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Abstract
    Assessment of early life history characteristics requires effective sampling methods for larval fishes. Different gears are suitable for different habitats and frequently select different sizes or life history stages. In this study, two passive sampling methods were compared for use in collecting larval freshwater and estuarine fishes in two tropical streams. Drift nets and light traps were identified a priori as the most appropriate gears for sampling in these systems based on stream characteristics (including accessibility, size, and morphology). Catches were compared between sampling methods. Additionally, catches under different environmental conditions (flow, tide stage, and moon phase), during different sampling periods (time of day and week of summer), and at different depths were compared using mixed-effects analysis of variance. A total of 2,156 fish were captured in 954 h of sampling. The significant ($\alpha < 0.05$) explanatory variables for total larval catch were stream, sampling method, week of summer, time of day, and moon phase. Eleven families were represented in the samples, with the families Gobiidae ($n = 948$), Eleotridae ($n = 391$), and Syngnathidae ($n = 276$) comprising 94.9% of the identifiable larvae collected. The variables that explained both the Gobiidae and Syngnathidae catches were gear, week of summer, and time of day. The Syngnathidae catch was highest in drift nets, but the Gobiidae catch was highest in light traps. Nighttime light trap sampling appeared to be the best overall technique for larval collection in these systems. However, a combination of light traps and drift nets would reduce the biases associated with size and species selectivity found for individual gears, and this approach would be preferable for sampling small tropical streams.

The early life history of fishes is a critical period for effective conservation and management, as many processes during this period influence survival and subsequent recruitment to larger size-classes (Chambers and Trippel 1997). This is particularly true for native tropical island stream fishes, many of which are reliant on migrations between marine and freshwater habitats (Erdman 1984; Neal et al. 2009). However, there is a paucity of published information regarding the natural life history of tropical stream fishes, especially regarding early life history characteristics, and the limited information available is often conflicting. For example, Nordlie (1979) first described the bigmouth sleeper *Gobiomorus dormitor* as anadromous, but 2 years later described it as catadromous (Nordlie 1981). Elsewhere in the literature, this species has been described as amphidromous (Winemiller and Ponwith 1998). Hernández-Saavedra et al. (2004) were unsure of spawning location and recommended further research. Whereas there are many threats to bigmouth sleeper and other tropical stream fishes (Neal et al. 2009), conservation efforts must rely on accurate characterizations of life history strategies if they are to be effective.

Assessment of early life history characteristics requires effective sampling methods for larval fishes. There are various active and passive sampling methods for collecting estuarine and freshwater fish larvae. Different gears are suitable for different habitats and frequently select different sizes or life history stages (Leis 2000). Active sampling methods include seining, trawling, electrofishing, plankton net tows, pumping (Kelso and Rutherford 1996), and lighted lift nets (Rooker et al. 1996). These sampling methods have been used since the early 1800s but have greatly improved in efficiency in recent decades. Active sampling methods, especially pumps, are known to damage larvae (Gale and Mohr 1978) and can require significant labor to operate. Active sampling can be high in fuel and equipment costs, or may be restricted by access or navigation in the environment to be sampled. Passive sampling methods include drift sampling, activity traps, and light traps. These passive gears are stationary, require less labor, and have lower associated operation costs (Kelso and Rutherford 1996).

Distributions of fish larvae vary spatially and temporally in nature (Chambers and Trippel 1997). The duration of spawning season can vary from a few days to many months. There can be seasonal variability of spawning intensity due to density-independent factors, such as rainfall, floods, and droughts (Kelso and Rutherford 1996). Spatial distributions of larvae can vary from the surface to the bottom of the water column, and larval abundance can vary with light, temperature, and flow (Kelso and Rutherford 1996). All of these factors, and more, can influence the choice of sampling season, time, and method. Factors to consider in a larval sampling design include spawning season, spawning location, larval buoyancy, and diel tendencies of the target species (Kelso and Rutherford 1996).

In this study, we compared two passive sampling methods for use in collecting larval freshwater and estuarine fishes in two tropical streams. Drift nets and light traps were identified a priori as the most appropriate sampling methods for sampling in these systems. This selection was based on stream accessibility, size, and morphology, as most streams on the study island of Puerto Rico are narrow, shallow, flashy, and often heavily vegetated along the margins; these streams are not navigable and access is usually limited to road crossings. Catches were compared between sampling methods. Additionally, catches under different environmental conditions (flow, tide, moon phase), during different sampling periods (time of day and week of summer), and during different depth sets were compared.

METHODS

Study area.—Puerto Rico is a self-governing country in a commonwealth with the United States located to the east of the Dominican Republic and to the west of the Virgin Islands. The Atlantic Ocean is located to the north, and the Caribbean Sea to the south. Puerto Rico is the smallest and easternmost island of the Greater Antilles. The island is approximately 180 km long (east to west) and 65 km wide (north to south). Puerto Rico is mostly mountainous and has many shallow streams that are largely nonnavigable. Larval sampling was conducted in 2007 in the Río Cañas and Río Guanajibo. The Río Cañas flows south to the Caribbean Sea through the coastal city of Ponce. The Río Guanajibo flows northwest to the Atlantic Ocean through the coastal city of Mayaguez.

Sampling methods comparison.—Two passive sampling methods, light traps and drift nets, were identified for comparison a priori as the most likely candidates for stream conditions in Puerto Rico. Active gears were not selected because the streams are not navigable and many obstructions are present within these systems. Light traps were modified quatrefoil traps (Aquatic Research Instruments, Hope, Idaho). The light traps consisted of an acrylic trapping assembly with an internal polycarbonate tube where the light source was located. The unit was 30 × 30 × 20 cm (length × width × height) and had a 10-mm gap on all four sides to allow organisms inside the trap. The drift net had a 30 × 45-cm opening, was 1 m in length, and utilized a mesh size of 363 μm.

Sampling was conducted from June–August 2007. This period has been reported to include peak spawning for several Caribbean stream fishes, including bigmouth sleeper (Nordlie 1981; Bacheler et al. 2004; Hernández-Saavedra et al. 2004; Harris 2007) and mountain mullet *Agonostomus monticola* (Aiken 1998). Sampling was conducted weekly for seven consecutive weeks. The two streams were sampled on consecutive nights whenever weather and scheduling allowed. The two sampling gears were fished concurrently at night (1800–0600 hours). Drift nets were also deployed during the day (0600–1800 hours). For both sampling methods, collected larvae were removed every 3 h to reduce larval damage and net clogging. The moon phase (new moon, first quarter moon, full moon, or last quarter moon) was noted for each week, and the tide stage (ebb, flood, high slack, or low slack) was noted for each 3-h sample period.

Two light traps were sampled concurrently: one anchored to the bottom, and one floating at the surface. The light source inside was a submersible, battery-powered light-emitting diode dive light. A battery-powered source of light was chosen over chemical light sticks due to diminishing luminance found in chemical light sticks for sampling periods exceeding 1 h (Kissick 1993). Larval fish were attracted to the light and collected at the bottom of the trap in a 250-μm mesh plankton sock. Contents of the sock during each 3-h period were preserved separately in a 5% solution of formalin for later identification (Conrow et al. 1990).

Two drift nets were sampled concurrently: one set at the water's surface, and the other set at the stream bottom. Nets were secured to the substrate with two rebar stakes in the Río Cañas mouth, and suspended from a bridge at the Río Guanajibo mouth. Flow measurements were taken at the beginning and end of each 3-h period (Robinson et al. 1998) using a Geopacks handheld digital flowmeter (Geopacks, Hatherleigh, Devon, UK). A flowmeter was also positioned in the center of the net mouth. Flowmeter data were used to calculate the water

volume (m³) sampled by drift nets during each 3-h period. Following collection, larval fishes were rinsed into a collection cup at the narrow end of the net. The contents of the drift nets during each 3-h period were preserved separately in a 5% solution of formalin (Conrow et al. 1990). Larval identification was based on larval descriptions from regional taxonomic guides and using reference specimens produced at the Maricao Fish Hatchery in Puerto Rico.

The influences of stream, sampling method, depth, time of day (eight 3-h periods during a day), week of summer (ordinal date), tide stage, and moon phase on total larval catch (all fish larvae collected during a 3-h period) and taxonomic group catch (all larvae from a given family collected during a 3-h period) were examined. Catch data were rank transformed due to nonnormality (Conover and Iman 1976). Catch data were first examined using mixed-effects repeated-measure design analysis of variances (ANOVAs; PROC MIXED; SAS 2004). Stream, sampling method, depth, and week of summer were fixed effects. Catch data were considered to have been measured repeatedly each time of day within each week of summer (i.e., week of summer was the subject). We used only data from 3-h periods between 1800 and 0600 hours in these analyses because we were comparing drift nets with light traps and light trap data were only collected at night. Catch data were then examined using three-factor mixed-effects design ANOVAs (PROC MIXED; SAS 2004), where time of day was the fixed effect and tide stage and moon phase were the random effects. We used only data from drift nets in these analyses. Drift net data were collected throughout entire 24-h periods, so those data encompassed a full tidal cycle. Additionally, the influences of the explanatory variables on bigmouth sleeper standard length (SL) were examined using the same two statistical approaches described above. Tukey's honestly significantly different tests were conducted post hoc for pairwise comparisons of means that were significantly different. An $\alpha = 0.05$ level of significance was used for these and all subsequent statistical tests.

Of the Eleotridae collected, only one individual was identified as a species other than bigmouth sleeper. Accordingly, analyses were conducted at the species level rather than the family level, which also allowed analysis of the influence of potential explanatory variables on mean length of bigmouth sleeper. Catch data were related to diel period (day or night) and water volume sampled. Light traps were only used at night, so analyses of the influence of diel period on catches and bigmouth sleeper mean standard length were conducted with drift net data alone. Day and night averages for total larval catch and taxonomic group catch were compared with standard t-tests. The relationships between water volume sampled (m³) in drift nets and catches were examined using a general linear model with a quadratic term.

Catch data were reclassified to reflect presence or absence of each taxon in samples. The influences of the same explanatory variables described above on presence or absence data for total larval catch and taxonomic group catch were analyzed using a logistic regression, assuming a binomial distribution, and using a logit link function (PROC logistic; SAS 2004).

RESULTS

A total of 2,156 fish larvae and juveniles were captured during 954 h of sampling. Unidentified juvenile fishes made up 6.5% of individuals caught, and less than 1.0% of larvae collected were damaged. Yolk sac larvae accounted for 14.5% of the catch and were not identified. The significant explanatory variables for total larval catch were stream, sampling method, week of summer, time of day, and moon phase (Table 1). The Río Cañas had higher total larval catch than the Río Guanajibo (1,780 versus 376 fish larvae). Light traps (54.6% of larvae; $n = 1,177$) caught more larvae than drift nets (45.4%; $n = 979$). Total larval catch in drift nets was highest at night ($t = 5.26$, df = 206, $P < 0.001$), peak in catch occurring just before dawn (Figure 1). Highest total larval catches in light traps were realized between

TABLE 1. Mixed-effects, repeated-measures ANOVA results for a comparison of larval sampling methods. The P-values for significant variables are given in bold italics.

| Explanatory variable	Total larval catch			Gobiidae catch			Syngnathidae catch			Bigmouth sleeper catch			Bigmouth sleeper SL		
	F-value	df	P-value	F-value	df	P-value	F-value	df	P-value	F-value	df	P-value	F-value	df	P-value
Stream	8.56	1	*0.0039*	13.98	1	*0.0002*	3.50	1	0.0630	0.05	1	0.8268	12.72	1	*0.0004*
Gear	6.93	1	*0.0092*	25.91	1	*<0.0001*	19.49	1	*<0.0001*	13.25	1	*0.0004*	117.80	1	*<0.0001*
Depth	2.12	1	0.1473	0.00	1	0.9547	2.54	1	0.1126	0.09	1	0.7655	14.73	1	*0.0002*
Week of summer	9.48	1	*0.0024*	7.50	1	*0.0068*	13.83	1	*0.0003*	0.62	1	0.4326	20.52	1	*<0.0001*
Time of day	5.51	7	*<0.0001*	2.52	7	*0.0169*	3.89	7	*0.0006*	1.05	7	0.3980	7.18	5	*<0.0001*
Tide stage	0.13	3	0.9439	1.11	3	0.3459	0.42	3	0.7404	1.11	3	0.3457	6.84	3	*0.0002*
Moon phase	3.83	3	*0.0108*	1.55	3	0.2032	5.51	3	*0.0012*	1.35	3	0.2580	12.45	3	*<0.0001*
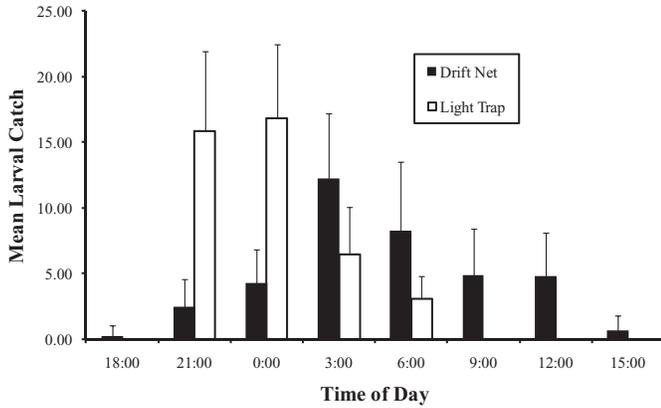
FIGURE 1. Mean larval catch per 3-h period in drift nets (black bars) and light traps (white bars) over the diel period. Light traps were fished only from 1800 to 0600 hours; error bars = SE.

FIGURE 3. Mean larval catch per 3-h hour period in drift nets and light traps among moon phases; error bars = SE.

1800 hours and midnight, and catch declined throughout the remainder of the night. Larvae were collected throughout the study in both drift nets and light traps, a clear peak occurring in late June to early July (Figure 2). Greater total larval catches of both gear types pooled occurred during the new quarter moon; however, sampling gears displayed opposite individual patterns (Figure 3).

There was no relationship between total larval catch in drift nets and water volume sampled. Water velocity readings from the Río Cañas ranged from –34.70 cm/s to 144.00 cm/s (average [SD] = 13.70 [22.30] cm/s). The negative values represent an incoming tide (i.e., upstream flow). At the Río Guanajibo, water velocity ranged from 5.40 to 61.00 cm/s (average [SD] = 18.50 [14.40] cm/s). Overall, drift nets sampled 55,077 m^3 of water, or 86.6 (71.0) m^3 of water per hour.

Predictors of larval presence or absence included stream, time of day, week of summer, and moon phase (Table 2). Larvae were more likely to be collected in Río Cañas samples and more likely to be collected during mid to late summer and at night. Moon phase influenced the presence of larvae, larvae being most
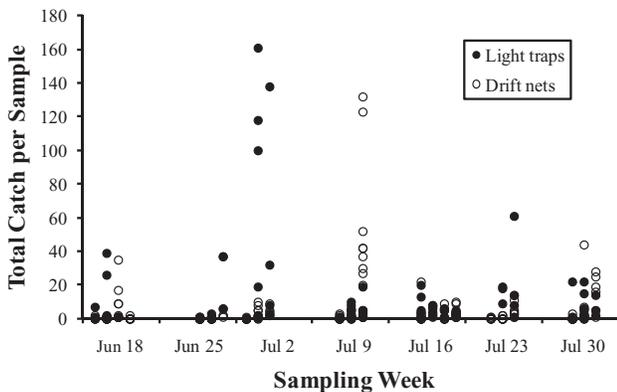
commonly present in samples during the first and last quarter moons.

Eleven families were identified during this study (Figure 4). The families Gobiidae ($n = 948$), Eleotridae ($n = 391$), and Syngnathidae ($n = 276$) comprised 94.9% of the identifiable larvae collected. Seven other families were collected but consisted of few individuals. These families were Achiridae ($n = 4$), Anguillidae ($n = 17$), Carangidae ($n = 4$), Clupeidae ($n = 1$), Elopidae ($n = 13$), Haemulidae ($n = 1$), Lutjanidae ($n = 13$), and Mugilidae ($n = 19$). Due to the low number of individuals collected from these families, they were not included in further analyses.

Variables that explained both Gobiidae and Syngnathidae catch rates were sampling method, week of summer, and time of day (Table 1). Gobiid larvae were more abundant in light traps, but syngnathid larvae were more abundant in drift nets

FIGURE 2. Total larval catch per 3-h period in light traps and drift nets throughout study (18 June 2007–1 August 2007).
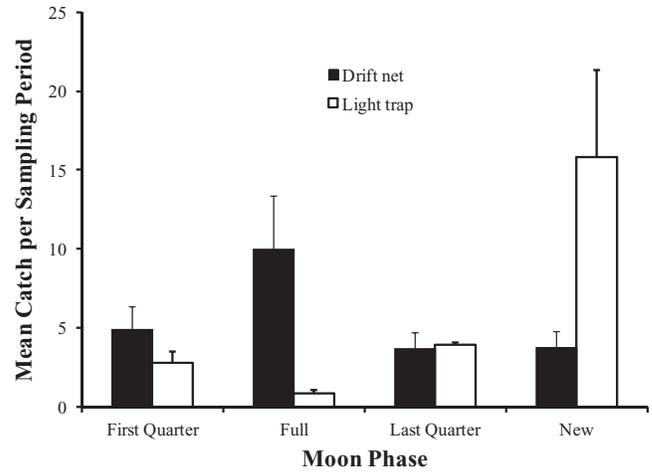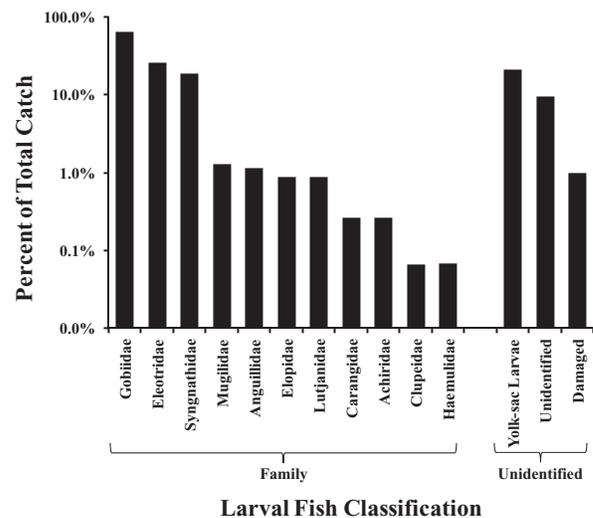

FIGURE 4. Percent total catch by family. Yolk sac larvae are presented separately due to difficulty of identification.

TABLE 2. Logistic regression model results for a comparison of larval sampling methods. Data were presence or absence; the *P*-values for significant variables are given in bold italics.

Explanatory variable	All larvae			Gobiidae			Syngnathidae			Bigmouth sleeper		
	χ^2	df	*P*-value	χ^2	df	*P*-value	χ^2	df	*P*-value	χ^2	df	*P*-value
Stream	11.20	1	***<0.001***	12.64	1	***<0.001***	17.59	1	***<0.001***	3.93	1	***<0.0010***
Gear	2.48	1	0.1152	2.31	1	0.1277	22.89	1	***<0.001***	9.49	1	***<0.0010***
Depth	3.52	1	0.0604	3.36	1	0.0666	1.81	1	0.178	0.00	1	0.9521
Week of summer	11.77	1	***<0.001***	11.45	1	***<0.001***	21.33	1	***<0.001***	0.01	1	0.9135
Time of day	27.63	7	***<0.001***	26.82	7	***<0.001***	21.10	7	***<0.001***	2.08	7	0.9552
Tide stage	0.95	3	0.8122	0.82	3	0.8441	2.58	3	0.4598	7.17	3	0.0666
Moon phase	17.54	3	***<0.001***	19.69	3	***<0.001***	27.23	3	***<0.001***	3.45	3	0.3260

(Figure 5). Larvae of these two families were collected in greater numbers as the week of summer progressed (Figure 6). Both groups were most likely to be collected at night (Gobiidae: 2100–0600 hours; Syngnathidae: 0000–0600 hours). Gobiidae had higher catch rates in Río Cañas. Syngnathidae had greater catches during the last quarter moon. Predictors of presence or absence for both Gobiidae and Syngnathidae (Table 2) were generally the same as the predictors of catch rates.

The only variable that explained bigmouth sleeper catch was sampling method, significantly more bigmouth sleeper being collected in light traps (*n* = 225) than in drift nets (*n* = 165; Table 1). When presence or absence data were analyzed (Table 2), bigmouth sleeper presence was affected by stream (presence was more likely in Río Cañas) and sampling method (presence was more likely in light traps). All seven variables influenced bigmouth sleeper SL. Larger bigmouth sleeper were collected in Río Cañas, in light traps, and at the surface. Larger bigmouth sleeper were caught later in the year, and at dawn and dusk when compared with the rest of the day. Larger bigmouth sleeper were collected during flood tides and during first quarter moons.

DISCUSSION

Light traps appear to be the most effective larval fish sampling method for tropical streams in Puerto Rico, although issues with size and species selectivity warrants a multiple-gear approach. The greatest catches of Gobiidae and Eleotridae were collected using light traps, and this gear captured more fish larvae overall. Attracting larval fish with light is a well-known sampling method (Lucas and Baras 2001), and the findings from this study are consistent with similar studies. For example, bigmouth sleeper larvae were successfully collected in the marine environment with night lights off the coast of Panama (Victor 2007), and another eleotrid, *Micropercops cinctus* (no English common name), was found to display positive phototaxis in laboratory trials (Makeeva 2002). The success of light traps in this study contrasts with findings of Hickford and Schiel (1999), which compared light traps with active sampling using a plankton net in inshore temperate waters and determined that plankton nets captured twice as many taxa from twice as many families as light traps. In the current study, greater catches of Syngnathidae were collected using drift nets, so light traps would not be the most efficient sampling method for members of this family.
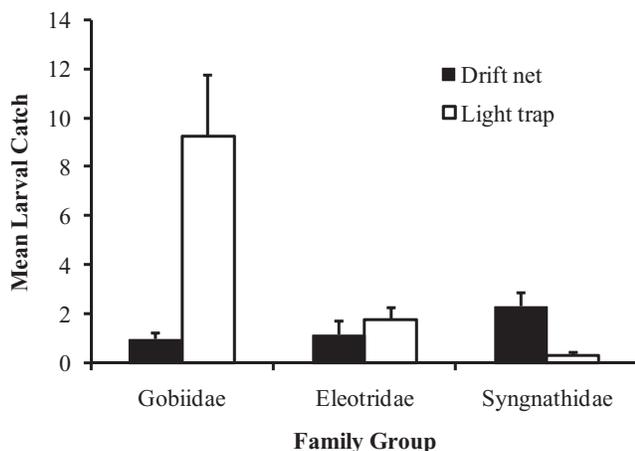

FIGURE 5. Mean larval catch per 3-h hour period in drift nets and light traps for the top three families caught; error bars = SE.
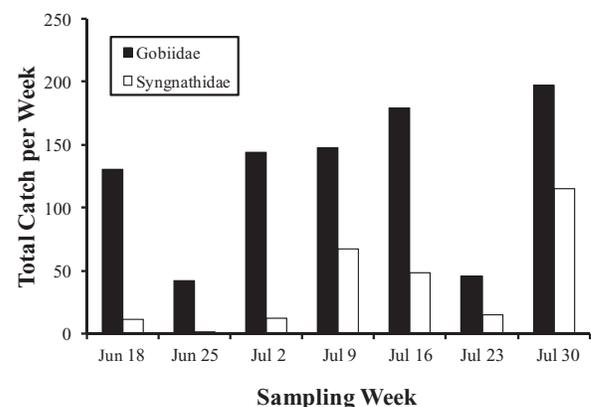

FIGURE 6. Weekly total larval catches from the families Gobiidae and Syngnathidae.

The propensity for smaller larvae, particularly yolk sac larvae, to be collected in drift nets instead of light traps might be a function of mobility. For example, bigmouth sleeper yolk sac larvae (about 2 mm) have limited fin development and are certainly less mobile than larger (>8-mm) bigmouth sleeper larvae, which have a full complement of fins and fin rays (Adelsberger 2009). Thus, larger larvae should be more capable of locomotion towards light traps, while smaller larvae are at the mercy of the current. Consequently, gear selection for future larval fish sampling in Puerto Rico streams should consider species and life stage targeted. For example, the dichotomous pattern in larval catch between gears during differing moon phases (Figure 3) suggests that larval behavior is critical to catch rate. It is likely that drift nets, which experienced peak catch during full moon periods, captured larvae as they were passively transported downstream. Light traps, in turn, may have captured more mobile and phototactic fish, possibly as they returned to the stream during new moon periods. Although it can be argued that light traps may have been less effective on brighter, full moon nights (Gehrke 1994), the size difference observed between larvae caught in each gear supports the idea that ontogenetic changes in behavior affects gear success.

The unpredictability of stream flow may have hampered the efficacy of drift nets, as flow rates were highly variable and negative flow was experienced at times of low discharge and incoming tides. Other complications of using drift nets in Puerto Rico streams included the propensity for gear loss during periods of high flow and the tendency for clogging when debris was abundant. Without constant vigilance, drift nets can be lost easily or rendered ineffective. In fact, two nets were lost during a flash flood event during this study, even with constant vigilance by the researchers. Light traps can be positioned outside of the primary flow and are less likely to be lost or inundated with debris. However, this may reduce their efficacy if larvae are utilizing stream currents for transport.

Total larval catch of drift nets was greatest during night samples. This is consistent with the basic understanding of stream drift as a nocturnal event and can be attributed to predator avoidance (Allan 1995). Since light traps are only effective at night and drift nets are more effective at night, larval fish sampling in tropical streams should be conducted between dusk and dawn. Obviously, factors including safety of researchers, security of gear, and availability of personnel would need to be considered on a case-by-case basis when determining a sampling protocol.

Gobiidae composed the majority of larvae collected during this study, which is consistent with larval fish community composition in many tropical waters. Gobiidae larvae tend to be a dominant component of community composition in estuarine and marine areas worldwide (Blaber 2000), including Puerto Rico (Neal et al. 2009). The lack of species other than bigmouth sleeper in the catch of Eleotridae was somewhat surprising given the occurrence of fat sleeper *Dormitator maculatus* and smallscaled spinycheek sleeper *Eleotris perniger* in Puerto

Rico (Neal et al. 2009). The smallscaled spinycheek sleeper has been collected in abundance in these two streams (Kwak et al. 2007). Likewise, low catches of Mugilidae were surprising given their abundance in these systems. Mountain mullet are especially abundant throughout Puerto Rico streams; total density estimates for mountain mullet of more than 155,000 fish/ha compare with estimates of about 38,100 fish/ha for Eleotridae and 134,000 fish/ha for Gobiidae (Kwak et al. 2007). The lack of larval mullet and other Eleotridae species may indicate that the sampling period did not include peak spawning times for these species or that these species are traveling outside of the stream mouth to spawn.

Conservation and management of stream fishes requires a complete understanding of their natural life histories. Information on the larval stages of several of these species is incomplete or conflicting. Larval fish sampling will be required to fill these data gaps. This study provides information that allows researchers to choose an appropriate sampling method. This study also shows the nature of environmental influences on larval fish catch rates in Puerto Rico streams. More broadly, the techniques assessed in the present study should be effective for sampling streams in other tropical environments which contain similar species assemblages.

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