD, USA).

Ages of sampled postlarvae were estimated as above, and the mean and standard deviation of length- and age-at-recruitment of all individuals from each taxon were calculated. The earliest and latest observed hatching times in the annual cycle were determined for all taxa by subtracting the age in days from the recruitment day of the year.

*Modeling post-larval recruitment phenology*

A periodic regression approach was adopted to model fluctuations in postlarval recruit abundances at river mouths over lunar and annual cycles. Periodic regression has been
demonstrated to be a robust and sensitive method for modeling cyclic patterns in species abundance and has previously been applied to model amphidromous postlarval recruit abundance at the annual scale (Bell, 1997; DeBruyn & Meeuwig, 2001). Poisson generalized linear models were fit to River Goby and Sirajo Goby count data with a log-link. Effort (volume of water sampled) was included as an offset term in all models. Mean CPUE (fish/m³) by lunar day was calculated for the four most numerous taxa to identify which taxa exhibited possible patterns of recruit abundances that were related to the lunar cycle. Recruitment phenology was modeled for Sirajo Goby and River Goby, because these taxa were most abundant in our samples (Table 1).

For each analysis, the day a sample occurred was assigned an angular equivalent (θ) by dividing the day of the cycle (0-28.5 for lunar and 0-364 for annual) by 2π (DeBruyn & Meeuwig, 2001). Then the following five classes of models were developed to describe periodicity in recruit abundances over the both scales of interest.

1. Models describing a single peak in recruitment over the cycle using the terms \( \sin(\theta) \) and \( \cos(\theta) \) as independent variables.

2. Models describing two even peaks in recruitment over the cycle using the terms \( \sin(2\theta) \) and \( \cos(2\theta) \) as independent variables.

3. Models describing two uneven peaks in recruitment over the cycle using the terms \( \sin(\theta) \), \( \cos(\theta) \), and \( \cos(2\theta) \) as independent variables.

4. Models describing two uneven peaks in recruitment over the cycle using the terms \( \sin(\theta) \), \( \cos(\theta) \), and \( \sin(2\theta) \) as independent variables.
5. Models describing no periodicity in recruit abundances.

Three additional variables that were hypothesized to influence the abundances of recruiting postlarvae were also considered for model inclusion as environmental covariates. The environmental covariates were a categorical variable that identified whether a sample was taken at dawn or dusk (DD), a categorical variable that identified the river of each sample (River), and the log_{10} transformed mean daily river discharge (Q) on the day of the sample. River discharge values were obtained from the lowest elevation U.S. Geological Survey (USGS) gauging station present on each river (http://nwis.waterdata.usgs.gov/nwis). The station numbers used for each river were 50029000, Río Grande de Arecibo; 50038100, Río Grande de Manatí; 50065500, Mameyes River; and 50067000, Sabana River. The environmental covariates were considered for model inclusion in all possible combinations with the each of the five classes of models described above.

No interactions among terms were modeled, so in total, 40 candidate models were developed for each species-temporal scale combination. Akaike’s Information Criterion (AIC) was used to select the best model from each suite of candidate models (Akaike, 1974; Burnham & Anderson, 2003). Once a model was selected it was used to predict the density of postlarvae over the scale of interest while holding all other independent variables at a constant level. A pseudo $R^2$ value—calculated as 1-(selected model deviance/null model deviance)—was determined as a measure of the predictive ability of each selected model (Cameron & Windmeijer, 1996).
Results

Postlarvae of all of Puerto Rico native amphidromous stream fish taxa were captured during both sampling regimes (Table 1). More River Goby postlarvae were caught in both annual-scale and lunar-scale samples than any other fish taxon, and Sirajo Goby was the second most abundant taxon. Relatively few Fat Sleepers and Mountain Mullet were captured, while intermediate numbers of Smallscaled Spinycheek Sleepers and Bigmouth Sleepers were sampled during both regimes. Plots of mean CPUE by lunar day of the four most abundant taxa revealed disproportionate amounts of Sirajo Goby and River Goby recruitment on specific days of the lunar cycle, while no such pattern emerged in plots of Smallscaled Spinycheek Sleeper and Bigmouth Sleepers (Figure 1). Moreover, the periods of elevated Sirajo Goby and River Goby recruitment coincided with the period of the lunar month when annual sampling was conducted. Therefore, periodic regression models of the catch of Sirajo Goby and River Goby postlarvae were fit to determine annual and lunar periodicity in the recruit abundances of both taxa.

Lunar-scale recruitment phenology

AIC strongly favored model 3a (i.e., two uneven recruitment peaks, all three environmental covariates) as the best model of both the River Goby and Sirajo Goby catches from the lunar sampling schedule (Table 2). These models represented a substantial decrease in deviance from null models— the pseudo-$R^2$ values of this model fit to River Goby and Sirajo Goby catches were 0.55 and 0.62, respectively. All of the terms in the models of both taxa were statistically significant ($\alpha = 0.01$) (Table 3). This indicates significant lunar periodicity in the catch of postlarvae of both goby taxa; and that goby postlarvae catch was
related to the river at which sampling occurred, the mean daily discharge on the day of sampling, and whether the fish were sampled at dawn or dusk.

The coefficients of model 3a fit to Sirajo Goby data indicated that, with other covariates held constant, recruitment would be expected to be highest at the Río Grande de Manatí, second highest at the Río Grande de Arecibo, third highest at the Mameyes River, and lowest at the Sabana River; this model also predicted greater recruit abundances at high discharge volumes and at dawn. The direction of the relationships between recruit abundances, discharge and sampling time described by model 3a fit to River Goby catches were the same as those for the Sirajo Goby model, but the relative expected abundances by river was distinct for the models of the two taxa. In the case of the River Goby model, if all other factors were held constant the highest expected recruit densities would occur at the Sabana River, followed by the Río Grande de Arecibo, then the Mameyes River; and they would be lowest at the Río Grande de Manatí.

The selected Sirajo Goby lunar-scale recruitment model describes two peaks in Sirajo Goby recruitment of very different amplitudes during each lunar cycle (Fig 2a). The major peak in Sirajo Goby recruitment is centered on the 23rd lunar day and spans days 20-28 of the lunar cycle. The location of the major peak in recruitment agrees with nominal mean CPUE values, which were also greatest on the 23rd day of the lunar cycle and were relatively high on days 22-26, and on day 28 (Figure 1). The maximum expected Sirajo Goby recruit abundances (at dawn at each river’s respective mean discharge) were approximately 30 fish/m³ at the Río Grande de Arecibo and Río Grande de Manatí; they were almost 3 fish/m³ at the Mameyes River, and nearly 1 fish/m³ in the Sabana river. A minor peak in Sirajo Goby
recruitment is centered on the 6th lunar day and is much lower in amplitude than the major peak. Expected recruit abundances at this point in the lunar cycle are less than 1 fish/m$^3$ at all rivers. Modeled Sirajo Goby recruit densities were near zero at all times in the lunar cycle outside of these two peaks in recruitment. In this projection of Sirajo Goby abundances over the lunar cycle the values at the Río Grande de Arecibo are slightly greater than at the Río Grande de Manatí, even though the coefficient for the Río Grande de Manatí is slightly greater. This occurred due to differences in the mean discharge values of the two rivers, which was greater in the Río Grande de Arecibo than in the Río Grande de Manatí.

The selected model of River Goby recruitment at the lunar scale describes a similar pattern as lunar-scale Sirajo Goby recruitment because there are two peaks in recruitment of very different amplitudes during each lunar cycle. However, both peaks were wider than those modeled for Sirajo Goby, and the expected mean recruit densities at the Río Grande de Arecibo were greater than 1 fish/m$^3$ throughout the lunar cycle. The major peak in River Goby recruitment was centered on the 22nd lunar day. Maximum expected recruit abundances at the Río Grande de Arecibo were over 200 fish/m$^3$, around 15 fish/m$^3$ at the Sabana river, and eight fish/m$^3$ at the Río Grande de Manatí. At the Mameyes River—the river with lowest predicted values at mean discharge—there were 6 fish/m$^3$. The minor peak in River Goby recruitment occurred between the 7th and 8th lunar day. During the minor peak in recruitment, expected recruit densities at the Río Grande de Arecibo were approximately 35 fish/m$^3$; they were two fish/m$^3$ at the Sabana and about 1 fish/m$^3$ at the Mameyes River and the Río Grande de Manatí. When projected at their mean discharge values, the expected recruit abundances are highest for the Río Grande de Arecibo and are
higher for the Río Grande de Manatí than the Mameyes River. This difference from the strength of the coefficients is again due to the effect of discharge on the modeled values.

**Annual-scale recruitment phenology**

Model 3a was also selected by AIC as the best model to describe both Sirajo Goby and River Goby catch at the annual-scale. The pseudo-\( R^2 \) values of the annual-scale Sirajo Goby and River Goby models were 0.48 and 0.54, respectively, which indicate substantial predictive ability of these models. All model terms for both species were significant at the \( \alpha = 0.01 \) level, so the recruit abundances of postlarvae of both goby taxa had significant annual periodicity and were also affected by the river, whether samples were taken at dawn or dusk, and daily discharge values. The coefficients of these models indicate the same direction of effects of dusk versus dawn and discharge on recruit abundances of both species that were found for these models at the lunar scale. These models also predict greater recruit abundances of both taxa at the Río Grande de Arecibo than at the Mameyes River.

The River Goby and Sirajo Goby annual-scale models describe patterns of recruitment in which postlarval densities are relatively low in the first half of the year (January through June) and relatively high during the second half (approximately July through December). Modeled Sirajo Goby densities were less than five fish/m\(^3\) from day 27 (January 26) to day 152 (May 31) in the Río Grande de Arecibo and less than 1 fish/m\(^3\) from days 12 (January 12) to 166 (June 15) in the Mameyes River. River Goby densities are expected to be less than five fish/m\(^3\) from day 10 (January 11) to day 156 (June 4) in the Río Grande de Arecibo and less than 1 fish/m\(^3\) from days 361 (December 26) to 164 (June 13) in the Mameyes River. Models of both taxa describe two distinct peaks of recruitment within
the July through January period of elevated recruitment. The highest peak in recruitment of both taxa is during late July, and the secondary peak is during late December for Sirajo Goby and late November for River Goby. Maximum Sirajo Goby recruitment was modeled to occur on day 204 of the year (July 23); mean density of Sirajo Goby postlarvae at this time is expected to be 41 fish/m$^3$ in the Río Grande de Arecibo and three fish/m$^3$ in the Mameyes River. Maximum River Goby recruitment was modeled on day 207 (July 27$^{th}$); modeled mean density of River Goby postlarvae at this time was 567 fish/m$^3$ in the Río Grande de Arecibo and 31 fish/m$^3$ in the Mameyes River. The secondary peak in Sirajo Goby recruitment was centered on day 341 of the year (December 7); modeled mean density of Sirajo Goby postlarvae at this time was 34 fish/m$^3$ at the Río Grande de Arecibo and almost 3 fish/m$^3$ in the Mameyes River. The secondary peak in River Goby recruitment was centered on day 330 of the year (November 25), and modeled mean density of River Goby postlarvae at this time was 64 fish/m$^3$ in the Río Grande de Arecibo, and 3 fish/m$^3$ in the Mameyes River.

The annual postlarval recruitment cycles of Puerto Rico gobies appears to be associated with the long-term precipitation. Mean daily rainfall in Puerto Rico is generally relatively high from the months of April through December and is low through the rest of the year. Increases in recruitment of both gobies began about 60 to 90 days after there is a notable increase in mean daily rainfall. Recruitment began to taper shortly after mean daily rainfall did and was low during the months of lowest rainfall (Fig 3).
**Length- and age-at-recruitment**

The length- and age-at-recruitment of 499 individuals from the four native gobiod taxa varied widely within and among taxa (Table 5). Sirajo Gobies were by far the largest of the four taxa at the time of recruitment (21.94 mm mean length); the minimum observed length of Sirajo Gobies was greater than the maximum length of the other three taxa. Sirajo Gobies also varied the most in size; they had the largest standard deviation and largest size range of any taxa measured. Smallscaled Spinycheek Sleepers were the second largest taxon at recruitment (15.75 mm mean length), River Gobies were third largest (14.91 mm mean), and Bigmouth Sleepers recruited at the smallest sizes (12.48 mm mean). Smallscaled Spinycheek Sleepers were the oldest of the four taxa at recruitment (65.17 days mean age); their mean age-at-recruitment and range of ages at recruitment was similar to those of River Gobies (63.55 days mean age). Sirajo Gobies were the third oldest of the four taxa at the time of recruitment, and Bigmouth Sleepers recruit at the youngest age.

**Discussion**

This is the first study to quantify the postlarval recruit abundances of all members of an island stream amphidromous fish assemblage, while determining the length- and age-at-recruitment of most of the assemblage. Gobies exhibited strong annual and lunar periodicity in recruitment to rivers and were more abundant at the postlarval stage than the other members of Puerto Rico’s amphidromous fish assemblage. Length and age at recruitment showed variation within and among amphidromous fish taxa, suggesting plasticity of pelagic larval duration.
Lunar-scale periodicity in recruit abundance

Maximum recruitment of both goby taxa occurs toward the end of the lunar cycle (days 21-28), during what is termed the last-quarter or third-quarter moon phase. Although a two peak model of lunar periodicity was the best fit to the Sirajo Goby recruitment data, it is apparent that almost all Sirajo Goby recruitment occurs during the the last-quarter moon phase, with maximum recruitment occurring around the 22nd to 23rd lunar day. The secondary modeled peak in Sirajo Goby recruitment occurred between the new and first-quarter moon phases was relatively small, and modeled recruitment at other times in the lunar cycle was near zero. Although the end of the lunar cycle is also clearly a major time of recruitment for River Goby postlarvae, recruitment of this species does not appear to be as restricted to the last-quarter moon phase as that of Sirajo Goby recruitment because the major peak of River Goby recruitment spanned several days beyond the last quarter. Moreover, substantial River Goby recruit density was predicted at the Río Grande de Arecibo early in the first-quarter moon phase, and mean density of River Goby was not modeled to approach zero at any point in the lunar cycle at this river.

These results corroborate the observations of Erdman (1961), who described mass recruitment episodes of Sirajo Goby postlarvae two days after the last lunar quarter began (i.e., 22nd and 23rd lunar day) in Puerto Rico. These findings also generally agree with Bell et al.’s (1997) study on the Caribbean island of Dominica, which identified the last lunar quarter as the main period of recruitment for Sicydium spp. However, Bell et al. (1997) concluded that maximum Sicydium recruitment occurred at a slightly different period in the lunar cycle than the present study of Puerto Rico fish. These authors observed maximum
recruitment four days after the last lunar quarter, which is three days after the modeled and nominal-mean maxima presented here. The differences between these two studies may be attributed to small latitudinal or interisland variation in recruit timing, or differences in sampling methods (e.g., locations of traps deployed by Bell et al. [1995]).

This research expands on the few prior studies of amphidromous fish recruitment in the Caribbean in several ways. First, it quantitatively models lunar periodicity in recruit abundances, allowing for explicit predictions of migration and recruitment timing. Although Bell et al. (1995) quantified recruit abundances of Sicydium spp. and posited lunar cycling in this species, no models of lunar periodicity were fit by these authors. The current study is also the first to fit a lunar-periodic model of River Goby recruitment, which demonstrates synchronicity in the lunar periodicity of River Gobies and Sirajo Gobies, and documents that River Goby postlarvae can reach even greater abundances than Sirajo Goby postlarvae. Furthermore, even with sufficient sampling, no obvious lunar cycling was revealed for any of the other Caribbean gobioid species.

Lunar periodicity in postlarval recruitment has been established in other species of amphidromous gobies (Nishimoto & Kuamo’o, 1997; Hoareau et al., 2007), catadromous eels (Sugeha et al., 2001; Jellyman & Lambert, 2003), and in coral reef fishes (McFarland et al., 1985; Robertson, 1992; Sponaugle & Cowen, 1994; Adamski et al., 2011). Adaptive mechanisms of lunar-periodic recruitment may include (1) favorable tidal patterns during the transition to adult habitats, (2) predation risk reduction during nighttime migration on dark moon phases, and (3) predation risk reduction through predator swamping. None of these adaptive mechanisms are necessarily mutually exclusive, but the plausibility of each of the
three as a mechanism behind the observed lunar periodicity of Caribbean goby recruitment is discussed below.

Caribbean amphidromous gobies conduct the majority of successful recruitment to rivers during neap tides (i.e., times of minimum differences between high and low tides), which occur on the first- and last-quarter moon phases. If adaptive mechanism (1, tidal patterns) had led to the lunar periodicities modeled here, then low tidal currents would confer greater recruitment success. This could happen if strong tidal currents interfere with postlarvae’s ability to locate and navigate through a river mouth. Negative rheotaxis is thought to direct upstream migration by postlarvae to adult habitats (Bell, 1994; Keith, 2003; Keith & Lord, 2011) and high flood tides could effectively dampen the river discharge signal that would cause negative rheotaxis, whereas strong ebbing tides may overwhelm the river discharge signal and cause gobies to swim toward shore but not necessarily navigate to a river mouth. The idea that rheotaxis plays a role in successful goby recruitment is further supported by the coefficients of the periodic regression models, which indicate that densities increase with increasing river discharge.

At first glance, it seems unlikely that adaptive mechanism (2, reduced nighttime predation) is related to the periodicity of Caribbean amphidromous goby recruitment, because maximum ingress occurred when about half of the moon is illuminated, rather than during the new moon when there is no lunar illumination. However, it remains possible that adaptive mechanism (2) is at play. For example, Sponaugle and Cowen (1994) speculated that mechanisms (1) and (2) may have worked in concert to yield maximum recruitment on the last-quarter moon phase in two reef gobies in Barbados. They reasoned that since the last-
quarter moon rises later in the night than the first-quarter moon, coinciding darkness during early evenings and neap tides could create optimal conditions for settlement from the plankton to the reef. Since amphidromous goby recruitment was greater at dawn than dusk in this study, it appears likely that more postlarvae are making the transition from ocean to river habitats at later times in the night, when the moon has already risen, which does not lend evidence to adaptive mechanism (2). However, if amphidromous gobies face substantial predation pressure from visual predators in nearshore areas of the ocean during an early-night approach to the river mouth, then adaptive mechanism (2) could lead their lunar periodicities. Adaptive mechanism (2) also gains credence when one considers the migration of these gobies beyond river ingress. After ingress, goby postlarvae undergo rapid metamorphosis to juveniles and continue to migrate upstream to adult habitats, which can be as far as 84 km from the river mouth and 702 m above sea level in Puerto Rico (Kwak et al., 2007). Further, the ideal habitats for goby species may be stream locations above natural barriers (e.g., waterfalls) that reduce or exclude predatory species (Cooney & Kwak 2013). Relatively little is known about the duration or diel patterns of upstream migration, but if migration occurs at night over the 7–14 days following river ingress, then adaptive mechanism (2) could be influential since lunar illumination continues to decrease for approximately a week and maximum lunar illumination is not reached until about two weeks following peak ingress.

By parsimony, it seems that adaptive mechanism (3, predator swamping) is the most likely to contribute to lunar synchrony since this would only require that the moon functions as cue for mass migration and a Hollings type-II interaction with river-mouth predators (Ims 1990). Moreover, diet sampling associated with the present study (Chapter 4) and
observations by other scientists (e.g., Clark, 1901) indicate that goby postlarvae undergo intensive predation by birds and fishes during these mass-ingress episodes.

To fully understand the adaptive mechanisms that drive patterns of lunar periodicity in amphidromous goby recruitment would require additional investigation. However, regardless of the drivers of lunar periodicity, it can be firmly concluded that the last-quarter moon phase is a key time for the population dynamics, conservation and management of amphidromous fishes in Puerto Rico. Furthermore, due to the agreement among the current study, the findings of Bell et al. (1995) on Dominica, and general fisher anecdotes (reviewed in Bell [1999]), this conclusion can be further generalized to state that the last-quarter moon phase is a key time for amphidromous gobies throughout the Caribbean.

Annual-scale periodicity in recruit abundance

In addition to lunar periodicity, these findings demonstrate annual periodicity in the recruitment of amphidromous gobies that ingressed to Puerto Rico rivers during the last-quarter moon phase. A high-recruitment season of approximately June through January is defined for both goby taxa, while lower recruitment is expected from late January to June. Annual periodic regression models describe two local recruitment maxima for both taxa within the general recruitment season. For both taxa the larger of the two maxima occurred in July; the secondary peak of recruitment was in December for Sirajo Goby and November for River Goby. It is possible that these are times of exceptionally high recruitment within a main recruitment season, but it is also plausible that these are simply manifestations of the variability in recruit abundances during last-quarter peaks over the two years that postlarvae densities were monitored.
The annual periodicity of Caribbean amphidromous fish recruitment demonstrated here both corroborates and expands upon the findings of Erdman (1961) and Bell et al. (1995), who used fishery-dependent data (barrels landed of mixed-taxon catches of amphidromous postlarvae purportedly dominated by *Sicydium punctatum* as a proxy for postlarval densities to fit an annual periodic regression model. These authors did not consider multipeak models, but the model that they fit and findings presented describe periods of low and high recruitment during approximately the same months as the models presented here (i.e., relatively low recruitment from January through June and relatively high recruitment from July through January). Similarly, Erdman described migration runs of postlarval Sirajo Gobies from July through January with the largest runs occurring from September to November. The results presented here expand upon this previous research by demonstrating annual periodicity in Sirajo Goby recruitment on Puerto Rico with a quantitative, fishery-independent dataset and by demonstrating similar annual periodicity in the recruitment of the River Goby.

It is likely that an underlying mechanism of annual recruitment periodicity is related to annual patterns of precipitation. Indeed, periods of increased goby recruitment coincide with a rainy season in Puerto Rico (Figure 3). Moreover, a marked increase in recruitment occurred approximately 60-90 days following a notable increase in mean daily precipitation, which approximates the typical pelagic larval duration of Puerto Rico amphidromous gobies (Table 1). One reason that recruitment success may be greater during times of increased precipitation is that river mouths in Puerto Rico are susceptible to blockage by a sand berm. These berms are formed by ocean wave action, and remain in place until a freshwater spate
opens them (Negrón-Gonzalez & Cintrón, 1979). River flows with sufficient magnitude to remove berms and maintain an open river mouth are presumably more frequent and of greater magnitude during periods of high precipitation. Thus, by temporally matching spawning and recruitment to high-rainfall periods, the probability that the river mouth will be open during larval export to the ocean and subsequent postlarval ingress to the river is enhanced. The notion that river flow plays a role in successful recruitment migration is further supported by the significant, positive relationship between goby recruitment and river discharge in all of the periodic regression models presented here.

Conclusions and conservation implications

The results and conclusions presented here identify the last-quarter moon phase during the months from June through January as especially critical periods for Caribbean amphidromous stream fish population dynamics. During these times, large pulses of amphidromous postlarvae form culturally valuable artisanal fisheries, represent a periodic food source for estuarine and riverine predators, and are a vector for the upstream transport of marine nutrients and energy. These temporal dynamics of fish recruitment clearly identify periods when Caribbean natural resource managers may focus protection of the integrity of stream ecosystems during the year and lunar-cycle. One clear way to achieve ecosystem integrity during key recruitment periods is to set goals for natural (or similar) instream flows, as they likely play a role in directing amphidromous migrations and maintaining open migration pathways to complete the complex amphidromous life cycle.
References


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Keith P., Lord C. & Lorion J. (2011) Phylogeny and biogeography of Sicydiinae (Teleostei:
Gobiidae) inferred from mitochondrial and nuclear genes. *Marine biology.*


Service F-4-7 Final Report.


Tables

**Table 1** The number of samples ($N$) and number of individuals of each taxon captured during each sampling schedule.

<table>
<thead>
<tr>
<th>Sampling Schedule</th>
<th>N</th>
<th>River Goby</th>
<th>Sirajo Goby</th>
<th>Bigmouth Sleeper</th>
<th>Smallscaled Spinycheek Sleeper</th>
<th>Fat Sleeper</th>
<th>Mountain Mullet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lunar-Scale</td>
<td>1,570</td>
<td>17,282</td>
<td>2,240</td>
<td>1,167</td>
<td>436</td>
<td>142</td>
<td>52</td>
</tr>
<tr>
<td>Year-Scale</td>
<td>382</td>
<td>17,462</td>
<td>3,012</td>
<td>102</td>
<td>526</td>
<td>26</td>
<td>31</td>
</tr>
</tbody>
</table>
Table 2 The top three (based on AIC) Poisson periodic regression models of postlarval recruit abundances for each taxon-scale-combination that was modeled (see methods for further description). Only the top three models from a suite of 40 models that were considered are displayed because in each instance the top model held all of the AIC weight. In the model descriptions \( \theta \) is either the lunar day in radians or the day of the year in radians, River is a factor for the river mouth where sampling occurred, DD is a factor that identifies if samples were taken at dawn or dusk and Q is \( \log_{10} \) transformed mean daily river discharge.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Scale</th>
<th>Model name</th>
<th>Model description</th>
<th>AIC ( \Delta )</th>
<th>( \Delta _i(AIC) )</th>
<th>( w_i(AIC) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sirajo Goby</td>
<td>Lunar</td>
<td>3a</td>
<td>( \cos(2\theta) + \cos(\theta) + \sin(\theta) + \text{River} + \text{DD} + Q )</td>
<td>7,132.77</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3b</td>
<td>( \cos(2\theta) + \cos(\theta) + \sin(\theta) + \text{River} + \text{DD} )</td>
<td>7,339.20</td>
<td>206.43</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3d</td>
<td>( \cos(2\theta) + \cos(\theta) + \sin(\theta) + \text{DD} + Q )</td>
<td>7,452.77</td>
<td>320.00</td>
<td>0.00</td>
</tr>
<tr>
<td>River Goby</td>
<td>Lunar</td>
<td>3a</td>
<td>( \cos(2\theta) + \cos(\theta) + \sin(\theta) + \text{River} + \text{DD} + Q )</td>
<td>7,324.29</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3d</td>
<td>( \cos(2\theta) + \cos(\theta) + \sin(\theta) + \text{DD} + Q )</td>
<td>7,695.23</td>
<td>3,703.64</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2a</td>
<td>( \cos(2\theta) + \sin(2\theta) + \text{River} + \text{DD} + Q )</td>
<td>7,776.22</td>
<td>4,513.93</td>
<td>0.00</td>
</tr>
<tr>
<td>Sirajo Goby</td>
<td>Annual</td>
<td>3a</td>
<td>( \cos(2\theta) + \cos(\theta) + \sin(\theta) + \text{River} + \text{DD} + Q )</td>
<td>7,704.22</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3b</td>
<td>( \cos(2\theta) + \cos(\theta) + \sin(\theta) + \text{River} + \text{DD} )</td>
<td>7,747.25</td>
<td>45.83</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4a</td>
<td>( \cos(\theta) + \sin(\theta) + \sin(2\theta) + \text{River} + \text{DD} + Q )</td>
<td>7,908.85</td>
<td>207.43</td>
<td>0.00</td>
</tr>
<tr>
<td>River Goby</td>
<td>Annual</td>
<td>3a</td>
<td>( \cos(2\theta) + \cos(\theta) + \sin(\theta) + \text{River} + \text{DD} + Q )</td>
<td>5,632.41</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3b</td>
<td>( \cos(2\theta) + \cos(\theta) + \sin(\theta) + \text{River} + \text{DD} )</td>
<td>5,724.68</td>
<td>923.26</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3d</td>
<td>( \cos(2\theta) + \cos(\theta) + \sin(\theta) + \text{DD} + Q )</td>
<td>57,397.73</td>
<td>1,077.32</td>
<td>0.00</td>
</tr>
</tbody>
</table>
**Table 3** Model parameters and coefficients (values in parenthesis are the standard deviations of the coefficients) of River Goby and Sirajo Goby lunar-scale Poisson periodic regression models.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sirajo Goby Model 3a</th>
<th>River Goby Model 3a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-12.90 (0.46)***</td>
<td>-5.76 (0.08)***</td>
</tr>
<tr>
<td>Cos(θ)</td>
<td>5.52 (0.30)***</td>
<td>-0.29 (0.03)***</td>
</tr>
<tr>
<td>Sin(θ)</td>
<td>-1.91 (0.13)***</td>
<td>-0.96 (0.02)***</td>
</tr>
<tr>
<td>Cos(2θ)</td>
<td>-5.14 (0.25)***</td>
<td>-2.10 (0.03)***</td>
</tr>
<tr>
<td>River: Mameyes</td>
<td>1.04 (0.38)**</td>
<td>-2.14 (0.09)***</td>
</tr>
<tr>
<td>River: Río Grande de Manatí</td>
<td>2.84 (0.37)***</td>
<td>-3.25 (0.09)***</td>
</tr>
<tr>
<td>River: Río Grande de Arecibo</td>
<td>2.51 (0.37)***</td>
<td>-0.83 (0.08)***</td>
</tr>
<tr>
<td>Dawn-Dusk: Dawn</td>
<td>3.54 (0.15)***</td>
<td>2.67 (0.04)***</td>
</tr>
<tr>
<td>Discharge</td>
<td>0.43 (0.03)***</td>
<td>1.08 (0.01)***</td>
</tr>
<tr>
<td>Pseudo R²</td>
<td>0.62</td>
<td>0.55</td>
</tr>
</tbody>
</table>

*** p < 0.001, ** p < 0.01, * p < 0.05
Table 4 Model parameters and coefficients (values in parenthesis are the standard deviations of coefficients) of River Goby and Sirajo Goby annual-scale poisson periodic regression models.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sirajo Goby Model 3a</th>
<th>River Goby Model 3a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-5.89 (0.32)***</td>
<td>-7.55 (0.12)***</td>
</tr>
<tr>
<td>Cos(2θ)</td>
<td>1.37 (0.05)***</td>
<td>2.88 (0.04)***</td>
</tr>
<tr>
<td>Cos(θ)</td>
<td>-0.10 (0.02)***</td>
<td>-1.24 (0.01)***</td>
</tr>
<tr>
<td>Sin(θ)</td>
<td>-2.11 (0.09)***</td>
<td>-5.50 (0.08)***</td>
</tr>
<tr>
<td>River: Río Grande de Arecibo</td>
<td>1.97 (0.10)***</td>
<td>1.83 (0.06)***</td>
</tr>
<tr>
<td>Dawn-Dusk: Dawn</td>
<td>4.31 (0.29)***</td>
<td>3.90 (0.08)***</td>
</tr>
<tr>
<td>Discharge</td>
<td>0.24 (0.04)***</td>
<td>0.48 (0.02)***</td>
</tr>
<tr>
<td>Psuedo R²</td>
<td>0.48</td>
<td>0.54</td>
</tr>
</tbody>
</table>

*** p < 0.001, ** p < 0.01, * p < 0.05
### Table 5

Length-and age-at-recruitment parameters including: mean, standard deviation (SD), minimum (min.) and maximum (max) observed values four all four gobiod taxa. Also shown are the minimum and maximum estimated hatch dates (i.e., calendar dates) and day-of-year (DOY) of hatching.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>River Goby (N=266)</th>
<th>Sirajo Goby (N=140)</th>
<th>Smallscaled Spinycheek Sleeper (N=36)</th>
<th>Bigmouth Sleeper (N=57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (d)</td>
<td>Mean (SD)</td>
<td>Min.-Max.</td>
<td>Mean (SD)</td>
<td>Min.-Max.</td>
</tr>
<tr>
<td></td>
<td>63.55 (9.03)</td>
<td>42-103</td>
<td>59.25 (10.44)</td>
<td>36-89</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>65.17 (10.52)</td>
<td>47-102</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>43.14 (6.74)</td>
<td>28-66</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>14.91 (0.77)</td>
<td>12.85-16.7</td>
<td>21.94 (1.76)</td>
<td>17.84-26.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.48 (0.57)</td>
<td>15.75 (0.74)</td>
<td>14.31-17.94</td>
</tr>
<tr>
<td>Hatch Date</td>
<td>----</td>
<td>2-Jan-11-Dec</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Hatch DOY</td>
<td>----</td>
<td>2-345</td>
<td>----</td>
<td>135-334</td>
</tr>
</tbody>
</table>

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Figures

![Graphs showing the mean catch per unit effort (CPUE) by lunar day for four gobioid taxa: Sirajo Goby, Smallscaled Spinycheek Sleeper, River Goby, and Bigmouth Sleeper. Error bars represent ± one standard error.](image)

**Fig 1.** Mean catch per unit effort (CPUE) by lunar day of four gobioi...
Fig 2. Modeled (lines) recruit abundances and observed catch per unit effort (CPUE, points) over the lunar cycle for all four rivers. The model values are the expected catch from 1 m$^3$ of water at dawn, at each river’s respective mean discharge value.
Fig 3. Mean daily rainfall for Puerto Rico averaged across all available gauges from a historic record including 1950-to present day (Menne et al., 2012). Modeled (lines) recruit abundances and observed catch per unit effort (CPUE, points) over a year for two rivers. The model values are the expected catch from 1 m$^3$ of water at dawn, at each river’s respective mean discharge value.
CHAPTER 2: Stable isotopes and contaminants in postlarval amphidromous fishes
reveal their role in nutrient and pollutant dynamics

Summary

1. Diadromous fishes are known biotransport vectors that move nutrients, energy, and contaminants in an upstream direction in stream ecosystems. This process has been demonstrated repeatedly in anadromous salmonids, but the role of other diadromous species, especially tropical taxa, as biotransport vectors is less studied.

2. Amphidromous fish species may be important biotransport vectors in their postlarval and juvenile stages with the potential to broadly and strongly impact tropical stream ecosystem functioning, but this role is practically unknown. Moreover, since some species are harvested in artisanal fisheries as postlarvae, heavy contaminant loads in them may present a human health concern.

3. This research incorporates stable isotope and contaminant analyses to infer the larval habitats of amphidromous fishes and the role of postlarvae as nutrient and contaminant biotransport vectors on the Caribbean island of Puerto Rico.

4. Recruiting postlarval amphidromous fishes were isotopically more similar to marine basal sources and food web components than those riverine and did not contain loads of anthropogenic pollutants that would be of ecological or human health concern.

5. These research findings are the first and strongest evidence that amphidromous postlarvae function as a marine subsidy to lotic ecosystems to transport marine nutrients into and up rivers without posing a health threat to natural predators or human consumers.
Introduction

Conceptual models of whole-stream ecology typically describe downstream or lateral directionality in the flows of energy, materials, and nutrients through streams and between adjacent ecosystems (Vannote, et al., 1980; Junk, Bayley & Sparks, 1989). Empirical research and models that account for human influences on streams describe the flow of anthropogenic pollutants as almost exclusively with the flow of water, from watershed surfaces to downstream receiving ecosystems (Williamson & Morrisey, 2000; Mason, 2002; Walsh et al., 2005; Larsen & Webb, 2009). However, some species of diadromous fishes have been shown to function as biotransport vectors that can move substantial quantities of nutrients and contaminants upstream and across ecosystem boundaries (Flecker et al., 2010). Therefore, the presence of diadromous fishes may alter stream ecosystem function in a manner that is not predicted by well-accepted conceptual models.

The majority of published studies on the function of diadromous fishes as upstream vectors of nutrients have been conducted on anadromous salmonids. There is good reason for the emphasis on salmonids, as they (especially Pacific salmon) are often major nutrient subsidies of marine derived nutrients (MDN) to the small-order streams and lakes where they spawn. As such, salmon spawning runs increase the primary and secondary production of these systems and even contribute to production in adjacent riparian environments (Naiman et al. 2002). Some anadromous clupeids and potamodromous fishes have also been empirically shown to be upstream vectors of nutrients (Winemiller & Jepsen, 1996; MacAvoy, Macko & Garman, 1998; Walters, Barnes & Post, 2009; Jones & Mackereth, 2016). Yet, compared to salmonids, few other studies have been conducted with the
expressed purpose of determining if these and other diadromous taxa are upstream vectors of nutrients. Moreover, the magnitude of nutrient transport functions of many species is considered severely diminished due to population declines, and thus, an ecological conservation concern (Waldman, 2003; Flecker et al., 2010).

The nutrient transport function of migratory fishes has been evaluated in only a few instances in the tropics (Winemiller & Jepsen, 1996; Sorensen & Hobson, 2005), and surprisingly few empirical studies of contaminant transport by any diadromous fishes have been conducted. Like research on nutrient transport, the majority of empirical examples of contaminant transport focus on salmonids, which have been shown to move contaminants from the marine environment to freshwater streams and lakes, and from the Laurentian Great Lakes to their tributary streams (Krümmel et al., 2003; Sarica et al., 2004; Flecker et al., 2010; Gerig et al., 2016). In some instances, the contaminants that salmon import to these systems move through the local food web and accumulate in resident species (Ewald et al., 1998). To the author’s knowledge there are no published studies of contaminant transport by non-salmonid diadromous fishes. Indeed, Flecker et al. (2010) identified the study of migratory fishes as contaminant vectors as a frontier of research on migratory fish subsidies, and they specifically pointed out the lack of research on this topic in tropical lotic systems.

Amphidromous fishes have the potential to function as nutrient vectors to upstream ecosystems in many locations. Amphidromy is a ubiquitous form of diadromy in the tropics, and amphidromous fishes are the dominant component of stream fish assemblages in multiple ecological contexts, but are especially prevalent on oceanic tropical islands (McDowall 2004, 2007; Thuesen et al., 2011). Some amphidromous species undergo
synchronous, pulsed mass-recruitment events, which represent a large influx of biomass to the river (Engman, Chapter 1; Jenkins et al. 2010). Furthermore, following recruitment to rivers, the postlarvae of some taxa such as sicydiine gobies rapidly migrate to upstream habitats, which can span substantial distance inland and elevation (Fitzsimons, Parham & Nishimoto, 2002; Keith, 2003; Cooney & Kwak, 2013). Therefore, the marine nutrients that they carry may be transported throughout a drainage basin and to low-order streams. The source of nutrition for amphidromous postlarvae, and thus, the nutrients that they transport during recruitment, has only been evaluated in a single study from Hawaii (Sorensen & Hobson, 2005), and the role of amphidromous fishes as nutrient transport vectors remains unresolved (Freeman et al., 2003).

Migrant amphidromous postlarvae also have the potential to transport contaminants across the freshwater–marine interface and to upstream habitats, a potential human and ecosystem health concern. Although the exact locations of the larval habitats of amphidromous fishes are currently unknown, they typically include estuaries or the nearshore marine environment (McDowall, 2007). Estuaries and the nearshore marine environment are known to accumulate anthropogenic pollutants carried to them by streams and rivers (Williamson & Morrisey, 2000; Larsen & Webb, 2009). Thus, amphidromous fishes may be exposed to larger quantities of environmental contaminants during larval growth and development than in the adult life stage. Amphidromous fishes are expected to experience a predator gauntlet during recruitment and upstream migration; and anecdotal evidence suggests that they are consumed by both fish and bird predators (Erdman 1961; Hein & Crowl, 2010). Additionally, amphidromous postlarvae are harvested for human consumption
in artisanal fisheries, which are globally distributed (Bell, 1999; Kwak et al., 2016). If amphidromous postlarvae are highly contaminated, they may serve as a direct path for the transfer of these contaminants to humans and riverine predators.

More information is needed on larval habitats and other characteristics of the larval phases of amphidromous fishes to determine their role as potential nutrient and contaminant vectors. For example, based on studies of larval tolerance to freshwater it was presumed that the larvae of amphidromous gobies require high-salinity environments to survive the larval duration (Bell & Brown, 1995; Iida et al., 2010). However, recent studies using otolith microchemistry have demonstrated examples of facultative amphidromy (Hogan et al., 2014; Smith & Kwak, 2014). To determine if postlarval amphidromous fish transport marine nutrients into and up freshwater streams and rivers, it is critical to know the rearing habitats of amphidromous larvae prior to river ingress.

In this paper we determine the role of amphidromous fish migration in nutrient and pollutant transport on the Caribbean island of Puerto Rico. First, we use stable isotope analyses to infer potential sources of nutrition and growth-habitats during the larval phase in several species of Caribbean amphidromous fishes. We also measure the concentrations of a suite of aquatic contaminants in the bodies of a fish that is harvested in a postlarvae fishery to evaluate safety for consumption by humans and wildlife. The findings of this research have important implications for tropical stream ecosystem functioning as well as human and ecosystem health.
Methods

Study site

We selected the Río Grande de Arecibo drainage, Puerto Rico, and the adjacent nearshore marine environment as our study system for two reasons. First, regular and abundant mass-recruitment pulses of amphidromous gobies occur at the mouth of this river during the last-quarter moon phase for over half of each year (Engman, Chapter 1). Second, the mouth of this river is one of the most well-known and important sites for an amphidromous postlarval goby fishery in Puerto Rico (Erdman, 1961; pers. obs.). Therefore, postlarvae that recruit to this specific river are subject to human consumption. The total area of the Río Grande de Arecibo drainage is approximately 997 km², and its geology is of mixed Karst and volcanic origin (Zamora, Corujo Flores & Cham, 1986). The total length of the stream system from its headwaters to the mouth in the city of Arecibo is 92 km (Zamora, Corujo Flores & Cham, 1986). Puerto Rico is an ideal island to study the early life history of amphidromous fishes, as nearly all of the native stream fishes are amphidromous and are widely distributed throughout the Caribbean and other regions (Neal, Lilyestrom & Kwak 2009).

Biota sampling

We sampled recruiting postlarval amphidromous fishes and components of the freshwater, marine, and estuarine food webs for stable isotope analysis at five locations as follows: (1) the nearshore marine environment [i.e., in the ocean, 0.6–1.3 km offshore (north) of the river mouth], (2) the river plume (i.e., a marine-freshwater mixing area ~0.1 km north of the river mouth), (3) the mouth of the Río Grande de Arecibo (i.e., the location where the
river channel ends and beach begins), (4) a large pool downstream of a low-head dam in the lower freshwater zone of the river that is approximately 2.64 river-km (rkm) upstream of the river mouth, and (5) a site in a main tributary of the Rio Grande de Arecibo called the Tanamá River that is ~7.77 rkm upstream of the river mouth. Whole seston samples (i.e., plankton, small nekton, and particulate organic matter) were collected from surface waters in the nearshore marine environment and from the river plume with a bongo-style net, which had two 0.3-m hoops, 500-µm mesh, a 6:1 tail-to-mouth ratio, and was fitted with solid cup ends (Smith & Hightower, 2012). Recruiting postlarval amphidromous fishes were collected at the river mouth and immediately downstream of the low-head dam with a 1 x 1m, 1-mm mesh, benthic kick net in a method analogous to a seine-haul (Engman, Chapter 1). Samples of benthic algae, snails of the genus Neritina, and riverine coarse particulate organic matter [CPOM (primarily leaves)] were also collected at the low-head dam site. Algae (primarily filamentous) was collected from cobble-sized substrate by scraping the algae with a scalpel; CPOM was collected by scooping with a D-shaped dipnet in depositional areas; and Neritina were collected by hand. Adult Sicydium spp. and benthic algae samples were collected in the Tanamá River and adult Sicydium were collected with a pulsed-DC backpack electrofisher (Smith-Root, Vancouver, WA). All samples collected for stable isotope analyses were temporarily stored in Whirl-paks (Nasco Science, Fort Atkinson, WI) on ice and in the dark while in the field, then were stored in a 20°C freezer until further processing.

For contaminant analyses, we collected samples of postlarval Sicydium spp. at the river mouth because Sicydium spp. are the target of the amphidromous postlarvae fishery, and nearly all fishing occurs at river mouths. Furthermore, samples were taken
simultaneously with the active fishery. *Sicydium* spp. were collected and handled as described above for stable isotope samples; they were sorted from other taxa in the field using food-grade utensil and stored in food-grade containers on ice before being frozen for later processing.

*Stable isotope analyses*

Seston samples that were analyzed for carbon and nitrogen stable isotope ratios were defrosted, inspected for any larval or postlarval fishes under a dissecting microscope and filtered onto pre-combusted (450°C x 4 h) 47-mm glass fiber filter (GF/F; Whatman) at low pressure vacuum. The filters were treated with 10% hydrochloric acid (HCl) and then were rinsed through with deionized (DI) water. A single seston sample to be used for sulfur isotopic analysis was processed in a similar manner to that of seston for carbon and nitrogen except that the sample was concentrated into a small volume above the filter then picked from this concentrate with forceps and rinsed with DI water before drying. All sampled postlarvae were sorted to species, or genus in the case of *Sicydium*, and were rinsed with deionized water (DI). The head and viscera of each postlarva was removed with a scalpel to prevent any stomach contents from affecting their isotopic signature and to remove the ossified otolith. Algae samples were inspected under a dissecting microscope and additional POM, macroinvertebrates, and large pieces of substrate were separated from the algae. Then, algae samples were rinsed free of sediment with DI water. Macroinvertebrates were also removed from CPOM samples, which were subsequently rinsed with DI water. Samples of *Neritina* were removed from their shells, foot muscle tissue was separated from the viscera, which was discarded, and muscle tissue was rinsed with DI water. Lateral musculature was
removed from skinned samples of adult *Sicydium*. Samples of adult *Sicydium* were processed and analyzed as individuals, while all other samples were composites of multiple individuals that were sampled at the same time and location. Composite samples of amphidromous postlarvae contained 7–30 individuals. All samples were dried to a constant mass at 60°C, and all samples except the GFF filters were ground to a fine powder with a mortar and pestle before analyses.

Stable isotope analyses were conducted at the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University in Flagstaff, Arizona. There, samples were combusted to gasses (CO₂, N₂ and SO₂), and their respective stable isotopes were measured with gas isotope-ratio mass spectroscopy. Isotopic ratios were calculated with the following formula:

$$\delta X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

where $R_{\text{sample}}$ is $^{13}\text{C} / ^{12}\text{C}$, $^{15}\text{N} / ^{14}\text{N}$, or $^{34}\text{S} / ^{32}\text{S}$ in the sample and $R_{\text{standard}}$ is $^{13}\text{C} / ^{12}\text{C}$, $^{15}\text{N} / ^{14}\text{N}$, or $^{34}\text{S} / ^{32}\text{S}$ in the standard. Analytical standards were atmospheric N₂ for nitrogen, Vienna Pee Dee Belemnite for carbon and Canyon Diablo Triolite for sulfur (Fry, 2006).

**Contaminant analyses**

Samples of *Sicydium* postlarvae were analyzed for organic and inorganic contaminants as composites of the whole body tissues of multiple individuals, because this is the manner of human consumption. We created replicate composite samples of *Sicydium* (each sample consisted of 103–263 individuals) collected on two dates (20 July, 2014, and 9
July, 2015) for analyses of organic contaminant and metal concentrations. We prepared samples for analyses by first homogenizing them in a stainless steel food processor and then storing them in a -80°C freezer in food-grade containers.

Samples were analyzed for a suite of organic contaminants including ten polychlorinated biphenyl congeners (PCBs, all Aroclor), 20 organochlorine pesticides (OCPs), and 27 semivolatile organic compounds [18 polycyclic aromatic hydrocarbons (PAHs), 7 current use pesticides (CUPs), 1,1'-Biphenyl, and Dibenzofuran] by Shealy Environmental Services, Inc., West Columbia, SC. Additionally, the percent lipids of each sample was measured by Shealy Environmental Services, Inc. Organic contaminant compounds were extracted from tissue samples using EPA method 3540 C (Soxhlet extraction). The concentrations of semivolatile compounds were measured by gas chromatography/mass spectrometry (GC/MS) following the protocol for EPA method 8270 D. The concentrations of PCBs were measured by GC following EPA analytical method 8082A, and the concentrations of OCPs were measured with EPA analytical method 8081B. The detection limits of organic compounds ranged 0.021–220 µg/kg wet weight (Table 1). Quality control was conducted by measuring the percent recovery of surrogate compounds in spiked samples. The percent recovery was always within the acceptance limits and all organic contaminants are reported as wet weight (w.w.) concentrations.

To further prepare Sicydium postlarvae homogenates for metal analysis, the tissues were weighed wet, freeze dried (lyophilized) and re-weighed to determine the percent moisture. Then they were analyzed for 22 metals at RTI International, Durham, NC. To measure the concentrations of all metals except for mercury, fish tissues were processed
following a modified version of the EPA 3050B method, then were analyzed with a Thermo X-Series II ICP-MS or a Thermo iCAP6500 ICP-OES depending on the concentration of the analyte present in the samples. Mercury was analyzed with the cold-vapor atomic absorption method using a Milestone DMA-80 mercury analyzer. Quality control on metal analyses included measures of percent recovery of laboratory control samples (LCS). Percent recovery was always 80–120%, which is an acceptable level of accuracy, and therefore, results were not corrected for percent recovery.

We summarized laboratory results for comparison with other studies of fish tissue concentrations and agency guidance for assessing chemical contaminant data in fishes. We first calculated the percent of detections for each contaminant (i.e., percent of samples that had concentrations above the detection limit). Next, we calculated the mean and standard error of the concentrations of each contaminant that was determined for all four of the samples. In the case that a specific analyte was detected in some, but not all, samples a concentration of 1/2 of the detection limit was assumed for the samples where the analyte was not detected for the purpose of calculating the mean and standard error. Mean and individual sample concentrations of each contaminant were compared to endpoints and thresholds provided for concentrations in the tissue of fishes or seafood by the U.S. Environmental Protection Agency (EPA) and the U.S. Food and Drug Administration (FDA). In the case that guidance from these two federal agencies was unavailable for a given analyte, we referred to the recommendations of the North Carolina Department of Public Health (NCDPH).
**Results**

*Isotopic composition*

We collected samples of *Sicydium* spp. and *Awaous banana* for stable isotope analyses in two morphological stages, and both stages had similar isotopic composition. Postlarvae at the river mouth were almost completely unpigmented and displayed the characteristics of the postlarval stage 1 (PL1) described by Keith *et al.* (2008) for *Sicyopterus lagocephalus*. Postlarvae at the dam had acquired pigmentation analogous to the postlarval stage 2 (PL2) described by the same authors. In the Río Grande de Arecibo, recruiting gobies were in the PL1 stage at the mouth of the river, then migrated to the dam, and transitioned to the PL2 stage in as little as 24 to 48 hours after river ingress (pers. obs). *Sicydium* spp. and *Awaous banana* from the mouth and dam stations had very similar δ³⁴S, δ¹³C, and δ¹⁵N signatures (Table 1), and mean and standard error of δ³⁴S, δ¹³C, and δ¹⁵N values of samples of the two goby taxa at both stages and locations were calculated as single values to illustrate the isotopic signature of these taxa during ingress to river mouths and subsequent upstream migration (Fig.1, 2).

Mean and individual δ³⁴S values of all of the migrant amphidromous postlarvae were much more similar to each other, the sample of marine seston, and known values for seawater sulfate than any of the riverine food web components. The δ³⁴S values of all riverine food web components were between 8.4 ‰ and 9.4 ‰ and even though riverine benthic algae had a relatively variable sulfur signature, its mean δ³⁴S value was more than one standard error below 10 ‰. δ³⁴S values (mean values where available) of all the amphidromous postlarvae were between 20.3 ‰ and 21.3 ‰ and were always one standard
error or more above 19.8 ‰ (Table 1, Fig. 1). These means and standard errors overlap, or are very close to the δ³⁴S value of the nearshore marine seston sample and global ocean water sulfate, which is uniformly ~ 21‰ (Rees, Jenkins & Monster 1978; Michener & Lajtha 2007).

The δ¹³C values of amphidromous postlarvae were generally much more enriched in ¹³C than the riverine food web components and slightly more enriched than nearshore marine seston samples, while seston samples from the plume had intermediate δ¹³C values. The mean δ¹³C values of all three taxa of amphidromous postlarvae were clustered together and spanned values of -19.4 ‰ (Agonostomus monticola) to -17.8 ‰ (Eleotris perniger) (Table 1, Fig. 1). Also, where calculable, the variability (as standard error) of the δ¹³C of postlarval migrants was relatively low, which suggests consistency in the basal resources utilized by larval goby taxa for growth (Table 1). Nearshore marine seston had slightly ¹³C-depleted and more variable δ¹³C values than amphidromous postlarvae, but was ¹³C-enriched and less variable than samples from the river plume. The mean δ¹³C values of all riverine food web components were lower than any migrant postlarvae or marine seston. Neritina had the most enriched δ¹³C signature of riverine food web components, while the river CPOM signature was the most depleted. Adult Sicydium had a depleted mean δ¹³C value that was relatively close to the mean value of benthic algae. The δ¹³C signature of riverine benthic algae was the most variable (SE = 7.3) of all food web components analyzed (Table 1); its mean ± 1 SE encompassed the values of both of its expected consumers [i.e., Neritina spp. and Sicydium adults (Erdman, 1961; March et al., 2002)] as well as values for all other food web
components, except for the mean of *Awaous banana* and the single value obtained for *Agonostomus monticola* postlarvae (Table 1, Fig. 1).

All taxa of amphidromous postlarvae exhibited similar, and relatively low (4.8 to 5.7 ‰) δ^{15}N values. Of the postlarvae, *Sicydium* spp. had the lowest δ^{15}N at 4.8 ‰, and *Agonostomus monticola* had the highest at 5.7 ‰ (Table 1). All of these values were below the mean values for nearshore marine seston, river algae, adult *Sicydium* spp., and *Neritina* spp. but above values for seston collected in the plume and river CPOM. *Neritina* spp. had the most enriched nitrogen isotopic signature of any of the sample types and was 3.8‰ above the mean of its expected basal food source, benthic algae. Adult *Sicydium* spp. is another known algivore, which had only a slightly more ^15^N-enriched mean nitrogen signature, and the error bars of these two consumers were overlapping (Fig. 1). Benthic algae had the most variable δ^{15}N values followed by marine seston (Table 1).

**Contaminants**

*Sicydium* postlarvae were low in lipid content (mean percent lipids =1.89%, SE=0.12); therefore, it was not necessary to correct contaminants concentrations. We detected 14 OCPs and three CUPs (all forms of Endrin) in the bodies of *Sicydium* postlarvae; levels of all other organic contaminants were below detection. No PCBs were at concentrations above the detection limits. Of the 17 organic contaminants detected, six (alpha-BHC, delta-BHC, dieldrin, Endosulfan I, Endosulfan II, Endrin, and 4-4′-DDT) were present at concentrations above detection limits in 100% of the samples analyzed (Table 2). All of the observed concentrations of organic contaminants were below known permissible
limits or health endpoints for concentrations in fish tissue that are recommended for human consumption (USFDA, 1998; USEPA, 2000; NCDPH, 2007).

Twenty of the 22 metals analyzed were present in the bodies of *Sicydium* postlarvae at concentrations above laboratory detection limits. With the exception of cobalt, lead, silica, and vanadium, all detected metals were present in 100% of the samples that were analyzed (Table 2). Arsenic, cadmium, chromium, lead, mercury, nickel, and selenium are classified as chemical hazards or target analytes for risk assessment and management with maximum levels or health endpoints identified for human consumption (USFDA, 1998; USEPA, 2000). All of the measured tissue concentrations of total arsenic in *Sicydium* were well below the FDA-designated limit for shellfish of 76 ppm total arsenic (USFDA, 1998). The EPA and many other agencies provide guidance for values of inorganic arsenic in edible fish tissues only because the organic form of arsenic is considered to be relatively nontoxic (USEPA, 2000). To compare our results with these values, we used information from a review by Neff (1997) that demonstrated that inorganic arsenic makes up between 0.5 and 1% of the total arsenic in most marine invertebrates and fishes. If we conservatively assume a value of 1% inorganic arsenic in the *Sicydium* spp. that we sampled, then the mean value of inorganic arsenic in our samples would be 0.05 µg/g w.w.; all but one individual sample would be below the EPA unlimited non-cancer health endpoint; and one sample would be in the range of the 16 meals/month consumption limit. Measured cadmium concentrations approached, but were below, the EPA non-cancer unrestricted health end point for fish tissues (0.088 µg/g w.w.). The mean of cadmium concentration in *Sicydium* spp. was 0.06 ± 0.01 µg/g w.w. (Table 2), with a maximum measured cadmium concentration of 0.075 µg/g w.w. The mean
tissue concentration of selenium in *Sicydium* spp. (1.45 ± 0.58 µg/g w.w.) was also close to, but below, the EPA non-cancer unrestricted health end point for fish tissues (1.5 µg/g w.w.); however, values of two of the individual samples (2.40 and 2.52 µg/g w.w.) exceeded the unrestricted health endpoint and were in the consumption range recommended for 16 meals per month (1.5 - 2.9 µg/g w.w.) (USEPA 2000, Table 2). The mean and individual values of all other metal concentrations were well below known and available thresholds.

**Discussion**

**Stable isotopes**

Sulfur isotopic composition of amphidromous migrant postlarvae provided clear evidence that they feed and grown in the marine environment prior to river ingress. Sulfur isotopes are useful for distinguishing reliance on marine or freshwater food webs in animals, because minimal fractionation occurs with trophic transfer, and marine primary producers are consistently and strongly distinct from terrestrial plants in δ³⁴S (Peterson & Fry, 1987). In the case of our study, the δ³⁴S signatures of migrant postlarvae were always more than twice as enriched in ³⁴S as primary producers, CPOM, and primary consumers in the freshwater environment, including adults of the same taxon. Postlarval δ³⁴S values were very similar to a sample of nearshore marine seston and universal seawater sulfate values (Rees *et al.*, 1978). Moreover, they were within the range of values (+17 to +21‰) that would be expected for marine plankton and seaweed on a global basis (Peterson & Fry, 1987). This strongly implies that oceanic primary producers or consumers of oceanic primary production are a likely food source for larval amphidromous fishes.
The results of the carbon isotope analysis provided additional evidence that amphidromous larval growth is based on production from marine sources, rather than riverine. Like, $\delta^{34}$S the $\delta^{13}$C values of amphidromous postlarvae exhibited minimal variation around mean values, and means or individual values were similar across taxa, which suggest reliance on a single, common source of primary production. Also, the $\delta^{13}$C values of amphidromous postlarvae were distinct from, and enriched in $^{13}$C compared to riverine or river plume food web components. This result agrees well with the findings of Coat et al. (2009, 2011), who also observed a pattern of increasing carbon isotopic enrichment with increased reliance on marine production in stream organisms on the Caribbean island of Guadeloupe.

The difference in mean $\delta^{13}$C of Sicydium spp. adults and postlarvae (8.8 ‰) is especially informative, because it indicates that this taxon depends on separate basal resources for larval and adult growth. Our carbon isotopic results indicate that the pelagic marine environment is the likely source of primary production that yields larval growth in Caribbean amphidromous fishes because their $\delta^{13}$C values were similar to those of a marine seston.

Although $\delta^{15}$N can be useful for distinguishing marine and freshwater food webs, we primarily interpreted our measured nitrogen isotopic composition as an indicator of trophic level. $\delta^{15}$N is a reliable indicator of trophic level because trophic-transfer fractionation of this isotope causes a predictable level of enrichment with each addition of a trophic level in a given food web (Peterson & Fry, 1987). We sampled two known consumers of benthic algae.
in the freshwater environment, both of which had higher δ^{15}N than algae. *Neritina* spp. was about 3.8‰ more enriched than the mean value for algae, which would be an expected difference for an algal consumer that is known to feed on benthic invertebrates as well (March *et al.*, 2011). Our measured δ^{15}N value was about 1‰ greater than the value reported by March *et al.* (2011) for *Neritina* spp. in a lowland reach of another Puerto Rico river. The difference in mean δ^{15}N values of algae and adult *Sicydium* spp. was not as great as that expected by trophic fractionation. Algae values were highly variable, which may suggest that anthropogenic inputs (e.g., wastewater effluent, fertilizer) have enriched the δ^{15}N values of basal resources in the streams that we studied on some of the sampling occasions (McClelland, Valiela & Michener, 1997). Furthermore, although we did inspect for and remove any macroinvertebrates from our samples of benthic algae, it is possible that some microinvertebrates remained in some samples, which would increase their δ^{15}N. Marine seston had a higher mean δ^{15}N signal than postlarval fish, which may indicate that these samples contained a mix of trophic levels, possibly including those above larval fishes. We anecdotaly observed ctenophores, chaetognaths and cnidarians in some samples (pers. obs.). The relatively high variability in marine seston δ^{15}N suggests that individual samples contained varying proportions of the different trophic levels. The value of postlarval δ^{15}N relative to seston appears to indicate that amphidromous larvae feed on the lower trophic levels of the marine pelagic food web, possibly consuming a combination of marine primary producers and primary consumers, such as zooplankton. However, virtually nothing is known about the food habits of amphidromous larvae in their pelagic phase.
Our stable isotope findings provide key insights on the environment that supports larval growth and development by several Caribbean amphidromous fishes. Specifically, we present evidence based on stable isotope analysis of multiple elements that *Sicydium* spp., *Awaous banana*, *Eleotris perniger*, and *Agonostomus monticola* utilize the marine environment during larval stages, rather than freshwater portions of a river or a freshwater-marine mixing zone near the river mouth. Carbon and sulfur isotopic signatures were useful for making this distinction, as the bodies of amphidromous postlarvae were consistently more similar in this aspect to components of the marine food web that those sampled in the river or the river plume. Additionally, the isotopic signatures of *Sicydium* spp. postlarvae were distinct from those of adults, which suggests differential habitat use and basal sources between these life stages. Furthermore, we found no evidence that migrant goby postlarvae that were captured in the freshwater portion of the river had distinct isotopic signatures from those captured at the river mouth. Although Smith and Kwak (2014) found evidence of deviation from amphidromy in small percentages of *Awaous banana*, *Agonostomus monticola*, and *Sicydium* spp. populations with otolith microchemistry analyses, these findings confirm their broader conclusion that amphidromy is the dominant life history of these species.

**Contaminants**

It does not appear that *Sicydium* spp.—a culturally-significant food fish in Puerto Rico and other Caribbean locations in the postlarval stage—accumulates high concentrations of most of the toxic contaminants that we measured during larval growth and transition to postlarvae. We detected several organic pollutants, including OCPs and CUPs in the bodies
of this fish at low concentrations, but these were always at levels far below levels of concern for human health. Twenty metals that were analyzed were at levels above detection limits. They included many that are essential nutrients and are only toxic at extremely high levels of consumption (e.g., sodium, magnesium, potassium, etc.). Other, more toxic metals present at levels above detection limits (e.g. chromium, mercury, lead, and nickel) were at concentrations that were well below known thresholds or levels of concern for human consumption.

Although *Sicydium* spp. contained nearly negligible amounts of most of the contaminants that we measured, three metals that are considered chemical hazards, cadmium, selenium, and arsenic, were present in *Sicydium* spp. postlarvae at concentrations that are close to the lowest levels of concern for human consumption. Cadmium concentrations were below known thresholds or guidelines in all samples, but our maximum measured value was within 0.013 µg/g w.w. of the EPA 16 meals per month consumption limit. Individual values of arsenic and selenium crossed EPA thresholds for recommending that consumers limit the consumption of postlarval *Sicydium* spp. to 16 meals per month, but mean concentrations of both of these metals were within recommended values for unlimited consumption. Furthermore, although selenium can have acute and chronic toxicity, it is also an essential nutrient with interactive effects that can reduce the toxicity of other metals such as mercury, lead, and arsenic (USEPA, 2000). As such, the consumption of *Sicydium* spp. does not appear to be of human health concern in this system. Because *Sicydium* spp. postlarvae are available for harvest during very limited time periods, and this food is a regional delicacy,
rather than a staple seafood (Engman, Chapter 1), it is very unlikely that the consumption of this food product is of any concern to human health, fish, or wildlife predators.

The sources and processes that resulted in the observed tissue concentrations of these metals are uncertain, but there is no conclusive evidence to indicate that *Sicydium* spp. are highly contaminated from anthropogenic sources. For example, the levels of total arsenic in marine organisms are known to be extremely variable and are often naturally much higher than those of freshwater fishes, and based on our calculations, the levels of inorganic arsenic in *Sicydium* postlarvae would be within the range of values (0.001 to 0.5 μg/g w.w.) expected for fish and invertebrates from uncontaminated marine environments (Neff, 1997; USEPA, 1997). Arsenic, cadmium, and selenium are naturally present in the earth’s crust and reach the oceans through natural transport routes such as weathering, erosion, volcanoes and rivers. However, all of these metals can also originate from anthropogenic sources and activities as well, such as smelting/mining, surface mine drainage, paints, alloys, batteries, plastics, pesticides, herbicides, and waste disposal operations (GESAMP, 1987; WHO 1992; Neff 1997; USEPA, 2000).

These findings indicate that amphidromous postlarvae do indeed transport marine nutrients and energy into and up rivers during recruitment migrations, but do so without heavy body-loads of anthropogenic contaminants. Therefore, if there is an efficient means for the transfer of these nutrients to freshwater or estuarine food webs (e.g., via predation or excretion), then amphidromous postlarval recruitment functions as a marine material subsidy to these systems. Furthermore, this subsidy is a relatively uncontaminated food source for local people and fish and bird predators. The magnitude of the subsidy depends on the
biomass of postlarval migrants, but these research findings are the first and strongest evidence of a marine subsidy to freshwater lotic ecosystems from the migration of amphidromous fishes.
References


Smith W.E. & Kwak T.J. (2014) Otolith microchemistry of tropical diadromous fishes:


USFDA (Food and Drug Administration). (1998) Action levels for poisonous or deleterious substances in human food and animal feed. *Industry Activities Staff Booklet*. Washington, DC.


pseudoharengus) contribute marine-derived nutrients to coastal stream food webs.

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Tables

**Table 1** Mean carbon, nitrogen, and sulfur stable isotopic composition of various marine, river plume, and riverine organic matter samples from the Río Grande de Arecibo and nearshore marine environment. Values shown are mean ± SE were calculable. Numbers in parentheses represent sample size, the number of composite samples for each item except for *Sicydium* spp. adults where it is the number of individuals sampled.

<table>
<thead>
<tr>
<th>Item</th>
<th>δ(^{13})C (‰)</th>
<th>δ(^{15})N (‰)</th>
<th>δ(^{34})S (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benthic algae</td>
<td>-26.1 ± 7.3 (3)</td>
<td>5.8 ± 0.7 (3)</td>
<td>8.3 ± 1.4 (2)</td>
</tr>
<tr>
<td>Particulate organic matter</td>
<td>-29.6 (1)</td>
<td>4.6 (1)</td>
<td>9.0 (1)</td>
</tr>
<tr>
<td><em>Neritina</em> spp.</td>
<td>-21.5 (1)</td>
<td>9.6 (1)</td>
<td>8.4 (1)</td>
</tr>
<tr>
<td><em>Sicydium</em> spp. adults</td>
<td>-27.6 ± 0.2 (7)</td>
<td>6.2 ± 0.1 (7)</td>
<td>9.4 ± 0.1 (7)</td>
</tr>
<tr>
<td><em>Sicydium</em> spp. (river mouth)</td>
<td>-18.8 ± 0.0 (3)</td>
<td>4.8 ± 0.3 (3)</td>
<td>20.3 ± 0.5 (3)</td>
</tr>
<tr>
<td><em>Sicydium</em> spp. postlarvae (dam)</td>
<td>-18.8 ± 0.1 (3)</td>
<td>4.8 ± 0.2 (3)</td>
<td>20.4 ± 0.2 (1)</td>
</tr>
<tr>
<td><em>Awaous banana</em> postlarvae (river mouth)</td>
<td>-18.4 ± 0.1 (2)</td>
<td>5.0 ± 0.1 (2)</td>
<td>20.6 (1)</td>
</tr>
<tr>
<td><em>Awaous banana</em> postlarvae (dam)</td>
<td>-18.8 ± 0.2 (2)</td>
<td>5.5 ± 0.1 (2)</td>
<td>20.4 ± 0.2 (2)</td>
</tr>
<tr>
<td><em>Agonostomus monticola</em> postlarvae</td>
<td>-17.8 (1)</td>
<td>5.7 (1)</td>
<td>21.3 (1)</td>
</tr>
<tr>
<td><em>Eleotris perniger</em> postlarvae</td>
<td>-19.4 (1)</td>
<td>4.9 (1)</td>
<td>20.4 (1)</td>
</tr>
<tr>
<td>Seston (nearshore marine)</td>
<td>-20.7 ± 0.6 (6)</td>
<td>6.2 ± 0.6 (6)</td>
<td>20.2 (1)</td>
</tr>
<tr>
<td>Seston (river plume)</td>
<td>-24.5 ± 1.3 (3)</td>
<td>4.7 ± 0.2 (3)</td>
<td>20.8 (1)</td>
</tr>
</tbody>
</table>
Table 2 Mean concentration and standard error (SE) of all contaminants that were detected in at least one sample of *Sicydium* spp. postlarvae from the Río Grande de Arecibo. Organic contaminant concentrations and minimum detections limits (MDL) are expressed as parts per billion wet weight. Metal concentrations are expressed as parts per million wet weight and metal MDLs are expressed as parts per million dry weight. Percent (%) detection is the percent of samples in which the contaminant was measured above the MDL.

<table>
<thead>
<tr>
<th>Class</th>
<th>Contaminant</th>
<th>Mean</th>
<th>SE</th>
<th>MDL</th>
<th>% Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organochlorine pesticides</td>
<td>Aldrin</td>
<td>0.04</td>
<td>0.01</td>
<td>0.04</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>gamma-BHC (Lindane)</td>
<td>0.07</td>
<td>0.03</td>
<td>0.08</td>
<td>25</td>
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<tr>
<td></td>
<td>alpha-BHC</td>
<td>0.12</td>
<td>0.01</td>
<td>0.05</td>
<td>100</td>
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<td></td>
<td>beta-BHC</td>
<td>0.60</td>
<td>0.25</td>
<td>0.04</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>delta-BHC</td>
<td>0.20</td>
<td>0.08</td>
<td>0.02</td>
<td>75</td>
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<tr>
<td></td>
<td>cis-Chlordane</td>
<td>0.07</td>
<td>0.03</td>
<td>0.03</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>trans-Chlordane</td>
<td>0.04</td>
<td>0.03</td>
<td>0.03</td>
<td>25</td>
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<tr>
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<td>4,4'-DDT</td>
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<td>0.01</td>
<td>0.07</td>
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<td>Dieldrin</td>
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<td>0.06</td>
<td>0.03</td>
<td>100</td>
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<td></td>
<td>Endosulfan I</td>
<td>0.26</td>
<td>0.08</td>
<td>0.04</td>
<td>100</td>
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<td></td>
<td>Endosulfan II</td>
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<td>0.06</td>
<td>0.03</td>
<td>75</td>
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<td></td>
<td>Heptachlor</td>
<td>0.16</td>
<td>0.09</td>
<td>0.06</td>
<td>25</td>
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<tr>
<td></td>
<td>Heptachlor epoxide</td>
<td>0.08</td>
<td>0.02</td>
<td>0.04</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Methoxychlor</td>
<td>0.18</td>
<td>0.08</td>
<td>0.07</td>
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<td>Current use pesticides</td>
<td>Endrin</td>
<td>0.14</td>
<td>0.04</td>
<td>0.02</td>
<td>100</td>
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<tr>
<td></td>
<td>Endrin aldehyde</td>
<td>0.05</td>
<td>0.02</td>
<td>0.03</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Endrin ketone</td>
<td>0.07</td>
<td>0.03</td>
<td>0.04</td>
<td>50</td>
</tr>
<tr>
<td>Metals</td>
<td>Aluminum</td>
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<td></td>
<td>Arsenic</td>
<td>4.98</td>
<td>2.53</td>
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<td>Barium</td>
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<td></td>
<td>Cadmium</td>
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<td>0.01</td>
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<td>Cobalt</td>
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Fig. 1 Mean (± SE) $\delta^{34}$S of organic matter samples, including migrant amphidromous postlarvae, riverine food web components, and marine seston from the Río Grande de Arecibo and nearshore marine environment. Points are individual sample values where error bars are absent.
**Fig. 2** Mean (± SE) δ¹³C and δ¹⁵N biplot for the Río Grande de Arecibo and nearshore marine environment. Organic matter sample material is indicated by symbols. Symbols are individual sample values where error bars are absent.
CHAPTER 3: The role of amphidromous fish recruitment in the trophic ecology of Caribbean freshwater–marine ecotones

Summary

1. Amphidromous fishes undergo pulsed mass-recruitment events that may function as an important input of marine-derived biomass to tropical island estuaries and streams and a potential food source for commercially and recreationally important fishes.

2. Amphidromous fishes and shrimps are expected to pass through a predator gauntlet during recruitment and upstream migration. This predator gauntlet is perceived to affect recruitment dynamics, migratory behavior, and the distribution of amphidromous species, but this has not been demonstrated by researching predation on amphidromous postlarvae across the freshwater–marine ecotone.

3. In this article, we determine the role of postlarval amphidromous fish recruitment in the fish assemblage structure and trophic ecology of a Caribbean freshwater–marine ecotone on the island of Puerto Rico.

4. Nearly all riverine and estuarine predator fish diets sampled contained amphidromous gobioids during an amphidromous fish mass-recruitment event. This event was related to major changes in the trophic ecology of several predator fish species, which experienced greater stomach or foregut fullness, feeding frequency, and diet shifts during the recruitment event. The fish assemblage structure and distribution of individual predator species in this ecotone remained stable throughout the mass-recruitment event.
5. Amphidromous fish and shrimp postlarvae indeed experience a predator gauntlet during recruitment and upstream migration and are important components of the diets of recreationally and commercially valuable estuarine and freshwater predator fishes. Amphidromous fish recruitment was confirmed as a periodically available, marine-derived material subsidy to tropical estuaries and streams. These new findings may guide estuarine and inland fisheries management and ecosystem conservation plans in the Caribbean to include recruitment of amphidromous postlarvae and related threats to amphidromous species.
**Introduction**

Diadromous, estuarine, and primarily marine fishes typically make up the majority of native inland fish richness on oceanic islands and in steep-gradient coastal continental drainages throughout the tropics (Winemiller, Agostinho & Caramaschi, 2008; Neal, Lilyestrom & Kwak, 2009; Thuesen et al., 2011; Kwak et al., 2016). In these types of systems, marine and estuarine species are lost from fish assemblages as distance from the ocean and gradient increase, which leaves middle and upstream stream sections dominated by diadromous fishes that can traverse high-gradient reaches (Lyons & Schneider, 1990; Neal, Lilyestrom & Kwak, 2009; Jenkins et al., 2010; Jenkins & Jupiter, 2011; Cooney & Kwak, 2013). Amphidromy is an especially prevalent form of diadromy on tropical islands where amphidromous fishes (predominately Gobiidae and Eleotridae [suborder Gobioidei]; Fitzsimons, Nishimoto & Devick, 1996; McDowall, 2004), shrimps, and snails are major components of stream faunal communities (Smith, Covich & Brasher, 2003).

Among diadromous fishes, less is known about amphidromous fishes than any other form (Omori et al., 2012), but recent findings have improved our understanding of their biology and roles in stream ecosystems. One finding to emerge from this line of research is that some amphidromous postlarvae recruit to streams in pulsed mass-recruitment events that are periodic at annual and lunar scales. This phenomenon has been noted anecdotally many times (Bell 1999); but has rarely been quantified (see Bell, 1997; Engman, Chapter 1). Recently, Engman (Chapter 1) confirmed that in Caribbean amphidromous gobioids, principally Sirajo Goby (*Sicydium* spp.) and River Goby (*Awaous banana*) conduct synchronous pulsed mass-recruitment events that are periodic on lunar and seasonal scales.
The postlarvae of amphidromous fishes (especially sicydiine gobies and galaxiids) are harvested in artisanal fisheries in locations globally, where these types of periodic mass-recruitment events occur (McDowall 2004). Although infrequently quantified, postlarval fishery harvests can be impressive (up to 20,000 metric tons/year), especially since individual goby postlarvae are typically less than 30 mm in length (Bell, 1999; Castellanos-Galindo et al., 2011; Kwak et al., 2016). Findings such as these imply that amphidromous fish recruitment is a periodically available and potentially substantial influx of biomass to estuarine and riverine food webs (Jenkins et al., 2010).

It is widely perceived that that postlarval and juvenile amphidromous fishes and shrimps must pass through a gauntlet of predatory fishes in the estuaries and lower reaches of streams when recruiting to adult habitats (Hein & Crowl, 2010). A predator gauntlet is a mechanism by which postlarval recruitment might impact freshwater and estuarine food webs. It also may regulate the migration behavior and distribution within the watershed of some amphidromous fishes and shrimps. Lower-trophic-level-species such as algivorous gobies and detritivorous shrimps continue upstream migration until they arrive at stream reaches that are above of waterfalls, which are barriers to predatory fishes (Fitzsimons, Parham & Nishimoto, 2002; Schoenfuss & Blob, 2007; Covich et al., 2009; Cooney & Kwak, 2013). The adaptive advantage of reaching predator-free habitats has even been postulated to have driven the evolution of specialized morphology and behaviors that yield waterfall climbing abilities in amphidromous gobies (Blob et al., 2010; Leonard et al., 2012). Although Hein & Crowl (2010) demonstrated that the presence of predatory fishes causes amphidromous shrimps to migrate upstream of waterfalls, the widely assumed predator
gauntlet has not yet been observed directly with directed research of the feeding ecology of the potential predators of amphidromous postlarvae.

If diadromous and estuarine fishes consume amphidromous postlarvae then recruitment may influence the trophic ecology and fish assemblage structure of tropical streams and their estuaries. Amphidromous recruitment pulses could alter freshwater–marine ecotone fish assemblage structure if marine predatory species migrate into and up rivers with the amphidromous food pulse; or if upstream-resident predators move downstream to feed during peak recruitment times. In addition, since recruitment of amphidromous postlarvae represents the movement of biomass from the marine environment to estuaries and freshwater systems it may function as a resource subsidy with the potential to augment secondary productivity in these ecosystems. As the majority of inland fishes in many tropical regions, amphidromous, estuarine, and marine species play major ecological roles in estuaries and streams and provide valuable ecosystem services to people in these regions (Jenkins et al., 2010; Kwak et al., 2016). Therefore, understanding the trophic interactions of amphidromous postlarvae with estuarine and riverine predator fishes is essential for tropical ecosystem management and conservation.

The inland native fish assemblages of Puerto Rico contain a species-poor diadromous fish component complemented by a suite of estuarine and primarily marine species. In Puerto Rico there are less than ten native freshwater fishes, which are all amphidromous with the exception of the catadromous American Eel (*Anguilla rostrata*), and at least 37 primarily marine or estuarine species that occur in inland water bodies (Neal *et al.*, 2009; Kwak *et al.*, 2016). Since all of these fishes can disperse through the marine environment, Puerto Rico
shares many species with other Caribbean islands and continental coasts (Lefrançois et al., 2011; Kwak et al., 2016).

Inland fisheries that target native species at multiple life stages are important providers of ecosystem services to the people of Puerto Rico and other tropical islands. An artisanal fishery for amphidromous postlarvae—colloquially known as cetí—occurs at the river mouths of several large drainages and is considered culturally significant (Erdman, 1961; Kwak et al., 2016). Recreational and subsistence fishing for adult fishes occurs throughout stream sections with ocean connectivity. Lowland rivers appear to be the most popular locations for these fisheries, as these locations provide anglers with the opportunity to target prized sport fishes such as Snooks (Centropomidae) and Tarpon Megalops atlanticus from shore (Kwak et al., 2016). Only two native sport fishes occur in upland stream reaches—Bigmouth Sleeper Gobiomorus dormitor and Mountain Mullet Agonostomus monticola—both of which are amphidromous predators (Neal et al., 2009).

Understanding the feeding ecology and structure of estuarine and freshwater fish assemblages is crucial to proper fisheries management and ecosystem conservation in the Caribbean. However, research on diets of estuarine fishes and amphidromous fishes in Puerto Rico is limited to few studies of limited scope (Aliaume, Zerbi & Miller, 2005; Nieves 1998; Bacheler, Neal & Noble 2004). Moreover, no study has been specifically designed to quantify predation on amphidromous postlarvae during recruitment and upstream migration in any location. In this article, we determine the role of postlarval amphidromous fish recruitment in the fish assemblage structure and trophic ecology of a Caribbean freshwater—
marine ecotone on the island of Puerto Rico. We accomplish this goal by addressing two questions:

(1) Do amphidromous fish recruitment events induce shifts in the fish assemblage structure of the freshwater–marine ecotone?

(2) Are there shifts in the diets of predatory fishes in the freshwater–marine ecotone in association with amphidromous mass-recruitment events?

Methods

Study system

We selected the Río Grande de Arecibo, Puerto Rico, as our study system. This study was conducted at locations throughout the freshwater–marine ecotone of the Río Grande de Arecibo, which extends from the river mouth (i.e., the location were river channel ends and beach habitat begins) to a low-head dam 2.62 km upstream from the mouth. Like other rivers on the north coast of Puerto Rico (Negrón-Gonzalez & Cintrón, 1979), the Río Grande de Arecibo in its lowest section is a salt-wedge estuary with variable salinities that depend on the magnitude and timing of freshwater river flows.

The mouth of the Río Grande de Arecibo is known as a central location for the amphidromous postlarval goby (cetí) fishery and receives recreational fishing pressure (Erdman, 1961; pers. obs.). Engman (Chapter 1) quantified postlarval recruitment to multiple rivers in Puerto Rico and found that the Río Grande de Arecibo mouth receives regular and relatively abundant mass recruitment pulses, or runs, of Sirajo Goby and River Goby postlarvae. Recruitment runs to the Río Grande de Arecibo mouth occur from June through January on the last quarter moon phase (days 21 through 28 of the lunar cycle) with peak
recruitment at the river mouth on lunar days 22-24. Sirajo Goby and River Goby exhibit strong lunar periodicity in recruitment, and therefore are the majority of the individuals in these runs, but eleotrid fish species also recruit throughout the lunar cycle in Puerto Rico, so the postlarvae of Smallscaled Spinycheek Sleeper (*Eleotris perniger*), Bigmouth Sleeper (*Gobiomorus dormitor*) and Fat Sleeper (*Dormitator maculatus*) may also be present in the Río Grande de Arecibo estuary during these times.

Temporal study design

To document the effects of amphidromous gobioid recruitment on the fish trophic ecology and assemblage structure of the Río Grande de Arecibo, we characterized fish assemblage structure and the diets of predatory fishes in the following two time periods: (1) a period immediately before an amphidromous fish recruitment run, 3 July to 8 July, 2015 (days 16-21 of the lunar cycle), which we refer to as before the run; and (2) a period during the amphidromous fish recruitment run, 9 July to 12 July, 2015 (days 22-25 of the lunar cycle), which we refer to as during the run. All sampling of the fish assemblage and predatory fish diets occurred from 3 July to 12 July, 2015 (i.e., days 16-25 of the lunar cycle).

Fish Assemblage Sampling

We used a 30.5 x 2.4 m beach-seine with a 2.4 x 2.4 x 1.8 m bag (25.4-mm mesh in wings, 12.7-mm mesh in bag), fitted with tow ropes, to sample fishes at the river mouth of the Río Grande de Arecibo. All beach-seine sampling was conducted at night to minimize behavioral avoidance of the gear. The beach seine was deployed from the hull of a Zodiac inflatable boat in a semi-circle pattern and hauled to shore by a minimum of four people. On
each sampling night, two non-overlapping seine-hauls were conducted at the river-mouth station (Station 1).

We sampled the fish assemblage at low-salinity locations of the freshwater–marine ecotone with daytime pulsed-DC boat-mounted electrofishing (Smith-Root GPP 2.5). Electrofishing was conducted at 11 stations between 1.5 and ~2.6 km upstream of the river mouth. The most upstream station (Station 12) was a large pool located below a low-head dam, which was fished in a pattern that covered the entire physical extent of the pool. The remaining 10 stations were fixed, 100-m reaches that were demarcated with flagging and fished on alternating banks during each day of the study. Electrofishing effort was recorded as the number of seconds of time the water was electrified, and catch per unit effort (CPUE) was standardized as the number of fish captured per hour (fish/h).

Fishes that were captured by electrofishing or seine were identified to species, measured (total length [TL], mm) and weighed (g). We quickly noted that Sailfin Catfishes *Pterygoplicthys* spp. were very abundant at electrofishing stations and their fin spines entangled dip nets, which interfered with normal electrofishing procedures. Due to the difficulty of handling these fishes and to maintain standard electrofishing effort, Sailfin Catfishes were counted at the surface rather than netted while electrofishing. Since these catfishes were not handled for identification, they were considered one taxon *Pterygoplicthys* spp. for the purpose of data summaries and analyses.

*Fish assemblage description and comparisons*

We characterized the overall fish assemblage of the Río Grande de Arecibo and explored longitudinal and temporal shifts in assemblage structure with multiple metrics and
analyses. First, we calculated the relative frequencies ([total individuals captured of a taxon/total fish captured × 100]) of all species in our total catch (electrofishing and seine combined) and determined the mean and standard error of the lengths and weights of each species (Table 1). Next, we used nonmetric multidimensional scaling (NMDS) to model spatial and temporal similarities among sites in the fish assemblage (Kwak & Peterson, 2007) of the Río Grande de Arecibo freshwater–marine ecotone. Separate NMDS analyses were performed on seine data and electrofishing data, because units of CPUE varied between gears. We stratified the section of the Río Grande de Arecibo that was sampled with electrofishing into three locations: a downstream location (Stations 2 through 6), an upstream location (Stations 7 through 11), and the dam pool location (Station 12). The electrofishing species-abundance data used for the NMDS analysis were the CPUE of each species at each location by date. The seine species-abundance data matrix was constructed by summing the catch of both seine hauls on each date. NMDS ordinations were conducted on Bray-Curtis similarity measures calculated for each matrix. The appropriate number of dimensions for each NMDS was selected based on visual evaluation of scree plots of stress values (McCune, Grace & Urban, 2002). Ordinations were conducted using the MetaMDS function in the Vegan package (maximum of 100 random starts; centering, principal components rotation; half-change scaling [Oksanen, et al., 2016]). Finally, we used Spearman’s rank correlation coefficients to further evaluate similarities in fish species composition along the longitudinal gradient of the freshwater–marine ecotone and between the two time periods (Kwak & Peterson, 2007). We calculated Spearman’s rank correlation coefficients and associated p-
values of species rank-abundances between all pairwise combinations of electrofishing locations and the river mouth, in both time periods with the statistical package R 3.3.1 (R Development Core Team, 2016). Very rare species (i.e. those that made up less than 0.25% of our total catch, 2 or fewer individuals) were eliminated for the purposes of the rank correlation analysis.

Diet sampling

We intensively sampled the diet of the six most abundant predator species in the Río Grande de Arecibo through both time periods, and seven additional predatory fishes only during the postlarval amphidromous fish run. We refer to the six most abundant species as the focal species of our diet study and use the diet data from these species to compare predatory fish trophic ecology between the two sampling periods. The diet samples from the seven additional fishes are considered supporting evidence, since no more than three individuals of each additional species was sampled.

The diet was sampled as stomach contents of captured predators that were extracted in the field with a series of acrylic tubes of different diameters (appropriate diameter based on fish size; Van Den Avyle & Roussel, 1980) or by stomach excision (Garvey & Chipps 2012). If a fish’s diet was sampled with an acrylic tube, the fish was first anaesthetized in aerated water with a buffered 80-mg/l tricaine methane sulphonate (MS-222) solution for 2 min (Smith & Kwak, 2014). Fish whose diet was sampled by excision were euthanized with an overdose of buffered MS-222. Diet samples were extracted by excision from all very small individuals or from species with esophageal morphologies that precluded effective extraction of diet with tubes [i.e., American Eel, Burro Grunt Pomadasys crocro, and
Mountain Mullet]. The use of acrylic tubes has been shown to be nearly as effective as other non-lethal methods of examining stomach contents in fish taxa with relatively large mouths and short stomachs such as black basses and salmonids (Kamler & Pope, 2001). As suggested by Kamler & Pope (2001) the tubes were also employed as a gastroscope after the initial extraction to determine if all contents were removed. If any diet items remained in the stomach after the initial tube extraction, which occasionally occurred if a fish consumed a very large food item relative to its body size, then the fish was sacrificed and the stomach was subsequently excised. After extraction, all diet items were immediately preserved in a 10% buffered formalin solution. Preserved diet items were identified to the lowest taxon feasible, sorted, blotted to remove excess moisture, and weighed wet (±0.01 g).

To validate the efficacy of the tubing procedure, a subset of fish (n = 27) was double-sampled with tubes and by stomach excision. First, a diet sample was extracted with an acrylic tube, and the stomach was immediately excised. The efficacy of the tube method was determined by comparison between the means of the total wet weight of the stomach contents from the tube and excision and the wet weight of the tube sample alone. A Welch’s two-sample t-test performed with the statistical package R 3.3.1 (R Development Core Team, 2016) revealed that the difference in mean values was not significantly different from zero (t = -1.622, P = 0.1166).

*Diet characterization*

We calculated the percentage of stomachs with food ([number of fish with food in stomach/total number fish examined ×100]) as an indicator of feeding frequency.
et al., 2002); and stomach fullness ([total stomach contents wet weight/fish wet body weight ×100]) as a measure feeding intensity (Gordon, 1977; Pine et al., 2005). The percentage of stomachs with food was calculated for the six focal species individually and for all six species pooled in each time period separately. We tested the hypothesis that the relative proportions of fish with food and with empty stomachs were independent of the run status (before run versus during run) with a two-tailed Fisher’s exact test on 2×2 contingency tables with the statistical package R 3.3.1 (R Development Core Team, 2016). We calculated the mean stomach fullness of each species in both time periods and conducted two-tailed t-tests with the statistical package R 3.3.1 (R Development Core Team, 2016) to test the hypothesis that mean stomach fullness differed by time period.

The diet composition of each species was quantified with two commonly employed indices. Mean percent composition by weight (%W) of each prey item

\[
\text{Mean } \%W = \frac{1}{P} \sum_{j=1}^{PP} \left\{ \frac{W_{ij}}{\sum_{i=1}^{Q} W_{ij}} \right\} \times 100
\]

where \( P \) is the number of fish with food in their stomachs, \( Q \) is the number of food item categories and \( W_{ij} \) is the weight of prey item \( i \) in fish \( j \); and the frequency of occurrence (\( \%F \)) of each prey item

\[
\%F = \frac{J_i}{P} \times 100
\]
where \( J_i \) is the number of fish containing prey item \( i \) and \( P \) is the number of fish with food in their stomachs. Both indices were calculated separately by predator species and time period.

To determine how the feeding habits of individual predator species responded to the amphidromous fish run, we performed permutational multivariate analysis of variance (PERMANOVA) in the *Vegan* package of R (Oksanen *et al.*, 2016) on all focal species for which there were sufficient sample sizes. In this analysis, \( \%W \) was the independent variable, each individual fish was treated as a replicate, and the time period (i.e., before the run versus during the run) was the treatment.

**Results**

*Fish assemblage dynamics*

We sampled a total of 1,200 adult fish from 31 species in the Río Grande de Arecibo freshwater–marine ecotone (Table 1). 1,073 individuals of 24 species were captured with boat electrofishing and 127 individuals from 20 species were captured with the beach seine. Nine fishes were numerically dominant in our samples and made up 91% of the combined catch (Table 1). The six focal species made up 47.3% of our total catch and included two primarily riverine predators (American Eel and Bigmouth Sleeper), and four estuarine predators (Tarpon Snook *Centropomus pectinatus*, Common Snook *Centropomus undecimalis*, Burro Grunt *Pomadasys croco*, and Bay Whiff *Citharichthys spilopterus*).

NMDS ordination allowed for visual evaluation of longitudinal gradients and temporal shifts in fish assemblage structure (Fig.1). Scree plots indicated that a three-dimensional (3-D) NMDS ordination was appropriate for the electrofishing ordination, while a 2-D NMDS ordination was sufficient for the river mouth ordination. Convergent solutions
with final stress values of 13.68% and 6.8% were solved in 20 iterations for the electrofishing and seine ordinations, respectively. A bi-plot of electrofishing NMDS axis one versus electrofishing NMDS axis two is provided in Fig. 1a. The other two possible bi-plots (axis one vs. axis three and axis two vs. axis three) were examined, but not included because they do not provide distinct patterns in the results. The convex hulls (smallest convex polygons) in Fig. 1 define the extent of the observed fish assemblage structure of each location-time period combination in multivariate space. Fig. 1a indicates some overlap in the fish assemblage structure of all three locations the Río Grande de Arecibo that were sampled with electrofishing. Visual inspection of this plot reveals greater overlap between adjacent locations (i.e., dam and upstream, and upstream and downstream) than between the two non-adjacent locations, which suggests a longitudinal gradient of fish assemblage structure. There was also within-location overlap between the two time periods in the polygons of each river location, and convex polygons in Fig. 1b indicate overlap between the fish assemblage structure at the river mouth between time periods.

The Spearman’s rank correlation analysis provided statistics that allowed us to compare the species composition at all locations in the freshwater–marine ecotone within and between time periods (Table 2). There were very strong and significant, positive ($r > 0.8$) correlations between the species compositions of all three of the electrofished locations in the before the run period. During the run, there were strong correlations between the species composition at the dam and the upstream and downstream locations, and very strong correlations between the upstream and downstream locations. This analysis indicates that the species composition of the mouth and the electrofished locations of the Río Grande de
Arecibo were distinct since there were no significant correlations between the river mouth station species composition and any of the electrofished locations. There were strong or very strong correlations between the species composition of all locations between time periods, suggesting that the occurrence of amphidromous run did not alter fish assemblage structure.

**Predator fish feeding**

Before the run began, most of the predatory fishes that we sampled had empty stomachs, but in contrast during the run, the majority of fish diets sampled contained prey items. The observed percentage of stomachs with food was greater during the run, for all species separately and when all species were pooled. Fisher’s exact tests revealed a lack of independence between the relative proportion of individuals with food in their stomachs and the run status for Tarpon Snook, Bigmouth Sleeper, American Eel, and all species pooled; but not for Common Snook, Bay Whiff, or Burro Grunt (Table 3). Before the run, Bay Whiff exhibited the highest percentage of stomachs with food (50.0%) and American Eel had the lowest (6.25%). During the run, the species with the highest percent of individuals with food in their stomachs was Burro Grunt (87.5%), while Common Snook was the lowest (38.1%).

Mean fullness values were generally very low before the run, compared to those during the run. Mean fullness was less than 1% for all six focal species before the run (Table 4). Bay Whiff had the highest mean fullness in this time period at 0.61%; while the mean fullness of Common Snook, Burro Grunt, and American Eel were each less than 0.10%. During the run, mean fullness was greater than 1% for Tarpon Snook and Burro Grunt. The lowest observed mean fullness during the run was 0.42% in Bay Whiff; the only species with lower observed mean fullness during the run than before it, but a t-test indicated that this
difference was not significant. T-tests confirmed significant differences in the mean fullness of Tarpon Snook, American Eel and Bigmouth Sleeper between the two time periods (Table 4), which indicates that these predatory species were exploiting the temporary food resource available during the amphidromous run.

*Diet composition by frequency of occurrence*

Postlarval amphidromous shrimps (Atyidae, Xiphocarididae, and Palaemonidae) were one of the most frequently encountered items in the diets of focal predators before the amphidromous postlarval fish run. Prior to the postlarval fish run, postlarval shrimp occurred in the diets of all focal species except for Burro Grunt and were the most frequently encountered item in four of the six species (Tarpon Snook, Common Snook, Bay Whiff, and American Eel, in which amphidromous shrimp were tied with freshwater macroinvertebrates in frequency). The frequency of occurrence of postlarval amphidromous shrimps in the diets of these four species before the run was at least 50%. Postlarval shrimps were especially prevalent in the diets of Tarpon Snook (77.8%) and American Eel (100%) before the run. Only one American Eel had any food in its stomach before the postlarval fish run, and freshwater macroinvertebrates were also encountered in that individuals’ stomach (Table 5).

Adult amphidromous shrimps and unidentified fishes were two other prey items that frequently occurred in focal predator diets before the postlarval run began. Adult amphidromous shrimps had the highest frequency in the diets of Burro Grunt and Bigmouth Sleepers (along with the Puerto Rican Freshwater Crab *Epilobocera sinuatifrons*), and were the item with the second highest frequency in Tarpon Snook and Bigmouth Sleeper diets (along with unidentified fishes). Unidentified fishes were the item with the second highest
frequency in Common Snook and Bay Whiff diets before the postlarval fish run. Bay Whiff was the only predator that was observed to consume amphidromous gobioid fishes before the run was initiated (Table 5).

Postlarval shrimps continued to be frequently encountered in the diets of Río Grande de Arecibo predators during the amphidromous postlarval fish run, but postlarval Sirajo Goby and postlarval gobioids were also frequently encountered in many focal predators’ diets. Postlarval shrimps was the item with the highest frequency in Tarpon Snook and Bay Whiff diets. Sirajo Goby and postlarval shrimps were tied for the highest frequency in Burro Grunt, Bigmouth Sleeper, and American Eel diets during the postlarval fish run. During the run, postlarval Sirajo Goby was found in the diets all the focal predator species and had the second highest frequency in Bay Whiff and Tarpon Snook. Postlarval gobioids occurred in the diets of three of the six predators during the run, were second in frequency in American Eel, third in Bigmouth Sleeper and were tied for third in Burro Grunt during this time period. Common Snook was the species with the lowest frequency in postlarval Sirajo Goby, postlarval gobioids, and postlarval shrimps categories during the run; unidentified fishes was the most frequently occurring item in Common Snook diets during this period (Table 5).

Diet composition by weight

Before the amphidromous postlarval fish run, postlarval amphidromous shrimps, unknown fishes, adult amphidromous shrimps, and the Puerto Rican Freshwater Crab were abundant diet components by weight of the six focal predators (Fig. 2). Postlarval amphidromous shrimps made up the greatest biomass of the diets of Tarpon Snook (69.7%), Common Snook (37.8%), and American Eel (77.8%); and were tied with unidentified fishes
for the largest mean biomass in the Bigmouth Sleeper diet (15.4%). Unknown fishes were the most abundant item by weight found in Bay Whiff diets (49.3%) and the second most abundant in for Common Snook diets (19.4%). Adult shrimps made up 100% of the observed diet of Burro Grunt and were the second most abundant item in Bigmouth Sleeper diets (24.7%). The Puerto Rican Freshwater Crab made up the greatest biomass of Bigmouth Sleeper diets before the postlarval fish run at 29.2%.

During the postlarval fish run, postlarval shrimps remained a major component of the diets of several Rio Grande de Arecibo predators, but were generally less abundant by weight than before the run (Fig. 2). On average, postlarval amphidromous shrimps made up the largest percent by weight in the diets of three species during the run, Tarpon Snook (38.1%), Bigmouth Sleeper (36.0%), and American Eel (32.8%). Postlarval shrimps made up the second most biomass in Bay Whiff diets (36.2%) and were the third most biomass in Burro Grunt diets (22.5% mean) during the run.

During the run, postlarval Sirajo Goby was the most abundant item by weight in the diets of two focal species and were major components of the diets of three other focal species. Postlarval Sirajo Goby was the item with the highest mean biomass in the diets of Bay Whiff (63.8%) and Burro Grunt (35.2%) during the run. In this time period, postlarval Sirajo Goby was nearly as abundant by weight as postlarval shrimps were in the diets of Tarpon Snook (36.5% mean), Bigmouth Sleeper (35.5%), and American Eel (29.7%). In addition, if all amphidromous gobioïds fishes were combined, they would make up the largest component by weight of the diets of all three of these species in this time period: Tarpon Snook (50%), Bigmouth Sleeper (38.5%), and American Eel (41.2%).
Common Snook was the only focal predator for which amphidromous postlarval fishes or shrimps were not a major component of the diet during the run. Instead, this species consumed mostly adult and juvenile fishes in this time period, and unknown fishes made up nearly 50% of their diet biomass; when summed, all adult and juvenile fishes made up 68.4% of the Common Snook diet biomass during the postlarval fish run. Unknown fishes also made up a large portion by weight of the diet of Burro Grunt during the run as well (28.6% mean), the second most abundant diet item for Burro Grunt.

Shifts in the overall diet composition of Bigmouth Sleeper \(F_{1,37}=3.8921, P=0.001\) and Tarpon Snook \(F_{1,68}=7.51, P=0.001\) between periods before and during the amphidromous postlarval fish run were confirmed by PERMANOVA, but were not significant for Common Snook \(F_{1,12}=1.7556, P=0.099\). A high proportion of empty stomachs precluded a PERMANOVA analysis on the diet compositions of Burro Grunt, American Eel, and Bay Whiff.

**Discussion**

*Fish assemblage dynamics*

The Río Grande de Arecibo freshwater–marine ecotone harbors a fish species assemblage that is typical of similar systems in the island of Puerto Rico and other islands of the greater Caribbean area. The observed richness (31 fish species) and species composition in this study is similar to the only account of a riverine estuary fish assemblage on the island of Puerto Rico. Smith, Corujo Flores & Pringle (2008) compared the contemporary fish assemblage of the Espiritu Santo River’s estuary with that of a historical survey and reported sampled richness values during the months of June and July of 31 species in 2004 and of 34
species in 1977. Nearly all of the native species that we observed in the Río Grande de Arecibo were present in at least one of the two surveys of the Espiritu Santo River. However, we found that Sailfin Catfishes, which can be highly invasive, were very abundant in the Río Grande de Arecibo and Smith, Corujo Flores & Pringle (2008) never captured these fishes in the Espiritu Santo. This occurrence, which is known widespread throughout the island, is a conservation concern, considering the ecosystem-level effects of loricariid catfish invasions in other systems (Nico et al., 2012; Kwak et al., 2016).

Our results do not support the hypothesis that fish assemblage structure in this system changes in response to mass recruitment events of amphidromous postlarval fishes. If the overall fish assemblage structure had been different during the run, we would expect the shift in assemblage structure to be reflected in the NMDS or Spearman’s rank-correlation analyses. Furthermore, we found no evidence for the movement or concentration of some of the most abundant predators in this system in response to the amphidromous fish run. Despite our observation of a stable fish assemblage across the freshwater–marine ecotone during the study period, this assemblage may change seasonally or even over the scale of the lunar cycle as has been shown for other estuarine systems in the neotropics (Stoner, 1986; Ramos et al., 2011).

**Predator Fish Trophic Ecology**

Despite stability in fish assemblage structure before and during the amphidromous fish run, we detected definite shifts in the trophic ecology of fish predators. One shift was an increase in fishes with food in their stomachs. This increase was statistically significant and pronounced in Tarpon Snook, American Eel, and Bigmouth Sleeper, which were all more
than twice as likely to have food in their stomachs during the run than before it (Table 3). We
could not statistically confirm the dependence of the frequency of Burro Grunt individuals
encountered with food on the run status; however, given the magnitude of the observed
differences in the percentages of individuals with food between the two time periods, we
attribute the lack of statistical significance to low sample size, rather than a true lack of
dependence. We interpret these increases in individuals with food in their stomachs as an
indicator of increased feeding frequency in Tarpon Snook, American Eel, Bigmouth Sleeper
and Burro Grunt.

With the increase in feeding frequency, this predatory fish assemblage went from one
that would be classified as having a very frequent occurrence of empty stomachs to one with
a moderately frequent occurrence of empty stomachs. According to a large-dataset analysis
of teleost fish species from North America, Africa, and the Neotropics, the mean percentage
of empty stomachs in diurnal piscivore fish species is about 35.2% and is generally less than
50% in piscivores that do not provide parental care (Arrington et al., 2002). Our observed
values before the run were over twice the aforementioned mean for predators as a whole in
this system (77.8%) and were never less than this value for any species, individually. During
the run, the overall percent of empty stomachs was still relatively high compared to the
findings of Arrington et al. (2002); however, one-third of our focal species had less than
35.2% empty stomachs, and when focal species were pooled, fewer than 50% of the
predators had empty stomachs.

The same predatory fishes that fed more frequently were also significantly more full
during the amphidromous fish run. Stomach fullness is considered an index of fish feeding
intensity (Hyslop 1980). This result has important implications for energy balance because a high frequency of empty stomachs alone does not necessarily imply a long-term negative energy balance (Arrington et al., 2002). Since we observed greater feeding intensity in conjunction with increased feeding frequency during the run, it is likely that the period of the amphidromous fish run was a time of a more positive energy balance for predatory species such as Tarpon Snook, American Eel, and Bigmouth Sleeper. Given our data, the only scenario by which a shift toward a more positive energy balance would not occur is if there are concurrent shifts in food quality or foraging energy that offset the observed increases in meal frequency and quantity, but this is unlikely, given the high-protein food sources consumed by predators during the amphidromous fish run.

The observed increases in feeding frequency and intensity are related to the consumption of recruiting postlarval fishes. Postlarval gobioids, especially Sirajo Goby, were major components of the diet of all the species who exhibited increased mean fullness and/or stomachs with food during the amphidromous fish run. Furthermore, when large numbers of amphidromous fishes became available as prey, significant changes in the overall diet composition of Tarpon Snook and Bigmouth Sleeper occurred. Even species that did not show significantly greater fullness or feeding frequency during the run consumed postlarval amphidromous fishes. Sirajo Gobies were the majority of Bay Whiff diets and made up more than one-tenth of Common Snook diets by weight during this period. When Sirajo Goby and other gobioid postlarvae are available in high densities they serve as forage for nearly all of the predatory fishes of the Río Grande de Arecibo freshwater–marine ecotone. In addition to their presence in the diets of all of our focal species, Sirajo Goby or other postlarval gobiods
were found in the diets of five (Lined Sole *Achirus lineatus*, Tarpon, Fat Snook *Centropomus parallelus*, Mountain Mullet, and Barbu *Polydactylus virginicus*) of the seven additional species that we sampled at low numbers during the run. We only sampled the diet of one individual of the other two additional species (Fat Snook and Scrawled Sole *Trinectes inscriptus*) during the run and each had an empty stomach, so it is plausible that these two species are also predators of amphidromous postlarval fishes when they feed.

Increased consumption of amphidromous postlarval shrimps during the gobioid mass recruitment event may also have contributed to the observed shifts in the trophic ecology of this system. Amphidromous shrimp postlarvae were substantial components of the diets of five of the six focal predator species during this period, and we observed increased mean biomass of this food item in the diets of three species. Although we did not monitor postlarval shrimp densities in the river, we visually observed increases in shrimp densities coinciding with the amphidromous fish run (pers. obs.). Moreover, studies suggest that upstream migration of some amphidromous shrimps is negatively correlated with moonlight intensity (*Kikkert, Crowl & Covich, 2009*); and other studies have documented observations of the temporal coincidence of mass migrations of postlarval shrimps and fishes (*Nordlie, 1981; Bell, 1999*). Thus, predatory fishes in the Río Grande de Arecibo may have fed more on amphidromous shrimps postlarvae during the run because they were also present at high densities.

We confirmed that both amphidromous fishes and shrimps undergo a predator gauntlet during recruitment to river mouths and upstream migration. This result supports many of the conclusions of past researchers about mechanisms of observed patterns of
amphidromous species distributions within watersheds and the evolution of waterfall climbing. Additionally, these findings corroborate a proposed driver of recruitment dynamics in amphidromous species—predator swamping—which is one adaptive explanation for pulsed migrations in amphidromous fishes and shrimps (Bell, 1999; Engman, Chapter 1).

**Ecological and management implications**

An amphidromous postlarval fish mass-recruitment event clearly affected the trophic ecology of a Caribbean island freshwater–marine ecotone, but did not alter fish assemblage structure. For some predatory fishes, trophic ecology shifts were manifest through increases in feeding frequency and intensity, and major changes in diet composition. Other species’ diets were more subtly affected but it appears that nearly all of the predatory fishes of this system consumed amphidromous postlarval fishes during the migratory run. Thus, amphidromous postlarval fishes serves as a periodically available and significant food source for predatory fishes in Caribbean island streams and their estuaries.

In addition to its role as a food source, amphidromous recruitment functions to transfer marine nutrients and energy to estuarine and freshwater food webs. Engman (Chapter 3) found that the $\delta^{34}S$ of postlarval Sirajo Goby and River Goby at the time of recruitment were around 20.4 ‰, which reflects a clearly marine, rather than freshwater source. Therefore, marine nutrients and energy are being transferred directly to the upper trophic levels of estuarine and freshwater food webs through predation. This demonstrates the ecological function of amphidromous recruitment as a marine material subsidy for tropical island estuaries and streams. The role of other diadromous fishes as material subsidies has been extensively studied in anadromous salmonids, and to a lesser extent in in
other groups of diadromous fishes (Flecker et al., 2010). However, our study is the first quantitative description of the role of amphidromous species as material subsidies (Freeman et al., 2003).

Flecker et al. (2010) suggested that it is unlikely that amphidromous fishes are important as material subsidies under the assumptions that amphidromous fishes survive for years following returns to freshwater, and they are small net influxes of biomass to streams. However, our findings and those of other researchers contradict this conjecture. Flecker et al. (2010) listed three conditions that maximize the potential for migratory fish to represent major nutrient subsidies, and our findings suggest that postlarval amphidromous fishes and their environments are likely to meet these conditions. In this article, we demonstrated one of Flecker et al.’s (2010) primary conditions—an efficient mechanism for liberating nutrients from recruiting by mortality through predation. A second condition is for the recipient system to have a low availability of nutrients and energy; the oceanic island streams and short coastal continental watersheds in the tropics where amphidromous fishes are prevalent are typically classified as oligotrophic and dependent upon allochthonous inputs from upstream forested reaches (Boulton et al., 2008; Teichert et al., 2014). The final condition is that the biomass of migrants be relatively high compared to ecosystem size. Although additional research is needed to accurately quantify the biomass influx that amphidromous recruitment represents, anecdotal evidence suggests that it may be relatively high, especially for oceanic island streams, which are typically small systems. Thus, amphidromous fishes are a seasonally and periodically available and exploited marine subsidy to Caribbean estuaries.
and freshwater streams. Further research, such as quantifying amphidromous recruit biomass is needed to determine the magnitude of this nutrient and energy subsidy.

Our findings may assist Caribbean island fisheries managers to consider how amphidromous fish recruitment positively influences inland fisheries and guard against threats to these ecosystem functions and services. Amphidromous recruits (shrimps and fishes) likely contribute to the growth and production of several recreationally and commercially important fisheries, including Tarpon Snook and Bigmouth Sleeper. These two species are especially relevant to fisheries management in Puerto Rico, since snooks are a valuable recreational and commercial fisheries resource in rivers, lagoons, and the marine environment (Aliaume et al., 2005); and the Bigmouth Sleeper is one of only two native sport fishes with wide distributions in freshwater streams (Neal et al., 2009). Postlarval recruits of amphidromous fishes and shrimps are a critically important, but threatened, food source of these valuable fisheries and constitute an often overlooked conservation concern in Puerto Rico and the greater Caribbean (Kwak et al., 2016).

Perhaps the most direct threats to the abundance of amphidromous postlarval recruits are the unmonitored and unregulated harvest of postlarvae, artificial instream barriers, and the alteration and loss of instream flows. In Puerto Rico, the impact of the artisanal postlarvae fisheries is unknown, because there is no monitoring of the cetí fishery, and it is largely unregulated. Nevertheless, given that there are no harvest limits or seasons in place, the potential for overexploitation of this fishery to have a negative impact merits further investigation. One may postulate that the mere existence of these fisheries is evidence that
current exploitation is at a sustainable rate, but the dynamics and trends of the fisheries remain unknown, so any conclusions would be purely speculative.

Large dams are known to extirpate amphidromous fish assemblages from whole river sections, which decreases habitat availability and spawning stock biomass (Cooney & Kwak, 2013). Dams and associated water withdrawals also have been shown to cause significant reductions in the biomass of downstream drifting amphidromous shrimp larvae (March et al., 1998), with similar effects presumed for fish larvae. Alterations to the volume and timing of instream flows may also reduce postlarval recruit abundances, even if larval export to the ocean is high. Recruit abundances of both shrimps and fishes in Puerto Rico are positively related to stream flow in Puerto Rico (Benstead, March & Pringle, 2000; Engman, Chapter 1). Low stream flows can even create a total loss of connectivity between oceans and freshwaters, which may result in major recruitment failure if they occur at critical annual or lunar cycle periods (Engman, Chapter 1). Due to the critical, ecological role of migratory stages of amphidromous fishes in the population dynamics and ecology of freshwater-to-marine environments, managers of Caribbean inland fisheries and ecosystems may incorporate our findings toward actions to conserve and protect these species from headwater streams to the ocean for the future benefit of multiple ecosystems and local societies.
References


Modelling 243, 89–94.


### Table 1

List of species captured in the Río Grande de Arecibo, mean sizes, total catch; and relative abundance in catch, by gear and total. Species are ordered by the relative abundance of the total catch. Values are shown ± SE where calculable.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>TL (mm)</th>
<th>Weight (g)</th>
<th>Electrofishing</th>
<th>Seine</th>
<th>Total</th>
<th>Electrofishing</th>
<th>Seine</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sailfin Catfish</td>
<td><em>Pterygoplichthys</em> spp.</td>
<td>471 ± 76</td>
<td>874 ± 68</td>
<td>282</td>
<td>3</td>
<td>285</td>
<td>26.3</td>
<td>2.4</td>
<td>23.8</td>
</tr>
<tr>
<td>Bigmouth Sleeper</td>
<td><em>Gobiomorus dormitor</em></td>
<td>197 ± 6</td>
<td>98 ± 9</td>
<td>230</td>
<td>0</td>
<td>230</td>
<td>21.4</td>
<td>0</td>
<td>19.2</td>
</tr>
<tr>
<td>Striped Mojarra</td>
<td><em>Eugerres plumieri</em></td>
<td>177 ± 4</td>
<td>78 ± 6</td>
<td>199</td>
<td>11</td>
<td>210</td>
<td>18.5</td>
<td>8.7</td>
<td>17.5</td>
</tr>
<tr>
<td>Tarpon Snook</td>
<td><em>Centropomus pectinatus</em></td>
<td>226 ± 6</td>
<td>141 ± 12</td>
<td>146</td>
<td>18</td>
<td>164</td>
<td>13.6</td>
<td>14.2</td>
<td>13.7</td>
</tr>
<tr>
<td>American Eel</td>
<td><em>Anguilla rostrata</em></td>
<td>454 ± 14</td>
<td>201 ± 21</td>
<td>76</td>
<td>0</td>
<td>76</td>
<td>7.1</td>
<td>0</td>
<td>6.3</td>
</tr>
<tr>
<td>Common Snook</td>
<td><em>Centropomus undecimalis</em></td>
<td>397 ± 31</td>
<td>1,172 ± 381</td>
<td>47</td>
<td>2</td>
<td>49</td>
<td>4.4</td>
<td>1.6</td>
<td>4.1</td>
</tr>
<tr>
<td>Zabaleta Anchovy</td>
<td><em>Anchovia clupeoides</em></td>
<td>120 ± 2</td>
<td>11 ± 0</td>
<td>1</td>
<td>28</td>
<td>29</td>
<td>0.1</td>
<td>22.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Burro Grunt</td>
<td><em>Pomadasys croco</em></td>
<td>342 ± 17</td>
<td>600 ± 86</td>
<td>26</td>
<td>1</td>
<td>27</td>
<td>2.4</td>
<td>0.8</td>
<td>2.3</td>
</tr>
<tr>
<td>Bay Whiff</td>
<td><em>Citharinchthys spiopterus</em></td>
<td>118 ± 7</td>
<td>20 ± 3</td>
<td>8</td>
<td>14</td>
<td>22</td>
<td>0.7</td>
<td>11.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Swordspine Snook</td>
<td><em>Centropomus ensiferus</em></td>
<td>164 ± 16</td>
<td>52 ± 13</td>
<td>20</td>
<td>1</td>
<td>21</td>
<td>1.9</td>
<td>0.8</td>
<td>1.8</td>
</tr>
<tr>
<td>White Mullet</td>
<td><em>Mugil curema</em></td>
<td>131 ± 34</td>
<td>216 ± 120</td>
<td>3</td>
<td>16</td>
<td>19</td>
<td>0.3</td>
<td>12.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Irish Mojarra</td>
<td><em>Diapterus auratus</em></td>
<td>101 ± 9</td>
<td>17 ± 5</td>
<td>0</td>
<td>15</td>
<td>15</td>
<td>0</td>
<td>11.8</td>
<td>1.3</td>
</tr>
<tr>
<td>River Goby</td>
<td><em>Awaous banana</em></td>
<td>147 ± 21</td>
<td>52 ± 17</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0.9</td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>Lined Sole</td>
<td><em>Achirus lineatus</em></td>
<td>128 ± 24</td>
<td>64 ± 20</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>0.5</td>
<td>1.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Mountain Mullet</td>
<td><em>Agonostomus monticola</em></td>
<td>93 ± 35</td>
<td>64 ± 12</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>0.4</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>Atlantic Thread Herring</td>
<td><em>Opisthonema oglinum</em></td>
<td>87 ± 3</td>
<td>7 ± 1</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>0.2</td>
<td>1.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Mozambique Tilapia</td>
<td><em>Oreochromis mossambicus</em></td>
<td>376 ± 7</td>
<td>985 ± 159</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>0.1</td>
<td>2.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Smallscaled Spinycheek Sleeper</td>
<td><em>Electris perriger</em></td>
<td>98 ± 23</td>
<td>13 ± 8</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>0.2</td>
<td>0.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Barbu</td>
<td><em>Polydactylus virginicus</em></td>
<td>110 ± 6</td>
<td>13 ± 2</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>2.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Broad-striped Anchovy</td>
<td><em>Anchoa hepsetus</em></td>
<td>69 ± 5</td>
<td>2 ± 1</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0.2</td>
<td>0</td>
<td>0.2</td>
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<tr>
<td>Horse-eye Jack</td>
<td><em>Caranx latus</em></td>
<td>89 ± 3</td>
<td>10</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Fat Snook</td>
<td><em>Centropomus parallelus</em></td>
<td>391 ± 5</td>
<td>701 ± 64</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0.2</td>
<td>0</td>
<td>0.2</td>
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<tr>
<td>Darter Goby</td>
<td><em>Ctenogobius boleosoma</em></td>
<td>44</td>
<td>N/A</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0.2</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>Flagfin Mojarra</td>
<td><em>Eucinostomus melanopterus</em></td>
<td>94 ± 8</td>
<td>9 ± 3</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Species</td>
<td>Scientific Name</td>
<td>Value 1</td>
<td>Value 2</td>
<td>Value 3</td>
<td>Value 4</td>
<td>Value 5</td>
<td>Value 6</td>
<td>Value 7</td>
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</tr>
<tr>
<td>Tarpon</td>
<td><em>Megalops atlanticus</em></td>
<td>590 ± 29</td>
<td>1,314 ± 147</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0.2</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>Midas Cichlid</td>
<td><em>Amphilophus labiatus</em></td>
<td>161</td>
<td>89</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.1</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>Butterfly Peacock Bass</td>
<td><em>Cichla ocellaris</em></td>
<td>372</td>
<td>745</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.1</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>Ladyfish</td>
<td><em>Elops saurus</em></td>
<td>370</td>
<td>220</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.8</td>
<td>0.1</td>
</tr>
<tr>
<td>Short-tailed Pipfish</td>
<td><em>Microphis brachyurus</em></td>
<td>88</td>
<td>NA</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.1</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>Whitemouth Croaker</td>
<td><em>Micropogonias furnieri</em></td>
<td>138</td>
<td>20</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.8</td>
<td>0.1</td>
</tr>
<tr>
<td>Scrawled Sole</td>
<td><em>Trinectes inscriptus</em></td>
<td>61</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.8</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Table 2 Fish assemblage species composition Spearman's rank correlations between sampling locations throughout the Río Grande de Arecibo freshwater–marine ecotone. Values in parenthesis are $P$-values.

<table>
<thead>
<tr>
<th>Location</th>
<th>Time Period</th>
<th>Dam</th>
<th>Upstream</th>
<th>Downstream</th>
<th>Mouth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>During</td>
<td>Before</td>
<td>During</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.79</td>
<td>(&lt;0.01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upstream</td>
<td>Before</td>
<td>0.87</td>
<td>0.59 (0.01)</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>0.87</td>
<td>(&lt;0.01)</td>
<td>0.68</td>
<td>(&lt;0.01)</td>
</tr>
<tr>
<td>Downstream</td>
<td>Before</td>
<td>0.76</td>
<td>(&lt;0.01)</td>
<td>0.63</td>
<td>(&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>(&lt;0.01)</td>
<td>(&lt;0.01)</td>
<td></td>
<td>(&lt;0.01)</td>
</tr>
<tr>
<td>Mouth</td>
<td>Before</td>
<td>-0.45</td>
<td>-0.13 (0.6)</td>
<td>-0.46</td>
<td>-0.03 (0.9)</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>(0.05)</td>
<td>(0.10)</td>
<td>-0.31</td>
<td>-0.21</td>
</tr>
</tbody>
</table>
Table 3: Number of diet samples (n) of Río Grande de Arecibo six focal predator fish species, number of individuals with prey items in their stomach, percent with prey items in stomach, and Fisher's exact test on independence of the frequency of empty stomachs and time period. Numbers in parenthesis are P-values from Fisher’s exact tests.

<table>
<thead>
<tr>
<th>Predator species</th>
<th>Time period</th>
<th>No. diets sampled</th>
<th>No. with food in stomach</th>
<th>Percent with food in stomach</th>
<th>Fisher's exact test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tarpon Snook</td>
<td>Before</td>
<td>64</td>
<td>18</td>
<td>28.1</td>
<td>0.20 (&lt; 0.01)</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>78</td>
<td>52</td>
<td>66.6</td>
<td></td>
</tr>
<tr>
<td>Common Snook</td>
<td>Before</td>
<td>21</td>
<td>6</td>
<td>28.6</td>
<td>0.66 (0.74)</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>21</td>
<td>8</td>
<td>38.1</td>
<td></td>
</tr>
<tr>
<td>Bay Whiff</td>
<td>Before</td>
<td>10</td>
<td>5</td>
<td>50.0</td>
<td>0.76 (1)</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>7</td>
<td>4</td>
<td>57.1</td>
<td></td>
</tr>
<tr>
<td>Burro Grunt</td>
<td>Before</td>
<td>4</td>
<td>1</td>
<td>25.0</td>
<td>0.07 (0.07)</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>8</td>
<td>7</td>
<td>87.5</td>
<td></td>
</tr>
<tr>
<td>American Eel</td>
<td>Before</td>
<td>16</td>
<td>1</td>
<td>6.3</td>
<td>0.01 (0.02)</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>16</td>
<td>7</td>
<td>43.8</td>
<td></td>
</tr>
<tr>
<td>Bigmouth Sleeper</td>
<td>Before</td>
<td>83</td>
<td>13</td>
<td>15.7</td>
<td>0.30 (&lt; 0.01)</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>67</td>
<td>26</td>
<td>38.8</td>
<td></td>
</tr>
<tr>
<td>Pooled</td>
<td>Before</td>
<td>198</td>
<td>40</td>
<td>22.2</td>
<td>0.26 (&lt; 0.01)</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>197</td>
<td>104</td>
<td>52.8</td>
<td></td>
</tr>
</tbody>
</table>
Table 4 Mean stomach fullness of six focal predator fishes during both time periods (before and during amphidromous postlarval fish run). Values are shown ± SE. T-test and corresponding P-value compare the means of stomach fullness between the two time periods.

<table>
<thead>
<tr>
<th>Predator species</th>
<th>Fullness (% body weight)</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>During</td>
<td></td>
</tr>
<tr>
<td>Tarpon Snook</td>
<td>0.31 ± 0.25</td>
<td>1.53 ± 0.37</td>
<td>2.71</td>
</tr>
<tr>
<td>Common Snook</td>
<td>0.08 ± 0.05</td>
<td>0.47 ± 0.25</td>
<td>1.53</td>
</tr>
<tr>
<td>Bay Whiff</td>
<td>0.61 ± 0.30</td>
<td>0.42 ± 0.27</td>
<td>-0.48</td>
</tr>
<tr>
<td>Burro Grunt</td>
<td>0.02 ± 0.02</td>
<td>1.3 ± 0.96</td>
<td>1.34</td>
</tr>
<tr>
<td>American Eel</td>
<td>0.00 ± 0.01</td>
<td>0.65 ± 0.23</td>
<td>2.78</td>
</tr>
<tr>
<td>Bigmouth Sleeper</td>
<td>0.19 ± 0.12</td>
<td>0.87 ± 0.30</td>
<td>2.15</td>
</tr>
</tbody>
</table>
Table 5 Percent frequency occurrence of each diet item of six focal predator fishes from the Rio Grande de Arecibo in time periods before and during the amphidromous postlarval fish run. Numbers in parenthesis are the number of diets sampled.

<table>
<thead>
<tr>
<th>Diet Item</th>
<th>Tarpon Snook</th>
<th>Common Snook</th>
<th>Bay Whiff</th>
<th>Burro Grunt</th>
<th>American Eel</th>
<th>Bigmouth Sleeper</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before (64)</td>
<td>During (78)</td>
<td>Before (21)</td>
<td>During (21)</td>
<td>Before (10)</td>
<td>During (7)</td>
</tr>
<tr>
<td>Postlarval Sirajo Goby</td>
<td>0</td>
<td>63.5</td>
<td>0</td>
<td>12.5</td>
<td>0</td>
<td>50.0</td>
</tr>
<tr>
<td>Other postlarval gobiods</td>
<td>0</td>
<td>25.0</td>
<td>0</td>
<td>0</td>
<td>40.0</td>
<td>0</td>
</tr>
<tr>
<td>Postlarval shrimps</td>
<td>77.8</td>
<td>65.4</td>
<td>50.0</td>
<td>0</td>
<td>60.0</td>
<td>75.0</td>
</tr>
<tr>
<td>Adult shrimps</td>
<td>16.7</td>
<td>5.8</td>
<td>16.7</td>
<td>25.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mountain Mullet</td>
<td>0</td>
<td>3.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Darter Goby</td>
<td>0</td>
<td>1.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Smallscaled</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Spinycheek Sleeper Fat Sleeper</td>
<td>11.1</td>
<td>0</td>
<td>0</td>
<td>12.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Broad-Striped Anchovy</td>
<td>0</td>
<td>0</td>
<td>16.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bigmouth Sleeper</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Unidentified fishes</td>
<td>5.6</td>
<td>9.6</td>
<td>33.3</td>
<td>62.5</td>
<td>40.0</td>
<td>0</td>
</tr>
<tr>
<td>Freshwater macroinvertebrates</td>
<td>5.6</td>
<td>0</td>
<td>16.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Puerto Rican Crab</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neritid snail</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Fig. 1 Plot of axis 1 versus axis 2 of a non-metric multidimensional scaling analysis for fish assemblage samples collected with (a) boat-mounted electrofishing at three locations and (b) beach seine at the river mouth, in the Río Grande de Arecibo in time periods before and during an amphidromous postlarval fish run. Each point represents a sample of the fish assemblage on a single date.
Fig. 2 The diet composition of six focal predator fishes by mean percent weight (%W) in both time periods before and during an amphidromous postlarval fish run at the Río Grande de Arecibo.
CHAPTER 4: Watershed characteristics and microhabitat use of Caribbean amphidromous gobioïd postlarvae

Summary

1. Amphidromous gobioïd fishes are threatened by many anthropogenic factors related to habitat loss and degradation. Despite the importance of successful recruitment to fish population dynamics, habitat use by postlarvae during recruitment from the marine environment to rivers has never been quantified.

2. In this article we compare postlarval densities in multiple rivers on the Caribbean island of Puerto Rico and explore how watershed-scale habitat characteristics are related to recruit abundance in two goby taxa. We also describe the riverine microhabitats that are utilized by postlarvae of the amphidromous gobioïd assemblage of Puerto Rico during recruitment and upstream migration.

3. Recruit abundances of two goby taxa were distinct among Puerto Rico rivers, and mean recruit densities of Sirajo Goby (*Sicydium* spp.) were correlated with river mouth location.

4. The depth, water velocity, substrate, and physical cover of microhabitats that are occupied most frequently by amphidromous postlarvae during recruitment and upstream migration are quantified, along with associated water physicochemical parameters. In general, postlarvae at river mouths occupied low velocity waters with downstream flow direction and low salinities and were found over sand substrates. River Goby at upstream locations occupied low velocity freshwater with upstream flow direction over sand substrates.
Our findings provide novel information on Caribbean gobioid recruitment and habitat requirements of postlarvae. This information may guide Caribbean stream ecosystem conservation and management to ensure the viability of amphidromous fish populations and enhance the sustainability of the ecosystem functions and services that they provide.
Introduction

Amphidromous fishes are major components of native stream fish assemblages in a variety of ecological contexts, but these species also face many threats. Amphidromous species from the Gobiidae and Eleotridae families (suborder Gobiodei) often make up the majority of native stream fish richness on oceanic tropical islands (Ryan, 1991; Fitzsimons, Nishimoto & Devick, 1996; McDowall, 2004; Keith & Lord, 2011) and are important components of the stream fish assemblage on some continental tropical islands, temperate island chains, and in tropical coastal continental areas (Shen & Tzeng, 2008; McDowall, 2010; Thuesen et al., 2011). Stream ecosystems where amphidromous fishes are typically prevalent are subject to disproportionately high anthropogenic pressure. For example, on oceanic tropical islands, human population densities are often very high, but surface water (i.e., streams) can be the only sources of freshwater, which leads to the overexploitation of aquatic resources (Ramírez et al., 2012; Kwak et al., 2016). Major threats to amphidromous fish populations include instream barriers to migration, alterations of stream flow from water abstraction or hydroelectric operations, fisheries that target multiple life stages, exotic species introductions, and habitat alteration by stream channelization and watershed urbanization (Holmquist et al., 1998; Brasher, 2003; Engman & Ramírez, 2012; Cooney & Kwak, 2013; Smith & Kwak, 2015; Kwak et al., 2016).

Information on the habitat requirements of amphidromous gobioids during their postlarval stage is needed to protect these prevalent and valuable species. Understanding habitat use throughout fish life history is critical for successful stream conservation and fisheries management, because habitat availability and quality specific to any one stage can
limit stream fish population sizes and vital rates (Rosenfeld, 2003; Rosenfeld & Hatfield, 2006). Research has been conducted on the habitats utilized by adult amphidromous gobioids in a broad set of locations, including Puerto Rico, Hawaii, Reunion Island, Japan, and Australia (Tamada, 2011; Engman & Ramirez, 2012; Kwak et al., 2013; McRae, McRae & Fitzsimons, 2013; Donaldson, Ebner & Fulton, 2013; Teichert et al., 2014). Investigators have also identified specific spawning microhabitat use by gobioids on Reunion Island and Japan (Onikura et al., 2008; Teichert et al., 2013). Moreover, fish otolith microchemistry and stable isotope analyses have been used to document gobioid larval habitats in several locations where information is available on adult habitat use (Sorensen & Hobson, 2005; Hogan et al., 2014; Smith & Kwak, 2014; Engman, Chapter 2). However, no research has been conducted to quantify the habitat use by amphidromous fishes during the postlarval stage, a critical period when recruitment to stream ecosystems occurs. Successful postlarval recruitment and upstream migration is critical to amphidromous fish population viability because recruitment determines local and metapopulation dynamics in species with marine larval phases (Caley et al., 1996). Furthermore, amphidromous postlarvae are harvested in globally dispersed artisanal fisheries, are a food source for estuarine predators, and transport marine nutrients into freshwater systems (Bell, 1999; Castellanos-Galindo et al., 2011; Lefrançois et al., 2011; Engman, Chapters 2 & 3). Therefore, successful postlarval recruitment is a key aspect of fisheries management and stream ecosystem functions and services, especially on oceanic tropical islands.

In this article we quantify habitat use by recruiting amphidromous gobioid postlarvae at two spatial scales on the Caribbean island of Puerto Rico. First, we compare postlarval
densities in multiple Puerto Rico rivers and explore how watershed-scale habitat characteristics are related to recruit abundance in two goby taxa. Next, we describe the microhabitats that are occupied by postlarvae of the amphidromous gobioid assemblage of Puerto Rico during recruitment and upstream migration.

**Methods**

*Study systems*

The island of Puerto Rico is an ideal location for study of the biology and ecology of amphidromous fishes, as it is centrally located in the Caribbean archipelago, and native stream fish assemblages are dominated by amphidromous gobioid species with broad distributions in the greater Caribbean region (Lefrançois *et al.*, 2011; Kwak *et al.*, 2016). The amphidromous gobioid fish assemblage of Puerto Rico includes Bighmouth Sleeper (*Gobiomorus dormitor*), Smallscaled Spinycheek Sleeper (*Eleotris perniger*)—family Eleotridae; and River Goby (*Awaous banana*) and Sirajo Goby (*Sicydium spp.*)—family Gobiidae (Kwak *et al.*, 2016). Engman (Chapter 1) determined that gobies recruited into river mouths in higher densities than the two eleotrid species, and that gobies followed strong lunar and annual cycles of recruitment in the Caribbean. Specifically, he established that the majority of River Goby and Sirajo Goby recruitment occurs during the last quarter moon phase of the lunar cycle from the months of June through January.

For this study we selected seven rivers that span the north coast of the island of Puerto Rico to evaluate the relationship between watershed-scale habitat characteristics and the abundance of River Goby and Sirajo Goby postlarval recruits. The area of each study watershed was ascertained from the Puerto Rico GAP database; watershed discharge was
quantified as the mean daily discharge of the lowest elevation U.S. Geological Survey (USGS) gauging station present on each river during the watershed-scale sampling period; and river mouth longitude was recorded with a Garmin GPS Model V (Gould et al., 2007; USGS, 2016; Table 1). A subset of four of the seven watersheds was further sampled to identify the microhabitats that gobioid postlarvae use during recruitment (i.e., river ingress) and upstream migration (Table 1).

**Sampling protocol and schedule**

Postlarval amphidromous fishes were captured with a 1x1 m benthic kick-net with 1-mm mesh, fitted with wooden brails and a bottom lead line. To sample fishes, a two-person team swept the net directly towards the bank with the lead line lightly scraping the substrate. A large tub was placed on shore to collect the catch at the end of the net-haul. Captured fishes were separated from excess sediment and organic material, and preserved in a 10% buffered formalin solution for laboratory identification. The depth of the starting point of each net-haul and its distance from shore was recorded in order to determine the volume of water sampled. Each sampling occasion was composed of five net-hauls conducted over a 10-20 m reach. The first net-haul was conducted at the down-stream end of the sampling reach, and the location of subsequent net-hauls was moved incrementally upstream. At times it was necessary to conduct less than five net-hauls on a sampling occasion due to inclement weather or logistical constraints, but no less than three net-hauls ever constituted a sampling occasion. Additional details of the methods that were used to capture, identify, and determine the density of gobioid postlarvae are described in detail in Engman (Chapter 1).
We conducted watershed-scale sampling to determine recruit densities [i.e., catch per unit effort (CPUE) as fish/m$^3$ water sampled] at the river mouth of each watershed—defined as the point where the river-channel ends and ocean habitat begins—and determined postlarval microhabitat use at two stations on each study system. The first station was the river mouth, and the second was at a location immediately downstream of the most downstream riffle on each river and immediately upstream of the maximum inland penetration of the salt-wedge of each river’s estuary.

Watershed-scale sampling of River Goby and Sirajo Goby recruit abundances occurred during known recruitment times in annual and lunar temporal cycles. We quantified River Goby and Sirajo Goby recruit densities on all study rivers from June 20 to October 18, 2014. During watershed-scale sampling, each river was sampled twice per month at the river mouth on days 22 to 24 of each month’s lunar cycle.

Sampling of gobiod postlarvae to determine microhabitat use occurred during two temporally extensive sampling periods and two intensive sampling periods that spanned the annual and lunar cycles. Four study rivers were sampled during the extensive sampling period and two rivers, the Río Grande de Arecibo and Mameyes, were sampled during the intensive sampling periods (Table 1). The extensive periods spanned dates from November 2013 to May 2014 and from November 2014 to April 2015. During the extensive sampling periods both rivers were sampled twice per month on the days 22 to 25 of the lunar cycle (i.e., the last quarter moon phase). We were unable to conduct sampling on December 2013, February 2014, and April 2014 due to logistical constraints. The intensive sampling periods were from June 18 to October 8, 2013 and from May 29 to October 17, 2014. In the 2013
intensive sampling period the Río Grande de Arecibo and Mameyes rivers were sampled
during the full period and the Sabana and Río Grande de Manatí rivers were sampled from
June 18 to July 11 only. In 2014, all four rivers were sampled throughout the entire intensive
sampling period. In the 2013 intensive sampling period, rivers were sampled only 8 times per
month at the mouth and upstream stations, and in 2014, rivers were sampled 12 times per
month at the mouth station. Supplemental sampling of River Goby microhabitat use was
conducted at multiple locations between the mouth station and the lower upstream station on
the Río Grande de Arecibo during June 2013.

We measured a suite of physical and chemical habitat parameters at locations where
postlarvae were captured. The total depth (m) at the starting point of each net-haul was
measured with a top-set wading rod and was divided by two to determine the median depth
(m) of each sample location. Water velocity at each sample location were measured using a
top-set wading rod and a Marsh-McBirney Flowmate Model 2000 meter. Bottom and mean
water column velocity was measured at three locations within the area of each net haul: the
starting point, the mid-point of the haul, and a point immediately adjacent to bank. The mean
value of both the bottom and mean-column point measurements were calculated to determine
a single bottom and mean water-column velocity for each sample-location. Mean water-
column velocity was measured at 60% of the total depth. Water velocity was recorded as
negative values at any location where the flow was oriented in an upstream direction, rather
than downstream. We also visually identified the substrate (bottom materials) and associated
physical cover types with greatest percent areal coverage in each net-haul area. Substrate
types were classified according to a modified Wentworth scale, which included silt, sand,
gravel, and cobble in each net-haul swath (Bovee & Milhous, 1978). Physical cover type categories included filamentous algae, aquatic macrophytes, cobble, fine woody debris, coarse woody debris, and artificial materials (e.g., concrete, trash, tires, etc.). Finally, we measured water physicochemical parameters [i.e., temperature (°C), salinity (parts per thousand), and dissolved oxygen (mg/L)] at the midpoint of the sampling station once per sampling occasion with a Yellow Springs Instrument (YSI) model 556 Multiprobe instrument.

Data compilation and analyses

A Kruskal-Wallis test was employed to determine if River Goby and Sirajo Goby postlarvae recruit abundances (fish/m³) varied among the seven rivers in our watershed-scale habitat study. We then used Dunn’s tests with the Holm correction for multiple comparisons between all river-pairs. Next, we calculated the mean (±1 SE) Sirajo Goby and River Goby recruit densities at each river over the entire watershed-scale sampling schedule to obtain a single index of postlarval recruitment for each river basin. Finally, we used Pearson’s product-moment correlation matrix to explore trends between mean goby postlarval recruit densities and the watershed-scale habitat characteristics that are presented in Table 1.

To determine taxon-specific microhabitat use, we summarized data for each measured parameter and compiled them from all locations where postlarval recruits of each of the four gobioïd taxa were captured. We calculated means (±1 SE) for each continuous variable and identified modal substrate and cover classes. Next, we constructed frequency histograms of mean column and bottom velocity, and median depth of occupied microhabitats for all taxa.
All statistical analyses described above were conducted in the statistical package R 3.3.1 (R Development Core Team, 2016).

**Results**

In total, we obtained 50 postlarval abundance samples at each river mouth during the watershed-scale sampling regime, except for the Espiritu Santo River, which was sampled 45 times. We attempted to sample amphidromous postlarvae to determine their microhabitat use 1,861 times at river mouth and 291 times at upstream stations of the four study river. However, because postlarval recruitment does not occur continuously through lunar or annual cycles and postlarvae move quickly through the mouth and lower river to upstream habitats, we only captured postlarvae of each study taxon in a fraction of these samples (Chapter 1, Chapter 3). Moreover, nearly all of our observations of Sirajo Goby, Smallscaled Spinycheek Sleeper, and Bignose Sleeper were at the river mouth station, so we limited our study of microhabitat use by these species to river mouth stations (Table 2, Fig. 2 & 3). However, we captured River Goby in 121 samples at upstream stations, so we describe microhabitat use by River Goby recruits at both locations (Table 2, Fig. 4).

**River basin comparisons**

Rivers on the north coast of Puerto Rico received distinctly abundant postlarval goby runs, and their mean postlarval recruit abundances varied with river mouth location. Kruskal-Wallis tests revealed that the densities [CPUE (fish/m³)] of both Sirajo Goby ($\chi^2 = 47.56, df = 6, P < 0.01$) and River Goby ($\chi^2 = 35.77, df = 6, P < 0.01$) postlarval recruits varied by river basin, and Dunn’s tests indicated between-river differences in postlarval goby abundances for some of the rivers studied (Table 2, Table 3). Specifically, the Rio Grande
Arecibo and Cibuco River received greater River Goby and Sirajo Goby recruit abundances than multiple other rivers, and the Sabana River received greater River Goby recruit abundances than the Espiritu Santo River (Table 2, Table 3). Overall mean River Goby recruit densities were highest in the Río Grande de Arecibo and lowest in the Espiritu Santo River while mean Sirajo Goby densities were highest at the Camuy River and lowest at the Espiritu Santo River. The Pearson’s product-moment correlation matrix revealed no strong or significant correlations between mean River Goby postlarvae density and watershed area ($r = 0.51$, $P = 0.25$), mean river discharge ($r = 0.56$, $P = 0.18$), or river mouth location (i.e., longitude) ($r = 0.34$, $P = 0.34$). Similarly, mean Sirajo Goby postlarvae densities were not strongly correlated with watershed area ($r = 0.57$, $P = 0.18$) or mean river discharge ($r = 0.49$, $P = 0.26$). However, the longitude of the river mouth was strongly and significantly correlated with mean Sirajo Goby density (Fig. 2).

**Microhabitat use**

At river mouth stations, all four taxa were most frequently found in locations where water velocities were near zero and oriented downstream. The river mouth station mean and bottom water velocity distributions at microhabitats occupied by all four taxa were strongly unimodal, centered on zero, and right-skewed [i.e., had longer and heavier tails in the downstream direction (Figs. 4, 5)]. Means of mean water-column and bottom velocity of microhabitats used by the four study taxa were always in the downstream direction at the river mouth. The means of mean water-column velocity use by the four taxa included values from 0.08 m/s (for Bigmouth Sleeper) to 0.18 m/s (for Sirajo Goby), and mean bottom water velocity use ranged from 0.03 m/s (for Bigmouth Sleeper) to 0.08 m/s (for Sirajo Goby).
river mouths, River Goby occupied the widest range of mean water-column and bottom velocities of the four taxa studied, Smallscaled Spinycheek Sleeper used the narrowest range of mean velocities, and Bigmouth Sleeper used the narrowest range of mean water-column velocities (Table 5).

At upstream stations, River Goby continued to occupy low-velocity habitats most frequently; however, River Goby microhabitat use distributions at these stations shifted toward locations where stream flow moved in the upstream direction. River Goby upstream station mean and bottom water velocity distributions were strongly unimodal, centered on zero, and left-skewed [i.e., had longer and heavier tails in the upstream-flow direction (Fig. 6)]. Also, the means of mean-column and bottom velocity occupied by River Goby at the upstream stations were in the upstream direction (Table 5). Finally, River Goby used microhabitats with higher maximum upstream flow velocities and lower maximum downstream flow velocities at upstream stations than at river mouth stations.

Depth of microhabitats occupied by most of the taxa was non-specific, but Sirajo Goby at the river mouth and River Goby at upstream stations exhibited higher proportions of habitat use at specific depths. Depths used by River Goby, Smallscaled Spinycheek Sleeper, and Bigmouth Sleeper were fairly uniformly distributed at river mouth stations, where mean depth use by all four taxa was about 0.3 m and included the entire range of values that they could have been sampled at, based on the limitations of our gear (from 0.1 to 0.5 m). Sirajo Goby occupied depth distribution at the river mouth was weakly unimodal, centered on 0.4 m, and the occurrence of this taxon at median depths of 0.5 m was relatively low (Fig. 3, Table 2). River Goby depth use at the upstream stations was also unimodal with the
maximum and mean number of positive observations occurring at about 0.2 m (Fig. 4, Table 2).

Mean water quality parameter measurements at occupied microhabitats were similar for all taxa at both stations, except for salinity values, which were lower at the upstream stations (as expected). Mean temperatures were ~26°C for all taxa at all stations. Salinity was generally low (~ 2 ppt), and all taxa were present when the mouth station salinity exceeded 30 ppt, except for Sirajo Goby which was captured at a maximum salinity of ~21 ppt. As expected, salinity at upstream stations was always relatively low, so River Goby sampled there were in low-salinity microhabitats. All taxa were captured over wide ranges of dissolved oxygen concentrations at the river mouth, including values near 2 mg/L for all taxa except Bigmouth Sleeper, which was captured over the narrowest range of values. River Goby at upstream stations were captured over an even narrower range of values, which were never below 5.28 mg/L (Table 3).

Discussion

Our results indicate that amphidromous goby recruitment to rivers across the north coast of Puerto Rico is not uniform, similar to the findings of prior sampling in these rivers (Engman, Chapter 1). In this study, we identified the Río Grande de Arecibo as a river that receives greater River Goby recruitment than others in Puerto Rico. Specifically, River Goby recruit densities at the Río Grande de Arecibo mouth were statistically greater than those at the Río Grande de Manatí and the Espiritu Santo rivers, and mean River Goby postlarvae density at the Río Grande de Arecibo was greater than that at all six other rivers. Statistical comparisons and mean recruitment values also indicated greater Sirajo Goby recruitment at
the Río Grande de Arecibo than the Sabana, Mameyes and Espiritu Santo rivers. These results agree with findings of Engman (Chapter 1), where modeled recruit densities of River Goby and Sirajo Goby postlarvae in the Río Grande de Arecibo were greater than those in the Río Grande de Manatí, Sabana, and Mameyes rivers. River Goby and Sirajo Goby recruitment was also relatively high in the Cibuco River, and mean Sirajo Goby recruit density was high at the Camuy River. These two rivers were not sampled in Engman’s (Chapter 1) study, and thus, cannot be compared.

The fact that recruitment is variable among rivers is likely to influence goby population dynamics in Puerto Rico. Local goby populations in rivers where postlarvae densities are low may be more limited by recruit-supply than those where postlarvae densities are consistently high as long as supply does not scale with adult habitat size. Indeed, the results of our correlation analysis do not provide any indication that goby recruit-supply scales to adult habitat size area in Puerto Rico because watershed area and total river discharge (two coarse metrics of habitat areas) were not significantly correlated to recruit abundances. However, correlation analysis indicated that rivers that were farther west on the island received greater Sirajo Goby recruit abundances than rivers toward the east side of the island. One possible explanation for this observation is that there is a net-western direction of Sirajo Goby larval dispersal on the north coast of Puerto Rico. If true, this scenario would imply that source-sink dynamics may influence Sirajo Goby population abundances. Although this hypothesis is somewhat speculative, Cook et al. (2009) used population genetic analyses of mitochondrial DNA to demonstrate that connectivity among river populations via larval dispersal of Sicydium spp. occurs in Puerto Rico. Future studies that
explore larval dispersal pathways with biophysical models or microsatellite markers would be informative to determine if there is a dominant direction of larval dispersal and a predictable pattern in source-sink dynamics among Puerto Rico rivers.

Regardless of the mechanisms that lead to distinct recruitment magnitude in the rivers of Puerto Rico, these differences have important implications for stream ecosystem functions and services, and fisheries and ecosystem management in Puerto Rico. Engman (Chapters 2, 3) demonstrated that goby postlarvae are an important food source for estuarine and riverine predators and sportfish during mass recruitment events, and that these events function as a marine subsidy to estuarine and riverine ecosystems. Moreover, in Puerto Rico there is a culturally valuable artisanal fishery that targets Sirajo Gobies, which is largely unregulated, and occurs on some of the rivers that we have identified as high-recruitment rivers (Kwak et al., 2016; pers. obs.). It is important to recognize that locations that receive greater recruit abundances are more likely to benefit from ecosystem functions and services and to consider measures, such as monitoring and regulation of the postlarvae fishery, to ensure their sustainability. In rivers where goby postlarvae density is low, it would be beneficial to evaluate if goby populations are limited by recruitment (e.g., development of stock-recruitment relationships). If so, regulation of postlarvae harvest or measures to enhance recruitment, such as flow regulations that ensure river–ocean connectivity and potential freshwater attraction plumes, are possible conservation actions.

Our habitat findings at two spatial scales provide additional information that will be useful for conservation and management of amphidromous fish populations and the ecosystem functions and services that they provide. The microhabitat use distributions that
we developed for native amphidromous fish recruits (Figs. 2-5) are typically employed as the only biological criteria included in instream flow modeling to determine optimal flow regimes and effects of altered flows in stream and river ecosystems (Bovee, 1986; Annear et al., 2004). These distributions may be especially useful as criteria for setting flow standards in the Caribbean region, where freshwater fish are considered the most important aquatic organism in regulating flows, but the ecology or habitat requirements of aquatic species are not always considered by resource managers in their instream flow determinations (Scatena, 2004).

We found that postlarvae of all taxa occupied microhabitats with low water velocities that were oriented downstream and areas with low salinity when recruiting to river mouths. These microhabitat characteristics are ideal for postlarvae that use rheotaxis or freshwater olfactory cues to navigate upstream, but avoid high velocity currents that are energetically costly to swim against (Keith, 2003; Keith & Lord, 2011). Two conditions that are conducive to low-salinity and low-downstream-velocity habitats at Puerto Rico river mouths are (1) freshwater spates that occur with sufficient frequency and magnitude to prevent the formation of sand berms that block river mouths, and (2) river mouths and upstream areas that have not been artificially constricted or armored. In addition to forming a physical barrier to migration, river mouths that are blocked by sand berms become fully lentic, hypersaline habitats (Zamora, Corujo Flores & Cham, 1986). Stream mouths in Puerto Rico with unrestricted, natural geomorphology typically have sand substrates and dynamic shapes, and they broaden under high flow conditions, effectively dispersing flow energy and creating low velocity microhabitats near banks (pers. obs.). River Goby at upstream locations tended
to use locations with low and negative or upstream velocities. Backwaters, eddies, and negative velocity habitats typically only occur in rivers with complex channel morphology. Channelization of rivers creates homogenous channel form and velocities and thus, reduce microhabitat diversity and abundance of microhabitat locations that River Gobies occupied most frequently (Smiley & Dibble 2005; White et al. 2009). Therefore the regulation of naturally variable flow regimes and minimizing river mouth armoring and stream channelization can protect the microhabitats that amphidromous gobies use during postlarval recruitment and migration toward sustainable native amphidromous fish assemblages and fisheries.
References


United Nations; Michigan State University; and The American Fisheries Society, Rome; East Lansing; and Bethesda.


Tables

**Table 1** Watershed characteristics and mean ± 1 SE Sirajo Goby and River Goby recruit density of each study river. Rivers are listed in east-to-west order of the location of the river mouth. The USGS ID column indicates the USGS gauging station data that were used to determine mean discharge. Rivers that were sampled for fish microhabitat use are indicated with an X.

<table>
<thead>
<tr>
<th>River name</th>
<th>Sirajo Goby (fish/m³)</th>
<th>River Goby (fish/m³)</th>
<th>Watershed area (km²)</th>
<th>USGS ID</th>
<th>Mean discharge (m³/s)</th>
<th>Mouth longitude (°W)</th>
<th>Microhabitat use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabana</td>
<td>0.24 ± 0.14</td>
<td>10.85 ± 3.87</td>
<td>1,865</td>
<td>50067000</td>
<td>0.36</td>
<td>65.71</td>
<td>X</td>
</tr>
<tr>
<td>Mameyes</td>
<td>1.37 ± 0.90</td>
<td>1.39 ± 0.51</td>
<td>4,031</td>
<td>50065500</td>
<td>1.21</td>
<td>65.7</td>
<td>X</td>
</tr>
<tr>
<td>Espíritu Santo Cibuco</td>
<td>0.23 ± 0.12</td>
<td>0.27 ± 0.17</td>
<td>6,780</td>
<td>50063800</td>
<td>1.3</td>
<td>65.8</td>
<td></td>
</tr>
<tr>
<td>Río Grande de Manatí</td>
<td>14.33 ± 7.04</td>
<td>0.64 ± 0.21</td>
<td>60,815</td>
<td>50038100</td>
<td>8.08</td>
<td>66.53</td>
<td>X</td>
</tr>
<tr>
<td>Río Grande de Arecibo</td>
<td>9.95 ± 4.10</td>
<td>26.67 ± 10.48</td>
<td>67,262</td>
<td>50029000</td>
<td>10.95</td>
<td>66.71</td>
<td>X</td>
</tr>
<tr>
<td>Camuy</td>
<td>18.27 ± 11.61</td>
<td>3.48 ± 2.02</td>
<td>15,995</td>
<td>50014800</td>
<td>2.54</td>
<td>66.84</td>
<td></td>
</tr>
</tbody>
</table>
Table 2 Dunn’s test for multiple pairwise comparisons of River Goby postlarvae density (CPUE, fish/m³) during the last quarter moon phase at the mouth of all seven study rivers. Numbers in each cell are the Z-statistic for the column to row comparison and the associated P-value is in parentheses. Cells with an asterisk indicate significant differences in the River Goby densities of the two rivers being compared.

<table>
<thead>
<tr>
<th></th>
<th>Río Grande de Arecibo</th>
<th>Camuy</th>
<th>Cibuco</th>
<th>Espiritu Santo</th>
<th>Mameyes</th>
<th>Río Grande de Manatí</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camuy</td>
<td>2.67 (0.05)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cibuco</td>
<td>-0.65 (0.52)</td>
<td>-3.32 (0.01)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Espiritu Santo</td>
<td>5.08 (&lt; 0.01)*</td>
<td>2.48 (0.07)</td>
<td>5.71 (&lt;0.01)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mameyes</td>
<td>2.74 (0.04)</td>
<td>0.068 (0.47)</td>
<td>3.39 (&lt;0.01)*</td>
<td>-2.42 (0.08)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Río Grande de Manatí</td>
<td>3.61 (&lt;0.01)*</td>
<td>0.94 (1)</td>
<td>4.26 (&lt;0.01)*</td>
<td>-1.57 (0.41)</td>
<td>0.87 (0.77)</td>
<td></td>
</tr>
<tr>
<td>Sabana</td>
<td>1.87 (0.28)</td>
<td>-0.80 (0.63)</td>
<td>2.51 (0.07)</td>
<td>-3.26 (0.01)*</td>
<td>-0.87 (0.96)</td>
<td>-1.74 (0.33)</td>
</tr>
</tbody>
</table>
Table 3 Dunn’s test for multiple pairwise comparisons of Sirajo Goby postlarvae density (CPUE, fish/m$^3$) during the last quarter moon phase at the mouth of all seven study rivers. Numbers in each cell are the Z-statistic for the column to row comparison and the associated $P$-value is in parentheses. Cells with an asterisk indicate significant differences in the Sirajo Goby densities of the two rivers being compared.

<table>
<thead>
<tr>
<th>River name</th>
<th>Río Grande de Arecibo</th>
<th>Camuy</th>
<th>Cibuco</th>
<th>Espiritu Santo</th>
<th>Mameyes</th>
<th>Río Grande de Manatí</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camuy</td>
<td>1.41 (0.56)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cibuco</td>
<td>-0.23 (1)</td>
<td>-1.64 (0.45)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Espiritu Santo</td>
<td>3.68 (&lt;0.01)*</td>
<td>2.30 (0.13)</td>
<td>3.90 (&lt;0.01)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mameyes</td>
<td>3.35 (0.01)*</td>
<td>1.94 (0.26)</td>
<td>3.59 (&lt;0.01)</td>
<td>-0.41 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Río Grande de Manatí</td>
<td>1.41 (0.48)</td>
<td>&lt;0.01 (0.50)</td>
<td>1.64 (0.40)</td>
<td>-2.30 (0.14)</td>
<td>-1.94 (0.29)</td>
<td></td>
</tr>
<tr>
<td>Sabana</td>
<td>3.89 (&lt;0.01)*</td>
<td>-2.48 (0.09)</td>
<td>4.13 (&lt;0.01)*</td>
<td>0.11 (0.9)</td>
<td>-0.54 (1)</td>
<td>2.49 (0.10)</td>
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</tbody>
</table>
Table 4 Summary of microhabitat use characteristics by amphidromous postlarvae during recruitment to the Sabana River, Mameyes River, Río Grande de Manatí, and Río Grande de Arecibo in Puerto Rico. Negative water velocities indicate flow in the upstream direction.

<table>
<thead>
<tr>
<th>Taxon with total sample size (N)</th>
<th>Station</th>
<th>Mean velocity (m/s)</th>
<th>Bottom velocity (m/s)</th>
<th>Median depth (m)</th>
<th>Temp. (°C)</th>
<th>Sal. (ppt)</th>
<th>DO (mg/L)</th>
<th>Substrate</th>
<th>Cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sirajo Goby (211)</td>
<td>River mouth</td>
<td>Mean or mode</td>
<td>0.18</td>
<td>0.08</td>
<td>0.32</td>
<td>26.58</td>
<td>2.87</td>
<td>6.06</td>
<td>Sand</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SE</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
<td>0.13</td>
<td>0.37</td>
<td>0.14</td>
<td>Macrophyte</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minimum</td>
<td>-0.18</td>
<td>-0.21</td>
<td>0.10</td>
<td>24.09</td>
<td>0.09</td>
<td>2.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maximum</td>
<td>0.87</td>
<td>0.43</td>
<td>0.50</td>
<td>29.11</td>
<td>21.32</td>
<td>10.70</td>
<td></td>
</tr>
<tr>
<td>Smallscaled Spinycheek Sleeper (189)</td>
<td>River mouth</td>
<td>Mean or mode</td>
<td>0.11</td>
<td>0.04</td>
<td>0.30</td>
<td>26.14</td>
<td>2.36</td>
<td>7.25</td>
<td>Sand</td>
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<tr>
<td></td>
<td></td>
<td>SE</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
<td>0.13</td>
<td>0.39</td>
<td>0.73</td>
<td>Macrophyte</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minimum</td>
<td>-0.18</td>
<td>-0.13</td>
<td>0.10</td>
<td>22.74</td>
<td>0.08</td>
<td>2.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maximum</td>
<td>0.83</td>
<td>0.36</td>
<td>0.50</td>
<td>30.71</td>
<td>33.99</td>
<td>10.70</td>
<td></td>
</tr>
<tr>
<td>Bigmouth Sleeper (131)</td>
<td>River mouth</td>
<td>Mean or mode</td>
<td>0.08</td>
<td>0.03</td>
<td>0.31</td>
<td>26.47</td>
<td>3.28</td>
<td>7.22</td>
<td>Sand</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SE</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
<td>0.17</td>
<td>0.48</td>
<td>0.83</td>
<td>Macrophyte</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minimum</td>
<td>-0.16</td>
<td>-0.12</td>
<td>0.10</td>
<td>22.96</td>
<td>0.08</td>
<td>4.10</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Maximum</td>
<td>0.93</td>
<td>0.34</td>
<td>0.50</td>
<td>30.71</td>
<td>33.99</td>
<td>9.10</td>
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<tr>
<td>River Goby PL1 (647)</td>
<td>River mouth</td>
<td>Mean or mode</td>
<td>0.11</td>
<td>0.04</td>
<td>0.27</td>
<td>26.46</td>
<td>2.70</td>
<td>6.99</td>
<td>Sand</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SE</td>
<td>0.01</td>
<td>0.00</td>
<td>&lt;0.01</td>
<td>0.07</td>
<td>0.26</td>
<td>0.35</td>
<td>Macrophyte</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minimum</td>
<td>-0.38</td>
<td>-0.26</td>
<td>0.10</td>
<td>24.09</td>
<td>0.08</td>
<td>2.30</td>
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<tr>
<td></td>
<td></td>
<td>Maximum</td>
<td>1.75</td>
<td>0.64</td>
<td>0.50</td>
<td>30.71</td>
<td>35.53</td>
<td>10.70</td>
<td></td>
</tr>
<tr>
<td>River Goby (121)</td>
<td>Upstream stations</td>
<td>Mean or mode</td>
<td>-0.03</td>
<td>-0.02</td>
<td>0.21</td>
<td>26.26</td>
<td>0.08</td>
<td>6.94</td>
<td>Sand</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SE</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.14</td>
<td>0.00</td>
<td>0.12</td>
<td>Fine woody debris</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minimum</td>
<td>-0.59</td>
<td>-0.36</td>
<td>0.10</td>
<td>23.66</td>
<td>0.02</td>
<td>5.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maximum</td>
<td>0.25</td>
<td>0.07</td>
<td>0.40</td>
<td>29.83</td>
<td>0.34</td>
<td>10.72</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1 Pearson’s product-moment correlation analysis ($r$) between mean Sirajo Goby postlarvae density (as CPUE) and river mouth longitude.
**Fig. 2** Distributions of mean water-column velocity of microhabitats occupied by Sirajo Goby, River Goby, Smallscaled Spinycheek Sleeper, and Bigmouth Sleeper postlarvae during recruitment at river mouths in Puerto Rico.
Fig. 3 Distributions of bottom water velocity of microhabitats occupied by Sirajo Goby, River Goby, Smallscaled Spinycheek Sleeper, and Bigmouth Sleeper postlarvae during recruitment at river mouths in Puerto Rico.
Fig. 4 Distributions of depth of microhabitats occupied by Sirajo Goby, River Goby, Smallscaled Spinycheek Sleeper, and Bigmouth Sleeper postlarvae during recruitment at river mouths in Puerto Rico. Median depth refers to the median depth of the kick-net haul in which fishes were captured.
Fig. 5 Distributions of mean-column and bottom velocity, and depth of microhabitats occupied by River Goby postlarvae during upstream migration in Puerto Rico rivers.