

# Freshwater Sport Fish Management and Enhancement

SFR Project F-53R

Final Report

October 2009-December 2013



J. Wesley Neal, Robert Kröger, Craig G. Lilyestrom, Milton Muñoz, Marie Prchalová, Dijar Lutz-Carrillo,  
Nick R. Peterson, M. Clint Lloyd, Karina Olivieri-Velázquez, Cynthia N. Fox, and Jason M. Bies

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*The data presented in this final report represent all research activities during a four-year collaborative research project in Puerto Rico. The report is divided by study, with individual chapters per study presented in manuscript form. Each study and chapters are listed below with the starting page number.*

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## SUMMARY OF KEY FINDINGS AND RECOMMENDATIONS

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### ***Study 1 – Reservoir stock enhancement with largemouth bass:***

1. No benefits of producing and stocking triploid largemouth bass have been observed to date. Therefore, it is recommended that this line of research be discontinued and no further introductions conducted. An alternative direction for research would be use of all female populations via gynogenesis to increase maximum size potential.
2. No pure Florida largemouth bass populations remain in Puerto Rico natural systems. Analyses of subspecies composition via 6 diagnostic microsatellite loci found that all 12 populations evaluated in Puerto Rico, as well as the Maricao Hatchery, were composed of intergrade (Florida x northern) largemouth bass. All populations showed a preponderance of Florida largemouth bass alleles (range: 0.77 to 0.95; mean: 0.85, SD: 0.07), Florida subspecific genotypes were variable (range: 0.03 to 0.64; mean: 0.29, SD: 0.22) among reservoirs, and no pure northern subspecific genotypes were detected.
3. Analysis of subspecies composition found differences in size distributions between Florida largemouth bass and intergrades among reservoir populations in Puerto Rico. Florida largemouth bass displayed a more dispersed size distribution and more fish attaining larger sizes than hybrids; however, intra-population differences were observed among reservoirs.
4. Genetic analyses of largemouth bass populations in Puerto Rico using 11 polymorphic microsatellite DNA loci found reduced levels of genetic diversity (i.e., number of alleles per locus and observed heterozygosity) compared to populations in the USA, and fixation of allele *Mdo6* in Cerrillos Reservoir and the Maricao Hatchery broodstock. Likely causes include founder events (i.e., introductions using too few individuals) and subsequent inbreeding, hatchery stocking procedures, genetic drift (i.e., random genetic change), or combinations of these factors.
5. Analyses of subspecies composition and genetic variation indicate that genetics could be limiting growth and survival of largemouth bass in Puerto Rico. Further importation of fish from Florida and comparison of growth with Puerto Rico largemouth bass is recommended. Further, more sophisticated genetic analyses would help to determine the significance of these limitations.
6. We recommend that genetic samples be periodically collected and archived for future testing to evaluate continuing changes in subspecific alleles, genetic diversity metrics, population dynamics (e.g., growth and longevity), and combined responses among largemouth bass populations in Puerto Rico to importations and continued stocking and natural selection.

### ***Study 2 – Evaluation of bigmouth sleeper sport fish potential in reservoirs:***

1. The optimum rearing temperature and salinity for newly hatched bigmouth sleeper larvae is 26°C at 0 ppt. However, ontogenetic changes may require progressive changes in culture salinities as larvae develop. Additional research along these lines is necessary.
2. There appears to be differences in preferred salinity for larval rearing between bigmouth sleepers collected from free-flowing island rivers and landlocked bigmouth sleepers from Carite Reservoir. If this observed difference is genetic, it may be evidence that natural selection is driving this landlocked populations towards becoming the first true freshwater fish species in Puerto Rico.
3. Choice of young-of-year (YOY) sampling gear depends on the objectives and target species of the research. Light traps were the preferred gear for general YOY studies, catching a greater

evenness of YOY fish community than push nets in offshore habitats. Push nets collected greater numbers and a larger size range of threadfin shad, and are the preferred gear for sampling YOY of this species.

4. Littoral and limnetic light traps sampled the YOY fish community effectively, collecting 10 and 8 YOY fish species, respectively. Redbreast tilapia, largemouth bass, threadfin shad and sailfin armored catfish composed 85% of the relative species abundance in littoral light traps, whereas channel catfish and threadfin shad composed 90% of the relative species abundance in limnetic light traps.
5. Determination of time of year and location to sample YOY fish communities should be based on the species of YOY fish in which the manager is interested. Centrarchid and cichlidae species would be best sampled during spring and early summer months with littoral light traps. Ictalurid sampling should occur in limnetic habitats and could effectively be sampled in the spring or fall months.

### **Study 3 – Evaluation of management regulations for Cerrillos Reservoir:**

1. Population estimates from 2010-2012 indicate that largemouth bass overcrowding continued to result in slow growth, particularly in the 200-380 mm size range. Failure of the protected slot limit resulted from lack of harvest, and that regulation has been replaced with a 10 fish creel limit and no minimum size requirement.
2. In an attempt to experimentally restructure the size distribution of the bass population, 20% of the total largemouth bass biomass (793 kg; 2,333 fish) in the crowded size range was removed using electrofishing in spring 2012, and 22% (618 kg, 1,993 fish) in the crowded size range was removed using electrofishing in spring 2013. The population responded to the management action with significant increases in total length and condition. Further, because size distributions were restructured, the reservoir supported a greater biomass and abundance in 2014 than prior to the removals.
3. Stock size largemouth bass grow slowly at an average rate of 0.18 mm/d. Growth rates are more rapid as juveniles, and slow as fish reach crowded size classes. Data collected in March 2014 indicate that growth rate in length has not yet increased following the removal efforts, but growth in weight and condition have both increased. This suggests that fish must improve body condition before allocating energy towards increased growth in length.
4. Based on the results of the experimental removal, we recommend that annual exploitation ( $\mu$ ), which currently is about 9%, be increased to 32% to maintain the improvements in population size structure and condition. Education of anglers and mandatory harvest during tournaments could be used to increase fishing harvest, and management removals can be used to make up the difference to reach this level of exploitation. If the current level of exploitation is maintained in Cerrillos Reservoir, we expect the population to revert back to a crowded, slow growing, poor condition state.
5. Year-round diet analysis confirmed that threadfin shad are the primary forage base for largemouth bass in Cerrillos Reservoir. Additionally, tilapia and sunfish species make up the other major portions of largemouth bass diets, specifically when shad densities decrease in the late summer and fall.
6. Acoustic telemetry showed that largemouth bass in Cerrillos Reservoir select for softer clay and sand banks where vegetation is able to grow during extended periods of low water. However, this cover is temporary. The addition of artificial structure could provide permanent dense cover to areas devoid of habitat and near fishing piers, enhancing angling opportunities.

7. Largemouth bass removed from Cerrillos Reservoir (N = 2,148) were marked and relocated to Guajataca Reservoir in April 2012. Only 69 mortalities (3.2%) were recorded during the 2012 transfer. In 2013, removed fish were transferred to both Guajataca and La Plata Reservoir. Recent sampling efforts in Guajataca Reservoir collected 30 largemouth bass and found that 27% were recent transfers from Cerrillos Reservoir, suggesting that the transfer was successful.

#### **Study 4 – Alternative sport fish species in Puerto Rico:**

1. Analysis of broodstock management practices at the Maricao Fish Hatchery has revealed several areas for potential improvement. The four most critical recommendations are 1) improve broodstock admission procedures to include use of passive integrated transponder (*PIT*) tags to improve record keeping, 2) use a commercially available feed with 35-40% protein content to ensure that the dietary requirements of broodfish are met, 3) improve feed storage conditions and reduce maximum storage time to improve nutritional content, and 4) maintain or improve genetic diversity of broodstock by importing unrelated individuals from within the native range of each species.
2. Artificial propagation of peacock bass is easily conducted by establishing broodstock in spawning ponds with live prey and vertical spawning substrates. Floor tiling works well, and can be easily removed and transported to the hatchery aquaria. Future propagation of this and potentially other *Cichla* species should use this passive approach.
3. Attempts to actively spawn channel catfish in controlled recirculation systems have not been successful. This species is reproducing successfully in many reservoirs, suggesting that passive spawning may be the simplest form of propagation.

#### **Study 5 – Threadfin shad prey production in tropical reservoirs:**

1. Trawls are the superior gear for sampling threadfin shad in Puerto Rico, as frame trawling collected nearly 9 times more threadfin shad than comparable fishing effort with gillnets, and was much less cumbersome to clean and was much more durable. By-catch was 26 times lower in the trawl versus gillnets, and predation on ensnared shad in gillnets may have been substantial. Trawls provided a much more accurate representation of threadfin shad size distributions than gillnets for 99% of the population, and appeared to underestimate only the top 1% of the length distribution. Variability in trawl catch was much less than in gillnet catch, suggesting that less trawling effort would be required to collect precise samples. Therefore, we recommend that all future study of threadfin shad in Puerto Rico use trawl sampling.
2. Spatial variability should be considered when designing a sampling protocol for threadfin shad. Clear lateral, longitudinal, and vertical differences were observed in this study, suggesting that a robust sampling design is needed to account for spatial variability in these reservoirs. Other studies have suggested and this study supports that threadfin shad are primarily concentrated in offshore habitats and that inshore sampling is not warranted. The low catch rates observed in the 6-9 m stratum are indicative of few threadfin shad at greater depths; therefore, it is suggested that sampling designs include at least the 0-3 and 3-6 m strata.
3. Sampling across seasons is required for threadfin shad, as populations varied markedly between seasons in these tropical reservoirs. Density estimates changed as much as 46-fold and biomass estimates changed up to 40-fold within a 3-month sampling interval within individual reservoirs. Greatest densities were collected in winter and spring, with much lower densities in summer and fall. Sampling season should be determined based on sampling objectives. For basic monitoring



studies, annual winter or spring sampling would provide information on threadfin shad recruitment as well as overall maximum density, whereas summer or fall sampling would be useful for determining prey limitations. For comprehensive studies of production, biomass, or reproduction, sampling should be conducted at least quarterly in order to account for rapid changes in threadfin shad density and size distributions throughout the year.

4. Three to five replicates are appropriate – This study used three trawl tows per depth stratum in each reservoir section, providing an overall sample size of 18 (27 for Dos Bocas Reservoir) and a mean CV across individual tows of 0.59. While this value was slightly greater than the target of  $CV \leq 0.5$ , it was sufficient to detect differences between seasons, sections, and depths given the magnitudes of the observed differences. However, when detecting changes on a finer scale, these sample sizes may not be sufficient. Using standard sample size calculation based on a power of 0.80, it appears that the 18 tows used in this study were capable of detecting changes in threadfin shad density of 45% or greater between sampling periods. If only sampling depth strata 0-6 m, the reduction in variability should allow greater precision.
5. Not all reservoirs are the same – Mean threadfin shad density varied as much as 30-fold between the most and least productive reservoirs. Lucchetti Reservoir displayed the greatest annual mean biomass, but this value was heavily influenced by an unusually large winter estimate ( $72.4 \pm 9.5$  kg/ha). Dos Bocas Reservoir exhibited the greatest biomass in 3 out of 4 seasons. Carite Reservoir consistently displayed the least threadfin shad biomass across seasons with an annual mean of  $1.6 \pm 1.0$  kg/ha. This variability in biomass and density across reservoirs was likely due to differences in primary productivity between these systems.
6. Comparison to data from temperate reservoirs suggested that Puerto Rico reservoirs may have threadfin shad populations that are generally less dense with less biomass, calling into question the previous assumption that prey limitation is not an issue in these tropical systems.

#### **Study 6 – Response of Toa Vaca Reservoir to destratification:**

1. Primary fish species in Toa Vaca Reservoir did not exhibit spatially explicit distributions on a macro-habitat scale during pre-destratification sampling, and catch rates of sport fish and locally targeted food fish were high.
2. Water quality throughout the reservoir was good during the pre-destratification sampling and should not hinder fish populations. However, poor water quality events may be episodic and our data do not describe seasonal changes in water quality or fish assemblages. Therefore, water quality improvement or fish management suggestions are not warranted at this time.
3. Continued assessment of fish assemblages is recommended to provide information on how destratification systems effect tropical fish populations.
4. Increased water quality sampling (quarterly or monthly) is suggested to provide information about how the reservoir changes through time. Such data could increase management efficiency by indicating when the oxygen recovery system is providing the greatest potential habitat.

## **Study 1:**

### **Reservoir stock enhancement with largemouth bass**



# Chapter 1: Comparison of Diploid and Triploid Largemouth Bass Growth and Maturation in Puerto Rico

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**Abstract:** Triploid largemouth bass may have potential in sport fish management and in food fish production as a means to eliminate reproduction and potentially increase somatic growth. To examine this potential, four cohorts of diploid and triploid largemouth bass were produced, tagged with binary coded wire tags, stocked into Lucchetti Reservoir, Puerto Rico, and recaptured during subsequent sampling events. The four stocking events occurred over a ten-year period, and marked fish were sampled until no recaptures occurred. Growth rates, condition (relative weight,  $W_r$ ), and reproductive investment (gonadosomatic index, GSI) were compared for diploid and triploid fish. Overall mean daily growth rate (MDG) did not differ ( $P \geq 0.050$ ) between diploids (MDG  $\pm SE = 0.75 \pm 0.02$  mm/d) and triploids (MDG  $\pm SE = 0.74 \pm 0.03$ ), but was slightly greater for diploids age-1 and older than triploids of same age (MDG  $\pm SE = 0.71 \pm 0.02$  mm/d versus  $0.67 \pm 0.03$ , respectively;  $P = 0.002$ ). Von Bertalanffy growth parameters were similar between ploidy groups (Diploid:  $L_\infty = 387.3$ ,  $K = 1.23$ ,  $t_0 = -0.24$ ; Triploid:  $L_\infty = 390.3$ ,  $K = 1.20$ ,  $t_0 = -0.32$ ). Diploid largemouth bass exhibited advanced reproductive development following maturation at age 1, while triploids did not. Mean GSI was higher for diploid (GSI = 1.98,  $SE = 0.81$ ) than triploid (GSI = 0.29,  $SE = 0.05$ ) females, although male mean GSI did not significantly differ. Relative weight was consistently greater for diploid largemouth bass ( $P < 0.008$ ), likely due to differences in reproductive investment. The lack of significant growth advantage in tropical environments precludes using triploid largemouth bass to enhance trophy bass potential in Puerto Rico reservoirs. However, triploid largemouth bass may have utility in systems where largemouth bass reproduction is unwanted.

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The concept of using sterile or same-sex fish to eliminate reproduction and to increase somatic growth is a growing research area (e.g., Wolters et al. 1982; Parson and Meals 1997; Neal and Noble 2008). One method to produce sterile fish is to manipulate chromosome number (Stanley 1979; Gervai et al. 1980; Thorgaard et al. 1981; Wolters et al. 1982). Polyploidy can be readily induced in many fish species by shocking eggs early in development with sharp temperature changes, increases in hydrostatic pressure, or chemical treatments (Thorgaard and Allen 1987; Ihssen et al. 1990). For triploidy production, the shock must be administered shortly after fertilization. This treatment causes the egg to retain the second polar body that is normally shed, increasing the number of chromatids to three, and presumably resulting in sterilization of the offspring (Thorgaard 1983).

Sterilization of fish has several possible applications, including increased growth potential (Wolters et al. 1982), creation of non-reproductive populations (Parsons and Meals 1997), and interference

with reproducing populations for control applications (Parsons 1993). Particularly applicable to fish management and husbandry is the potential for faster growth, which theoretically results from the reduction in reproductive investment often observed with triploid individuals (e.g., Wolters et al. 1982; Parsons and Meals 1997). When reproductive development is reduced or foregone, energy ordinarily used for gonad development can be redirected to somatic growth, thereby increasing growth potential (Allen and Stanley 1978).

In Puerto Rico, growth of juvenile largemouth bass *Micropterus salmoides* is rapid, with fish usually reaching maturity by age 1 (Neal and Noble 2002). However, growth slows considerably after maturation, and is nearly negligible by age 2. Neal and Noble (2006) suggested that multiple spawning events per spawning season and extended reproductive period result in reduced growth rates of largemouth bass, and hypothesized that sterile triploid largemouth bass could be a solution. Pressure shock has been effective at

inducing triploidy in largemouth bass (Garrett et al. 1992; Neal et al. 2004), and this technique has been effectively applied in tropical systems (Neal et al. 2004).

Neal and Noble (2008) compared the first two years at large of a single stocking of diploid and triploid largemouth bass in Lucchetti Reservoir, Puerto Rico. That study did not find initial differences in growth between triploid and control largemouth bass, but postulated that more time post-maturation was needed. Also, sample sizes were low due to the inherent difficulties of producing triploid largemouth bass and, consequently, due to relatively low rates of recapture. In the current study, these limitations are overcome by combining data from Neal and Noble (2008) with three additional cohort stockings to compare growth, reproductive development, and condition of diploid and triploid largemouth bass in tropical reservoirs through age 3.

## Methods

*Study Reservoir* – Lucchetti Reservoir is a 108-ha impoundment in the mountain region of southwestern Puerto Rico. The area receives an average rainfall of 198 cm annually and was originally tropical forest, although much of the landscape is now used for agriculture. Lucchetti Reservoir has been categorized from mesotrophic to eutrophic on the basis of nutrients, physical limnology, chlorophyll *a*, and phytoplankton biomass data (Churchill et al. 1995). The maximum depth is 22.2 m (Neal et al. 1999). The primary function of the reservoir is water storage for irrigation, but the creation of the Lucchetti Field Station and associated facilities has improved recreational access and increased reservoir popularity among boating anglers (Churchill et al. 1995).

*Triploid Production* – Two cohorts of triploid largemouth bass were produced in 2000, and data from one cohort through age 1 were reported in Neal and Noble (2008). Two additional cohorts were produced in 2008 and 2009 using the following procedures. Broodstock used to produce experimental fish were collected from Lucchetti Reservoir using boom-mounted boat electrofishing at 7-8 A and 60 pps DC. Male and female fish were sorted based on external reproductive appearance (free-flowing gametes or extended abdomen with swollen, reddened urogenital pores), and only fish with free-flowing gametes or advanced gonadal development were transported to hatchery facilities in Maricao, Puerto Rico.

Fish with naturally free-flowing gametes were immediately spawned. Largemouth bass with advanced gonadal development but without free-flowing gametes were induced to release gametes using

hormone injections. Both males and females received Ovaprim® injections of 0.1 mL/kg initial and 0.5 mL/kg resolving dose (8 hours post-initial dose). Once gametes were free flowing, individual females were placed in an anesthetic bath of buffered MS-222 solution. A male largemouth bass was euthanized by overdose with MS-222 and the testes were removed and macerated, and about 10 mL of 0.3% NaCl irrigation solution was added to increase volume. Females were stripped into a mixing bowl with no water, and the macerated testes were mixed with water and poured through a fine mesh over the eggs to remove testicular tissue. Fertilization was considered instantaneous. Eggs were stirred for 1 minute with a feather before being divided into control (diploid) and treatment (triploid) groups.

Eggs from the treatment group were placed into a mesh basket and the basket was inserted into a water-filled pressure chamber. At 5 min post-fertilization, eggs were subjected to 563 kg/cm<sup>2</sup> (8,000 p.s.i.) for 1 minute (Garrett et al. 1992). Control eggs were not subjected to pressure shock. Eggs from both groups were placed on incubation mats within aerated 37.8-L hatching aquaria. Water temperatures were about 23°C during spawning and hatching.

Hatching began within 48 h, and swim-up and first feeding followed about 3 days post-hatching. Fry were fed live brine shrimp *Artemia gracilis* twice daily to satiation before being moved to a natural prey base in grow-out ponds. When juveniles were large enough (at least 40 mm total length), they were tagged with decimal coded wire tags that differentiate between treated and control fish. These fish were then transported to and released in Lucchetti Reservoir. This reservoir was chosen for this study because there is a historical database on largemouth bass population dynamics for this system. Four separate stocking events were conducted (Table 1). Ploidy of the two cohorts in 2000 was assayed using flow cytometry with methods previously described (Kerby and Harrell 1990; Neal and Noble 2008). Ploidy of the 2008 and 2009 cohorts was determined using the erythrocyte size technique described in Neal et al. (2004).

*Field Comparison of Ploidy Groups* – Following each stocking event, experimental largemouth bass were recaptured periodically beginning one month post-stocking and continuing for up to four years post-stocking for comparison of diploid and triploid largemouth bass. A boom-mounted electrofishing unit at 7-8 A and 60 pps DC was used to collect fish. Sampling was conducted in conjunction with other periodic sampling activities, so sampling times and target numbers varied.

Field verification of ploidy status of recaptured largemouth bass was performed using erythrocyte length (Neal et al. 2004). Blood samples were taken from all tagged bass using a large-bore syringe inserted into the blood sinuses located behind the gill arches. A small (< 0.5 cc) sample of blood was diluted using one drop of 0.5% NaCl solution, and analyzed within 6 hours of collection. All microtagged fish collected were euthanized, placed on ice, and returned to the laboratory for analysis. Total length, weight with stomach contents removed, and gonad weight (g) were recorded.

Periodic sampling of largemouth bass in Lucchetti Reservoir provided estimates of growth rate, condition ( $\geq 150$  mm TL), and reproductive development. Mean daily growth (MDG) rates of individual fish were determined by dividing the difference between mean size at stocking and individual size at recapture by the time at large. Relative weight ( $W_r$ ) was used as an index of condition, which is based on the log-linear intercept and slope parameters determined for largemouth bass by Wege and Anderson (1978). Relative weight compares the weight of an individual fish to the length-specific standard weight predicted by a weight-length regression constructed to represent the species (Anderson and Neumann 1996). Gonadosomatic index (GSI), which is the gonad weight expressed as a percentage of body weight (excluding stomach contents), was used as an index of reproductive development. Threshold limits for GSI of 0.2 for males and 2.0 for females were used to identify sexually mature individuals (Gran 1995). Largemouth bass with values exceeding these gender-specific limits were considered reproductively mature.

Comparison of mean daily growth rates of diploid and triploid largemouth bass across stockings, age class, and ploidy group was performed using a general linear model approach (PROC GLM, SAS Version 9.2). Von Bertalanffy growth equations were fit using

Fishery Analysis and Simulation Tools (FAST) Version 3.0 (Copyright Auburn University). For GSI comparison, a *t*-test was used after testing confirmed that normality assumptions were met. A general linear model (PROC GLM, SAS Version 9.2) was used to compare  $W_r$  for diploid and triploid groups, as ontogenetic changes in condition needed to be considered. All tests used an alpha level of 0.05. All statistics were calculated using SAS 9.2.

## Results

Diploid (N = 2,543) and triploid (N = 2,442) largemouth bass were successfully produced, tagged, and stocked into Lucchetti Reservoir during four separate stocking events (Table 1). Flow cytometry or erythrocyte length analysis confirmed the ploidy status of sub-samples of both diploid and triploid largemouth bass, with an overall triploid induction success of 96%. Due to natural growth variability in the fingerling production ponds, individual cohorts varied on average by 1.7 to 16.6 mm, although the average sizes during all stocking events combined were nearly identical (2.3 mm difference).

A total of 98 diploid and 47 triploid largemouth bass were recaptured. All recaptures were individually verified for ploidy and 100% correctly matched their initial stocking treatment (no diploid fish from the triploid treatment were recaptured). Most recaptures occurred during year 1 (age 0) and year 2 (age 1) following each stocking, and triploid largemouth bass began to disappear rapidly thereafter. Only 3 triploid largemouth bass were recaptured in year 3 post-stocking (age 2), compared to 18 diploid fish. By year 4 (age 3), no triploids were collected, and only two diploids were recaptured.

Both diploid and triploid largemouth bass grew rapidly and reached approximate size at maturity (~275 mm TL; Gran 1995) by age 1 in this study (Figure 1). Von Bertalanffy growth models were fit to treatment

TABLE 1. Stocking history of diploid and triploid largemouth bass in Lucchetti Reservoir, Puerto Rico. Stocking date, numbers stocked, mean total length, standard error, and percent triploid of the treatment group are presented.

| Stocking date | Diploid      |             |         | Triploid     |             |         |            |
|---------------|--------------|-------------|---------|--------------|-------------|---------|------------|
|               | N            | TL (mm)     | SE (mm) | N            | TL (mm)     | SE (mm) | % Trip (N) |
| May 2000      | 487          | 46.7        | 1.30    | 477          | 63.3        | 1.41    | 100% (23)  |
| June 2000     | 535          | 54.0        | 0.48    | 537          | 52.3        | 1.04    | 82% (50)   |
| June 2008     | 973          | 66.6        | 0.29    | 896          | 56.9        | 1.04    | 100% (100) |
| May 2009      | 548          | 70.7        | 0.39    | 532          | 74.5        | 1.43    | 100% (100) |
| <b>Total</b>  | <b>2,543</b> |             |         | <b>2,442</b> |             |         |            |
| <b>Mean</b>   |              | <b>59.5</b> |         |              | <b>61.8</b> |         | <b>96%</b> |

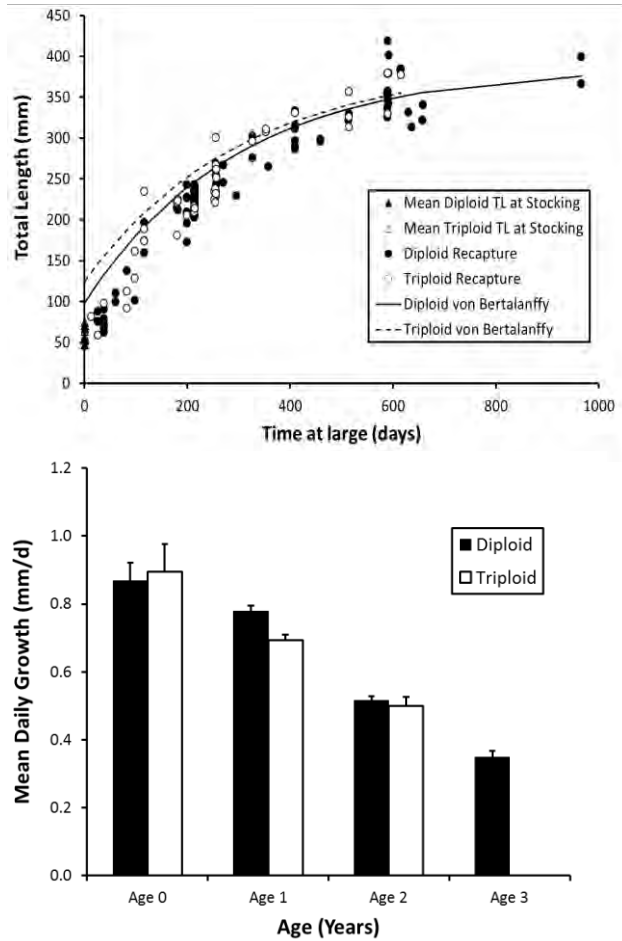


FIGURE 1. Top: Growth in total length of diploid and triploid largemouth bass stocked in Lucchetti Reservoir. Overall mean length at stocking for each group is displayed. Bottom: Mean daily growth rate by age for diploid and triploid largemouth bass calculated by dividing total length by total time at large. Error bars are one SE.

groups and parameter estimates, particularly  $L_{\infty}$ , were similar between groups (Diploid:  $L_{\infty} = 387.3$ ,  $K = 1.227$ ,  $t_0 = -0.238$ ); Triploid:  $L_{\infty} = 390.3$ ,  $K = 1.198$ ,  $t_0 = -0.32$ ). Mean daily growth rates ( $\pm SE$ ) for age 0 fish were  $0.87 \pm 0.05$  mm/d for diploids and  $0.89 \pm 0.08$  mm/d for triploids, and declined for older fish (Figure 2). The general linear model for mean daily growth did not detect growth differences between diploid and triploid largemouth bass overall ( $F_1 = 0.28$ ,  $P = 0.599$ ) or by age class ( $P = 0.074 - 0.951$ ).

Condition differed substantially between diploid and triploid largemouth bass ( $F_1 = 7.42$ ,  $P = 0.008$ ), with diploid largemouth bass having overall greater condition estimates. However, least square means procedures demonstrated that this difference was primarily driven by smaller size classes, with the only

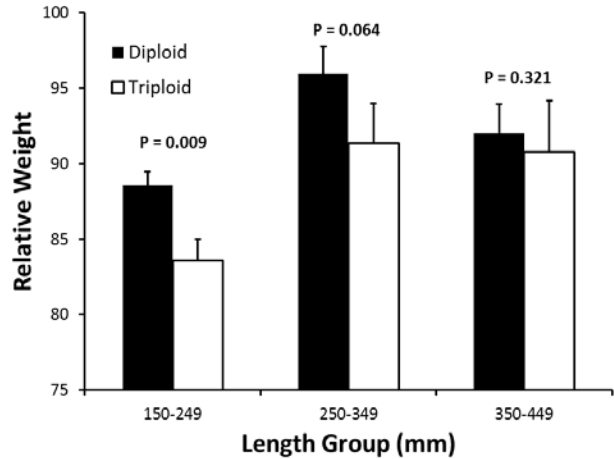


FIGURE 2. Mean body condition as measure by relative weight ( $W_r$ ) index of diploid and triploid largemouth bass stocked in Lucchetti Reservoir. Error bars are one SE, and P-values are comparisons between ploidy groups within size classes.

statistical difference between fish 150-249 mm TL (Figure 2). Condition of both ploidy groups of largemouth bass was greater at larger sizes.

Both ploidy groups reached approximate size at maturity (275 mm total length) during late-winter to early-spring (age 1) following stocking. Elevated gonadosomatic index (GSI) values indicative of maturity were observed in diploid fish beginning March at sizes as small as 228 mm for males and 265 mm for females. Female diploids had significantly higher GSI values than female triploids at age 1 ( $t_{21} = 2.52$ ,  $P = 0.010$ ; Figure 3), and no triploid females displayed maturing ovaries. Similar differences were observed between males of each ploidy group, with diploid males having greater GSI values ( $t_{43} = 2.76$ ,  $P = 0.004$ ), and several triploid males displayed GSI values marginally indicative of maturation. The highest GSI values for females and males were observed in diploid individuals.

## Discussion

The hypothesis that sterile triploid largemouth bass would experience a growth advantage due to lack of reproductive investment was not supported in this study. No differences in growth rate or maximum size were observed, despite reduced energy allocation to gonadal development by triploid largemouth bass. Reduced reproductive investment and increased growth rates have been demonstrated for some fish species, but the results are conflicting. For example, triploid and diploid channel catfish *Ictalurus punctatus* reared indoors differed in both GSI and weight by 8 months of age, with triploids being significantly less sexually developed and heavier in weight (Wolters et al. 1982).

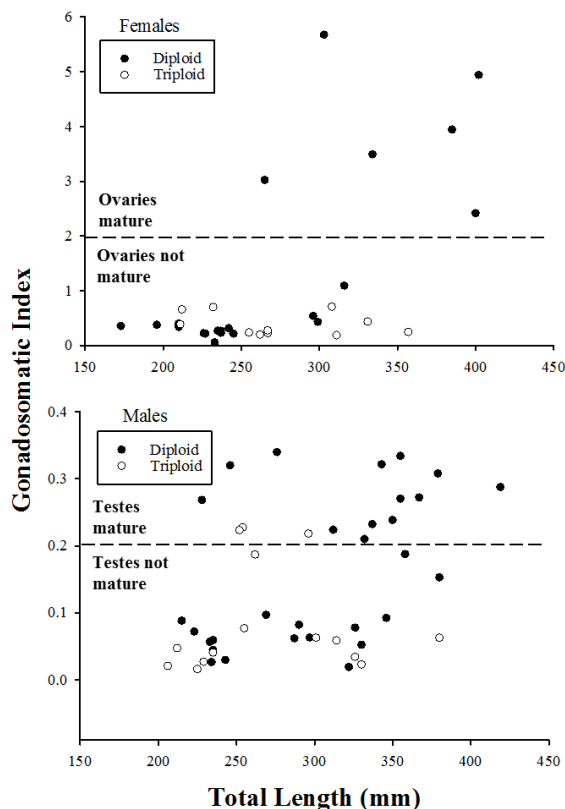


FIGURE 3. Reproductive development of diploid and triploid largemouth bass females (Top) and males (Bottom) as determined using the gonadosomatic index (GSI). Values of GSI exceeding 2.0 for females and 0.2 for males are considered mature (Gran 1995). Only fish collected during the spawning season (January-June) are presented.

Yet, when diploid and triploid channel catfish were raised outdoors at high densities, no differences in weight were detected, but significantly higher GSI values were observed for diploids (Wolters et al. 1991).

Diploid largemouth bass underwent greater reproductive development than triploid largemouth bass. Male triploids demonstrated some reproductive maturation, but gonadosomatic index values were reduced when compared to fully-developed diploid largemouth bass. These results are similar to studies with other species. For example, reduced reproductive development was reported for male triploid rainbow trout *Oncorhynchus mykiss* (Simon et al. 1993), suggesting that male triploids invest less in reproduction than normal diploids. Female largemouth bass triploids showed no apparent increase in ovarian size following maturity, while similar-size diploids often produced well-developed ovaries. These results are consistent with results for other fish species in which triploid females did not undergo sexual

maturation (e.g., Parsons 1993; Simon et al. 1993), and suggest that the triploid females do not invest significant energy into reproductive development.

Diploid largemouth bass were consistently more robust than triploid largemouth bass. This may be at least partially attributed to the differences in gonadal development, although the greatest differences were detected for fish 150-249 mm TL, which primarily included sub-adult fish. The difference in condition lessened as fish matured, and although mean diploid condition was somewhat larger than mean triploid condition at larger sizes, statistically there was not difference. However, because triploid fish were not expending energy on products of reproduction, they theoretically should have been converting more, not less, energy to biomass in the form of growth or fat storage.

Survival also appeared to be reduced for triploid largemouth bass. Despite similar stocking rates, less than half as many triploids as diploids were recaptured. It is possible that immediate post-stocking survival influenced recapture rates, as the two ploidy groups were often transported and stocked separately, and the first two cohorts were obtained from different facilities (triploids produced in earthen ponds in the warmer Lajas Valley; diploids produced in lined ponds in the much cooler Maricao area). However, handling and stocking conditions do not explain why most triploids disappeared by age 2 and none were recaptured at age 3, while diploids were collected.

Neal and Noble (2008) proposed that, although no differences were observed between ploidy groups through age 1, these differences might become apparent for older fish with greater reproductive opportunity. This was not the case, as growth rates converged to a nearly identical maximum size for both ploidy groups. These data imply that triploid largemouth bass do not have utility for trophy management in tropical reservoirs, where reduced longevity results in rapid mortality of stocked fish. It might be postulated that triploid largemouth bass may have greater potential in temperate systems, where longer lifespans provide more time for differences to be realized. However, this is unlikely because temperate largemouth bass generally spawn once or a few times during the spring (Heidinger 1975), and must contend with water temperature extremes that reduce growth during winter and summer (e.g., Rice et al. 1983). Conversely, largemouth bass in Puerto Rico spawn multiple times for up to six months (Gran 1995), and water temperatures are near optimal for growth year-round (Neal and Noble 2006). Therefore, the theoretical energetic advantage of foregoing reproduction should be greater in tropical systems.



Although this study concludes that triploid largemouth bass do not have efficacy for trophy management in tropical systems, they may be useful for creating non-reproductive populations where reproduction is not warranted. This is particularly true for systems with a history of limited largemouth bass harvest. Using triploid fish in this situation could allow the establishment of populations without the likelihood of overpopulation and stunting. Another application could be the creation of largemouth bass fisheries in areas where establishment of this species is not desired. However, ploidy verification of all fish stocked will be required to ensure that no reproductive fish are introduced, and this added cost may limit application of this management tool.

### Acknowledgements

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## Chapter 2: Subspecies Composition of Largemouth Bass in Puerto Rico Reservoirs

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**Abstract:** Largemouth bass *Micropterus salmoides* in Puerto Rico are the result of a few introductions from mainland North America over the last century. Initial introductions came from populations within the native range of the northern subspecies *M. s. salmoides*, while more recent introductions came from populations within the native range of the Florida subspecies *M. s. floridanus*. We determined the subspecies composition of largemouth bass populations among reservoir and hatchery populations in Puerto Rico and evaluated the relationship between subspecies composition and population metrics (length-frequency distributions, proportional size distribution [PSD], and mean total length) to provide recommendations for future broodstock collections for the Maricao Hatchery. Largemouth bass (n=418) were sampled from 12 reservoir populations and the contemporary hatchery stock on the island, and were genotyped at 6 diagnostic microsatellite loci. Among the reservoir populations, the proportion of Florida subspecific alleles ranged from 0.77 to 0.95 (mean 0.85, SD 0.07). Florida subspecific genotypes ranged from 0.03 to 0.64 (mean 0.29, SD 0.22) and no northern subspecific genotypes were recovered. All populations showed lower levels of Florida subspecific alleles and genotypes than the hatchery stock (alleles 0.98, genotypes 0.80), which could be a result of artificial selection of larger individuals during broodstock collections from Cerrillos Reservoir. Length-frequency distributions differed between Florida largemouth bass and intergrades (Florida x northern;  $P=0.026$ ), as populations frequently contained proportionally more large Florida largemouth bass. The prevalence of the Florida subspecies and hybrids in all populations on the island could be a derivative of genetic signatures from founding populations, represent the effects of natural selection, or be due to recent hatchery releases, although further research is needed to discriminate between these mechanisms. Since Florida largemouth bass displayed a more dispersed size distribution and more fish attaining larger sizes among populations, we recommend that future introductions should consist of only verified Florida largemouth bass.

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Largemouth bass have been separated into two subspecies (Bailey and Hubbs 1949), the northern largemouth bass (NLMB) *Micropterus salmoides salmoides* and the Florida largemouth bass (FLMB) *M. s. floridanus*. Each subspecies displays distinct yet overlapping native ranges with a large area of introgression (Philipp et al. 1983), and distinct physiological and morphological characteristics have been reported for the two subspecies (Hart 1952; Thrasher 1974; Parker et al. 1985; Philipp et al. 1985a; Williamson and Carmichael 1986; Carmichael et al. 1988). Many differences have been observed between subspecies regarding spawning periodicity (Isely et al. 1987; Rodgers et al. 2006), temperature tolerances

(Cichra et al. 1982; Guest 1982, 1985; Philipp et al. 1985b; Fields et al. 1987; Koppelman et al. 1988), survival (Wright and Wigtil 1982; Philipp and Whitt 1991; Neal and Noble 2002), and growth and catchability (Clugston 1964; Addison and Spencer 1971; Inman et al. 1976; Zolczynski and Davies 1976; Kleinsasser et al. 1990). The Florida subspecies has displayed greater growth potential than the northern subspecies at southern latitudes (Crawford et al. 2002; Lutz-Carrillo et al. 2006), which has resulted in vast dispersions of this subspecies by federal and state agencies over the last few decades. Supplemental stocking and introductions have successfully introduced FLMB into pre-existing populations of

NLMB throughout the United States including California (Bottroff 1967; Smith 1971; von Geldern and Mitchell 1975; Bottroff and Lembeck 1978; Pelzman 1980), Texas (Inman et al. 1976; Maceina et al. 1988), and Oklahoma (Reiger and Summerfelt 1978), as well as many other locations around the world (Corral 1936; Toots 1972; Heidinger 1976; Godinho and Ferreira 1993; Kitagawa et al. 2000; Kitagawa et al. 2005).

Stocking records indicate that largemouth bass *Micropterus salmoides* were introduced to Puerto Rico from the United States as early as 1915 (Johnson 1915; O'Malley 1916; Robbins and MacCrimmon 1974); however, establishment was considered unsuccessful (Table 1). A second introduction was attempted in 1946 when 88 adults and 1,067 juveniles were transported from Georgia and Mississippi to the Maricao Fish Hatchery and, subsequently, their offspring were propagated and distributed to the island's reservoirs (Erdman 1984; Neal et al. 2004). A third introduction was recorded in 1957 from the Welaka National Fish Hatchery, Florida, which consisted of only 30 adults that are believed to have originated from the St. John's River and Lake George (Welaka Hatchery personnel, pers. comm.). Philipp et al. (1983) found many introgressed Florida x northern largemouth bass (ILMB) populations throughout the southeastern United States; therefore, introductions of largemouth bass to Puerto Rico from Georgia and Mississippi were likely ILMB. Introductions from the Welaka Hatchery, on the other hand, likely represented non-introgressed FLMB, although recent collections indicate low levels of introgression in these populations (Barthel et al. 2010). Following introduction, largemouth bass became established as the most successful predator and most highly sought-after sport fish in reservoirs and farm ponds throughout the island (Erdman 1984; Neal et al. 2004).

Studies of largemouth bass dynamics in Puerto Rico indicate that, unlike their dynamics in temperate reservoirs, most bass do not survive to age 4 and rarely reach sizes considered preferred by anglers (Neal et al. 2002). This short life span and small size occurs in reservoirs throughout the island despite an apparent abundance of prey and year-round growing season (Alicea et al. 1997; Neal 1999). Following rapid growth as juveniles, growth slows commensurate with first maturation (Gran 1995). Largemouth bass in Puerto Rico exhibit an extended spawning season of up to 6 months, with multiple spawning events per individual. This increased reproductive activity may require an energetic shift from growth to reproduction,

leading to reduced growth and early mortality (Gran 1995; Neal 2003).

Previous research on somatic growth and mortality of largemouth bass in Puerto Rico has considered differences among subspecies. Neal et al. (1999) evaluated the subspecies composition of largemouth bass in Puerto Rico reservoirs using 3 diagnostic allozyme loci and discovered that only two of ten primary reservoirs contained a non-introgressed FLMB population (Cerrillos and Guajataca Reservoirs), while all but one (La Plata Reservoir) showed a preponderance of FLMB subspecific alleles. However, the sample sizes for several populations were less than ten individuals (Table 2) and procedures (i.e., development of microsatellite markers; Lutz-Carrillo et al. 2008) for determining introgression have been greatly refined in recent years (Wright and Bentzen 1984). Neal and Noble (2002) found that FLMB displayed greater longevity (i.e., 76% of total recaptures at age 2 and 100% at ages 3 and 4) than ILMB in Lucchetti Reservoir. Therefore, they recommended that the Maricao Hatchery collect broodstock from only pure FLMB populations, specifically Cerrillos Reservoir, to ensure production and propagations of pure FLMB to island reservoirs (Neal and Noble 2002).

Although FLMB have been shown to survive longer than ILMB in one Puerto Rico reservoir (Neal et al. 1999), a comprehensive evaluation of trends between subspecies composition and population metrics (length-frequency distributions, proportional size distribution [PSD], mean total length) could better elucidate whether or not these trends are apparent between subspecies and/or intergrades among other island populations. Whereas microsatellite markers provide enhanced detail of levels of introgression (i.e., a larger number of diagnostic loci and alleles; see Lutz-Carrillo et al. 2008) over other techniques (i.e., allozymes; Philipp et al. 1983), and sample size used in previous genetic studies were insufficient for unambiguous subspecies recognition (Neal et al. 1999), it's unclear whether or not Cerrillos Reservoir is currently providing non-introgressed FLMB to the Maricao Hatchery. If not, hatchery operations may want to consider collecting broodstock from alternative source populations, which may help to augment genetic variation, reduce the potential of genetic bottlenecks, and perhaps increase fitness (Allendorf and Luikart 2007).

Our objectives were to 1) determine the subspecies composition of largemouth bass among reservoirs and

TABLE 1. Importation and stocking history of largemouth bass in Puerto Rico from 1915 to 2010. *Micropterus salmoides* represents suspected introgressed largemouth bass genotypes, and *M. s. floridanus* represents the Florida largemouth bass genotype, although some introgression cannot be ruled out. Numbers of fish, size range (total length; mm), and source/recipient population data are given where available (n/a is not available; derived from Erdman 1984 and Neal et al. 2004).

| Year | Species  | Number  | Size      | Source/recipient  |
|------|--|---------|-----------|---|
| 1915 | <i>Micropterus salmoides</i>                       | 600     | n/a       | Mariaco Hatchery  |
| 1916 | <i>Micropterus salmoides</i>                       | 600     | n/a       | Mariaco Hatchery  |
| 1946 | <i>Micropterus salmoides</i>                       | 1,067   | n/a       | From Georgia and Mississippi  |
|      | <i>Micropterus salmoides</i>                       | 88      | adults    |   |
|      | <i>Micropterus salmoides</i>                       | 10,538  | 19-76     | From Maricao Hatchery to island's reservoirs; La Plata River  |
| 1947 | <i>Micropterus salmoides</i>                       | 22,977  | n/a       | From Maricao Hatchery to island's reservoirs  |
| 1948 | <i>Micropterus salmoides</i>                       | 8,866   | n/a       | From Maricao Hatchery to island's reservoirs  |
| 1949 | <i>Micropterus salmoides</i>                       | 298     | n/a       | From Maricao Hatchery to island's reservoirs  |
| 1950 | <i>Micropterus salmoides</i>                       | 7,455   | n/a       | From Maricao Hatchery to island's reservoirs  |
| 1951 | <i>Micropterus salmoides</i>                       | 100     | n/a       | From Maricao Hatchery to island's reservoirs  |
| 1957 | Florida Largemouth bass<br><i>M. s. floridanus</i> | 30      | n/a       | From Waleka Hatchery, Florida to Mariaco Hatchery   |
| 1994 | <i>Micropterus salmoides</i>                       | 3,966   | 38.1      | Loiza Reservoir   |
| 1995 | <i>Micropterus salmoides</i>                       | 64,653  | 25-63.5   | Carite, Carraizo, Cidra, Dos Bocas, Guayabal, Guayo, Patillas, Ponce I, Ponce II, Ponce III, Prieto, Toa Vaca reservoirs; Tortuguero Lagoon |
| 1996 | <i>Micropterus salmoides</i>                       | 49,467  | 38-76     | Carite, Dos Bocas, La Plata, Lucchetti, Toa Vaca reservoirs   |
|      | <i>M. s. floridanus</i>                            | 1,412   | n/a       | Lucchetti Reservoir   |
| 1997 | <i>Micropterus salmoides</i>                       | 38,026  | 38-76     | Carite, Carraizo, Cidra, Dos Bocas, Garzas, Las Curias, Lucchetti, Toa Vaca reservoirs; Tortuguero Lagoon; private pond                     |
|      | <i>M. s. floridanus</i>                            | 48,520  | 25-76     | Cerrillos, Guajataca, and Lucchetti reservoirs  |
| 1998 | <i>Micropterus salmoides</i>                       | 41,114  | 25-76     | Dos Bocas, Guayabal, La Plata, Las Curias, Patillas, Toa Vaca reservoirs; private ponds   |
| 2000 | <i>M. s. floridanus</i>                            | 193,024 | 38-69     | Carraizo, Cidra, Dos Bocas, Garzas, Guajataca, Guayabal, Guayo, La Plata, Matrullas, Patillas, Toa Vaca, reservoirs; Tortuguero Lagoon      |
| 2001 | <i>M. s. floridanus</i>                            | 133,421 | 25-171    | Carraizo, Cidra, Dos Bocas, Guajataca, Guayabal, La Plata, Las Curias, Lucchetti, Toa Vaca, reservoirs; private ponds                       |
| 2002 | <i>M. s. floridanus</i>                            | 84,419  | 44.4-63.5 | Caonillas, Cidra, Dos Bocas, Garzas, Guajataca, Guayo, La Plata, Loco reservoirs; private ponds   |
| 2003 | <i>M. s. floridanus</i>                            | 70,474  | 37-62     | Cidra, Dos Bocas, Guajataca, La Plata, Toa Vaca, reservoirs; private ponds  |
| 2004 | <i>M. s. floridanus</i>                            | 62,514  | 37-62     | Caonillas, Carite, Dos Bocas, Guajataca, La Plata, Lucchetti reservoirs; private ponds  |

TABLE 1 cont.

|      |                         |        |       |   |
|------|-------------------------|--------|-------|---|
| 2005 | <i>M. s. floridanus</i> | 41,675 | 37-62 | Cidra, Dos Bocas, Guajataca, Lucchetti reservoirs; private ponds                  |
| 2006 | <i>M. s. floridanus</i> | 57,767 | 37-62 | Caonillas, Carite, Cidra, Dos Bocas, La Plata, Patillas reservoirs; private ponds |
| 2007 | <i>M. s. floridanus</i> | 64,297 | 37-62 | Cidra, Caonillas, Carraizo, Curias, Guajataca, Guayabal reservoirs; private ponds |
| 2008 | <i>M. s. floridanus</i> | 44,360 | 37-62 | Caonillas, Carraizo, Dos Bocas, Guayabal, Lucchetti reservoirs; private ponds     |
| 2009 | <i>M. s. floridanus</i> | 47,380 | 37-62 | Caonillas, Guajataca, La Plata reservoirs; private ponds                          |
| 2010 | <i>M. s. floridanus</i> | 35,308 | 37-62 | Garzas, Guajataca, La Plata reservoirs  |

TABLE 2. Allele frequencies of three major diagnostic loci for largemouth bass collected from ten reservoirs in Puerto Rico. Under genetic status, I represents an introgressed population and F represents a pure Florida subspecies population (derived from Neal et al. 1999).

| Reservoir | N   | AAT   |       |       | IDH   |       |       | MDH   |       | Genetic Status |
|-----------|-----|-------|-------|-------|-------|-------|-------|-------|-------|----------------|
|           |     | 1     | 3     | 4     | 1     | 3     | 4     | 1     | 2     |                |
| Caonillas | 33  | 0.167 | 0.652 | 0.182 | 0.212 | 0.788 | 0.000 | 0.182 | 0.818 | I              |
| Carite    | 7   | 0.000 | 0.571 | 0.429 | 0.214 | 0.786 | 0.000 | 0.000 | 1.000 | I              |
| Cerrillos | 25  | 0.000 | 0.760 | 0.240 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | F              |
| Cidra     | 9   | 0.611 | 0.278 | 0.111 | 0.222 | 0.778 | 0.000 | 0.333 | 0.667 | I              |
| Dos Bocas | 7   | 0.000 | 0.857 | 0.143 | 0.286 | 0.714 | 0.000 | 0.286 | 0.714 | I              |
| Guajataca | 6   | 0.000 | 0.583 | 0.417 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | F              |
| Guayabal  | 30  | 0.267 | 0.233 | 0.500 | 0.177 | 0.883 | 0.000 | 0.317 | 0.683 | I              |
| Guayo     | 120 | 0.091 | 0.341 | 0.577 | 0.025 | 0.975 | 0.000 | 0.525 | 0.475 | I              |
| La Plata  | 7   | 0.571 | 0.286 | 0.143 | 0.071 | 0.857 | 0.071 | 0.500 | 0.500 | I              |
| Toa Vaca  | 6   | 0.167 | 0.167 | 0.666 | 0.000 | 1.000 | 0.000 | 0.167 | 0.833 | I              |

in the contemporary Maricao Hatchery broodstock in Puerto Rico, 2) compare population metrics (length-frequency distributions, proportional size distribution [PSD], mean total length) between FLMB, NLMB, and/or ILMB among reservoir populations, and 3) provide genetically-based recommendations for future hatchery broodstock collection protocols in Puerto Rico. Ultimately, these objectives were developed to determine which subspecies is most prevalent among populations in Puerto Rico and to provide the subspecific genetic framework for future studies.

## Methods

**Study Sites** – This study included 12 reservoirs located throughout the tropical island of Puerto Rico, as well as the Maricao Hatchery. Reservoirs were

chosen using three criteria: 1) reservoirs that contained a largemouth bass population, 2) management importance as designated by the Puerto Rico Department of Natural and Environmental Resources (PRDNER; Lilyestrom and Neal 2004), and 3) accessibility. Reservoirs ranged in surface area (ha) and elevation (m) above sea level 37.2-405.0 ha and 46.0-743.1 m, respectively (Table 3). The Maricao Hatchery is located at 454 m above sea level and is the only freshwater fish hatchery on the island operated by the PRDNER.

**Sample Collection and Processing** – At least 30 stock size largemouth bass ( $\geq 200$  mm) were collected from each reservoir except Guayabal Reservoir (N=18) and Carite Reservoir (N=27), and from the

TABLE 3. Locations, surface area (ha), and elevation (m) above sea level of the Maricao Fish Hatchery and 12 reservoir populations from which largemouth bass were sampled in this study.

|     | Collection (code)      | Latitude  | Longitude  | Surface Area (ha) | Elevation (m) |
|-----|------------------------|-----------|------------|-------------------|---------------|
| 1.  | Maricao Hatchery (Mhy) | 18°10'12" | -66°59'11" | -                 | 454           |
| 2.  | Guayo (Gyo)            | 18°11'58" | -66°50'00" | 118               | 445           |
| 3.  | Lucchetti (Luc)        | 18°05'31" | -66°51'51" | 106               | 174           |
| 4.  | Garzas (Gar)           | 18°08'13" | -66°44'32" | 44                | 742           |
| 5.  | Dos Bocas (Dbo)        | 18°19'59" | -66°40'04" | 254               | 89            |
| 6.  | Caonillas (Cao)        | 18°15'56" | -66°39'17" | 280               | 253           |
| 7.  | Matrullas (Mat)        | 18°12'26" | -66°28'46" | 37                | 743           |
| 8.  | Cerrillos (Cer)        | 18°05'20" | -66°34'44" | 249               | 137           |
| 9.  | Toa Vaca (Tov)         | 18°06'14" | -66°28'52" | 342               | 164           |
| 10. | Guayabal (Gyb)         | 18°05'42" | -66°30'13" | 131               | 105           |
| 11. | La Plata (Lap)         | 18°20'22" | -66°13'57" | 405               | 46            |
| 12. | Carite (Car)           | 18°04'29" | -66°06'09" | 133               | 545           |
| 13. | Patillas (Pat)         | 18°01'32" | -66°01'27" | 137               | 66            |

contemporary Maricao Hatchery broodstock during June and July 2011. For standardization purposes, six shoreline sites were established *a priori* in each reservoir. Subsequently, three out of the six sites were randomly selected in each reservoir and individually electrofished for 900 seconds using a boom-mounted electrofisher at 240-V pulsed DC. All largemouth bass were collected at each site, measured for total length (mm) and weighed (g). Additional collection sites were used if predetermined sample size (N = 30) was not collected initially.

For each fish collected, a section (~25 mm<sup>2</sup>) of the pectoral fin was removed, immediately placed in 2-ml storage vials, preserved in 70% ethanol, and stored at room temperature. Samples were then transported to the Texas Parks and Wildlife Department A.E. Wood Laboratory, San Marcos, Texas, for genetic processing and analyses during summer and fall of 2011.

Deoxyribonucleic acid (DNA) was extracted from a portion of each fin clip following a modified version of the Puregene protocol for extraction from fish tissue (Gentra Systems, Inc., Minneapolis, Minnesota). Briefly, 3-5 mm<sup>3</sup> of fin tissue was placed in 300 µL of cell lysis solution (10 mM tris-HCl, 10 mM EDTA [pH 8.0], and 2% sodium dodecyl sulfate) with 3 µL of proteinase K (20 mg/mL) and incubated at 55°C for 1.5-2.0 h. After incubation, 120 µL of ammonium acetate (7.5 M) was added and mixed. The solution was then incubated at 0°C for 10-15 minutes followed by centrifugation at 13,000 x gravity for 5 minutes. The supernatant was then added to 1,000 µL of 100% ethanol, stored at -80°C for 10 minutes, and

recentrifuged at 13,000 x gravity for 10 minutes. The supernatant was then decanted and dried at room temperature for 15 minutes. The pellet was resuspended in 200 µL of deionized H<sub>2</sub>O and left to rehydrate at room temperature for 24 hours. Recovered DNA was quantified using a Nanodrop<sup>®</sup> ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE) and adjusted to a concentration of 50 ng/µl using TLE buffer to ensure consistency in subsequent genotyping.

Two multiplex reactions (MPX8 and MPX9) encompassing six diagnostic microsatellite loci (TPW111, TPW112, TPW169, *Msa21*, *Mdo6*, and *Msa29*; Lutz-Carrillo et al. 2008) were used to analyze subspecies composition. Multilocus genotyping was performed using the polymerase chain reaction (PCR) to isolate and amplify the individual loci in all sampled individuals. The PCR products were denatured by heat and formamide and analyzed on an NEN 4200 Global IR2 DNA Sequencer (LICOR) using standard 50-350 base pair and 50-700 base pair ladders. Allele sizes were estimated using BioNumerics v. 6.5 (Applied-Maths, Belgium). All genotypes were confirmed visually and recorded in a master database in Microsoft Excel 2010 with the raw data consisting of observed genotypes and allele counts.

*Statistical Analyses* – For analysis of subspecies composition, at least 30 individuals per population were evaluated, except Guayabal Reservoir (N=18) and Carite Reservoir (N=27). Multiple loci were used to assign genotypes (FLMB, NLMB, ILMB) at the individual level based on the distribution of alleles



within and among loci in Microsoft Excel® (Microsoft Corporation, Palo Alto, California). Hybrid index values, defined as the proportion of FLMB alleles detected at each diagnostic locus for each individual, were calculated for each fish. Mean hybrid index values, the mean proportion of FLMB alleles among all loci examined, were then calculated for each population to represent the probability that a randomly selected individual was derived from a FLMB ancestor.

To test the null hypothesis of no differences in length-frequency distributions between FLMB, NLMB, or ILMB, length-frequency distributions were pooled among all populations and compared using a two-way Kolmogorov-Smirnov test and the NPAR1WAY procedure in SAS (SAS Institute, Cary, North Carolina). Length-frequency distributions between FLMB and ILMB were not compared within individual populations because sample sizes were inadequate to provide assumptions with an adequate level of confidence (Miranda 1997). Mean total length was determined for FLMB and ILMB within each population and compared using the GLM procedure in SAS (SAS Institute, Cary, North Carolina). To avoid the effects of gear bias, only stock-size (200 mm; Gabelhouse 1984) or larger largemouth bass were used to compare length-frequency distributions and mean total length among populations. Before analyses, total

length values were log-transformed to stabilize variance to mean ratios.

A chi-square test and the frequency procedure (FREQ) in SAS (SAS Institute, Cary, North Carolina) were used to test the null hypothesis that the frequency of observations among proportional size distribution (PSD) size groups (proportional size distribution of stock-to-quality [PSD-S-Q], quality-to-preferred [PSD-Q-P], preferred-memorable [PSD-P-M], memorable-to-trophy [PSD-M-T] size; Gabelhouse 1984) was independent of subspecific composition. Independent Wilcoxon rank-sum tests and a nonparametric one-way analysis of variance procedure (NPAR1WAY) in SAS (SAS Institute, Cary, North Carolina) were then used to determine if the frequencies of fish among and within PSD size groups differed between FLMB and ILMB. Significance was established at an alpha level of  $P \leq 0.05$  for all tests.

## Results

All 12 reservoir populations, as well as the Maricao Hatchery broodstock, consisted of ILMB (Figure 1). Among reservoir populations, the proportion of FLMB alleles ranged from 0.77 to 0.95 (mean 0.86, SD 0.07), with least and greatest proportions observed in Garzas and Cerrillos Reservoirs, respectively (Table 4; Figure 1).

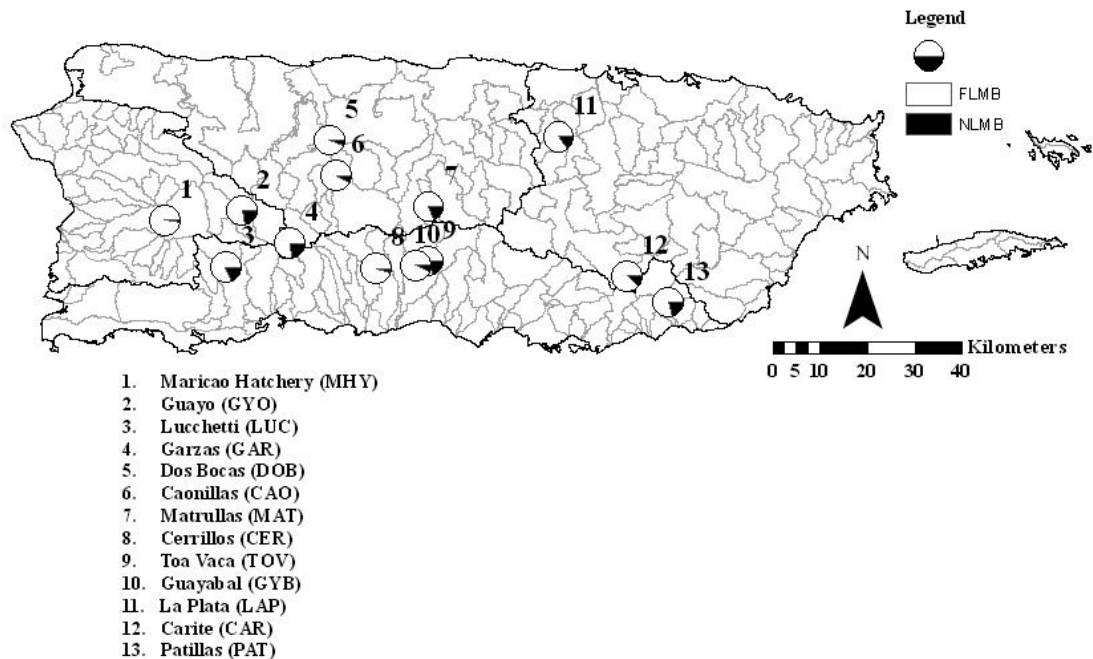


FIGURE 1. Locations of twelve reservoirs and one hatchery population in Puerto Rico sampled for this study; pie charts depict the frequency of northern largemouth bass and Florida largemouth bass alleles at six diagnostic microsatellite loci (Msa29, Msa21, Mdo6, TPW111, TPW112, TPW115) within populations. The black lines delineate the major hydrologic units designated by the U.S. Geological Survey, and the gray lines indicate watershed boundaries.

TABLE 4. Proportion of Florida largemouth bass alleles observed at 6 diagnostic microsatellite loci for 12 reservoir and the Maricao Fish Hatchery in Puerto Rico. The collective mean proportion of Florida bass alleles (FLMB) among loci and standard deviation (SD) for each population is presented.

|                  | N   | Microsatellite locus |       |      |        |        |        | FLMB | SD   |
|------------------|-----|----------------------|-------|------|--------|--------|--------|------|------|
|                  |     | Msa29                | Msa21 | Mdo6 | TPW111 | TPW112 | TPW115 |      |      |
| All Populations  | 418 | 0.81                 | 0.82  | 0.93 | 0.86   | 0.85   | 0.90   | 0.86 | 0.07 |
| Caonillas        | 33  | 0.91                 | 0.88  | 0.92 | 0.88   | 0.95   | 0.94   | 0.92 | 0.12 |
| Carite           | 27  | 0.83                 | 0.87  | 0.89 | 0.81   | 0.92   | 0.94   | 0.88 | 0.11 |
| Cerrillos        | 43  | 0.73                 | 0.98  | 1.00 | 1.00   | 0.99   | 1.00   | 0.95 | 0.06 |
| Dos Bocas        | 36  | 0.93                 | 0.95  | 0.98 | 0.95   | 0.91   | 0.94   | 0.94 | 0.10 |
| Garzas           | 32  | 0.73                 | 0.70  | 0.85 | 0.79   | 0.75   | 0.78   | 0.77 | 0.18 |
| Guayabal         | 18  | 0.81                 | 0.89  | 0.97 | 0.94   | 0.94   | 1.00   | 0.93 | 0.09 |
| Guayo            | 32  | 0.72                 | 0.77  | 0.94 | 0.73   | 0.72   | 0.80   | 0.78 | 0.13 |
| La Plata         | 32  | 0.88                 | 0.84  | 0.78 | 0.93   | 0.71   | 0.94   | 0.85 | 0.12 |
| Lucchetti        | 30  | 0.80                 | 0.68  | 0.95 | 0.75   | 0.83   | 0.83   | 0.81 | 0.13 |
| Maricao Hatchery | 40  | 0.91                 | 0.99  | 1.00 | 0.99   | 1.00   | 1.00   | 0.98 | 0.04 |
| Matrullas        | 31  | 0.68                 | 0.77  | 0.92 | 0.72   | 0.77   | 0.98   | 0.81 | 0.11 |
| Patillas         | 31  | 0.77                 | 0.73  | 0.93 | 0.73   | 0.82   | 0.84   | 0.80 | 0.15 |
| Tao Vaca         | 33  | 0.89                 | 0.62  | 0.97 | 0.91   | 0.77   | 0.77   | 0.82 | 0.12 |

The proportion of pure Florida largemouth bass genotypes within individual reservoirs ranged from 0.03 to 0.64 (mean 0.29, SD 0.22), with least and greatest proportions observed in Garzas and Dos Bocas Reservoir, respectively (Table 5). Among all largemouth examined, 33% (139/418) were assigned a pure FLMB genotype. Only 2 of 12 populations contained an individual with <50% FLMB influence (Garzas: N=3; Toa Vaca: N=1), and no pure NLMB genotypes were recovered (Table 5; Figure 2). All populations showed lower levels of FLMB alleles and genotypes than the Mariaco Hatchery broodstock (Tables 4 and 5).

Length-frequency distributions were different ( $P=0.026$ ) between FLMB and ILMB (Figure 3). Cumulative distribution functions for FLMB and ILMB were similar from 200 to 350 mm, and then distributions separated at 350 mm and higher. The length of greatest differentiation among cumulative distribution functions was observed around 400 mm. Introgressed largemouth bass were 16% more frequent than FLMB at lengths less than 400 mm, while FLMB were 16% and 8% more frequent than ILMB at lengths greater than 400 and 500 mm, respectively (Table 6).

Collectively, differences ( $F=2.17$ ;  $df=11$ ;  $P=0.016$ ) were observed between mean total length of FLMB (mean=378.6; SD=60.2; SE=17.4) and ILMB

(mean=358.1; SD=47.7; SE=13.8). Mean total length of FLMB was greater than ILMB in 9 out of 12 populations (Figure 4). Mean total length of FLMB ranged from 298.0 to 490.7 mm. Mean total length of ILMB ranged from 311.1 to 481.4 mm. Overall, a difference ( $\chi^2=8.28$ ,  $df=3$ ,  $P=0.041$ ) was observed between the frequency of FLMB and ILMB among PSD size groups (Figure 5). The majority of the overall  $\chi^2$ -value was explained within FLMB PSD Q-P, PSD P-M, and PSD M-T size groups, respectively. No trophy (PSD-T) FLMB or ILMB were collected.

## Discussion

Florida largemouth bass alleles and genotypes dominated all largemouth bass populations in Puerto Rico. The prevalence of FLMB alleles could be a genetic artifact from founding populations, the effects of recent hatchery releases, natural selection, or a combination of all of these mechanisms.

The Florida and northern subspecies of largemouth bass have unique genetic signatures (e.g., molecular markers; Philipp et al. 1983; Lutz-Carrillo et al. 2008) that can be passed from parents to offspring. However, the prevalence of subspecific markers can be greatly influenced by genetic processes (i.e., mutation, migration, genetic drift, selection) and environmental and artificial selection (e.g., stocking). Furthermore,

TABLE 5. Proportion of Florida largemouth bass genotypes (FLMB Genotypes) observed at 6 diagnostic microsatellite loci (Msa29, Msa21, Mdo6, TPW111, TPW112, TPW115; Lutz-Carrillo et al. 2008) for 12 largemouth bass populations in Puerto Rico. Standard deviation is presented.

| Population      | N   | FLMB Genotypes | SD   |
|-----------------|-----|----------------|------|
| All Populations | 378 | 0.32           | 0.22 |
| Caonillas       | 33  | 0.64           | 0.12 |
| Carite          | 27  | 0.30           | 0.11 |
| Cerrillos       | 43  | 0.49           | 0.05 |
| Dos Bocas       | 36  | 0.64           | 0.11 |
| Garzas          | 32  | 0.19           | 0.18 |
| Guayabal        | 18  | 0.44           | 0.09 |
| Guayo           | 32  | 0.03           | 0.13 |
| La Plata        | 32  | 0.19           | 0.12 |
| Lucchetti       | 30  | 0.17           | 0.13 |
| Matrullas       | 31  | 0.03           | 0.11 |
| Patillas        | 31  | 0.13           | 0.15 |
| Tao Vaca        | 33  | 0.09           | 0.12 |

hatchery practices can greatly alter the genetic composition and genetic processes in wild fish populations (Busack and Currens 1995).

Largemouth bass have been introduced to Puerto Rico from mainland North America from the native range of the northern subspecies and the Florida subspecies. Offspring of these imports were produced at the Maricao Hatchery and released to many reservoirs throughout the island, often without documentation. Starting in the year 2000, stocking records increased, and most documented stocking events consisted of fish presumed to be pure FLMB, although results from the current study suggest some introgression. Since hatchery practices can greatly alter the genetic composition of wild fish populations (Busack and Currens 1995), it is very likely that the current prevalence of FLMB alleles is a product of stocking pure (or mostly pure) FLMB from the Maricao Hatchery into introgressed populations. However, the genetic composition of previous hatchery brood fish is not known, and historical records of individual stocking events from the Maricao Hatchery to island reservoirs are vague or absent. Therefore, the influence of past stocking events on subspecific

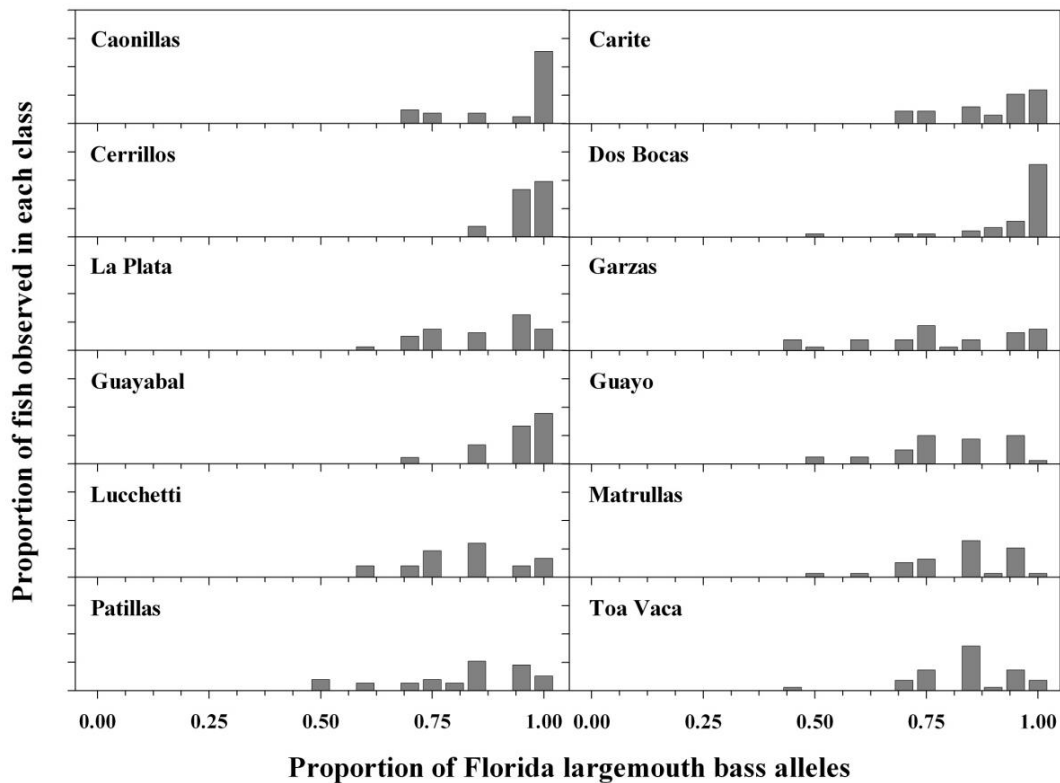


FIGURE 2. Hybrid index frequency histogram for Florida largemouth bass x northern largemouth bass intergrade populations in Puerto Rico. Estimates of introgression are based on 6 diagnostic microsatellite loci (Msa29, Msa21, Mdo6, TPW111, TPW112, TPW115; Lutz-Carrillo et al. 2008). The scale ranges from 0.0 (individuals with all largemouth bass alleles) to 1.0 (individuals with all Florida largemouth bass alleles) via one-allele increments.

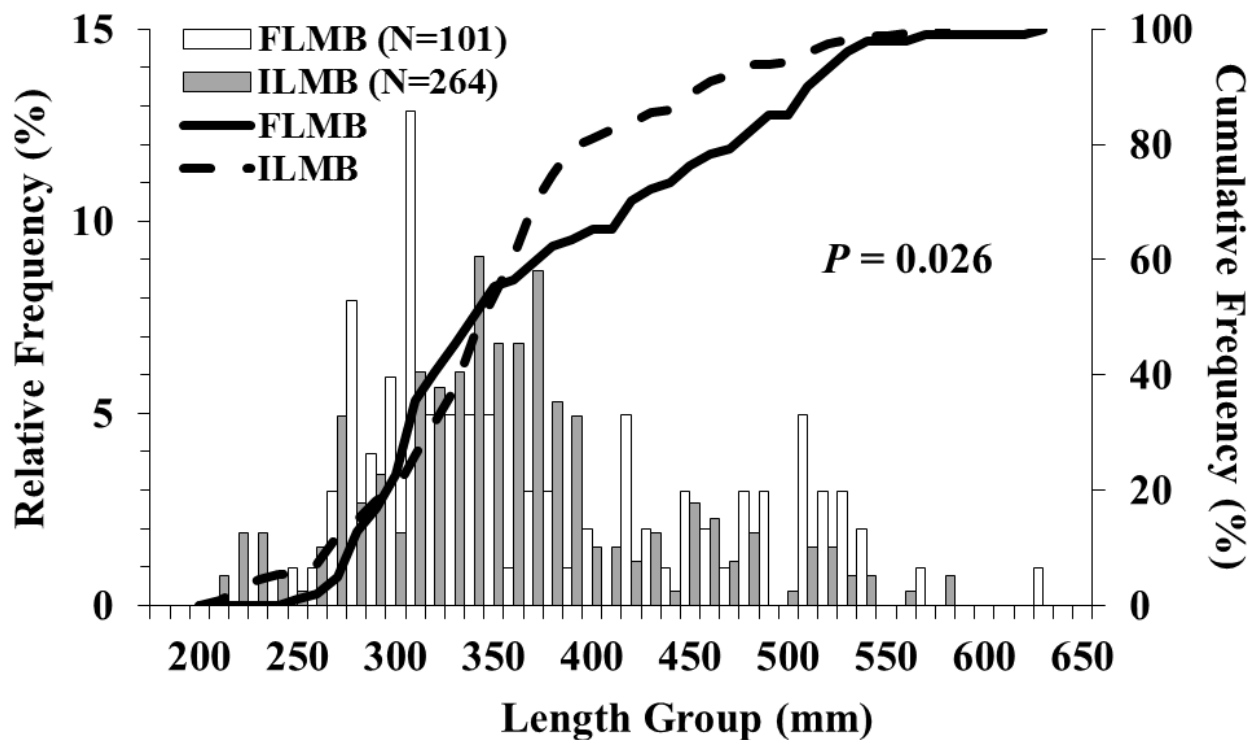


FIGURE 3. Relative length-frequency histogram (%; bars) and cumulative frequency (%; lines) for pure Florida largemouth bass (FLMB) and introgressed (Florida x northern) largemouth bass (ILMB) from 12 reservoirs in Puerto Rico.

TABLE 6. Overall percent frequency by length group for all largemouth bass, pure Florida largemouth bass (FLMB), and intergrade (Florida x northern) largemouth bass (ILMB) collected from 12 largemouth bass populations in Puerto Rico. Frequencies of each proportional size distribution length increment and cumulative frequencies by length class are presented.

| Size Group | Percent Frequency |            |            |                        |
|------------|-------------------|------------|------------|------------------------|
|            | All (365)         | FLMB (101) | ILMB (264) | Difference (FLMB-ILMB) |
| PSD S-Q    | 17.81             | 16.83      | 18.18      | -1.35                  |
| PSD Q-P    | 48.77             | 42.57      | 51.14      | -8.56                  |
| PSD P-M    | 25.21             | 25.74      | 25.00      | 0.74                   |
| PSD M-T    | 7.95              | 13.86      | 5.68       | 8.18                   |
| PSD-T      | 0.00              | 0.99       | 0.00       | 0.99                   |
| >300       | 82.19             | 83.17      | 81.82      | 1.35                   |
| >400       | 24.93             | 36.63      | 20.45      | 16.18                  |
| >500       | 8.49              | 14.85      | 6.06       | 8.79                   |
| >600       | 0.27              | 0.99       | 0.00       | 0.99                   |

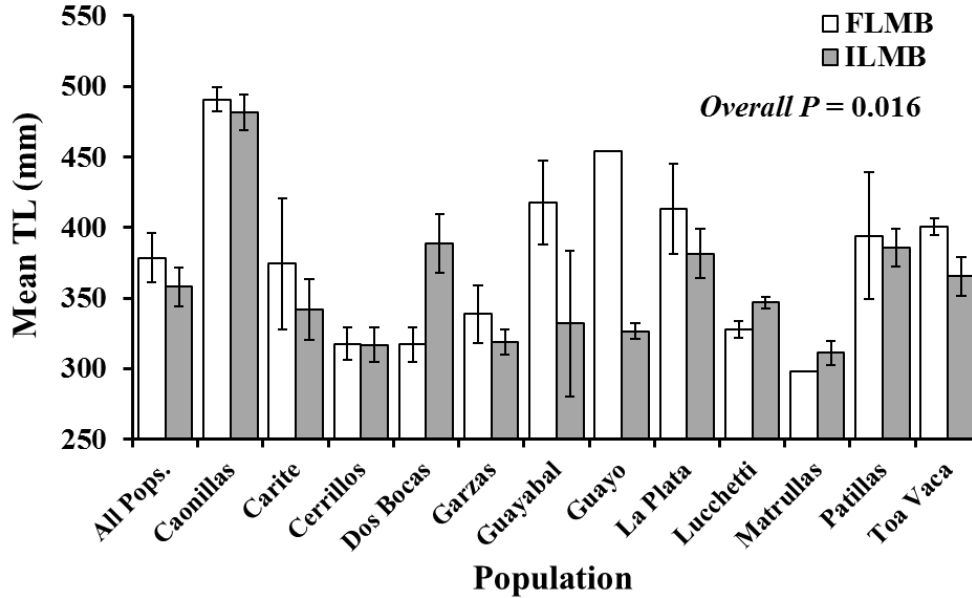


FIGURE 4. Mean total length (mm) of Florida largemouth bass (FLMB) and introgressed (Florida x northern) largemouth bass (ILMB) from 12 populations in Puerto Rico. The Overall  $P$ -value indicates a test of mean total length of FLMB vs. ILMB pooled among all populations. Error bars represent standard error.

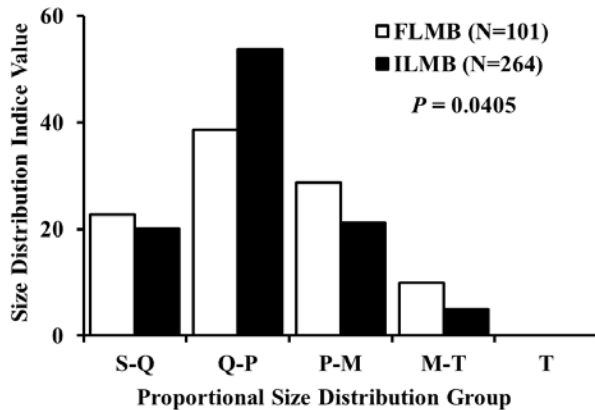


FIGURE 5. Proportional size distribution (PSD) value by incremental size categories for pure Florida largemouth bass (FLMB) and introgressed (Florida x northern) largemouth bass (ILMB) collected from twelve reservoirs in Puerto Rico. Proportional size distribution (PSD) categories represent fish 200-299 mm (PSD-S-Q), 300-379 mm (PSD-Q-P), 380-510 mm (PSD-P-M), 510-630 mm (PSD-M-T), and 630 mm or greater (PSD-T) determined from proposed length categories for largemouth bass (Gabelhouse 1984).

composition cannot be determined. Furthermore, subspecific alleles and genotypes provided by each importation event and precise populations of origin are not known. Therefore, evaluation of why one genotype would be most prevalent would be indefinite without further genetic analyses (i.e., measures of genetic variation and phylogenetic analyses).

Compared to previous genetic analyses (Neal et al. 1999), we found that 4 out of 8 populations increased

in the proportion of FLMB influence, whereas the other populations did not appreciably change. Excluding potential effects of hatchery stocking, an increase in the proportion of FLMB influence may suggest that natural selection favored FLMB genotypes in these populations. Natural selection is the differential contribution of genotypes to the next generation that results from improved survival and reproductive success of those genotypes, or co-adapted gene complexes, under the given environmental conditions. Both environmental conditions and genetic factors (e.g., genetic drift, migration) can influence the efficiency and direction of selection (Allendorf and Luikart 2007). Environmental conditions are fairly homogeneous in Puerto Rico (USGS 2005), with stable, warm temperatures and moderated photoperiods much like those found in the FLMB native range. If natural selection was highly influenced by genetic factors and favored FLMB alleles and genotypes, we would expect to see this unidirectional pattern of selection (e.g., an increase in FLMB influence) among populations over time. However, since this study was not developed to address the mechanisms (e.g., survival, reproduction, or environmental conditions) for the increase in FLMB influence, we cannot definitively say FLMB are the favored genotype in Puerto Rico. Furthermore, there may be issues with comparing these two studies (e.g., sample size and use of different genetic techniques and markers).

Due to longevity differences among subspecies of largemouth bass in Puerto Rico, it was previously suggested that Cerrillos Reservoir, then considered a

pure FLMB population (Neal et al. 1999), be designated as the solitary broodstock source for the Maricao Hatchery (Neal and Noble 2002). Our results indicate that the Cerrillos Reservoir population contains a small proportion of NLMB alleles. Although the subspecies status of Cerrillos Reservoir has changed, it continues to contain the highest mean proportion of FLMB alleles among all reservoir populations, and no other pure FLMB populations were discovered in the present study (Figure 1). Thus, at present, we recommend continuation of hatchery broodstock collections from Cerrillos Reservoir.

This study identified three reservoir populations (Caonillas, Dos Bocas, and Guayabal Reservoirs) with similar proportions of FLMB alleles and genotypes as Cerrillos Reservoir (Tables 4 and 5), and these populations could serve as alternative source populations for broodstock collection. In fact, Caonillas and Dos Bocas Reservoir contained a greater proportion of pure FLMB individuals in their populations than all other reservoirs (Table 5). Although the management objective of the Maricao Fish Hatchery is to maintain and propagate pure FLMB, we found that many current broodstock are introgressed and regular genetic verification is not currently conducted on Hatchery broodstock. If regular genetic testing is implemented, new broodstock collections could target populations in Cerrillos, Caonillas, and Dos Bocas Reservoir, and pure FLMB could be identified from these collections and retained as broodstock. Other factors, including reservoir access, travel and transport distance, and largemouth bass catch rates should be considered in broodstock site selection.

The existing Maricao Hatchery broodstock contained a higher mean proportion (98%) of FLMB alleles than all reservoir populations (Table 4), despite being collected exclusively from island reservoirs. This may be indicative of unintentional artificial selection from hatchery practices over the last two decades. Hatchery broodstock collections from the current broodstock source on the island, Cerrillos Reservoir, usually consist of large (>350 mm), mature individuals (Maricao Hatchery personnel, pers. comm.). In this study, FLMB genotypes were observed 11.5% more frequently than ILMB among largemouth bass that were greater than 350 mm in Cerrillos Reservoir. The selection of larger fish during broodstock collection events may have resulted in the high proportions of FLMB alleles and genotypes found at the Maricao hatchery.

For all reservoir data pooled, FLMB displayed a more dispersed size distribution with more fish attaining larger sizes (e.g., 16% more frequent at

lengths greater than 400 mm, greater size distribution indices in the P-M and M-T size groups) than ILMB. Furthermore, mean total length (mm) was greater for FLMB than ILMB overall and in 9 out of 12 populations. However, there were distinct differences within individual populations (Figure 4). These results support previous findings that FLMB provide enhanced survival potential in Puerto Rico, as Neal and Noble (2002) found that FLMB displayed greater longevity (i.e., 76% of total recaptures at age 2 and 100% at ages 3 and 4) than ILMB, but no differences in mean growth rates. Their study was conducted in only one small reservoir (Lucchetti Reservoir), whereas the current study included 12 reservoirs distributed throughout Puerto Rico. However, the current study lacks reservoir-specific growth or mortality estimates that would help distinguish a favored genotype on the island.

A central management goal of the PRDNER is to improve size structure (i.e., growth and longevity) among largemouth bass populations. The Maricao Fish Hatchery currently collects broodstock from one island reservoir, but these collection procedures can have negative consequences (see Miller and Kapuscinski 2003). Genetic changes are expected to occur from hatchery procedures (Hindar et al. 1991) based on the relative extent of genetic differentiation (i.e., measures of genetic variation, extent of genetic population structure) among sampled populations (Carvalho and Cross 1998), and can have strong selective effects (Doyle and Talbot 1986; Doyle and Talbot 2003). Continuous collections and propagations from one population over time can have inadvertent effects (Keller and Waller 2002), especially on small populations (Leberg 1991), and result in inbreeding depression, the reduction in fitness of progeny from matings between related individuals relative to the fitness of progeny between unrelated individuals (Allendorf and Luikart 2007). However, the extent of genetic population structure and genetic variation among populations on the island is currently unknown.

This study recommends that future hatchery broodstock collections in Puerto Rico target only large (>400 mm) individuals from the populations with high proportions of FLMB alleles and genotypes (i.e., Caonillas, Cerrillos, Dos Bocas, Guayabal). The propagation of offspring from large individuals from several populations could increase growth, longevity, and fitness, and augment genetic variation (Leberg 1990; Leberg 1993). Artificial selection for fish with more rapid growth or greater longevity in hatchery operations can increase fitness and survival among wild populations; however, this trend is not always apparent (Gross 1991; Hindar et al. 1991). Therefore, future genetic analyses are needed to determine the

relative extent of genetic differentiation (i.e., genetic variation, genetic population structure) among populations in Puerto Rico and to monitor the efficiency of this selective hatchery management approach over time.

The dominance of FLMB alleles among populations may support that FLMB are a more viable option than ILMB for establishing and maintaining largemouth bass populations in Puerto Rico. Since this study was unable to distinguish the mechanisms contributing to the prevalence of FLMB genotypes among populations, or differences in size structure between pure FLMB and ILMB, future studies are warranted and should be supported by reservoir-specific growth or mortality estimates.

Overall, the observed differences in size structure between FLMB and ILMB in Puerto Rico support the need to maintain pure FLMB in the Maricao Hatchery. Supplemental stocking of offspring from pure FLMB broodstock could increase FLMB genetic influence, which may improve size structure among the ILMB populations in Puerto Rico. Therefore, we recommend that all current and future largemouth bass utilized as broodstock for the Maricao Hatchery be genetically evaluated and verified as pure FLMB before being used as broodstock. Furthermore, supplemental importations of pure FLMB from the United States could be used to establish and maintain pure FLMB at the Maricao Fish Hatchery, and offspring produced could help increase FLMB influence among populations and potentially augment genetic variation and increase fitness (Allendorf and Luikart 2007) in Puerto Rico.

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## **Study 2:**

# **Evaluation of bigmouth sleeper sport fish potential in reservoirs**



### Chapter 3: Effects of Temperature and Salinity on Bigmouth Sleeper Early Hatchery Production

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**Abstract:** The bigmouth sleeper has potential to be a managed native species in Puerto Rico reservoirs, but this would require artificial propagation and supplemental stocking. Development of protocols for hatchery production has been limited by poor larval hatching and rearing success, presumably due to lack of information on temperature and salinity preferences. In this chapter, we investigate the effects of temperature and salinity on hatch success, growth, development, and survival of bigmouth sleeper *Gobiomorus dormitor* yolk sac larvae. Hatch success was greater at lower temperatures (23°C) and salinities (0, 5, 10 ppt), while larval survival and growth was greatest at 0 ppt and 26°C. Larvae reared at 29°C developed very rapidly but rarely survived 48-h post. Moreover, larvae at 23°C survived up to 120-h post hatch, but grew and developed very slowly. Salinity adversely affected immediate survival, as larvae cultured at salinities  $\geq 5$  ppt did not survive beyond 12-h post hatch. Larval size at hatch and growth was also significantly affected by salinity ( $P < 0.001$ ), with larvae at 0 ppt salinity significantly larger than larvae hatched or reared at salinities  $\geq 5$  ppt. Regardless of temperature, yolk sac reserves were completely depleted before the transition from yolk sac larvae to the larval stage could be achieved. We concluded that the optimum rearing temperature and salinity for newly hatched larvae is 26°C at 0 ppt, but suggest that ontogenetic changes may require progressive changes in culture salinities.

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The bigmouth sleeper *Gobiomorus dormitor* is the largest species of the family Eleotridae in the Western Hemisphere. This species is thought to be diadromous, requiring unimpeded access between freshwater and marine environments (Holmquist et al. 1998), and likely utilizes both freshwater and saltwater throughout its life history (Gilmore 1992; Hernández-Saavedra et al. 2004). However, the limited published literature regarding natural life history characteristics of bigmouth sleeper is conflicting, particularly for reproductive behavior. Nordlie (1979) first described this species as anadromous, yet two years later found that it is capable of completing its life cycle when restricted to freshwater (Nordlie 1981). Winemiller and Ponwith (1998) classified this species as amphidromous, which implies that fish migrate between fresh and saltwater during their life history, but not to spawn.

Reproduction in freshwater lakes has been observed in Lake Yojoa, Honduras (Darnell 1962), Lake Xiloá (McKaye et al. 1979) and Lake Masaya (Bedarf et al. 2001), Nicaragua, and Carite Reservoir in Puerto Rico (Bacheler et al. 2004a). This suggests that estuarine or marine migrations are not always necessary for effective reproduction. Thus, bigmouth sleepers appear to have some plasticity in their life-history strategies and may not require an uninterrupted passage

to and from marine systems (Nordlie 1981; Harris et al. 2011).

Because of its migratory behavior, the bigmouth sleeper is especially sensitive to anthropogenic changes in rivers, particularly river impoundment (Bacheler et al. 2004a). This species has suffered detrimental effects from dam construction in many places, including Florida, where it is considered threatened (Gilmore 1992; Hernández-Saavedra et al. 2004), and Puerto Rico, where it has been eliminated from most upstream river reaches above high dams (Holmquist et al. 1998; Kwak et al. 2007; Kwak et al. 2013). Many of Puerto Rico's rivers and streams are dammed for irrigation, hydroelectric power generation, drinking water and flood control, and currently few rivers have functional fish passage devices (Benstead et al. 1999; Kwak et al. 2013). In fact, river modification in Puerto Rico is so extensive that the number of large dams built per unit land over the last century has outpaced the state of California, which is well known for its extensive hydrologic engineering, by an average of 2 to 1 (Greathouse et al. 2006).

Management of native fish species in Puerto Rico's reservoirs is hindered by the absence of truly freshwater native species, with only a few euryhaline species that require open access to the marine

environment. These species are impacted by the construction of impassable dams on rivers, which effectively interrupt migrations between freshwater and marine environments (Holmquist et al. 1998; Neal et al. 2004; Kwak et al. 2013). In response to the elimination of native species above impoundments, exotic fish species (e.g., largemouth bass *Micropterus salmoides* and peacock bass *Cichla ocellaris*) have been introduced to create sport fishing opportunities in Puerto Rico reservoirs (Erdman 1984; Neal et al. 2004). However, interest remains in using native species for sport fishing to reduce impacts from introduced species (Clarkson et al. 2005). Despite their limited reproductive capacity in reservoirs, bigmouth sleepers grow well in lentic freshwater systems when they are present, and anglers frequently target them for sport and food (Neal et al. 2001; Bacheler 2002). In fact, Carite Reservoir has sustained a relatively abundant population, with estimates of 14 ( $\pm$  7 SE) adult fish per hectare in 2000 and 27 ( $\pm$  15 SE) adult fish per hectare in 2001 (Bacheler et al. 2004a).

Evidence of natural reproduction in reservoirs (Bacheler et al. 2004a), recent management preferences for native species (Kassler et al. 2001; Clarkson et al. 2005), and the fact that anglers currently target bigmouth sleeper (Bacheler et al. 2004b) suggest high potential for this species to serve both sport and food interests in Puerto Rico and throughout its range. Likewise, declines in bigmouth sleeper abundance within its range warrant directed conservation efforts for this species (Holmquist et al. 1998; Warren et al. 2000). However, bigmouth sleepers appear to have limited reproductive capacity in most reservoirs. Therefore, introductory and maintenance stocking of hatchery produced fish would be required to create and maintain these fisheries (Harris et al. 2011). This necessitates the development of spawning and rearing protocols.

Recent strides have been made in the propagation of bigmouth sleepers. Active spawning techniques using hormone injections followed by hand stripping (Neal et al. 2009), and allowing broodfish to spawn on their own in tanks (Harris et al. 2011), has proven successful at producing tens of thousands of larvae per breeding pair (Neal et al. 2009). However, larval rearing has proven more difficult than anticipated. Larvae rarely survived more than 48 to 72 hours, and larvae had not fully developed mouth or eye structures at the time of mortality (Neal et al. 2009). The failure of previous rearing attempts suggests that environmental conditions employed at the hatchery were not appropriate for this species.

Understanding the environmental preferences of larvae is crucial to the development of any species for

aquaculture (Shi et al. 2010). The larval stage of a teleost is considered to be the most sensitive period to environmental stressors (Berlinsky et al. 2004; Shi et al. 2010), and the most critical environmental factors in early ontogeny of fish are photoperiod, temperature, and salinity (Hart et al. 1995a; Kamler 2002; Shi et al. 2010). Water temperature and salinity may be particularly important to bigmouth sleeper, given their tendency to migrate between cool freshwater streams and warm brackish or marine environments. Restoration and sport fish management efforts for the bigmouth sleeper would clearly benefit from more complete natural life history information.

In this chapter, we examine the effects of water temperature and salinity on bigmouth sleeper hatch success, growth and survival. The primary goal is to determine optimal salinity and temperature regimes for bigmouth sleeper early ontogeny. The objectives are to determine 1) hatch success, 2) survival, and 3) growth and development of *G. dormitor* eggs at different temperatures (23, 26 and 29°C) and salinities (0, 5, 10, 15 and 20 ppt).

## Methods

*Fish Source and Husbandry* – Bigmouth sleeper broodfish were collected from Carite Reservoir, a 124-hectare impoundment located near the town of Cayey in mountainous south-central Puerto Rico. The reservoir is situated at 18°04'N, 66°05'W at 543.6 m above sea level (full pool). Annual rainfall in the region is 210 cm and the average air temperature is 23.5°C. Carite Reservoir was impounded in 1913 by construction of an earthen dam, with the original primary purpose of water storage for the irrigation of sugar cane (Erdman 1984). Carite Reservoir is the uppermost of a series of impoundments on the La Plata River. The reservoir drains 21.2 km<sup>2</sup> of primarily forested terrain (Carvajal-Zamora 1979). Maximum depth in Carite is 19.5 m with an average depth of 10 m at full pool (Carvajal-Zamora 1979).

Fish collection was conducted using mounted boom electrofishing at a target output power of 3,500 W and 60 Hz along the shoreline. In the field, bigmouth sleepers were tagged (Biomark PIT tags; 12.5 mm, 134.2 kHz ISO), measured for total length (TL) to the nearest millimeter, weighed (wet weight to the nearest 1 g) and sexed. Sex was determined by examining genital papillae; males have a long thin papilla with a terminal pore and females have a circular papilla with bristles on the posterior edge and a flap partially covering the genital pore (Bacheler 2002). Broodstock were transported from Carite Reservoir to the Maricao Fish Hatchery (MFH) located in Maricao, Puerto Rico, in a divided 560-L hauling tank with constant aeration.

At the hatchery, fish were placed in a recirculating system composed of four 1,665-L round polyethylene tanks with constant aeration. Broodfish were held in spawning tanks with submersible heaters with integrated sensors (Hydor<sup>®</sup> Theo Submersible Heaters, Aquatic Eco-systems, Inc.) to maintain water temperature at 26°C throughout the course of the study. This temperature was chosen to hold broodfish because it was the median temperature used in each experiment. Three males and three females were stocked into each tank for spawning. Broodfish were fed 2 to 3% of their body weight (live *Tilapia* sp. or *Oreochromis* spp.) on alternate days throughout the course of this study.

Spawning tanks were equipped with two spawning structures (PVC 40.64 cm L × 20.32 cm diameter, color grey; Vassallo Industries Inc., Cotto Laurel, Puerto Rico), the inner surface was covered with a flexible (203 mm x 292 mm) transparency film CG6000 (3M Visual Systems Division, Austin, Texas) to provide a spawning surface capable of being removed and manipulated. Spawning containers were monitored daily for presence of eggs.

*Experimental Design* – This study was divided into three independent trials, each using the same temperature-salinity design described below. Trial 1 determined temperature/salinity effects on hatch success (objective 1), trial 2 determined the effects of temperature/salinity on survival rates (objective 2), and trial 3 determined the effects of temperature/salinity on daily growth and development (objective 3).

Salinity treatments were chosen based on previous research findings. Neal et al. (2009) observed that larval survival was greatly improved at salinities between 6 ppt and 12 ppt. Also, larvae subjected to 24 ppt and 35 ppt treatments did not survive past 12 hours post-hatch. The salinities for this study were chosen to represent hatchery water (0 ppt) as well as different conditions found in brackish waters (5, 10, 15, 20 ppt). These salinities simulate salt concentrations that bigmouth sleeper larvae may encounter as they move from fresh water into estuarine environments.

Temperatures for this study were chosen to represent the mean ambient hatchery water temperature at the Maricao Fish Hatchery (23°C), and a range of temperatures that are present in bigmouth sleeper habitats during spawning season (26 and 29°C). For example, Harris (2007) reported that adult bigmouth sleepers held in warm ponds (~ 27.8°C) demonstrated earlier development than wild fish in cooler rivers (~23°C). In Carite Reservoir, peak reproductive development was intermediate to ponds and rivers with surface temperature averaging 26 °C (Bacheler 2002).

Eggs and larvae were obtained from bigmouth sleeper broodstock during the peak spawning period from May to September 2012 (Bacheler 2002; Harris 2011). After eggs were deposited and fertilized, the film substrate was removed from the spawning container, cut into several pieces with a scalpel then placed in a dissecting tray with water and low aeration. For trial 1, each piece was placed under a Leica EZ-4D stereo-microscope with integral digital camera (Leica Microsystems, Ltd. Switzerland) to further divide eggs and cut the sheet into pieces that contained 100 eggs each. For trials 2 and 3, sections of the substrate containing about equal numbers of eggs were placed in each of 15 hatching containers, which were maintained at each temperature-salinity combination. Using larvae from these containers after hatch reduced temperature and osmotic stress on the larvae when stocked into the experiment. For each trial, eggs or larvae from a single spawn of a single broodstock pair were used to avoid potential genetic differences confounding the results.

The experimental temperature-salinity system was designed as follows. Incubation units consisted of 45 1,000-mL clear glass beakers (Fisherbrand<sup>®</sup>, Thermo Fisher Scientific Inc.) filled with 900 mL of water of the assigned salinity. Three 341-L polyethylene tanks served as water baths (23, 26, and 29 °C); each tank had 2 submersible heaters with integrated sensors (Hydor<sup>®</sup> Theo Submersible Heaters, Aquatic Eco-systems, Inc.). To maintain homogeneous temperatures throughout the water column, two PVC pipes (15 cm L X 4 cm diameter; Vassallo Industries Inc., Cotto Laurel, Puerto Rico) were connected to an airline and placed at the bottom of each tank for constant water flow. Fifteen beakers (three per salinity treatment) were assigned to each water bath using a completely randomized design (Figure 1), for a total of three replicates for each treatment. A constant photoperiod of 12-h light and 12-h dark was provided (Martínez-Palacios and Salgado-García 2008), which represents the mean photoperiod in Puerto Rico.

Artificial seawater (Instant Ocean synthetic sea salt, Aquarium Systems) was used for the salinity treatments and was monitored daily using an optical refractometer (ATAGO Co., Ltd.). Five glass aquaria (52.2-L) served as water reserves for each of the target salinities. If target salinities were not met, water was changed by siphoning out approximately 25 to 50% of water with flexible PVC tubing (2.54 cm inner diameter) and adding new water from the aquaria containing the corresponding salinity.

For each trial, 100 eggs (trial 1) or 100 larvae (trials 2 and 3) were stocked per beaker. Beakers were



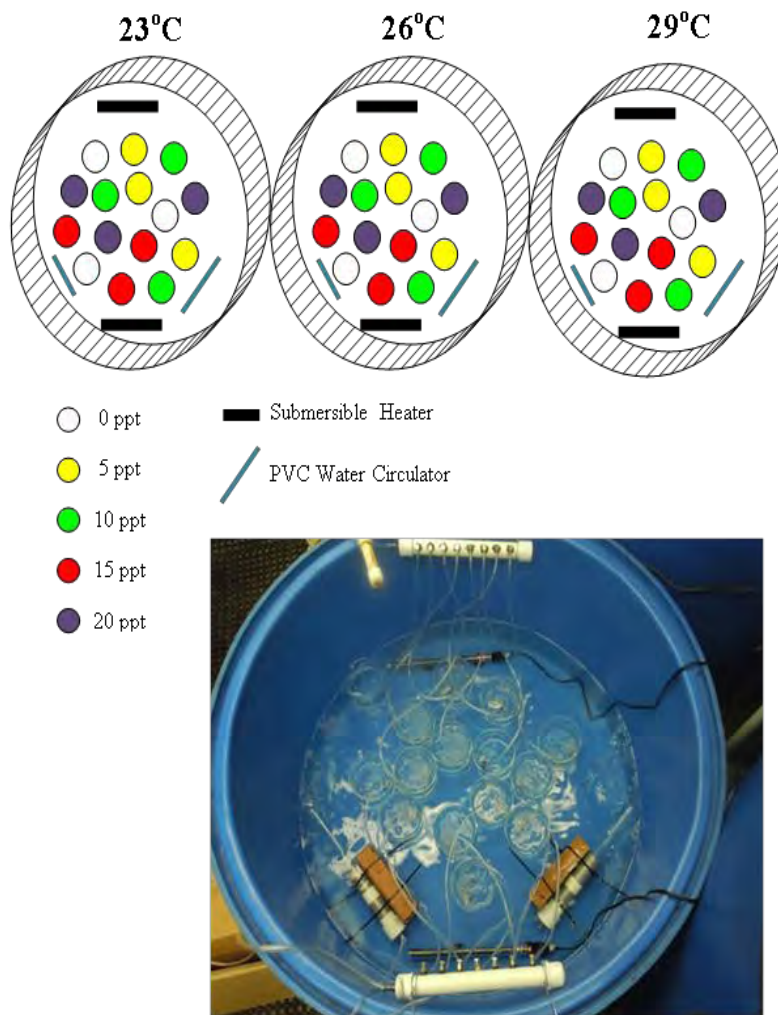


FIGURE 1. The experimental design used for each of the three temperature/salinity experiments comparing bigmouth sleeper hatching, survival, and development at the Maricao Fish Hatchery in 2012.

aerated individually with small (3.81 cm L x 1.27 cm W) airstones (Sweetwater® Air Diffusers, Aquatic Eco-systems, Inc.). The film sections containing adhered eggs were tethered to the airline to provide stability prior to eclosion.

Water quality parameters such as salinity, temperature, dissolved oxygen, pH, and total ammonia were monitored daily. Target dissolved oxygen content was  $\geq 5 \text{ mg}\cdot\text{L}^{-1}$ , pH levels were maintained between 6.5 and 8, and ammonia was kept at concentrations  $< 0.02 \text{ mg}\cdot\text{L}^{-1}$ . In cases where these target values were not met (e.g., ammonia accumulation  $> 0.02 \text{ mg}\cdot\text{L}^{-1}$ ) and extreme pH fluctuations within the beakers, water was changed by siphoning out approximately 10-25% of water with a flexible PVC tubing (2.54 cm inner diameter) and

adding new water of the same temperature and salinity. Salinity (ppt), temperature ( $^{\circ}\text{C}$ ), dissolved oxygen ( $\text{mg}\cdot\text{L}^{-1}$ ) and pH were measured with an YSI (Yellow Springs Instrument, Model 51B YSI Inc., Yellow Springs, Ohio). Total ammonia ( $\text{mg}\cdot\text{L}^{-1}$ ) was analyzed with a Hach test kit FF-1A (Hach Company, Loveland, Colorado).

*Trial 1: Hatch Success* – Fertilized eggs ( $n=100$ ) were exposed to five salinity treatments (0, 5, 10, 15 and 20 ppt) at three temperatures (23, 26 and  $29^{\circ}\text{C}$ ), with three replicates for each treatment combination. Film sections containing 100 eggs were randomly distributed into the beakers with specific temperature-salinity combinations, where they remained for 24 h post stocking.

Hatch success was calculated as the percent of stocked embryos that hatched after 24 h post-stock. I used a two-way analysis of variance (ANOVA) to determine differences among temperature-salinity treatment combinations. The assumption of normality was tested using a quantile-quantile (Q-Q plot) on the residuals of the replicate means. After validating the assumption of normally distributed populations, F-test statistics were calculated ( $\alpha=0.05$ ). Tukey's studentized range (HSD) test was used as a multiple comparison *post hoc* test to determine which treatments differed. Results from this trial were plotted to illustrate relationships between temperature/salinity and hatch success. Calculations were performed with SAS 9.3 (SAS Institute Inc., Cary, NC, USA.).

*Trial 2: Survival* – Larvae (n=100) were randomly assigned to five salinity treatments (0, 5, 10, 15 and 20 ppt) at three temperatures (23, 26 and 29°C), with three replicates for each treatment. Larvae remained in the temperature-salinity combination until 100% mortality or fully developed mouth structures were observed. Survival rates were calculated as the percentage of survivors by day and analyzed with a two-way analysis of variance (ANOVA) with repeated measures to determine difference among temperature-salinity treatment combinations. The assumption of normality was tested using a quantile-quantile (Q-Q plot) on the residuals of the replicate means. After validating the assumption of normally distributed populations, F-test statistics were calculated ( $\alpha=0.05$ ). Tukey's studentized range (HSD) test was used as a multiple comparison *post hoc* test to determine if any treatments differed. Results from this trial were plotted to illustrate relationships over time between temperature and larval survival. Calculations were performed with SAS 9.3 (SAS Institute Inc., Cary, NC, USA.).

*Trial 3: Growth and Development* – For this trial, larvae (n=100) were randomly assigned to five salinity treatments (0, 5, 10, 15 and 20 ppt) at three temperatures (23, 26 and 29°C), with three replicates for each treatment. Five live larvae from each replicate were randomly sampled (through siphoning) daily until there were no larvae remaining. Dead larvae were removed daily for the duration of this trial.

Growth (total and notochord length, TL and NL, respectively), maximum body height (mm), yolk sac volume (mm<sup>3</sup>) and development of main morphological and functional structures were recorded daily for each larvae (Lahnsteiner et al 2012).

Yolk sac volume was calculated using the formula:

$$V = (\pi/6) \cdot LH^2,$$

where  $V$  is yolk sac volume (mm<sup>3</sup>),  $L$  is the length of the yolk sac (mm), and  $H$  is the height (mm) of the

yolk sac (Blaxter and Hempel 1966; Hart and Purser 1995b).

Larvae were observed, photographed and measured with a Leica EZ- 4D stereo-microscope with integral digital camera, software version 1.8.0. The main morphological and functional structures were described and the days of development were determined considering the moment of hatching as 0 h. All growth variables were analyzed with a mixed model analysis of variance (ANOVA), to account for the random effects of time and determine differences among temperature-salinity treatment combinations. The assumption of normality was tested using a quantile-quantile (Q-Q plot) on the residuals of the replicate means. After validating the assumption of normally distributed populations, F-test statistics were calculated ( $\alpha=0.05$ ). Tukey's studentized range (HSD) test was used as a multiple comparison *post hoc* test to determine which treatments differed. Results from this trial were plotted to illustrate relationships between temperature and growth. Calculations were performed with SAS 9.3 (SAS Institute Inc., Cary, NC, USA.). Results are reported as mean  $\pm$  SE.

## Results

*Water Quality* – For all trials, water quality parameters, including salinity, temperature, dissolved oxygen, pH and total ammonia, were maintained within target ranges. Temperatures ranged 23  $\pm$  0.2°C, 26  $\pm$  0.2°C and 29  $\pm$  0.1°C, per respective trial. Dissolved oxygen content averaged 6.8  $\pm$  1.5 mg·L<sup>-1</sup>, mean ( $\pm$  SE) pH was 7.0  $\pm$  1.5, and total ammonia never exceeded 0.01 mg·L<sup>-1</sup>.

*Trial 1: Hatch Success* – Hatch success was affected by temperature ( $F_{2, 44}=7.967$ ,  $P=0.002$ ). Tukey's HSD comparisons revealed a difference between temperatures 26°C and 23°C; however, no other paired comparisons between temperature treatments were different (Table 1). Salinity concentrations did not affect bigmouth sleeper larval hatch success ( $F_{4,44}=0.699$ ,  $P=0.599$ ). However, the combination of lower temperatures (23°C) and salinity concentrations 0, 5 and 10 ppt increased hatch success (87.0  $\pm$  5.3,  $P<0.001$ ; 76.5  $\pm$  9.5,  $P=0.041$ ; and 66.0  $\pm$  4.0,  $P=0.016$ , respectively; Figure 2). In addition to the statistical differences observed in hatching success, a qualitative difference in the larvae produced was observed. Larvae hatched at 0 ppt salinity displayed much greater motility, with active swimming in the water column immediately following hatch. Conversely, larvae hatched at higher salinities displayed reduced motility or were completely immobile following hatch.

*Trial 2: Survival* – Salinity affected larval survival ( $P<0.001$ ). Larvae held at 5, 10, 15, and 20 ppt did not survive past 8 to 12-h post hatch. Larval survival at 0 ppt salinity was not affected by temperature ( $F_{2,35} = 0.150$ ,  $P=0.219$ , Figure 3). However, despite lack of statistical significance in larval survival between temperature treatments, there appeared to be a tradeoff between survival and development. Survival was prolonged for larvae set at 23°C, but larvae appeared underdeveloped at time of mortality (120-h post hatch) compared to the other treatment groups. Survival was similar at 26°C up to 120-h post hatch (Figure 3), and larvae exhibited greater development than at 23 °C. However, fully developed mouth structures were not observed at time of mortality (120-h). Larvae set at 29°C did not survive past 96-h post hatch, the yolk sac was completely absorbed, and mouth structure was evident but not fully developed. For all temperature treatments at 0 ppt salinity, larvae displayed a swim burst pattern, swimming vigorously towards the surface then drifting head first towards the bottom; however, swimming intensity varied with temperature, as temperature increased, intensity decreased.

*Trial 3: Growth and Development* – Daily growth of bigmouth sleeper larvae was affected by temperature in terms of total length (TL,  $P<0.001$ ) and notochord length (NL,  $P<0.001$ ). Mean total lengths at 26 and 29°C were greater than 23°C (Table 2), however there were no differences between 26 and 29°C (Figure 4). Notochord mean lengths at 29°C were greater than at 23°C (Table 2 and Figure 5).

Yolk sac volume was affected by temperature ( $P<0.001$ ), but the observed differences were primarily driven by the 24 h post hatch sample ( $P<0.001$ ). There was no difference in means beyond this point in time (Figure 6). Overall, there was no significant correlation between temperature and yolk sac volume ( $R= -0.099$ ,  $N=48$ ,  $P=0.544$ ). There was a negative correlation between yolk sac volume and TL and NL ( $R= -0.274$ ,  $N=48$ ,  $P=0.088$  and  $R= -0.185$ ,  $N=48$ ,  $P=0.253$ , respectively).

Maximum body height was affected by temperature ( $P=0.010$ ). Mean body heights were greater in larvae reared at 26 and 29°C (Figure 7). Additionally, there were significant positive correlations between body height, total length (TL,  $R= 0.661$ ,  $N=48$ ,  $P<0.001$ ), and notochord length (NL,  $R=0.601$ ,  $N=48$ ,  $P<0.001$ ), as total length and notochord length increased, body height increased.

Salinity greatly inhibited survival, and larvae did not survive beyond 12 h post hatch at salinities  $> 0$  ppt. Furthermore, salinity strongly affected size at hatch and growth up to 12 h post hatch ( $P<0.001$ ). Size at hatch was greatest for 0 ppt larvae, and no changes in length and yolk sac volume were observed for larvae following hatch at higher salinities (Table 3). Moreover, larvae held at greater salinities did not display the same swim pattern observed for larvae held at 0 ppt. Instead, larvae remained motionless on the bottom in a curled position, and rapidly began to lose their translucence. Evidence of yolk sac rupture was also observed.

TABLE 1. Tukey’s studentized (HSD) results for bigmouth sleeper hatch success (trial 1) at varying temperatures (23, 26, 29°C) and salinities (0, 5, 10, 15, 20 ppt) conducted at the Maricao Fish Hatchery, Puerto Rico, from May to September, 2012. Means with the same or grouped letters are not significantly different.

| Tukey grouping          | Mean (%) | N  | Temperature |
|-------------------------|----------|----|-------------|
| <b>Total length</b>     |          |    |             |
| A                       | 65.1     | 12 | 23          |
| B                       | 47.4     | 12 | 29          |
| B                       | 35       | 12 | 26          |
| <b>Notochord length</b> |          |    |             |
| A                       | 56.1     | 12 | 0           |
| A                       | 51.7     | 12 | 10          |
| A                       | 49.8     | 12 | 5           |
| A                       | 47.8     | 12 | 20          |
| A                       | 40.6     | 12 | 15          |

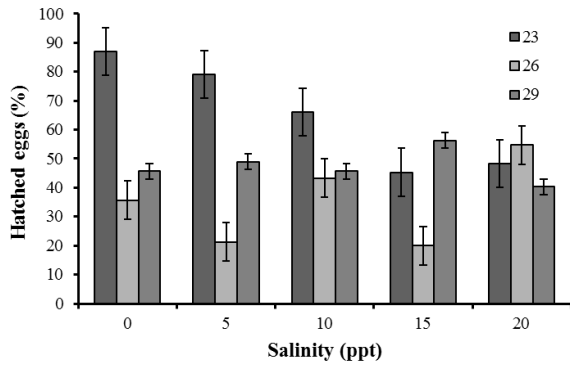


FIGURE 2. Percent of hatched larvae ( $\pm$ SE) for all temperatures (23, 26 and 29°C) and salinity concentrations (0, 5, 10, 15, 20 ppt) during trial 1 conducted at the Maricao Fish Hatchery from May to September 2012.

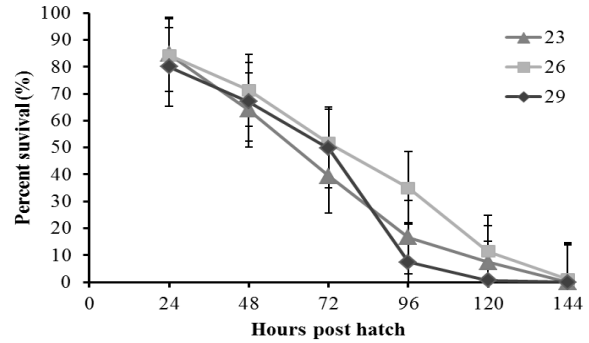


FIGURE 3. Mean survival ( $\pm$ SE) of bigmouth sleeper larvae for all temperatures (23, 26 and 29°C) at 0 ppt during trial 2 conducted at the Maricao Fish Hatchery from May to September 2012.

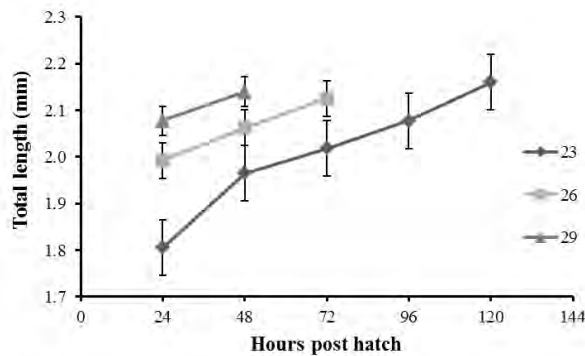


FIGURE 4. Mean total length ( $\pm$ SE) of bigmouth sleeper larvae for all temperatures (23, 26 and 29°C) at 0 ppt during trial 3 conducted at the Maricao Fish Hatchery from May to September 2012.

**Larval Morphological Development** – Newly hatched larvae from the 0 ppt treatment measured 1.00 to 1.48 mm TL ( $1.34 \pm 0.06$  mm, N=12) at 23°C, 1.76 to 1.89 mm TL ( $1.83 \pm 0.02$  mm, N=12) at 26°C, and 1.90 to 1.97 mm TL ( $1.91 \pm 0.05$  mm, N=12) at 29°C. Larvae had a large translucent yolk sac, ranging from 0.46 to 0.51 mm in diameter, which contained one or multiple oil globules (that coalesce within the first 12 to 24-h post hatch), and yellow-green melanophores on

the posterior periphery of the yolk sac. The mouth and anus were not yet formed. Yellow-green melanophores were also observed on the snout and over the eyes, near the anus and ventrally between the third and fifth pre-anal myomeres and the seventh and ninth post anal myomeres. The lens was formed but poorly defined and was unpigmented. The otic capsules containing the otoliths were visible in all treatment groups.

Twenty-four hours post hatch, larvae reached 1.66-1.90 mm TL ( $1.801 \pm 0.05$  mm, N=12) at 23°C, 1.94 to 2.09 mm TL ( $1.99 \pm 0.03$  mm, N=12) at 26°C and 2.05 to 2.12 mm TL ( $2.08 \pm 0.01$  mm, N=12) at 29°C. Yolk sac decreased in size with increasing temperature and lengths (Figure 8). The eye structure was more defined and protruded on larvae reared at 29°C than those set at 26 and 23°C (Figure 8). Larvae reared at 23°C were smaller in size and less developed. Larvae in all treatment groups had a small pericardial sinus and beating hearts.

Two days post hatch, larvae ranged from 1.89-2.01 mm TL ( $1.97 \pm 0.02$  mm, N=12) at 23°C, 2.03-2.10 mm TL ( $2.06 \pm 0.01$ , N=12) at 26°C, and 2.11-2.18 mm TL ( $2.14 \pm 0.02$  mm, N=12) at 29°C. Yolk sac was greatly reduced across all temperature treatments

TABLE 2. Tukey's studentized (HSD) results for bigmouth sleeper larval growth (trial 3) at varying temperatures (23, 26, 29°C) and salinities (0, 5, 10, 15, 20 ppt) conducted at the Maricao Fish Hatchery, Puerto Rico, from May to September, 2012. Means with the same or grouped letters are not significantly different.

| Tukey grouping          | Mean (mm) | N  | Temperature |
|-------------------------|-----------|----|-------------|
| <b>Total length</b>     |           |    |             |
| A                       | 2.11      | 12 | 29          |
| A                       | 2.06      | 12 | 26          |
| B                       | 1.99      | 12 | 23          |
| <b>Notochord length</b> |           |    |             |
| A                       | 0.298     | 12 | 29          |
| B                       | 0.289     | 12 | 26          |
| B                       | 0.249     | 12 | 23          |

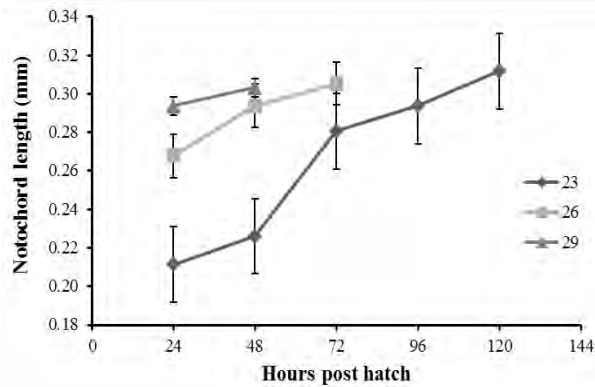


FIGURE 5. Mean notochord length ( $\pm$ SE) of bigmouth sleeper larvae for all temperatures (23, 26 and 29°C) at 0 ppt during trial 3 conducted at Maricao Fish Hatchery from May to September 2012

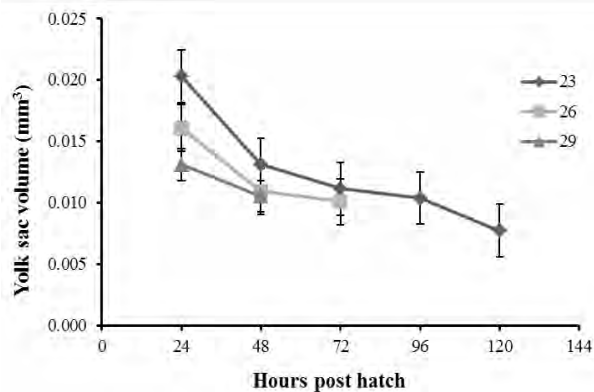


FIGURE 6. Mean yolk sac volume ( $\pm$ SE) of bigmouth sleeper larvae for all temperatures (23, 26 and 29°C) at 0 ppt during trial 3 conducted at Maricao Fish Hatchery from May to September 2012.

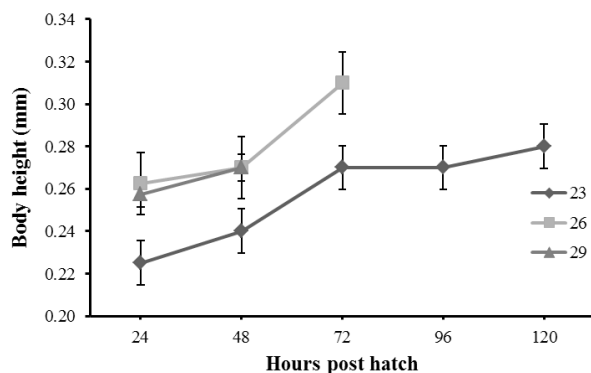


FIGURE 7. Mean maximum body height ( $\pm$ SE) of bigmouth sleeper larvae for all temperatures (23, 26 and 29°C) at 0 ppt during trial 3 conducted at Maricao Fish Hatchery from May to September 2012.

(Figure 9). For all three temperature treatments, growth in length and development of eye structures and pericardial sinuses were apparent. Warmer temperature treatments displayed additional development. At 26°C, the mouth cleft was visible and intestinal tract was starting to develop. For larvae held at 29°C, brain division into prosencephalon, mesencephalon and metencephalon was observed. Mouth, intestinal tract and buccal cavity were evident but not fully developed. Also the pectoral fin fold was visible. Larvae from this treatment group did not survive past 48-h post hatch, and yolk sac was completely resorbed at the time of mortality.

Three days post hatch larvae were  $2.02 \pm 0.04$  mm TL at 23°C and  $2.125 \pm 0.01$  mm TL at 26°C. No larvae remained from the 29°C treatment group. At 23°C, yolk sac was further reduced and larvae increased in size and had a more defined eye, the mouth cleft was visible, and intestinal tract was starting to develop. Brain divisions were vaguely discernable. Larvae held at 26°C had completely consumed the yolk and were surviving on oil globule reserves. The fore-brain, mid-brain, and hind-brain were present. Mouth, intestinal tract, and buccal cavity development were evident but not fully developed. The pectoral fin fold was visible (Figure 8). Larvae from this treatment group did not survive past 72-h post hatch, and the yolk sac was completely reabsorbed before digestive tract and mouths were fully developed.

Larvae set at 23°C grew to  $2.077 \pm 0.01$  mm TL at 96-h and were surviving on oil globule reserves. The fore-brain, mid-brain, and hind-brain were present. Mouth, intestinal tract and buccal cavity development were vaguely evident. At the time of mortality (120-h post hatch), larvae measured up to  $2.16 \pm 0.02$  mm TL, yolk sac was completely consumed, and the only noticeable change between 96-h and 120-h larvae was total length.

## Discussion

Understanding the environmental requirements of fish is crucial to the development of any species for aquaculture (Shi et al. 2010). Three of the most critical environmental factors in early ontogeny of fish are photoperiod, temperature, and salinity (Hart et al. 1995a; Kamler 2002; Shi et al. 2010). For bigmouth sleeper, water temperature and salinity are believed to be primary factors driving reproductive behavior in the wild, given this species' tendency to migrate between cooler freshwater streams and warmer brackish or marine environments. However, limited and often conflicting literature regarding bigmouth sleeper environmental requirements has previously inhibited development of hatchery protocols for this species.

TABLE 3. Mean total length, notochord length, and body height (mean  $\pm$  SE, N=12) of bigmouth sleeper at hatch and 12-h post hatch across temperature (23, 26 and 29°C) and salinity (0, 5, 10, 15, 20 ppt) treatments. Studies conducted at the Maricao Fish Hatchery, Puerto Rico, from May to September, 2012.

| Temperature     | Salinity | Total length    | Notochord length | Body height     |
|-----------------|----------|-----------------|------------------|-----------------|
| 0-h post hatch  |          |                 |                  |                 |
| 23              | 0        | 1.34 $\pm$ 0.06 | 1.26 $\pm$ 0.07  | 0.23 $\pm$ 0.03 |
|                 | 5        | 1.25 $\pm$ 0.05 | 1.14 $\pm$ 0.04  | 0.22 $\pm$ 0.02 |
|                 | 10       | 1.16 $\pm$ 0.04 | 1.06 $\pm$ 0.04  | 0.19 $\pm$ 0.01 |
|                 | 15       | 1.13 $\pm$ 0.03 | 0.99 $\pm$ 0.05  | 0.19 $\pm$ 0.02 |
|                 | 20       | 1.02 $\pm$ 0.03 | 0.96 $\pm$ 0.02  | 0.20 $\pm$ 0.01 |
| 26              | 0        | 1.83 $\pm$ 0.02 | 1.69 $\pm$ 0.05  | 0.25 $\pm$ 0.05 |
|                 | 5        | 1.54 $\pm$ 0.04 | 1.41 $\pm$ 0.08  | 0.24 $\pm$ 0.01 |
|                 | 10       | 1.46 $\pm$ 0.06 | 1.35 $\pm$ 0.05  | 0.20 $\pm$ 0.01 |
|                 | 15       | 1.19 $\pm$ 0.06 | 1.05 $\pm$ 0.03  | 0.17 $\pm$ 0.02 |
|                 | 20       | 1.09 $\pm$ 0.03 | 0.90 $\pm$ 0.04  | 0.16 $\pm$ 0.03 |
| 29              | 0        | 1.91 $\pm$ 0.05 | 1.86 $\pm$ 0.02  | 0.28 $\pm$ 0.04 |
|                 | 5        | 1.78 $\pm$ 0.07 | 1.69 $\pm$ 0.01  | 0.20 $\pm$ 0.04 |
|                 | 10       | 1.52 $\pm$ 0.06 | 1.28 $\pm$ 0.05  | 0.19 $\pm$ 0.02 |
|                 | 15       | 1.19 $\pm$ 0.07 | 0.97 $\pm$ 0.07  | 0.18 $\pm$ 0.03 |
|                 | 20       | 0.96 $\pm$ 0.08 | 0.90 $\pm$ 0.06  | 0.16 $\pm$ 0.08 |
| 12-h post hatch |          |                 |                  |                 |
| 23              | 0        | 1.80 $\pm$ 0.05 | 1.72 $\pm$ 0.65  | 0.24 $\pm$ 0.04 |
|                 | 5        | 1.68 $\pm$ 0.06 | 1.56 $\pm$ 0.08  | 0.22 $\pm$ 0.03 |
|                 | 10       | 1.48 $\pm$ 0.06 | 1.37 $\pm$ 0.07  | 0.19 $\pm$ 0.02 |
|                 | 15       | 1.34 $\pm$ 0.03 | 1.18 $\pm$ 0.06  | 0.18 $\pm$ 0.01 |
|                 | 20       | 1.12 $\pm$ 0.02 | 1.06 $\pm$ 0.01  | 0.20 $\pm$ 0.03 |
| 26              | 0        | 1.99 $\pm$ 0.03 | 1.79 $\pm$ 0.03  | 0.28 $\pm$ 0.03 |
|                 | 5        | 1.84 $\pm$ 0.08 | 1.71 $\pm$ 0.10  | 0.24 $\pm$ 0.04 |
|                 | 10       | 1.47 $\pm$ 0.08 | 1.35 $\pm$ 0.06  | 0.20 $\pm$ 0.02 |
|                 | 15       | 1.21 $\pm$ 0.07 | 1.07 $\pm$ 0.08  | 0.17 $\pm$ 0.03 |
|                 | 20       | 1.11 $\pm$ 0.01 | 0.94 $\pm$ 0.70  | 0.16 $\pm$ 0.01 |
| 29              | 0        | 2.08 $\pm$ 0.01 | 1.99 $\pm$ 0.05  | 0.27 $\pm$ 0.04 |
|                 | 5        | 1.80 $\pm$ 0.09 | 1.70 $\pm$ 0.11  | 0.21 $\pm$ 0.02 |
|                 | 10       | 1.53 $\pm$ 0.05 | 1.30 $\pm$ 0.35  | 0.19 $\pm$ 0.02 |
|                 | 15       | 1.19 $\pm$ 0.06 | 0.97 $\pm$ 0.37  | 0.19 $\pm$ 0.02 |
|                 | 20       | 0.98 $\pm$ 0.03 | 0.92 $\pm$ 0.29  | 0.16 $\pm$ 0.01 |

Therefore, the goal of this study was to investigate the effects of temperature and salinity on bigmouth sleeper larval ontogeny in order to determine optimal culture conditions. The overall conclusion of this research determined that bigmouth sleeper hatch success, survival, and larval development were predictably affected by environmental conditions in this study.

The effect of water temperature and salinity on bigmouth sleeper hatch success was synergistic. Neither factor appeared to singularly control hatch rate in bigmouth sleeper eggs, but the combination of low temperature (23°C) and lower salinity (0, 5, 10 ppt) produced the most successful hatches. Hart and Purser (1995b) reported a similar trend towards higher hatch rates in combinations of low temperature/low salinity and high temperature/ high salinity in greenback flounder *Rhombosolea tapirina*. The current study supported this finding on the low temperature/salinity end, but did not find an increase in hatch success at the upper end of the temperature/salinity range. Despite the tendency for greater hatch success at lower temperatures and salinities, this research demonstrated that bigmouth sleeper eggs will hatch successfully over a range of water temperatures and salinities.

Following hatching, salinity was the primary factor determining immediate survival, with no larvae surviving beyond 12-h post hatch in salinities greater than 0 ppt. This rapid mortality was most likely associated with the energy cost of osmoregulation. Metabolic demand of larvae increases in hyperosmotic environments as they attempt to maintain homeostasis of body fluids. Therefore, larvae in hyperosmotic environments need to divert more energy into osmoregulation rather than growth. Temperature can exacerbate osmoregulatory processes and salinity tolerance in fish (Overton et al. 2008), but the rapid 12-h post-hatch mortality experienced in all salinity treatments exceeding 0 ppt prevented analysis of potential temperature/salinity interactions. However, within the 0 ppt salinity treatment, warmer water temperatures resulted in more rapid yolk sac depletion, leading to more rapid mortality.

Salinity also affected size at hatch. Newly hatched larvae at 0 ppt salinity were significantly larger than larvae reared at salinities >5 ppt. The reduced growth of larvae in hyperosmotic environments have been documented for several species, such as sea bass *Dicentrarchus labrax*, (Johnson and Katavic 1986), Australian snapper *Pagrus auratus* ( Fielder et al. 2005), and obscure puffer *Takifugu obscurus* (Shi et al. 2010). Although the bigmouth sleeper is normally a diadromous species, the greater size at hatch for larvae reared in freshwater may be related to the source of

broodfish in this study. In Carite Reservoir, where this species is completely landlocked, successful spawning, hatching, and rearing occurs exclusively 0 ppt salinity. Optimum propagation salinities are generally similar to natural spawning conditions, as has been demonstrated for some other fish species including *Scophthalmus maximus* (Kulhmann and Quantz 1980), tawny puffer *Takifugu flavidus* (Zhang et al. 2010), and pomfret *Pampus punctatissimus* (Shi et al. 2008). However, salinity preferences may vary with developmental stages and should be further explored.

The survival results from this study contradict previous findings for this species, which reported low larval survival in freshwater. In 2008, 48-h survival of larvae held in freshwater was near 0%, while 90-95% of larvae held at 6 and 12 ppt salinity survived the period (Neal et al. 2009). Those studies were repeated in 2009, and found equally good survival between 0, 4, 5, 6, and 12 ppt salinities through the first 72-h post hatch. Conversely, the current study found that only larvae reared in freshwater (0 ppt) survived beyond the first 12-h. This lack of consensus between salinity trials is perplexing. Broodstock for all of these trials were collected from Carite Reservoir, which is an unusual system in that it has a landlocked population of the normally diadromous bigmouth sleeper. Because this species has been landlocked for over 90 years, which may represent 30 to 45 generations, it is possible that selective pressures are slowly increasing the prevalence of genes that allow freshwater spawning. Whereas survival was similar across replicates, the results appear to represent genetic differences between each cohort of larvae. Larvae within each of these studies were progeny from a single bigmouth sleeper pair, with a different pair used per trial. Thus, differences between larval cohorts may represent genetic differences within the parent population. It may be possible that there is a recessive gene that supports freshwater spawning and survival that is becoming more prevalent in the Carite Reservoir population. Although this is a speculation based on the observed variability in survival, further study of this possibility is warranted.

Larval development was positively correlated to water temperature, but at the expense of yolk sac reserves. Temperature plays a dominant role in the efficiency with which yolk is converted to body tissue (Blaxter 1969; Johns et al. 1981). Generally, yolk sac utilization is improved at an intermediate range of temperatures within the tolerance zone (Johns et al. 1981). At temperatures above and below the tolerance range, metabolic requirements are proportionally greater than those contributing to tissue production,

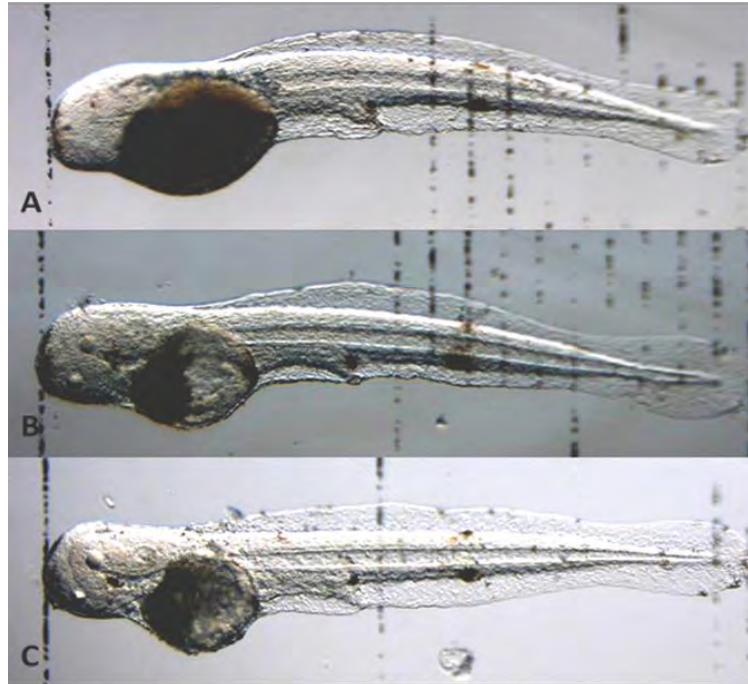


FIGURE 8. Development of bigmouth sleeper larvae at 24-h post hatch for temperature treatments a) 23, b) 26, and c) 29°C at 0 ppt salinity during trial 3 conducted at the Maricao Fish Hatchery from May to September 2012.

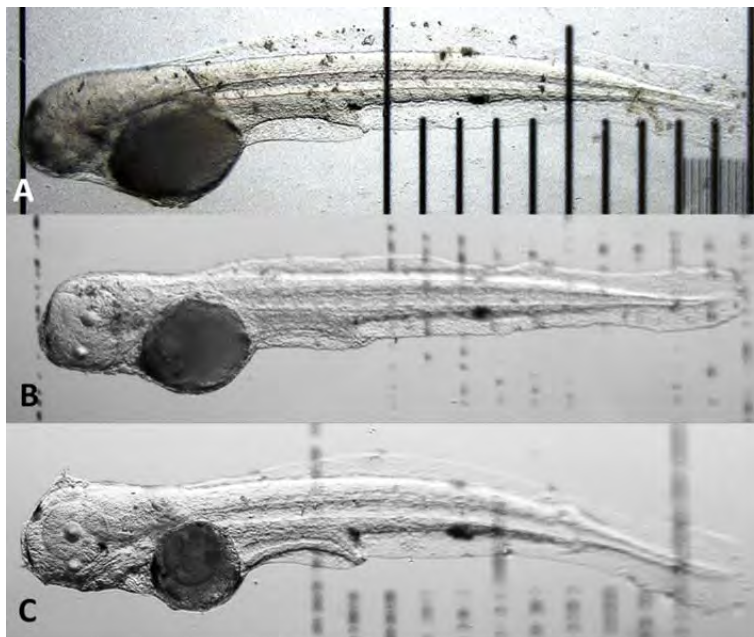


FIGURE 9. Development of bigmouth sleeper larvae at 48-h post hatch for temperature treatments a) 23, b) 26, and c) 29°C at 0 ppt salinity during trial 3 conducted at the Maricao Fish Hatchery from May to September 2012.



resulting in smaller larvae (Blaxter 1969; Johns et al. 1981). In this study, the rate of yolk absorption and the timing of the appearance of external morphological structures were accelerated at higher temperatures. This is evident in larvae reared at 26°C, in which the rate of yolk absorption and the timing of appearance of morphological structures were slightly slower than larvae set at 29°C. This suggests that development and metabolic demands were not as high, which in turn prolonged larval survival an additional 24 to 48-h. Larvae reared at 29°C developed very rapidly within the first 48-h post hatch and, at time of mortality, larvae possessed a more complete digestive tract and a visible jaw structure. On the other hand, larvae held at 23°C survived up to 120-h post hatch, but grew and developed very slowly. Regardless of temperature, no fully developed mouth structure was observed among any of the treatment groups, but the yolk sac had been completely consumed at the time of mortality.

Bigmouth sleeper larvae yolk sac quality and utilization may have been affected by deficiencies in essential vitamins (A & C) and essential fatty acids during the pre and post spawning season. Many of the deficiencies and problems encountered during the early rearing phases of newly hatched finfish larvae are directly related to the diet quality and feeding regime of the broodstock (Izquierdo et al. 2001). A recent assessment on broodstock management at the Maricao Fish Hatchery revealed nutritional deficiencies in bigmouth sleeper broodstock diet, by feeding live prey that were fed poor quality feeds. Embryo survival has been shown to be affected by deficiency of vitamin C content of broodstock diets and vitamin A is important for embryo and larval development due to its important role in bone development, retina formation, and differentiation of immune cells (Izquierdo et al. 2001). Broodstock nutrition is especially important for obtaining healthy larvae and fry, as broodstock nutrition directly influences hatchability and survival of larvae (Izquierdo et al. 2001; Pillay and Kutty 2001). Clearly, rearing protocols for bigmouth sleeper yolk sac larvae would benefit from studies that determine nutritional requirements to ensure optimal yolk sac quality.

Water temperature appeared to influence larval behavior and activity level, particularly swim patterns. As temperature increased, swimming intensity decreased, and larvae reared at lower temperatures displayed a more vivid swim pattern. This does not support findings of Fukuhara (1990), who reported that locomotive activity reached a maximum earlier with increasing temperature, and the level of activity was greater at higher temperatures for the black porgy *Acanthopagrus schlegeli*, the japanese anchovy

*Engraulis japonica*, the red seabream *Pagrus major*, and the olive flounder *Paralichthys olivaceus*.

Salinity also affected larval behavior and has a direct effect on survival through its influence on the amount of energy needed for osmoregulation (Bell and Brown 1995; Howell et al. 1998). Larvae held at 15 and 20 ppt across all temperature treatments were very weak and remained motionless on the bottom of the beakers prior to mortality. Bell and Brown (1995) observed that larvae (0 to 5-d post hatch) of the spotted algae-eating goby *Sicydium punctatum* initially minimized exposure to salinities >10 ppt, but later (5 to 8-d) occupied increasingly saline water. Furthermore, larvae held exclusively in freshwater or seawater treatments ceased activity at 4 to 5 d. This suggests that bigmouth sleeper larvae may require increasing salinity concentrations over time until they are capable of exogenous feeding. This hypothesis is supported by the normally diadromous life history strategy of this species, but it fails to explain why bigmouth sleepers can sometimes complete their life cycle exclusively in freshwater.

A defining and somewhat distinctive feature of the life history of the bigmouth sleeper is that, immediately following hatching, larvae are actively swimming within minutes (Bell and Brown 1995; McDowall 2009), with vigorous bursts towards the surface then drifting head first towards the bottom. This pattern was primarily evident at low salinity. Newly hatched larvae of the spotted algae-eating goby are negatively buoyant but avoid settling to the bottom by active swimming during drift to the sea (Bell and Brown 1995). The swim-up/sink-down behavior has also been observed in several related gobies: the scribbled goby *Awaous guamensis* (Ego 1956), the pacific fat sleeper *Dormitator latifrons* (Todd 1975), and the lyre goby *Evorthodus lyricus* (Foster and Fuiman 1987). This movement is most likely involuntary (McDowall 2009), and contributes to larval downstream transport. As larvae swim up to the surface, they are passively transported towards coastal estuaries. The burst swimming observed for bigmouth sleeper at low salinity likely evolved for the same purpose.

Although no formal description of bigmouth sleeper yolk sac larvae previously existed, studies have been conducted on the development of other larval eleotrid species (Adelsberger 2009). Makeeva (2002) described the development of *Micropercops cinctus* yolk sac larvae, and Yamasaki and Tachihara (2006) described the development of the goby *Stiphodon percnopterygion*, which have similar developmental patterns as bigmouth sleeper larvae. Adelsberger (2009) expressed difficulty in the identification of bigmouth sleeper yolk sac larvae, primarily due to its

small size (0.88-2.04 mm SL), limited meristic information, and a lack of information about larval stages of Eleotrids in literature. Adelsberger based her description on size and myomere counts, as these larvae lacked eye pigment, mouths, and all fins. This study provides additional developmental information for the time between hatching and formation mouth and digestive tract, eye structure, otic capsules (containing the otoliths), pericardial sinus and beating hearts, the divisions of the brain, and pectoral finfold. Although the transition from yolk sac larvae to the larval stage was not achieved, this study provides a basis for future comparisons and further research on the bigmouth sleeper larval early ontogeny.

The scale of this experiment may have limited larval development and survival. The difficulty of egg production and the complexity of the temperature/salinity treatments necessitated use of small sample sizes and limited water volumes. Increasing the number of larvae and water volume may increase the probability of obtaining bigmouth sleeper larvae capable of exogenous feeding.

Even though larvae were not reared to first feeding, this research provided valuable insight into the early life history of bigmouth sleepers and suggests several techniques for improving future propagation success. First, 26°C is the ideal hatching and rearing temperature for both maximizing hatch success and improving early survival of larvae. Thus, propagation of this species at the Maricao Fish Hatchery will require use of heated water or modification to the infrastructure in order to provide this temperature consistently. Second, despite that bigmouth sleepers are normally diadromous, 0 ppt salinity was the most appropriate rearing salinity in this study. However, because research on related species suggests that larvae may experience ontogenetic changes in salinity, experimentation with incremental salinity regimes would be appropriate. Finally, broodstock nutrition should be examined to determine if the developmental issues of the larvae can be resolved via improvements at the broodfish level. Broodstock nutrition directly influences hatchability and survival of larvae (Izquierdo et al. 2001; Pillay and Kutty 2001), and improvements in broodstock nutrition have been shown to greatly improve egg and sperm quality, fecundity, yolk sac quality, and hatching success (Duray et al. 1994). A high quality, nutrient-rich feed must be provided at appropriate feeding regimes. Further research should define the appropriate nutrition requirements for bigmouth sleeper.

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## Chapter 4: Comparison of Active and Passive Young-of-Year Fish Sampling Gears in a Tropical Reservoir

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**Abstract:** Young-of-year fish sampling is an important tool for predicting recruitment success and year-class strength of cohorts within managed fish populations. In Puerto Rico, limited research has been conducted on young-of-year sampling with no studies addressing reservoir systems. In this study, we compared the efficacy of passively-fished light traps and actively-fished push nets for determination of diversity and relative abundance of limnetic fishes in tropical reservoirs. Diversity of catch between push nets and light traps were similar, although species composition of catches differed between gears ( $F = 33.42$ ;  $P < 0.001$ ) and among seasons ( $F = 4.29$ ;  $P < 0.006$ ). Push net catches were dominated by threadfin shad, comprising 94.2% of total catch. Conversely, light traps collected primarily channel catfish (76.8%), with threadfin shad a distant second (13.8%). Light trap catches had greater species evenness in comparison to push nets, although their efficiency may be limited to presence/absence of species. Thus, gear selection should be based on research goals, with push nets an ideal gear for threadfin shad YOY sampling, and light traps more appropriate for community presence/absence sampling. The use of both gears concurrently would give a more complete picture of young-of-year fish communities, as well as help to alleviate existing selectivity biases.

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Young-of-year (YOY) fish sampling can be conducted with active and passive sampling methods, and there are a number of different gears that are used for both methods. Active larval sampling methods include seining, trawling, electrofishing, plankton net tows, and pumping (Kelso and Rutherford 1996). Though efficiency of these methods has improved greatly since the beginning of use in the early 1800's, some drawbacks to active sampling include the possibility of damaging YOY specimens and high operational cost, including significant labor requirements and increasing fuel costs. Passive YOY sampling methods include drift sampling, activity traps, and light traps. Passive gears are less expensive and require less operational costs than active gears (Kelso and Rutherford 1996). However, passive gears are stationary and selective, requiring YOY to encounter the gear through normal activity or by attracting the specimens.

Push nets, mounted to the bow of the boat and actively fished, can be used to effectively sample both limnetic and littoral YOY fish communities (Claramunt et al. 2005). When compared to traditionally towed nets of the same size, push nets show higher efficiency

and effectiveness in collecting YOY fishes (Claramunt et al. 2005; Overton and Rulifson 2007; Fryda et al. 2008). Light traps are a passive gear that has been described as "selective but useful devices" for sampling YOY fishes (Doherty 1987). While light trap utility may be limited in determining associated YOY fish densities, they can be an effective means of determining species presence or absence and relative abundance (Niles and Hartman 2007). Furthermore, Neal et al. (2012) successfully used light traps to sample YOY in Puerto Rico streams, including larvae and juveniles of native fish species.

Due to the paucity of information regarding gear selectivity by YOY fishes within Puerto Rico reservoirs, this research was designed to determine the efficacy of push nets and light traps in sampling the YOY fish community in Carite Reservoir. This research aimed at setting the foundation of YOY fish sampling in Puerto Rico by giving insight into gear selectivity and seasonality of YOY fish distributions within limnetic habitats of a tropical reservoir.

## Methods

*Study Site* -- Located in the mountainous south-central region of Puerto Rico, Carite Reservoir is a 124-ha impoundment on the La Plata River, formed by the construction of an earthen dam in 1913. The reservoir is situated at 18°04'N and 66°05'W at an elevation of 543.6 m above sea level at full pool. The drainage area of Carite Reservoir is comprised of mainly forest and encompasses 21.2 km<sup>2</sup> (Carvajal-Zamora 1979). Today Carite Reservoir provides water and electricity to meet the demands for domestic, industrial and agricultural needs in the area, and has light sport and consumptive fishing pressure.

*Sampling Gears* – One active and one passive gear type was chosen for YOY sampling comparison. Light traps were used as a passive gear and push nets were used as an active gear. Light traps were chosen because of their previously reported success for sampling Puerto Rico fishes (Neal et al. 2012), and push nets were chosen based on higher efficiencies and ease of use by a two-member crew (Claramunt et al. 2005; Overton and Rulifson 2007). The selected light traps were modified quatrefoil traps (Aquatic Research Instruments, Hope, ID, USA) that consisted of an acrylic trapping assembly with an internal polycarbonate tube where the light source was located. The units were 30 × 30 × 25 cm (length-width-height) and had a 7-mm gap on all four sides to allow organisms inside the trap (Figure 1). Young-of-year fish were collected at the bottom of the trap in a 250- $\mu$ m mesh plankton sock. The light source inside was a submersible battery powered LED dive light. A battery-powered source of light was chosen over chemical light sticks due to diminishing luminance found in chemical light sticks for sample periods exceeding 1 h (Kissick 1993).

The push nets were paired 0.5-m diameter bongo-style plankton nets. Nets were composed of 200- $\mu$ m mesh and measured 2 m in length. This mesh size was chosen for the possibility of catching yolk-sac larvae. The terminal end of the net was fitted with an 11-cm diameter removable PVC collection cup, also fitted with 200- $\mu$ m mesh. The push-nets were deployed from a boom system 1.5 m off the bow of the boat, and approximately 1 m below the surface of the water. The boom was designed to allow the push-nets to be pulled from the water to empty the collection cups after a push sample was completed. Push net hauls were conducted at a speed of 1 m/s in an attempt to preserve yolk-sac larvae (Colton et al. 1980; Claramunt et al. 2005). Colton et al. (1980) showed that sampling bongo-style push nets at slower towing speeds, which in turn lessens filtration pressure, increased undamaged yolk-sac larvae by almost fifty percent.



FIGURE 1. Modified quatrefoil light trap.

*Pilot Study* – When estimating larval and juvenile fish population abundances, it is important that these estimates be both accurate (unbiased) and precise (low variance; Cyr et al. 1992). Therefore, a preliminary study was conducted in December 2010 to determine the coefficient of variation (CV) for different time intervals of push net hauls. Push net hauls were conducted for time intervals of 1, 2.5, 5, 7.5, 10, and 15 min. The order in which the time intervals were sampled was chosen at random. Three replicates of each time interval were conducted throughout the night. Paired push nets were treated independently for each of the 18 hauls (N=36).

Young-of-year fish were only caught in 5, 10 and 15-min hauls. Of the 36 total replicates, a total of 8 YOY fish were collected. Low catch rates were

presumably due to time of year, which was likely outside of the spawning seasons of most species present. Though this is not a large number of YOY, standard error from the mean and CV were still possible. Duration of bongo-push net sampling was set at 10 minutes because the CV during the pilot study was less than 0.5 for 10 minute hauls (Figure 2; Cyr et al. 1992).

*Young-of-year Collection* – Young-of-year fish samples were collected from Carite Reservoir biweekly over the course of one year (N= 26). The reservoir was stratified into three sections: a lower section at the dam and two upper arms. Both gear types were sampled in limnetic habitats within each reservoir section. Limnetic sites were chosen in open water habitats greater than 25 m from shore, with a total depth > 5 m.

Twelve sites were chosen for gear comparison *a priori* for limnetic habitats within each section of reservoir (Figure 3). Prior to each sampling event, two sites were randomly chosen in each section of reservoir for light trap sampling, and one site was chosen at random in each section for push net hauls.

Young-of-year fish collection was conducted at night for proper function of light traps and to reduce visual avoidance of push nets (Tischler et al. 2000; Fryda et al. 2008). Light traps were set at sunset (1800 h), and samples were collected at 6 h intervals, with the first collection at midnight (0000 h) and the final retrieval at sunrise (0600 h). One push net haul was conducted per section during each 6-h light trap set. GPS coordinates were recorded at the start and end of each haul to give a total distance traveled, allowing for an estimate of the total volume of water filtered through push nets.

Young-of-year fish were sacrificed, fixed in 10% formalin and returned to the field station for analysis (Kelso and Rutherford 1996). Specimens were counted, measured for total length and identified to the species using larval and juvenile taxonomic keys (Auer 1982; Wang and Reyes 2008). Various morphological (e.g., myomere and fin ray counts) and meristic (e.g., photophore and melanophore pigmentation) measurements were used to identify YOY fish (Auer 1982). There are multiple periods to be considered in the early stages of fish development; from yolk-sac to pre- and post-flexon larvae through finfold absorption and fin ray development in juvenile fish. For this study, all of these stages are considered YOY fishes (Ahlstrom et al. 1976; Hardy et al. 1978).

*Analysis* – Catch rates of light traps and push-nets could not be compared directly because the volume of water that light traps sample is unknown. As an alternative, frequencies of relative species composition

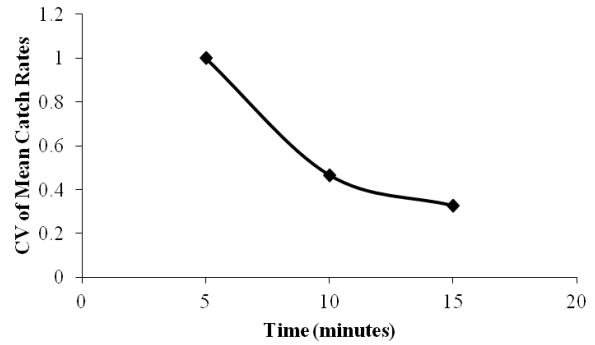


FIGURE 2. Coefficient of variation of mean catch rates of YOY fish at different tow durations during December 2010 in Carite Reservoir, Puerto Rico.

of catch, species diversity using the Shannon-Wiener Index, and species evenness were compared to determine efficacy of sampling gears.

Relative species composition matrices were constructed by summing the total number of fish by species, caught by a particular gear, within each section of reservoir, by season. Total number of species was then divided by the total sum of fish caught by each gear, per section, per season. This resulted in matrices constructed of relative percent of fish species within each section of reservoir for each season. Biweekly samples were combined into four seasons; spring (March-May), summer (June-August), fall (September-November) and winter (December-February).

Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis similarities was used to visually depict similarities in relative percent frequency of species composition between gears and among seasons. NMDS is an indirect gradient analysis that maximizes rank-order correlations between distance measures and distance in ordination space. Permutational multivariate analysis of variance (PERMANOVA) was used to determine differences between gears and seasons based on relative percentage of species compositions (R 2.15.1). Generalized linear mixed models were constructed to determine differences in diversity indices and evenness of catch between gears and among seasons and reservoir sections (SAS 9.2).

## Results

Light traps and push nets each collected a total of eight species of YOY fish. All sunfish were combined and counted as *Lepomis spp.*, due to overlapping meristic and morphometric measurements of the genus. Push nets and light traps collected a total of 1,231 and YOY fish, respectively, from June 2011 to June 2012.



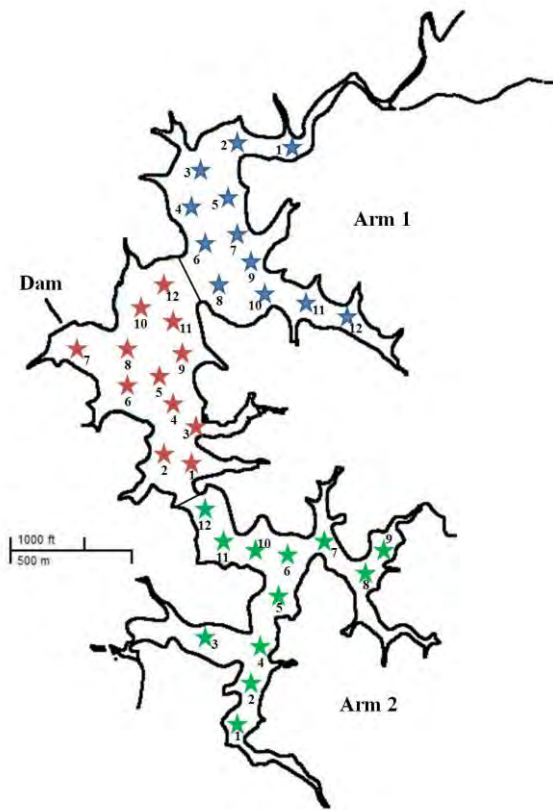


FIGURE 3. Limnetic sampling sites in Carite Reservoir, Puerto Rico.

Push nets sampled a total volume of 19,237 m<sup>3</sup> of water, 289 averaging 64.3 ± 0.4 m<sup>3</sup> per net haul. Although push nets and light traps sampled the same species, the relative frequency of species composition of catches differed between gears (F = 33.42; P < 0.001) and among seasons (F = 4.29; P < 0.006). Push net catches were dominated by threadfin shad *Dorosoma petenense*, comprising 94.2% of total catch, with channel catfish *Ictalurus punctatus* having the next largest percentage of total catch at 2.9% (Table 1). Light trap catches were dominated by channel catfish (76.8%) and threadfin shad (13.8%), which comprised approximately 90% of the total catch (Figure 4).

Young-of-year fish communities showed strong grouping of gear types in the NMDS ordination (Figure 5). Push nets grouped tightly on axis 1 and axis 2 with axis scores ranging from 0.06 to 0.20 (range = 0.14) and -0.07 to -0.01 (range = 0.06), respectively. Threadfin shad had a 0.90 correlation with axis 1 in the positive direction that strongly influenced the push net grouping. Centrarchids (*Lepomis spp.* and largemouth

bass *Micropterus salmoides*) only correlated 0.30 and 0.24 on axis 1, due to low numbers caught in either gear, but potentially influenced the push net grouping.

Light traps did not show as tight a group with axis scores ranging from -0.24 to 0.20 (range = 0.44) and -0.06 to 0.37 (range = 0.31) on axes 1 and 2, respectively. Channel catfish had a strong correlation of 0.97 on axis 1 in the negative direction, and had a strong influence on light trap grouping. White catfish *Ictalurus catus*, redbreast tilapia *Tilapia rendalli*, tiger barb and Amazon sailfin catfish also correlated with axis 1 (-0.28, -0.24, -0.11, and -0.10, respectively). The multitude of species influencing light traps did not allow as tight a grouping as push nets.

Shannon-Wiener diversity indices also differed between gears (F = 10.97; P < 0.001), but showed no difference among seasons (F = 3.36; P > 0.07), reservoir sections (F = 0.48; P > 0.64) or when the effects of gear and season were combined (F = 1.54; P > 0.20). Push nets had greater diversities during the summer (P < 0.005) and fall (P < 0.013; Figure 6). Light traps, however, consistently caught a greater variety of the larval fish community and showed greater evenness for spring (P < 0.001), summer (P < 0.001) and winter (P < 0.032; Figure 7).

## Discussion

Push nets and light traps successfully captured the same eight species, albeit in different proportions. Push nets collected almost four times the total number of YOY fish, although both gears had low diversities due to threadfin shad and channel catfish comprising over 90% of the total catch. Push nets primarily collected threadfin shad and less than 6% of the total catch was the remaining seven species. Diversities were greater for push nets compared to light traps in summer and fall seasons, but this could be attributed to light traps being 80% empty during the summer and catch being comprised mostly of channel catfish in the fall.

Diversity in this study was much lower than reported in Neal et al. (2001), who sampled littoral fish communities in Carite Reservoir using boom-mount and held-held electrofishing systems. Excluding leptomids, which were not identified to species, five of the seven species collected by these two gears represented only 45% of the non-leptomid species reported previously. There were six species collected in 2001 that were not found in the present study, bigmouth sleeper *Gobiomorus dormitor*, Mozambique tilapia *Oreochromis mossambicus*, butterfly peacock bass *Cichla ocellaris*, southern platyfish *Xiphophorus maculatus*, western mosquitofish *Gambusia affinis*, and rosy barb *Pethia conchonius*. Conversely, the current study collected two species that were not reported in

TABLE 1. Total catch and relative percent composition of young-of-year fish caught in light traps and push nets in limnetic habitats in Carite Reservoir, Puerto Rico, from June 2011 through June 2012.

| Taxon                            | Light Traps |      | Push Nets |      |
|----------------------------------|-------------|------|-----------|------|
|                                  | n           | %    | n         | %    |
| <b>Centrarchidae</b>             |             |      |           |      |
| <i>Micropterus salmoides</i>     | 4           | 1.4  | 3         | 0.2  |
| <i>Lepomis spp.</i>              | 1           | 0.4  | 15        | 1.2  |
| <b>Cichlidae</b>                 |             |      |           |      |
| <i>Tilapia rendalli</i>          | 8           | 2.8  | 1         | 0.1  |
| <b>Clupeidae</b>                 |             |      |           |      |
| <i>Dorosoma petenense</i>        | 40          | 13.8 | 1,159     | 94.2 |
| <b>Cyprinidae</b>                |             |      |           |      |
| <i>Puntius tetrazona</i>         | 1           | 0.4  | 2         | 0.2  |
| <b>Ictaluridae</b>               |             |      |           |      |
| <i>Ictalurus punctatus</i>       | 222         | 76.8 | 36        | 2.9  |
| <i>Ameiurus catus</i>            | 4           | 1.34 | 1         | 0.1  |
| <b>Loricariidae</b>              |             |      |           |      |
| <i>Pterygoplichthys pardalis</i> | 9           | 3.1  | 14        | 1.1  |
| <b>All taxa</b>                  | 289         |      | 1,231     |      |

the previous study, these species were Amazon sailfin catfish *Pterygoplichthys pardalis* and tiger barb *Puntius tetrazona*. The discrepancies between the two studies were likely due to differences in life stage and habitat sampled, as the current study targeted exclusively YOY fishes in limnetic habitat. However, the absence of Amazon sailfin catfish and tiger barbs in 2001 may indicate that these two species have more recently colonized the reservoir.

Threadfin shad are considered the principal prey species to sport fish in Puerto Rico reservoirs (Neal et al. 2011), and push nets were the clear choice for collecting this species. Push nets collected about 29 times more threadfin shad than light traps with considerably less effort. Furthermore, bycatch was much lower in push nets compared to light traps (5.9% versus 86.2%, respectively). Prchalová et al. (2012) reported similar findings when they compared frame trawls, an active gear, to gillnets, a passively fished gear. Although seasonal differences were not statistically significant for push nets, this was likely due to large variability in catch. Distinct trends of greater catch of age-0 threadfin shad during the spring season that sequentially decreased through the following seasons were apparent, with an eight-fold decrease in absolute catch between spring and

fall/winter seasons combined. These trends were similar to Neal and Prchalová (2012), who reported greatest abundances of YOY shad in the spring in Puerto Rico reservoirs. Based on the findings of the current study, targeted sampling of threadfin shad YOY should utilize push nets during the spring for maximization of YOY catch.

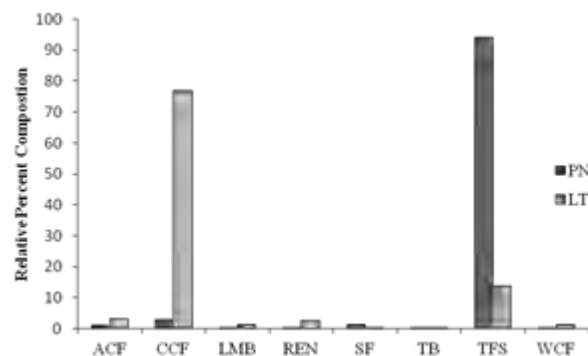


FIGURE 4. Relative percent composition of total catch from limnetic push nets and light traps from June 2011 through June 2012, in Carite Reservoir, Puerto Rico. Abbreviations are Amazon sailfin catfish (ACF), channel catfish (CCF), largemouth bass (LMB), redbreast tilapia (REN), *Lepomis spp.* (SF), tiger barb (TB), threadfin shad (TFS), and white catfish (WCF).

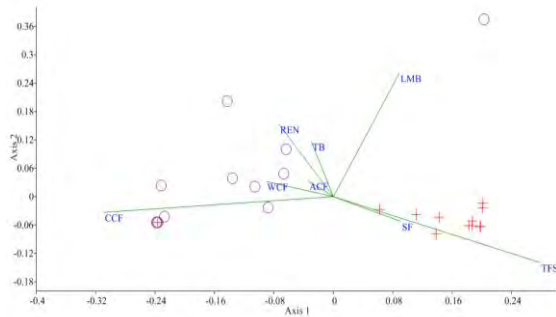


FIGURE 5. NMDS ordination of the relative percent composition of limnetic YOY fish communities sampled with light traps (circles) and push nets (crosses) in Carite Reservoir, Puerto Rico, from June 2011 through June 2012 (Stress: 0.0816;  $R^2$  Axis 1: 0.7271 Axis 2: 0.1723).

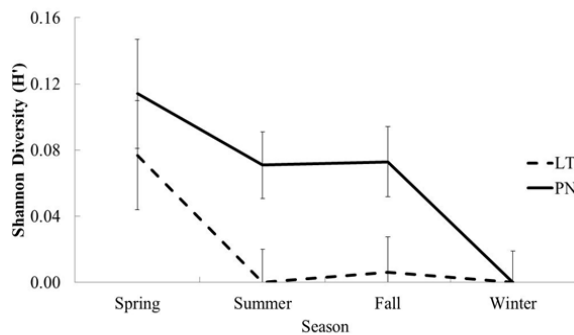


FIGURE 6. Mean  $\pm$  S.E. diversity of limnetic YOY catch for light traps and push nets sampled in Carite Reservoir, Puerto Rico.

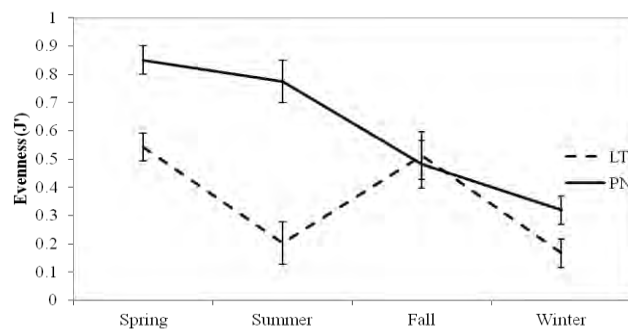


FIGURE 7. Mean  $\pm$  S.E. evenness of limnetic YOY catch for light traps and push nets sampled in Carite Reservoir, Puerto Rico.

Light trap catches had greater species evenness in comparison to push nets, although their efficiency is limited to presence/absence of species because the numbers of YOY fish caught would not be sufficient for attempting abundance estimates (Doherty 1987). As with push nets, trends in light trap catch indicated greater numbers of YOY fish being caught in spring and summer seasons, concurrent with primary

spawning seasons of many species. There is, however, an exception to these trends, with channel catfish catch peaking in both spring and fall seasons, with greatest abundances being caught during fall. This is inconsistent with the literature in that typically channel catfish start spawning earlier in the year (March) at lower latitudes and later in the year (July) at higher latitudes, but do not display more than one spawning season (Stevens 1959; Hubert and O'Shea 1991). Further discussion of this phenomenon is presented with spatiotemporal analyses in Chapter 5.

There are potential biases with sampling the YOY fish community with push nets. Visual avoidance of the gear has been determined to affect sizes of YOY fish captured with push nets (Brander and Thompson 1989; Hickford and Schiel 1999), and although samples were collected at night to lessen avoidance, targeted fish potentially possessed the ability to avoid the nets. Back filtration pressure within the nets could also contribute to possible gear bias. Total amount of water filtered by push nets was estimated using area of net mouth and distance traveled to simplify CPUE computations; however, large numbers of phytoplankton were often collected that could have potentially lessened filtration ability of the nets causing back pressure to build. A flow meter, mounted inside the mouth of the nets would provide greater accuracy of the volume of water filtered as well as indicate if nets were filtering at their greatest capacity (Claramunt et al. 2005).

Both gears efficiently sampled different aspects of the YOY fish community within Carite Reservoir. Due to the style of gear (active or passive) and the proportions of the YOY fish community sampled, these two gears are really not comparable. Selection and use of either of these gears should be based on the research goals and questions to be answered from fisheries managers in Puerto Rico. As suggested by this research and others, the use of both gears concurrently would give a more complete picture of YOY fish communities that inhabit limnetic habitats, as well as help to alleviate existing selectivity biases (Gregory and Powles 1988; Hickford and Schiel 1999).

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## Chapter 5: Spatiotemporal Trends of the Young-of-Year Fish Community in a Tropical Reservoir

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**Abstract:** Understanding spatial and temporal variability in young-of-year fish communities is critical for proper design of sampling protocols and determination of factors influencing recruitment. However, this life stage is rarely sampled and there is a paucity of information regarding distributions of young-of-year freshwater fishes in general. In tropical systems, these data are largely absent. In this study, we examined spatiotemporal trends of YOY fishes across one year using larval light traps. No longitudinal differences were detected between the dam and two arm sections. However, substantial differences occurred spatially between littoral and limnetic light traps ( $F = 9.32$ ;  $P < 0.001$ ), and temporally between seasons ( $F = 5.88$ ;  $P < 0.001$ ). Limnetic light traps were generally dominated by channel catfish (76.8%) and threadfin shad (13.8%), whereas littoral catch was composed of redbreast tilapia (39.5%), largemouth bass (21.4%), Amazon sailfin catfish (12.7%), and threadfin shad (12.1%). Most species displayed peak YOY abundances in late-winter to summer, correlating to known or expected spawning periods. The exception was channel catfish, which displayed a uniquely bimodal YOY periodicity and limnetic habitat usage that contradicts previous descriptions for this species. Based on these findings, we recommend spring and summer littoral light traps for general YOY sampling, and limnetic light traps in spring and fall when channel catfish are the species of interest. Light traps are not recommended for sampling threadfin shad, as this species is more effectively and quantitatively sampled using active gears such as frame trawls and push nets.

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Distributions of fish larvae and juveniles vary spatially and temporally in nature (Chambers and Trippel 1997). Duration of spawning season can vary from a few days to many months, and there can be seasonal variability of spawning intensity due to density independent factors, such as rainfall, floods, and droughts (Kelso and Rutherford 1996). Furthermore, abiotic and biotic factors can have profound effects on the productivity of lentic systems and their biota, and these factors vary through space and time. These chemical and physical characteristics create the abiotic framework in which different species can survive, grow, and reproduce. All of these factors, and more, can and should influence the decision making process of researchers and managers regarding when, where, and how to sample young-of-year (YOY) of individual fish species or the community as a whole.

Temperature, for example, is a physical characteristic that limits where a species can exist. Changes in water temperature, coupled with changes in other abiotic factors, such as hydrology, can induce certain fish species to spawn or influence the success of

recruitment by the YOY fishes (Kjesbu 1994; Nunn et al. 2007). Temperature can also limit growth or survival in YOY fishes, ultimately affecting recruitment success (Saksena et al. 1972; Uphoff 1989).

Another factor to consider is reservoir productivity, which is determined by nutrient availability. Productivity determines plant growth and, subsequently, biomass availability at higher trophic levels. Thus, eutrophic conditions provide greater biomass of forage for planktivorous organisms (Gonzalez et al. 2010), and this energy can move via a 'bottom-up' effect, increasing biomass of organisms at higher trophic states (Allen et al. 1999). Conversely, excessively elevated nutrient input and associated trophic state can lead to water quality deterioration and reduced habitat suitability for many species (Smith et al. 1999; Boesch 2002).

Date of hatch can affect YOY growth rates and survival. A major source of mortality for fish during early life stages is predation (Kim and DeVries 2001;

Legler et al. 2010), and faster-growing YOY may be better able to avoid predation and realize stronger recruitment success (Mills and Mann 1985). Santucci and Wahl (2003) suggested that predation is an important mechanism regulating the recruitment success of young bluegill *Lepomis macrochirus*, and that fish spawned earlier in the season experienced higher mortality than those spawned later. Furthermore, competition can greatly affect the survival and distributions of YOY fishes, as organisms share both space and resources within a system. Hence, competition within a species (intraspecific) and among different species (interspecific) is inevitable. Intense competition among YOY fishes can suppress prey populations, increasing variability in growth and survival of YOY fishes (Mittelbach 1988; Garvey and Stein 1998; Welker et al. 1994). Both predation risk and competitive interactions are regulated, in part, by hatching chronology and growth rates.

Fisheries managers need to have accurate information on spatial and temporal pattern of YOY occurrence in order to better manage recruitment in fisheries. Young-of-year fish sampling can be conducted with active and passive sampling methods, and there are a number of different gears that are used for both methods. Active YOY sampling methods include seining, trawling, electrofishing, plankton net tows, and pumping (Kelso and Rutherford 1996). Passive YOY sampling methods include drift sampling, activity traps, and light traps. Passive gears are less expensive and require less operational costs than active gears (Kelso and Rutherford 1996). However, passive gears are stationary and selective, requiring YOY to encounter the gear through normal activity or by attracting the specimens.

Despite the need for spatiotemporal information of YOY fishes, this life stage is rarely sampled and there is a paucity of information regarding distributions of YOY freshwater fishes in general. In tropical systems, these data are largely absent. Neal et al. (2012) compared YOY sampling methods for Puerto Rico streams, but no directed YOY sampling has occurred in Puerto Rico reservoirs. In the current study, the research was designed to determine spatial and temporal trends in YOY fish community in Carite Reservoir, Puerto Rico. This research aimed at setting the foundation of YOY fish sampling in Puerto Rico by giving insight into habitat selectivity and seasonality of YOY fish distributions within littoral and limnetic habitats of a tropical reservoir.

## Methods

*Study Site*—Located in the mountainous south-central region of Puerto Rico, Carite Reservoir is a 124-ha impoundment on the La Plata River, formed by

the construction of an earthen dam in 1913. The reservoir is situated at 18°04'N and 66°05'W at an elevation of 543.6 m above sea level at full pool. The drainage area of Carite Reservoir is comprised of mainly forest and encompasses 21.2 km<sup>2</sup> (Carvajal-Zamora 1979). Today Carite Reservoir provides water and electricity to meet the demands for domestic, industrial and agricultural needs in the area.

Carite Reservoir has historically been one of the least productive reservoirs in Puerto Rico, and catch rates of all fishes have been consistently low (Carvajal-Zamora 1979; Neal et al. 2001). Neal et al. (2001) found 14 fish species in Carite Reservoir, of which the bigmouth sleeper *Gobiomorus dormitor* was the only native species. Though populations of Bigmouth Sleeper have been documented in a few other reservoirs on the island, Carite Reservoir contains the only suspected land-locked reproducing population in Puerto Rico (Bacheler et al. 2004).

*Sampling Gear* – Light traps were used to sample the YOY fish community in littoral and limnetic habitats. Light traps were chosen because of their previously reported success for sampling Puerto Rico fishes (Adelsberger 2009). The selected light traps were modified quatrefoil traps (Aquatic Research Instruments, Hope, ID, USA) that consisted of an acrylic trapping assembly with an internal polycarbonate tube where the light source was located. The units were 30 × 30 × 25 cm (length × width × height), and had a 7-mm gap on all four sides to allow organisms inside the trap (Chapter 4). Young-of-year fish were collected at the bottom of the trap in a 250- $\mu$ m mesh plankton sock. The light source inside was a submersible battery powered LED dive light. A battery-powered source of light was chosen over chemical light sticks due to diminishing luminance found in chemical light sticks for sample periods exceeding 1 h (Kissick 1993).

*Young-of-year Collection* – Young-of-year fish samples were collected from Carite Reservoir every other week over the course of one year (N= 26). The reservoir was stratified into three sections: a lower section at the dam and two upper arms. Littoral and limnetic habitats were compared within each section of the reservoir. Littoral habitat sites were chosen within 5 m of shore, at a water depth of  $\leq$  2 m (Vasek et al. 2006). Limnetic sites were chosen in open water habitats greater than 25 m from shore, with total depth > 5 m. Twelve sites were chosen *a priori* for each habitat type, within each section of reservoir for YOY sampling (Figures 1 and 2). Prior to each sampling event, two sites were randomly chosen for each habitat type, in each section of reservoir for light trap sampling. Young-of-year fish collection was conducted

at night for proper function of light traps (Tischler et al. 2000). Light traps were set at chosen sites at sunset (1800 h) and samples were collected at 6 h intervals, with the first collection at midnight (0000 h) and the final retrieval at sunrise (0600 h).

Young-of-year fish samples were sacrificed, fixed in 10% formalin and returned to the field station for analysis (Kelso and Rutherford 1996). Young-of-year were counted, measured for total length and identified to the species level using YOY taxonomic keys (Auer 1982; Wang and Reyes 2008). Various morphological (e.g., myomere and fin ray counts) and meristic (e.g., photophore and melanophore pigmentation) measurements were used to identify YOY fish (Auer 1982). There are multiple periods to be considered in the early stages of fish development; from yolk-sac to pre- and post-flexion larvae through finfold absorption and fin ray development in juvenile fish. For this study, all of these stages are considered YOY fishes (Ahlstrom et al. 1976; Hardy et al. 1978).

*Water Quality* – *In situ* water quality parameters were measured concurrently with YOY sampling. Dissolved oxygen (DO; mg L<sup>-1</sup>), pH, specific conductivity (μS cm<sup>-1</sup>), temperature (°C), and turbidity (NTU) measurements were collected at each sample location. Water quality parameters were collected with a Manta™ II Water Quality Multiprobe when each light trap was set and every 6 h throughout the night when light trap samples were collected. The water quality multiprobe was used to record water quality

parameters at the surface and at 1 m at each light trap location.

*Analysis* – Catch of YOY was compared between light traps set in the littoral zone and in the limnetic zone of the reservoir. Relative percent frequency of species composition, species diversity using Shannon-Wiener Index, and catch per effort were (CPUE) compared between the two habitats to determine habitat use by YOY fish. Percent frequency of species composition, species diversity and CPUE comparisons were also conducted temporally, providing insight into seasonality of YOY fish distributions between habitats.

Relative species composition matrices were constructed by summing the total number of fish by species, caught by a particular gear, within each section of reservoir, by season. This number was divided by the total sum of fish caught by each gear, per section, per season. This resulted in matrices constructed of relative percent of fish species within each section of reservoir for each season. Biweekly samples were combined into four seasons; spring (March-May), summer (June-August), fall (September-November) and winter (December-February).

Nonmetric multidimensional scaling (NMDS) of Bray-Curtis matrices was used to determine similarities in species compositions spatially and temporally. NMDS is an indirect gradient analysis that maximizes rank-order correlations between distance measures and distance in ordination space. Permutational

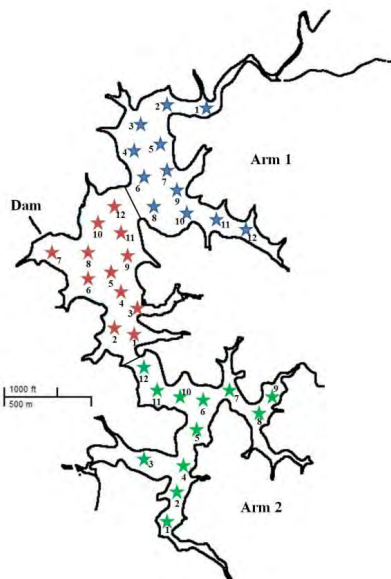


FIGURE 1. Limnetic sampling sites in Carite Reservoir, Puerto Rico.

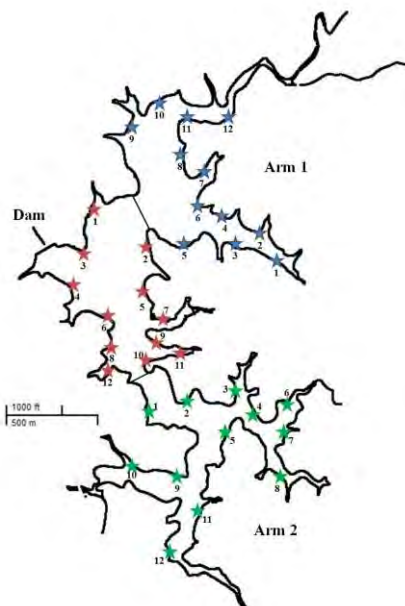


FIGURE 2. Littoral sampling sites in Carite Reservoir, Puerto Rico.



multivariate analysis of variance (PERMANOVA) was conducted on relative species compositions to determine differences in community abundances between habitats and among seasons. Shannon-Wiener diversity indices and catch per unit effort (log transformed) were fit to generalized linear mixed models to determine differences between habitats and among seasons and reservoir sections.

Water quality parameters were averaged per habitat and season. These data were combined with relative percent frequency matrices of the YOY fish community and analyzed with canonical correspondence analysis (CCA). CCA is a direct gradient analysis that ordines species abundances with environmental variables.

## Results

*Habitat Comparison* – A total of 826 YOY fish were collected in the light traps with 537 in the littoral light traps and 289 in the limnetic light traps. Ten species were collected with littoral light traps, which was two more than were caught with limnetic traps (Table 1). Limnetic light trap catch was dominated by two species, channel catfish and threadfin shad, comprising greater than 90% of total catch (76.8% and

13.8%, respectively; Figure 3). Littoral light trap catch was more evenly distributed among the YOY fish community with 90% of total catch being comprised of redbreast tilapia, largemouth bass, Amazon sailfin catfish, threadfin shad and tiger barb (39.5, 21.4, 12.7, 12.1 and 5.0%, respectively).

Young-of-year fish communities showed grouping of different habitats and seasons, mainly among limnetic traps in the NMDS ordination (Figure 4). Limnetic traps grouped more than littoral traps, indicating more similarity of the YOY fish collected. Spring and fall limnetic light traps grouped on axis 1 in the negative direction with a strong influence from channel catfish (-0.90). Spring littoral traps, as well as both littoral and limnetic traps set during summer, grouped on axis 1 in the positive direction. This group was influenced by a multitude of fish species. Redbreast tipalia, largemouth bass, Amazon sailfin catfish and western mosquitofish all influenced this grouping on the positive first axis (0.74, 0.71, 0.57 and 0.32, respectively). Fall littoral traps showed some grouping on the second axis in the negative direction with an influence from tiger barbs (-0.59). PERMANOVA of the relative percent composition of the YOY fish community also supports the different

TABLE 1. Total catch and relative percent composition of young-of-year fish caught with light traps set in littoral and limnetic habitats in Carite Reservoir, Puerto Rico, from June 2011 through June 2012.

| Taxon                            | Littoral |      | Limnetic |      |
|----------------------------------|----------|------|----------|------|
|                                  | n        | %    | n        | %    |
| <b>Centrarchidae</b>             |          |      |          |      |
| <i>Micropterus salmoides</i>     | 115      | 21.4 | 4        | 1.4  |
| <i>Lepomis spp.</i>              | 25       | 4.7  | 1        | 0.4  |
| <b>Cichlidae</b>                 |          |      |          |      |
| <i>Cichla ocellaris</i>          | 2        | 0.4  | 0        | 0.0  |
| <i>Tilapia rendalli</i>          | 212      | 39.5 | 8        | 2.8  |
| <b>Clupeidae</b>                 |          |      |          |      |
| <i>Dorosoma petenense</i>        | 66       | 12.1 | 40       | 13.8 |
| <b>Cyprinidae</b>                |          |      |          |      |
| <i>Puntius tetrazona</i>         | 27       | 5.0  | 1        | 0.4  |
| <b>Ictaluridae</b>               |          |      |          |      |
| <i>Ictalurus punctatus</i>       | 11       | 2.1  | 222      | 76.8 |
| <i>Ameiurus catus</i>            | 2        | 0.4  | 4        | 1.4  |
| <b>Loricariidae</b>              |          |      |          |      |
| <i>Pterygoplichthys pardalis</i> | 68       | 12.7 | 9        | 3.1  |
| <b>Poeciliidae</b>               |          |      |          |      |
| <i>Gambusia affinis</i>          | 9        | 1.7  | 0        | 0.0  |
| <b>All taxa</b>                  | 537      |      | 289      |      |

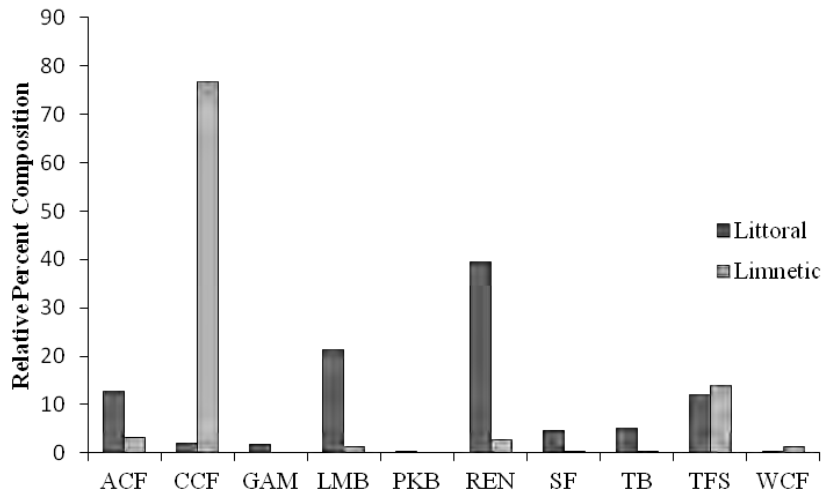


FIGURE 3. Relative percent composition of total catch from light traps set in both littoral and limnetic habitats in Carite Reservoir, Puerto Rico. Abbreviations are Amazon sailfin catfish (ACF), channel catfish (CCF), western mosquitofish (GAM), largemouth bass (LMB), butterfly peacock bass (PCB), redbreast tilapia (REN), *Lepomis spp.* (SF), tiger barb (TB), threadfin shad (TFS), and white catfish (WCF).

groupings observed in the NMDS ordination. The YOY fish community differed between habitats ( $F = 9.32$ ;  $P < 0.001$ ), among seasons ( $F = 5.88$ ;  $P < 0.001$ ), as well as when the effects of habitats and seasons are combined ( $F = 5.00$ ;  $P < 0.001$ ). Limnetic traps were strongly influenced by channel catfish in both spring and fall because this species made up greater than 76% of total catch. Littoral traps were highly influenced by redbreast tilapia and largemouth bass but did not group as tightly due to a greater species richness of the YOY community.

Shannon-Wiener diversity indices differed between habitats ( $F = 29.54$ ;  $P < 0.001$ ), among seasons ( $F = 7.99$ ;  $P < 0.009$ ) and when the effects of habitats and seasons are combined ( $F = 7.53$ ;  $P < 0.001$ ), but showed no difference among reservoir sections ( $F = 0.70$ ;  $P > 0.53$ ). Littoral light traps had greater diversity than limnetic light traps during both the spring ( $P < 0.001$ ) and summer ( $P < 0.001$ ) seasons (Figure 5). However, diversity was low and did not differ between habitats for fall and winter seasons ( $P > 0.6$ ;  $0.7$ , respectively).

Catch per unit effort differed between habitats for spring, summer and fall seasons. Littoral light traps had greater CPUE for spring and summer seasons ( $P < 0.001$ ;  $< 0.002$ , respectively). However, limnetic catch rates were greater during the fall season ( $P < 0.007$ ; Figure 6).

**Water Quality** – The results from the CCA indicate axis 1 had an eigenvalue of 0.2374 and explained 46.7% of the variability among sites and species in relation to water quality parameters, whereas axis 2 had

an eigenvalue of 0.1427 and explained 28.1% of the variance among sites and species, in relation to water quality parameters (Figure 7). Turbidity, pH and temperature correlated with sites and species on axis 1 in the negative direction (-0.60, -0.60 and -0.58, respectively). Dissolved oxygen (0.24) and specific conductivity (0.55) correlated with sites and species on axis 1 in the positive direction. As for axis 2, temperature, specific conductivity and dissolved oxygen negatively correlated with sites and species on the axis (-0.18, -0.38 and -0.52, respectively), while pH and turbidity correlated with sites and species in the positive direction (0.02 and 0.23, respectively).

## Discussion

This research identified clear temporal trends of the YOY fish community in Carite Reservoir. Greatest abundances of YOY fish were caught during spring and summer within littoral habitats. Largemouth bass and redbreast tilapia first appeared in littoral light traps late in the winter season. This is consistent with previous studies that indicate a prolonged period of spawning for largemouth bass in Puerto Rico reservoirs beginning as early as January (Ozen 2002; Neal 2003). Largemouth bass numbers peaked during the spring season, whereas redbreast tilapia had greater abundances late in the spring and throughout the summer seasons. Both of these species are sought after as sport or food fish, and YOY redbreast tilapia are an important food source for YOY largemouth bass (Alicea et al. 1997). Thus, sampling for YOY of these species would be best conducted during spring and summer seasons within littoral habitats.

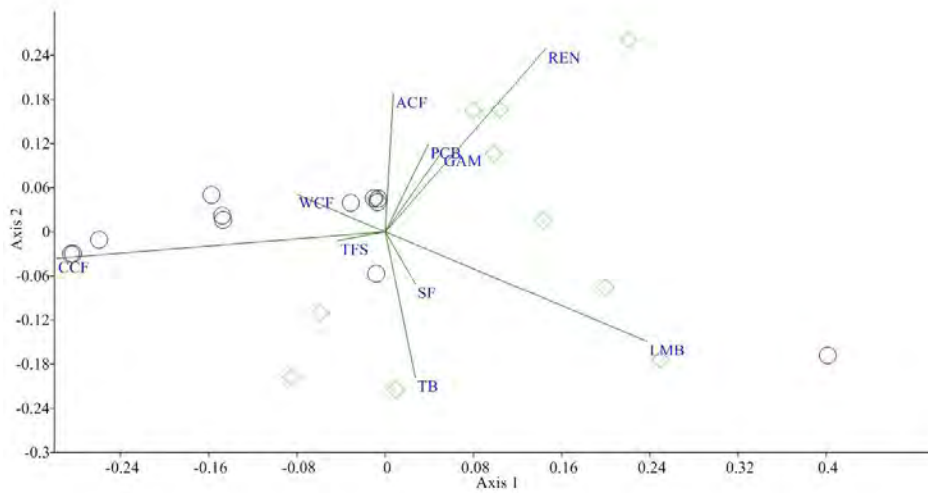


FIGURE 4. NMDS ordination of the relative percent composition of YOY fish communities sampled with light traps within littoral (diamonds) and limnetic (circles) habitats in Carite Reservoir, Puerto Rico, from June 2011 through June 2012 (Stress: 0.1382;  $R^2$  Axis 1: 0.6298 Axis 2: 0.3655).

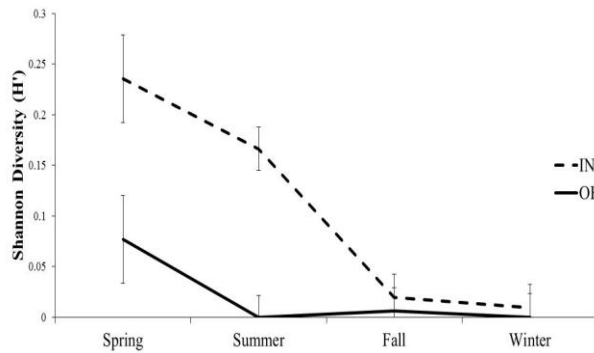


FIGURE 5. Mean  $\pm$  S.E. diversity comparing littoral and limnetic YOY catch for light traps sampled in Carite Reservoir, Puerto Rico.

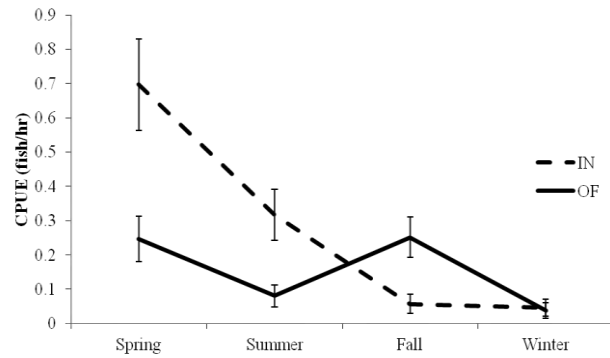


FIGURE 6. Mean catch per unit effort  $\pm$  S.E. comparing littoral and limnetic YOY catch for light traps sampled in Carite Reservoir, Puerto Rico.

Amazon sailfin catfish were present primarily in the late spring and early summer months, indicating that this period is the likely spawning season. This species is considered invasive, therefore knowledge regarding different life history stages could prove useful in potential control efforts for the species (Bunkley-Williams et al. 1994). Due to later appearance in spring and summer, control via predation by piscivorous species presents an opportunity for management of this invasive species. Early spawned and faster growing piscivores, such as largemouth bass, would have the ability to consume YOY Amazon sailfin catfish as they are produced, lessening recruitment into the adult population.

Threadfin shad are considered a primary forage species in Puerto Rico reservoirs (Neal and Prchalova

2012), and were collected in light traps set in both littoral and limnetic habitats. However, the low total catch and relatively low percent composition of this species, combined with its mobility and schooling tendencies, reduces the utility of light traps for making inferences regarding population dynamics. Threadfin shad are a schooling limnetic species and therefore a passive gear such as light traps may not be appropriate for sampling this species (Schael et al. 1995). The patchiness of shad distributions require schools to encounter the trap, whereas encountering schooling shad would have a higher probability with an active sampling gear (Prchalova et al. 2012), such as Bongo-style push nets (Chapter 4).

Channel catfish catch peaked in both spring and fall seasons indicating two separate spawning periods

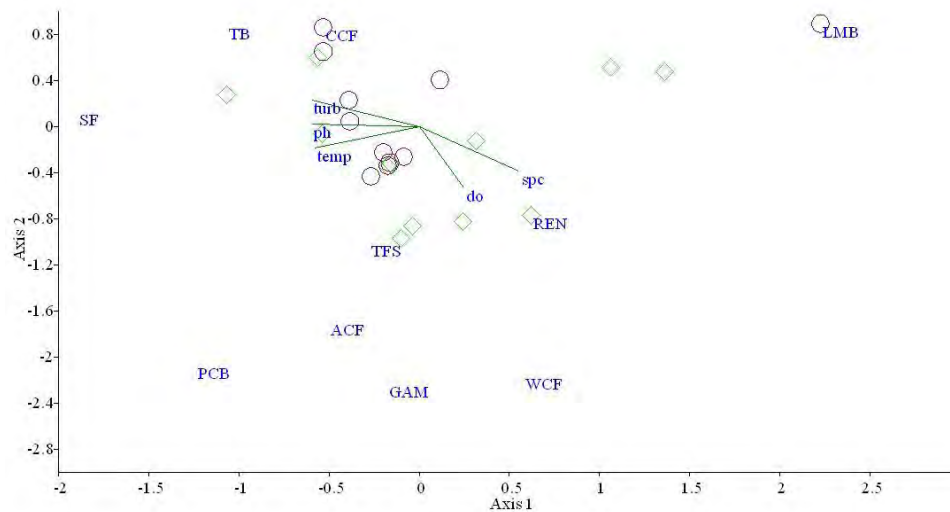


FIGURE 7. CCA of site (littoral [diamonds] and limnetic [circles]) and species matrices correlated with water quality parameters from Carite Reservoir, Puerto Rico, from June 2011 through June 2012.

during the year. The length distribution among all seasons was the same with catfish measuring between 15 – 20 mm, further indicating protracted spawning. In temperate climates where channel catfish are native, these fish typically spawn once per year in late spring (Hubert 1999) when temperatures reach 21-29°C, with 27°C being considered optimal for spawning (Clemens and Sneed 1957; Walsh and Lindberg 1999). During this study, temperatures ranged from 20-29°C over the course of the year. The water temperature reached its lowest value during late winter and began to rise in early spring, entering into the ideal spawning range for channel catfish. This increase into the spawning range could have initiated the first spawn and subsequent spring peak. Temperatures continued to increase gradually, and during late summer and fall, temperatures reached and remained at 27°C across multiple sampling events. These temperatures coincided with the fall peak in YOY channel catfish. This may be the first report of bimodal spawning by this species, as no evidence of natural bimodal spawning by channel catfish was found in the literature.

Spatial patterns in YOY catch were only detected for comparisons of littoral versus limnetic light traps, and no differences were detected between reservoir basins. However, the scale of this research was limited by cost of gear and manpower required to collect samples. Only two replicates per basin were collected for littoral and limnetic habitats, limiting statistical power. Furthermore, variability between habitats and individual sampling stations likely masked any

potential differences. These two factors could have led to Type II statistical error, in which an actual difference between basins would not be detected.

Despite lack of longitudinal differences, habitat differences were observed. Littoral traps caught greater numbers and exhibited greater diversities than limnetic traps and had 90% of total catch composed of redbreast tilapia, largemouth bass, Amazon sailfin catfish, threadfin shad, tiger barbs, and sunfish. Redbreast tilapia, largemouth bass, and Amazon sailfin catfish are generally considered littoral species, which spawn in nest depressions or spawning cavities, so it was not surprising that abundance of YOY of these species were greatest in littoral habitats. Conversely, channel catfish dominated limnetic samples, accounting for nearly 77% of the total catch. However, this species is also cavity spawner (Hubert 1999). This suggests that YOY of channel catfish migrate from littoral spawning sites to limnetic open water immediately after leaving parental production. Chapman (2000) noted that channel catfish YOY are guarded by the male for several days to several weeks, and then move to shallow water to begin feeding. Clearly, YOY of this species were not utilizing shallow littoral waters in this study, suggesting that they may employ a different life history strategy in tropical reservoirs. Further study is recommended.

Of the frequently collected species, only threadfin shad had similar catch between the two habitats. This was not surprising, as this schooling species appears equally at home in both littoral and limnetic environments (Neal and Prchalová 2012). *Dorosoma*

species are generally positively phototaxic (Shelton and Stephens 1980), so they are attracted to light traps in both habitats. However, the low catch rates and high effort associated with light traps as compared to push nets (Chapter 4) and trawls (Prchalová et al. 2012), excludes this gear as a viable sampling technique for this species.

Littoral light traps provided valuable information on species presence/absence and perhaps relative abundance. Although NMDS groupings of littoral light traps were not as tight as groupings of limnetic traps, this was due to the greater diversities caught within littoral traps, which led to less similarity among sites in the ordination. Conversely, limnetic light traps grouped much tighter due to the predominance of channel catfish in catches. Although limnetic light traps successfully captured eight different species, only channel catfish were caught in great enough numbers to elicit the use of this gear in limnetic habitats.

Water quality tends to vary spatially within aquatic systems, offering an alternative means to analyze spatial trends. Water quality variables had limited effect on YOY fish distributions, though some inferences can be made. Channel catfish, lepomis, and tiger barbs were influenced by turbidity, pH and temperature. The greater abundance of channel catfish in limnetic habitats potentially coincided with phytoplankton blooms or large suspended solids events, as evidenced by elevated turbidity. Greater turbidity could have given YOY channel catfish protection from predation (Miranda 1999), or it could have given the species an advantage during foraging activities (Daugherty and Sutton 2005). Redbreast tilapia and threadfin shad were influenced by dissolved oxygen and conductivity, whereas the rest of the YOY fish community did not correlate with water quality variables.

Based on the results of this study, the following recommendations are suggested for using light traps to sample YOY fish in Carite Reservoir: 1) littoral light traps should be used to provide general community information and seasonality of YOY fish species, 2) limnetic light traps have utility in characterizing channel catfish YOY dynamics, and 3) threadfin shad are most effectively sampled using active gears such as

push nets or trawls (Table 2). Although this research focused exclusively on this one reservoir, these recommendations should be applicable to other tropical reservoir systems with similar habitat, physicochemical, and fish community characteristics. Furthermore, this research detected interesting anomalies regarding channel catfish early life history, and further study is warranted to better understand these unique characteristics.

### Acknowledgments

The authors would like to express their deepest gratitude to C. Fox and M. Munoz for endless nights on the reservoir and for making this research possible, as well as colleges K. Olivieri, N. Peterson and S. Garcia. Special thanks to Z. Loman and S. Miranda for guidance with statistical analysis. We would also like to recognize the Puerto Rico Department of Natural and Environmental Resources, in addition to the U.S. Fish and Wildlife Sport Fish Restoration for funding this project.

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TABLE 2. Young of year sampling recommendations for Puerto Rico reservoirs.

| Species                | Season        | Gear       | Habitat  |
|------------------------|---------------|------------|----------|
| Largemouth Bass        | Spring        | Light Trap | Littoral |
| Redbreast Tilapia      | Spring-Summer | Light Trap | Littoral |
| Amazon Sailfin Catfish | Spring-Summer | Light Trap | Littoral |
| Channel Catfish        | Spring/Fall   | Light Trap | Limnetic |
| Threadfin Shad         | Spring-Summer | Push Net   | Limnetic |

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## **Study 3:**

# **Evaluation of management regulations for Cerrillos Reservoir**





## Chapter 6: Development of a Crowded Largemouth Bass Population in a Tropical Reservoir

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**Abstract:** This case study examines the development of an overcrowded largemouth bass population following initial stocking in a tropical reservoir and efforts to correct crowding with a protected slot length limit. Cerrillos Reservoir is a relatively new impoundment (filled 1996) in Puerto Rico and is one of only two reservoirs that have been stocked exclusively with pure Florida largemouth bass (*Micropterus salmoides floridanus*). Largemouth bass were first stocked in Cerrillos Reservoir in 1997 and the population quickly expanded. Within three years, relative weight declined from above 100 to about 80 and the population displayed characteristics of overcrowding, with much of the population composed of fish  $\leq 350$  mm. This condition was most likely the result of limited harvest, as angling was not allowed during the first three years of the fishery. In 2000, the reservoir was opened to angling, but access remained limited and unpredictable. A protected slot limit (356–508 mm) was implemented in 2003 to encourage harvest of smaller largemouth bass, to protect the intermediate-sized bass, and allow for occasional harvest of a trophy bass. Population sampling in 2010 indicated that overcrowding persists in Cerrillos Reservoir, with 91% of stock-size largemouth bass less than 400 mm, and 81% of those below the protected slot. The failure of the protected-slot limit in reducing largemouth bass crowding is believed to be due to limited angling pressure and unwillingness of anglers to keep smaller fish. The slot has since been removed and research has begun to address best management strategies for this reservoir.

*Previously Published:*

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Voluntary release of legal-size largemouth bass (*Micropterus salmoides*) has become common practice in recent years (Quinn 1996; Noble 2002; Myers et al. 2008; Willis et al. 2010). Allen et al. (2008) estimated that average fishing mortality rates for largemouth bass throughout most of the United States decreased by nearly half since 1990, likely the result of voluntary release by anglers. This raises concerns among managers that this behavior is reducing the effectiveness of harvest regulations (Bonds et al. 2008), which are one of the primary tools for largemouth bass management (Noble 2002).

Length limits can be used to alter the size structure of targeted species (Gabelhouse 1987; Perry et al. 1995). Largemouth bass are traditionally managed using either a minimum length limit or a protected slot length limit. Minimum length limits are most effective when recruitment is limited and adults must be

protected until they have spawned at least once (e.g., Hunt 1974). However, this regulation may not provide any benefit if recruitment is not limited, exploitation is low, growth is slow, or anglers remove sub-legal fish (e.g., Austen and Orth 1988). Protected slot limits usually are designed to take advantage of surplus recruits and to increase growth of mid-sized fish. However, when anglers fail to harvest fish under a protected slot length limit, the regulation becomes ineffective (Noble and Jones 1999).

Failure to adequately harvest largemouth bass populations can lead to an overcrowded population when recruitment is high and stable (Willis et al. 2010). Crowding results when fish densities at a particular size range exceed prey availability for that size range. Consequently, prey consumption decreases and growth slows for that size class. Smaller fish, which can utilize an alternative prey base, grow rapidly until reaching

sizes where prey availability is limited. This results in stockpiling of fish at that size class, exacerbating the crowding effect. Few fish escape the bottleneck and grow to larger sizes. While this phenomenon has been well-described for a variety of species in temperate systems (Byrd and Crance 1965; McHugh 1990; Hansen et al. 1998; Olive et al. 2005), it has been less commonly observed for tropical systems. The case study presented here provides an account of the development of a crowded largemouth bass population in a relatively new tropical reservoir. Furthermore, it examines the attempt to remedy the situation using a protected slot length limit.

## Methods

*Study Site* – The study was conducted in Puerto Rico, a commonwealth of the United States located to the east of the Dominican Republic and west of the Virgin Islands. Puerto Rico is the smallest and easternmost island of the Greater Antilles and is approximately 180 km long (east to west) and 65 km wide (north to south). Cerrillos Reservoir is a relatively new impoundment located in the mountains northeast of Ponce, Puerto Rico, in the southwestern region of the island. Construction of the reservoir began in 1982 and was completed in 1992, but filling was not completed until 1996. It is a 249-ha reservoir with mostly steep rocky shorelines and a maximum depth of over 80 m. Cerrillos Reservoir is mostly an oligotrophic system with very low nutrient concentrations (Neal et al. 2010) and no submerged vegetation. The primary purposes for the impoundment are flood control, hydropower, agricultural and drinking water supply, and recreation (USGS 2008). Annual fluctuations of 10 m or more in water level are not uncommon in Cerrillos Reservoir. The watershed is mostly comprised of coffee plantations (active and abandoned) and forested terrain.

Prey species were stocked in 1996, including threadfin shad (*Dorosoma petenense*), tilapia (*Tilapia* and *Oreochromis* spp.), and redear sunfish (*Lepomis microlophus*). Florida largemouth bass (*M. s. floridanus*) were stocked the following year. From 1997 to 2000, the reservoir was not open to the public for fishing. In 2000 the reservoir was opened three to four days a week, although long periods of closure were common. In 2003, a management station and access facility was constructed at the reservoir, and management biologists from the Puerto Rico Department of Natural and Environmental Resources (DNER) were stationed at the facility to provide reliable access Thursday to Sunday each week. Due to the steep rocky shoreline and surrounding terrain, bank angling is negligible except at the management facility, and most angling occurs in boats.

*Largemouth Bass Sampling* – The reservoir was sampled twice per year (spring and fall) each year from 1997–2001 using a boom-mounted electrofishing unit set at 60 pulses per second to achieve a current of 7–8 amps DC. Five sampling stations were electrofished for 15 minutes of pedal-down time per site. Sites were selected based on reservoir morphology to yield samples representative of all habitat types. All samples were collected during daylight hours. At each site, all largemouth bass encountered were collected, measured in total length (TL; mm), and weighed (g). Condition was determined on all largemouth bass ( $\geq 150$  mm TL) using the relative weight ( $W_r$ ) index (Anderson 1980). Catch per unit of effort (CPUE; in fish/hr) was calculated for all largemouth bass (total CPUE) and stock-size bass (CPUE of fish  $\geq 200$  mm TL) and used as an index of population density. Proportional size distribution (PSD) was used to describe largemouth bass population size structure within the reservoir.

No sampling occurred in Cerrillos Reservoir between summer 2001 and spring 2010. In spring 2010, intensive largemouth bass population sampling was conducted to determine abundance, biomass, size structure, and condition. The entire shoreline and available off-shore habitats were sampled on 22–25 March 2010 and all largemouth bass collected were measured and weighed, and all fish  $\geq 200$  mm were marked by clipping the left pelvic fin prior to release.

Marked fish were given sufficient time to reintegrate into the population prior to recapture on 19 April 2010. During the recapture sampling, the entire shoreline and available off-shore habitat was sampled using boat-mounted electrofishing, and all largemouth bass were collected, measured, and examined for marks. The number of stock-size largemouth bass was estimated using Chapman's modification of the Petersen index (Chapman 1951), with a target 95% confidence interval of  $\pm 25\%$  of N (Robson and Regier 1964). Estimated population size was multiplied by the mean weight of stock-size largemouth bass to estimate total stock-sized biomass. Furthermore, mark-recapture data were divided into stock, quality, preferred, and memorable size classes as defined by Gablehouse (1984) and population size and biomass were estimated for each size class. Preferred-sized fish were further subdivided into fish in this size class that were above and below the lower slot limit of 356 mm and population and biomass estimates were made to estimate the proportion of the largemouth bass population that was below the slot limit.

## Results

Cerrillos Reservoir showed substantial changes in largemouth bass population metrics from the initial stocking until spring 2001 (Figure 1). Largemouth bass

were first collected only a few months after the initial stocking of about 20,000 fingerlings in early summer 1997. Catch rates of largemouth bass rapidly increased over the next couple of years, and anecdotal evidence suggested that growth of largemouth bass from the initial stocking was rapid, with fish > 400 mm TL collected the second year after stocking. Additionally, rapidly increasing values of adult CPUE and presence of juvenile fish showed that successful reproduction was occurring. By April 1999 largemouth bass relative weight was 90 which suggested a balance among largemouth bass prey populations. However, electrofishing catch rates continued to increase, and relative weight continued to decline. By 2000, catch rates of stock-sized largemouth bass had stabilized around 60 fish/h and mean relative weight was stable near 80. The population was displaying characteristics of overcrowding at intermediate sizes, evident from the relationship of relative weight and total length (Figure 2).

Cerrillos Reservoir was opened to fishing in fall 2000. Following the opening of the fishery, the electrofishing catch rates of largemouth bass exceeding 200 mm TL decreased by about 40% (Figure 1). With the cessation of population growth, the declining condition stabilized and even suggested a slight increase. Although only one sample was collected after the fishery was opened, the initial trends suggested potential management through selective harvest. A protective slot length limit of 356–508 mm was implemented in 2003 to encourage harvest of smaller largemouth bass, to protect intermediate fast-growing bass, and to allow for occasional harvest of a trophy fish. This regulation allowed for a liberal harvest of 10 largemouth bass a day, but with the restriction of only one bass > 508 mm.

Population sampling in spring 2010 estimated the number of stock-size largemouth bass in Cerrillos

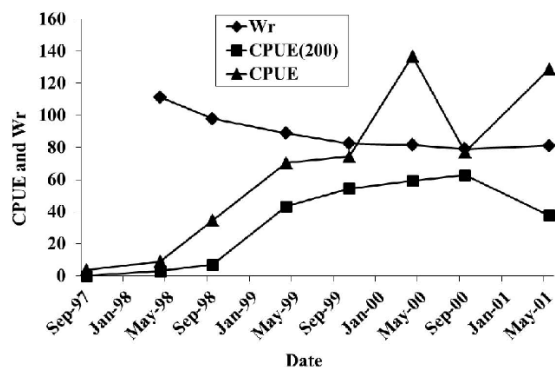


FIGURE 1. Overall catch-per-unit-effort (CPUE), CPUE of stock-size largemouth bass ( $\geq 200$  mm TL), and mean condition (relative weight; N ranged = 5 - 162) of largemouth bass from fall 1997 to spring 2001.

Reservoir to be  $9,790 \pm 2,053$  ( $\pm 21\%$ ). The data suggested that largemouth bass stockpiling and crowding was not affected by the protected slot length limit, with 91% of stock-size bass still < 400 mm TL. In fact, 81% of the population was below the protected slot of 356–508 mm and available for harvest (Figure 3). The maximum catch rates of stock-size largemouth bass during the study was  $64 \pm 5.3$  fish/hr, observed in September 2000. Proportional size distribution appeared to increase from spring 2000 to spring 2010; however, PSD-P and PSD-M both remained low over this same time frame (Table 1) indicating a lack of larger fish in the population.

Condition of largemouth bass in Cerrillos Reservoir dropped precipitously upon reaching 200 mm, and only a few fish showed increased condition after reaching 450 mm (Figure 2). Mean relative weight of largemouth bass in Cerrillos Reservoir was lower than that observed in southeastern U.S. waters in all four size classes (Table 2). Total biomass of the entire population was estimated to be 4,149 kg, yielding a relative biomass of  $16.7 \pm 3.5$  kg/ha. Largemouth bass

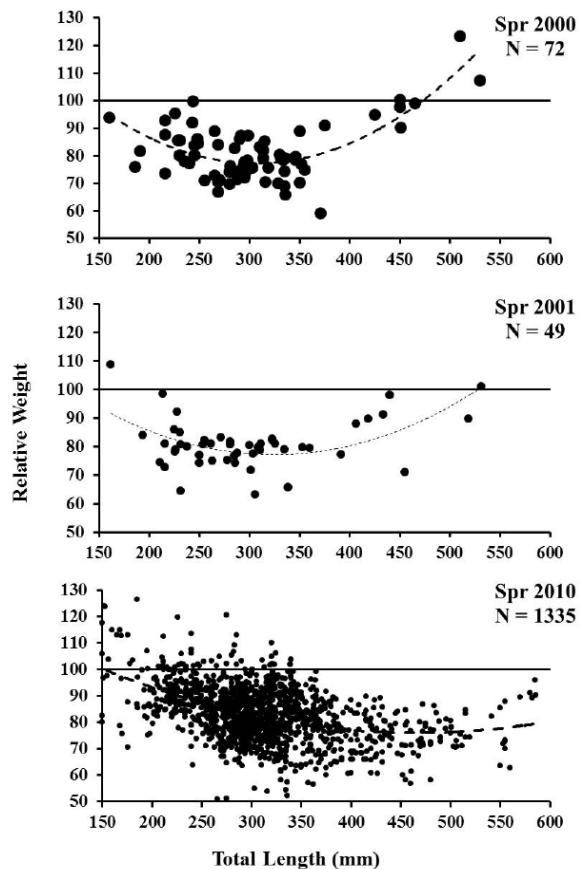


FIGURE 2. Relative weights for largemouth bass collected in Cerrillos Reservoir during spring 2000, spring 2001 and spring 2010. Dotted line in each graph is polynomial trend line. Solid line indicates optimum relative weight of 100.

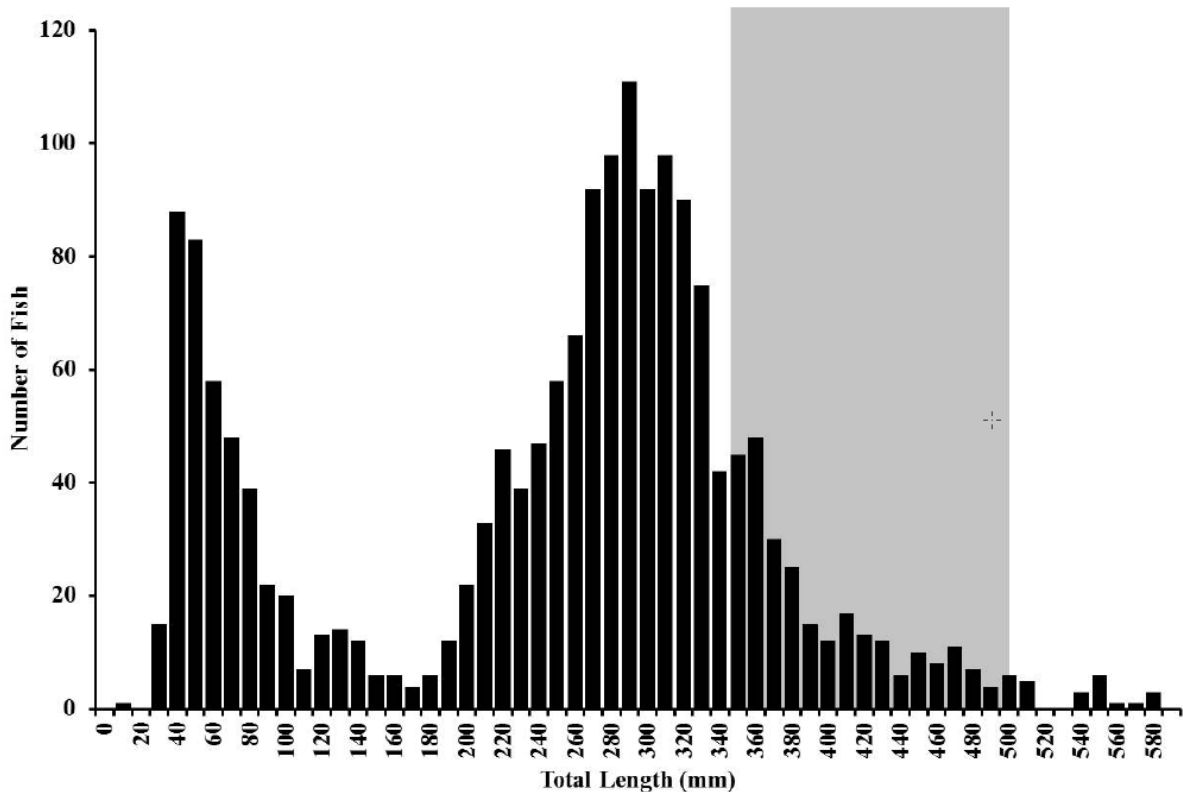


FIGURE 3. Largemouth bass (N = 1751) length distribution in Cerrillos Reservoir during marking procedures in March 2010. Shaded area indicates protected slot limit (356–508 mm).

≤ quality size composed the majority of the biomass in Cerrillos Reservoir (Figure 4), and bass smaller than the protected slot length limit composed 57% of the biomass (Table 3).

### Discussion

Largemouth bass have been widely introduced into freshwater systems around the world, including many tropical environments, yet most management models for largemouth bass are designed for temperate areas. Although the knowledge base for largemouth bass management in Puerto Rico reservoirs is expanding (Neal et al. 2002; Waters et al. 2005; Neal and Noble 2006; Neal et al. 2008), many uncertainties still exist. Unlike in temperate regions, tropical largemouth bass grow rapidly and usually reach maturity in less than one year (Gran 1995). Spawning is prolonged up to six months with multiple spawning events (Dadzie and Aloo 1990; Gran 1995), and growth slows as a consequence. Longevity is greatly truncated, with few fish surviving beyond age 3 (Neal and Noble 2006). Thus, reservoirs typically have populations composed of many mid-sized fish and a few trophy fish that, for unknown reasons, somehow defy slow growth or early mortality.

Cerrillos Reservoir does not follow typical population dynamics of other Puerto Rico reservoirs. Instead, the largemouth bass population displays the classic pattern of a stunted bass population common to temperate ponds and lakes. Catch rates of stock-sized bass were considerably higher than lakes in the southeast United States (Bonar et al. 2009), but were lower than more productive reservoirs on Puerto Rico (e.g., mean ± SE of catch rates of largemouth bass ≥ 250 mm TL in Lucchetti Reservoir was 101.9 ± 13.2 from 1996–1999; Neal et al. 1999). Furthermore, overall PSD was lower than PSD values from other major reservoirs across the island (Neal et al. 1999). Thus, the population was composed of mostly small largemouth bass ≤ 350 mm, with a few intermediate sized and larger bass (>500 mm). Surber (1949), Swingle (1950), and Olive et al. (2005) have all shown that lakes or impoundments with high numbers of small largemouth bass typically have low numbers of large bass. As with catch rates, largemouth bass relative biomass in Cerrillos Reservoir was greater than the average in southeastern U.S. systems (Bonar et al. 2009), yet less than observed in other, more productive reservoirs in Puerto Rico (e.g., mean ± SE relative biomass in Lucchetti Reservoir from 1996–1999

TABLE 1. Proportional size distribution (PSD) of largemouth bass in Cerrillos Reservoir in spring 2000, 2001, and 2010.

| Sample period | PSD-Q | PSD-P | PSD-M | PSD-T |
|---------------|-------|-------|-------|-------|
| Spring 2000   | 44    | 10    | 3     | 0     |
| Spring 2001   | 47    | 17    | 4     | 0     |
| Spring 2010   | 55    | 13    | 2     | 0     |

TABLE 2. Mean ( $\pm$  SE) relative weight for each size class (Gabelhouse 1984) of largemouth bass from Cerrillos Reservoir and lakes in the southeast United States (Bonar et al. 2009). NA indicates that no data are available.

| Length (mm) | Cerrillos    | Southeast US |
|-------------|--------------|--------------|
| 150–199     | 96 $\pm$ 2.5 | NA           |
| 200–299     | 86 $\pm$ 0.4 | 90 $\pm$ 1.1 |
| 300–379     | 82 $\pm$ 0.4 | 89 $\pm$ 1.1 |
| 380–509     | 75 $\pm$ 0.6 | 93 $\pm$ 1.2 |
| 510–629     | 79 $\pm$ 2.2 | 97 $\pm$ 2.1 |
| 630+        | NA           | NA           |

TABLE 3. Number collected (n), mean weight (WT, g), total biomass (TBM, kg), and percent biomass (% BM) by size class (mm, Gabelhouse 1984) of largemouth bass during marking procedures in March 2010. Quality size class is separated into two length classes to indicate fish below (300–355 mm) and within (356–379 mm) the protective slot length limit.

| Size class  | Stock | Quality |         | Preferred | Memorable | Trophy |
|-------------|-------|---------|---------|-----------|-----------|--------|
| Length (mm) | 200   | 300–355 | 356–379 | 380       | 510       | 630    |
| n           | 578   | 446     | 94      | 145       | 20        | 0      |
| Mean WT     | 222.5 | 406.4   | 581.6   | 939.5     | 2142.1    | 0      |
| TBM         | 128.6 | 181.3   | 54.7    | 136.2     | 42.8      | 0      |
| % BM        | 24    | 33      | 10      | 25        | 8         | 0      |

was 37.0  $\pm$  3.5 kg ha<sup>-1</sup>, Neal et al. 1999). Furthermore, the condition of largemouth bass in Cerrillos Reservoir declined sharply as fish enter the growth bottleneck. All of these factors point towards a high-density, slow-growing population.

Interestingly, the pattern of relative weight appears to have shifted from the period of initial crowding to the most recent sample. In the 1999–2001 data, the poorest conditions were observed for lengths around 300 mm, and relative weight increased rapidly for larger fish. Conversely, in 2010 condition was least for fish > 400 mm and only increased minimally in the largest fish. This suggests that prey populations have been depleted to a point that recruitment of prey to larger sizes is no longer sufficient to afford a foraging advantage to large largemouth bass (Swingle 1950; McHugh 1990; Guy and Willis 1995; Schindler et al.

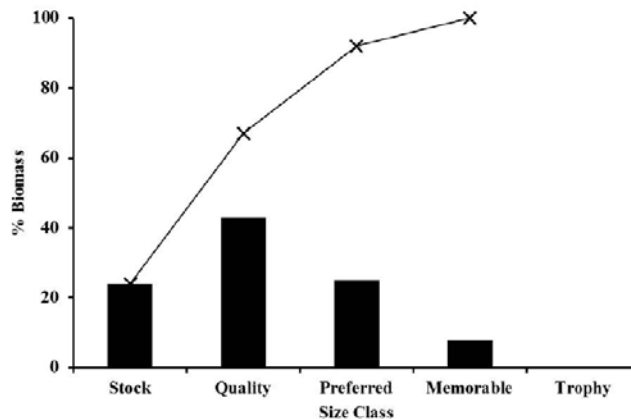


FIGURE 4. Percent biomass (columns) and cumulative biomass (x) by size class (Gabelhouse 1984) of largemouth bass during marking procedures in March 2010.

1997). Another possible cause of the low condition of larger fish could be energy partitioning. Since largemouth bass in Puerto Rico reach maturity by age 1 and spawning periods can last up to six months with individuals spawning multiple times in a single season (Dadzie and Aloo 1990; Gran 1995; Waters and Noble 2004), it is possible that more energy is being allocated towards reproduction than growth. For fish that are already in poor condition, this continued allocation of energy to gonadal growth instead of somatic growth may prohibit improvement in condition.

The current study was limited by the lack of accurate age estimates. Age and growth determination is difficult in tropical reservoirs because the fish are not subjected to major seasonal differences like fish in temperate regions. Although otoliths typically provide age information for many fishes (Welch et al. 1993; Soupier et al. 1997; Clayton and Maceina 1999), they do not provide reliable estimates of age for adult largemouth bass in Puerto Rico reservoirs (Neal et al. 1997). Length-frequency analyses (Guy and Brown 2007) can be used to estimate age of tropical largemouth bass because they grow rapidly for the first few years and their lifespan is usually less than four years. However, the Cerrillos Reservoir population stockpiles by age 1 into a nearly unimodal distribution, prohibiting length frequency analysis (Neal et al. 1997; Neal and Noble 2002). Although length-at-age keys have been used successfully to age largemouth bass in Puerto Rico, known age fish are not available to create length-at-age keys for Cerrillos Reservoir. Despite the fact that age and growth analyses were not included in this study, it is apparent that Cerrillos Reservoir largemouth bass are stockpiling at less than desirable sizes.

Many largemouth bass populations across the United States have responded to length limits as a form of management (Wilde 1997). Typically, imposing a slot length limit to increase harvest of small fish would restructure a largemouth bass population by freeing up resources thereby increasing growth of remaining bass (Wilde 1997; Willis et al. 2010). However, that was not the result of the protected slot length limit regulation on the largemouth bass population in Cerrillos Reservoir, and several factors may have interacted to prevent the success of this management tool. First, the reservoir has historically experienced limited angling effort due to periodic closures and limited accessibility. Restricted reservoir access following the initial introduction and subsequent population expansion allowed a largemouth bass crowded situation to become established, and limited angling effort and a truncated angling week have helped to maintain it. Second, anglers have appeared unwilling to remove small largemouth bass and do not support the protected slot length limit (D. Lopez-Clayton, Puerto Rico DNER, personal communication), despite that the majority of the largemouth bass in this reservoir were available for harvest, with only 17% protected by the regulation.

In order to succeed, fisheries management requires integrated information on the environment, the organisms, and the people (Nielsen 1999). Harvest regulations must be based on sound biological data, but also must have the potential to achieve the desired population-level effect while simultaneously generating support and compliance among the angler base. Due to its ineffectiveness and lack of support, the protected slot length limit was lifted in 2011. Also, the days of operation at the management station were extended to Wednesday-Sunday to increase public use. Shoreline access also has been minimally expanded with the addition of boardwalks and fishing docks to some areas of the shoreline. Research has been initiated to examine largemouth bass population abundance, size structure, prey resources, growth and mortality rates in Cerrillos Reservoir, with the objective of developing management strategies for this system.

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## Chapter 7: Largemouth Bass Habitat Selection and Home Range in Cerrillos Reservoir

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**Abstract:** Largemouth bass *Micropterus salmoides* behavior and survival can be substantially impacted by the availability and composition of littoral habitats. Whereas habitat selection studies for tropical populations of largemouth bass have been limited to greater productivity systems, this study seeks to characterize littoral zone features and habitat selection of largemouth bass in Cerrillos Reservoir, a deep (>80 m), steep-sided reservoir that is mesotrophic to oligotrophic. Littoral zone features were delineated into reaches of dominant slope, substrate, and structure, with the majority of the shoreline being characterized by steep slopes, rocky substrata, and rocky outcrops as the dominant structure. To assess selection of these habitat characteristics and compare home range sizes, 100 largemouth bass (>300 mm total length) received internal acoustic telemetry tags in May 2011 and were relocated bimonthly. Log-likelihood  $\chi^2$  tests revealed that largemouth bass selected for certain habitat characteristics ( $P<0.05$ ), but habitat use was heterogeneous among individuals ( $P<0.05$ ). However, on average largemouth bass demonstrated selection, as use was disproportionate to availability for all three factors assessed (substrata:  $P<0.05$ ; slope:  $P<0.05$ ; structure:  $P<0.05$ ). Selection ratios indicated largemouth bass select for areas clear of structure and avoid sites with silt substratum or rock piles. Although not significant, trends indicate that sites with clay or sand substrata or with moderate slopes were preferred by largemouth bass. Selection for areas with clay or sand substrata may be related to inundation of terrestrial vegetation that grows in these areas during prolonged low-water periods. Kernel density estimates of home range and core-use areas (95% and 50% probability of occurrence, respectively) varied from 0.37 to 96.93 ha (mean=9.65 ha; SE=3.34), and 0.06 to 19.98 ha (mean=1.65 ha; SE=0.59). The distribution of largemouth bass and patterns of habitat use within Cerrillos Reservoir suggest that the population may be subdivided into a normal littoral zone subpopulation, and an active limnetic subpopulation. If this is the case, current sampling protocols are underrepresenting the latter subpopulation.

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Littoral areas are important habitats for all life stages of many fish species. Under ideal conditions, littoral zones provide a diversity of structure, including aquatic macrophytes, coarse woody debris, and rock piles, which provides cover for smaller fish and ambush sites for predators. However, reservoirs often suffer from either insufficient or impaired littoral habitat, particularly due to variability in hydrology (Miranda and Bettoli 2010). Fluctuations in water level prevent the establishment of aquatic macrophytes, limit woody debris availability, and result in an ever-changing littoral zone. Slope is an important physical characteristic of habitat, and determines, in part, both the extent and dynamic nature of the littoral zones (Randall et al. 1996). Shallow slopes, although providing large flats desired by many species, are heavily affected by drawdowns and wave action, while steep slopes reduce the overall availability of littoral habitat. Further, physical structure tends to be scarce in steep sided (>45°) reservoirs, where littoral habitats are narrow and generally of poor quality.

Largemouth bass *Micropterus salmoides* have life histories closely associated with the littoral zone (Annett et al. 1996). They are highly selective of

nesting habitats in shallow water (Hunt et al. 2002; Waters and Noble 2004), the quality of which can have major impacts on recruitment (Hunt and Annett 2002). Dense structure such as brush and inundated terrestrial vegetation serve as refugia from predation and foraging areas for juveniles (Werner et al. 1977; Crowder and Cooper 1982; Savino and Stein 1982). Therefore, the composition and availability of littoral habitats can have substantial impacts on the behavior and survival of largemouth bass.

Habitat selection of largemouth bass has been studied in tropical systems (e.g., Lilyestrom and Churchill 1998; Waters and Noble 2004). However, these studies focused on productive reservoirs with abundant prey and limited water clarity. Cerrillos Reservoir differs from most reservoirs in Puerto Rico in that it is mesotrophic to oligotrophic, steep sided and exceptionally deep (>60 m), and is generally very clear (Soler-Lopez 2011). Furthermore, the largemouth bass population in this reservoir has been skewed towards a crowded, slow growing population since shortly after impoundment (Chapter 6). Thus, this chapter explores habitat selection and potential habitat limitations for largemouth bass in this reservoir. The objectives were

to 1) describe available littoral habitat within Cerrillos Reservoir, and 2) observe whether largemouth bass are selecting specific habitat characteristics.

## Methods

*Acoustic Telemetry* – Largemouth bass were captured using electrofishing in multiple areas of the reservoir, with 100 fish receiving surgically implanted transmitters. To reduce the likelihood of tag related behavioral changes or mortality, only fish greater than 300 mm total length (TL) were selected for the study (transmitter weight <3% body weight). Because Cerrillos Reservoir is exceptionally deep, ultrasonic transmitters (Sonotronics CT-05-36-I, length 63mm, weight 10g, 36 month battery life) were used instead of radio transmitters, as radio signals attenuate with depth (Winter 1983). Prior to surgery, fish were held in aerated water bath dosed with buffered tricaine methanesulfonate (MS-222; 80 mg/L, Argent Labs) until loss of equilibrium. Fish were transferred to a surgery trough with gills submerged into aerated water with a lighter dose (40 mg/L) of MS-222. Tags were rinsed in betadine prior to insertion into the ventral cavity via a small incision posterior to the pelvic fins. Two to three cross sutures with polyglycolic absorbable sutures (2-0, sterile, Safil, Braun, Loveland, CO) closed the incision. Following surgery, fish were held in a holding cage in freshwater and observed for several hours before being released.

To allow fish to reintegrate into the population and reduce any behavioral effects associated with surgeries, bi-monthly tracking events began one month after release. Initially 33 “stop locations” were selected, numbered 1-33 beginning at the dam (site #1) and ending in the river (site #33). In October 2011, two new sites were added by splitting sites #19 and #21 into two additional sites (#19A, #19B, #21A and #21B). The separations occurred because some tags had not been detected from the previously selected sites, but were eventually located during an intense shoreline and open water free-style sampling, indicating they were not detectable from specified sites.

During location sampling events, a GPS was used to navigate to stops. At each location, a hydrophone attached to PVC pipe was lowered into the water and rotated 360 degrees for each frequency. If a tag was detected, individual locations were determined by moving toward the signal with the hydrophone in the water until the direction to tag was reversed or the fish swam away. Fish locations were marked during each tracking event using a handheld GPS unit and also marked on a detailed reservoir map. Location description (open water, right shore, or left shore), depth (m), and distance from shore (<5 m, 5-10 m, or

>10 m) were also estimated and recorded for each tag detection. During each tracking event, attempts were made to locate and record all tagged fish regardless of perceived mortality status. To exclude locations of dead fish or shed tags, fish were determined to be dead if the individual failed to move between repeated relocations (Hightower et al. 2001; Waters et al. 2005). Although mortalities were usually apparent after 2 or 3 detections, additional detections confirmed mortality designations. Locations recorded after a fish was determined to be dead were excluded from analyses.

*Habitat Delineation* – Physical components of habitat were assessed based on structure, substrate, and slope (Table 1; Annett et al. 1996; Neal et al. 2001). Littoral zone features were systematically characterized visually from a boat; the reservoir shoreline was broken down into reaches of dominant slope, substrate, and structure. Starting and ending locations were recorded for every reach using a handheld GPS. To better account for fluctuations in water level, exposed habitat (168-174 m) and the first few meters of inundated habitat (<168 m) was assessed while the reservoir was 6 m below full pool. The linear distance along the shore was used to determine proportional availability of habitat characteristics.

*Analysis* – To assess selection of physical habitat characteristics, fish locations (excluding those described as open water during tracking events) were plotted as a layer on the delineated habitat using ArcGIS 10.1 (Environmental Systems Research Institute). Locations within each habitat reach were linked with that habitat category, and occurrences for individual tags in each category of slope, substrate, and structure were tabulated using statistical program R (R Core Team 2012). To prevent pseudoreplication, individual fish were the sampling unit (Hurlbert 1984). Statistical tests for selection performed in FishTel 1.4 (Rogers 2002; Rogers and White 2007) followed methods described by Manly et al. (1993). Log-likelihood  $\chi^2$  tests were used to assess resource selection in three ways: 1) testing heterogeneity of resource use among individuals, 2) testing selection for habitat characteristics based on availability, and 3) testing the interaction between fish heterogeneity and habitat selection. The third test compared differences in the first two test statistics with ( $I-1$ ) degrees of freedom, where  $I$  is the number of categories, and tested if fish utilized habitat categories in proportion to availability independent of variability in selection among tagged fish. Only tags with >20 littoral zone detections were used for these analyses. Once selection was established, selection ratios were calculated for categories of slope, substrate, and structure to elucidate selection and avoidance. Ratios were calculated following Manly et al. (1993) with Bonferroni

TABLE 1. Microhabitat characteristics used to characterize habitat in Cerrillos Reservoir, Puerto Rico. Habitat and substrate categories are modified from those presented in Annett et al. (1996) to be more characteristic of Puerto Rico reservoirs (Neal et al. 2001).

| Category                  | Description, particle diameter, or slope |
|---------------------------|--|
| <b>Habitat structure</b>  |  |
| Coarse Woody Debris (CWD) | Felled trees, stumps, inundated trees,   |
| Rock piles                | Boulders, rocks                          |
| Clear                     | No observable structure                  |
| <b>Substrate</b>          |  |
| Silt                      | Fine organic                             |
| Clay                      | Clay base                                |
| Sand                      | Less than 5 mm                           |
| Gravel                    | 5-65 mm                                  |
| Cobble                    | 65-256 mm                                |
| Boulder                   | More than 256 mm                         |
| Bedrock                   | Solid                                    |
| <b>Slope</b>              |  |
| Shallow                   | Less than 20 degree incline              |
| Moderate                  | 20-45 degree incline                     |
| Steep                     | Greater than 45 degree incline           |

confidence intervals (CI) constructed at the 100(1- $\alpha$ /I)% ensure the CI contain the true parameter (Thomas and Taylor 1990).

Home range estimates were calculated using plug-in kernel density estimates following Wand and Jones (1994). This method provides a non-parametric estimation of usage intensity over a given area (Worton 1989). Home range and core areas were estimated as the space with a 95% and 50% probability of occurrence. Only tags with >30 detections (n=37) were utilized in order to reduce bias and variance (Seaman et al. 1999). To reduce the bias due to sample size inequality, tag locations were subsampled to standardize them to the minimum number of detections (31).

## Results

*Tag Implantation and Relocation* – Implantation of ultrasonic tags occurred over two days in May 2011; no surgery took longer than 15 min (from anesthesia application to placement in recovery cage) with an average time of 8 min. A total of 100 fish (85 females, 15 males) were implanted with tags, ranging in TL 304 – 574 mm (mean=391.2 mm; SE=4.5 mm) and weight 344 – 2,622 g (mean=743.0 g; SE=37.2 g; 11 fish were not weighed due to equipment failure). Few males were caught that measured above the minimum length (300 mm) required to receive a tag, which is why a 50:50 male to female ratio was not used.

A total of 64 tracking events were conducted over more than two years post-implantation. Fifteen fish were ruled surgical mortalities or tag failures in the month between surgery and the start of the tracking, including six tags that were never detected. Excluding tags with <20 detections and detections that were

considered post mortality, 40 fish were located 1,925 times, with detections per fish ranging from 24 to 62 (mean = 48.13; SE = 1.66). The majority (84%) of these locations were less than 10 m from shore, while 11% were in open water and 5% were within the river in areas not defined in our habitat surveys. Locations were primarily distributed towards upper sections of the reservoir.

*Habitat Selection* – The littoral zone of Cerrillos Reservoir was characterized by steep slopes, rocky substrata, and rocky outcrops as the dominant structure (Figure 1). The extent of shallow slopes, gravel, sand and silt substrata, and coarse woody debris (CWD) increased with declining water levels. Largemouth bass selected for certain habitat characteristics, including substrata ( $\chi^2=2,677.2$ ; df=246; P<0.05), slope ( $\chi^2=1,288.8$ ; df=82; P<0.05), and structure ( $\chi^2=1,917.2$ ; df=82; P<0.05). However, habitat use was heterogeneous among fish selecting for substrata ( $\chi^2=2,463.7$ ; df=240; P<0.05), slope ( $\chi^2=1,240.8$ ; df=80; P<0.05), and structure ( $\chi^2=1,641.4$ ; df=80; P<0.05). Although not all largemouth bass used the littoral zone in the same manner, on average bass demonstrated selection, as use was disproportionate to availability for all three factors assessed (substrata:  $\chi^2=213.4$ , df=6, P<0.05; slope:  $\chi^2=47.95$ , df=2, P<0.05; structure:  $\chi^2=275.8$ ; df=2, P<0.05). Selection ratios indicate selection for areas clear of structure and avoidance of silt and rock piles by largemouth bass (Figure 2). Also, although not statistically different, trends indicate sites with clay or sand substrata or with moderate slopes were preferred by bass.

*Water Level Variability* – Cerrillos Reservoir experiences substantial water level fluctuations, which can affect available habitat. Annual water level fluctuations of at least 3 m occur every year, and water level declines 10 m are not uncommon. Between 2011 and 2013, annual range in water level change was 5.5 m, 5.0 m, and 9.2 m, respectively (Figure 3). In early 2014 at the time of this report, water level was in rapid decline at a consistent rate of 7.1 cm/d, and had fallen 6 m in the first three months of the year.

*Home Range* – Home (95%) and core use (50%) ranges varied greatly in size and were heavily right skewed. Home ranges varied from 0.37 to 96.93 ha (mean=9.65 ha; SE=3.34), while core use areas ranged from 0.06 to 19.98 ha (mean=1.65 ha; SE=0.59). The majority of home ranges were located in the upper half of the reservoir, and there were no significant trends in home range size and reservoir position (Figure 4). Two fish, tags #64 and #73, had estimates of home range areas much larger than the others, with core areas of 19.98 and 10.63 ha, and 95% areas of 96.93 and 85.65 ha, respectively. A closer inspection of the movement

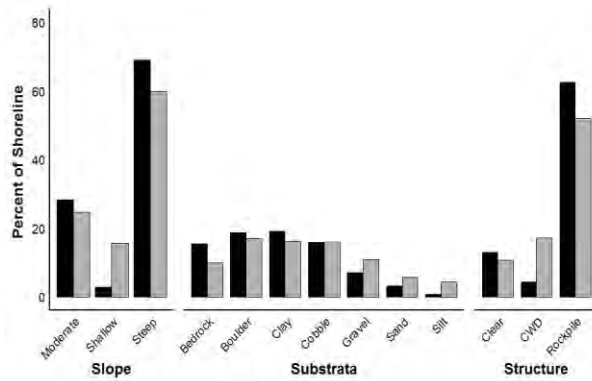


FIGURE 1. Percent of available shoreline by habitat characteristic. Dark and light bars represent available habitat between 168-174 m (full pool) and below 168 m, respectively.

detected a number of times in upper sections of the reservoir, but was also observed in multiple locations around the reservoir, including near the dam. Fish #71 was also observed in multiple areas around the reservoir, but was not nearly as far ranging as fish #64. If these two roaming fish are removed, the average core use area and home range is reduced to  $0.87 \text{ ha} \pm 0.16 \text{ SE}$  and  $4.99 \text{ ha} \pm 0.75 \text{ SE}$ , respectively.

## Discussion

Extreme variability in water levels can impact fish at all life stages, and is particularly deleterious during spawning and subsequent early life stages. Sharp declines in water level can expose largemouth bass nests and cause nesting males to leave the spawning grounds (Waters and Noble 2004), and consistently low water can affect recruitment via habitat availability. For instance, Neal et al. (2001) found a strong link between January-June water level and recruitment to age 1 in Lucchetti Reservoir. That study suggested that habitat availability, especially structural cover and inundated terrestrial vegetation, was a primary driver in the relationship.

The importance of CWD as habitat for largemouth bass is well established (Annett et al. 1996; Hunt et al. 2002; Ahrenstorff et al. 2009). As water level declines, shoreline characteristics in Cerrillos Reservoir transition towards predominance of shallow sloping silt flats with increasing availability of CWD. Neal et al. (2001) reported somewhat conflicting findings in Lucchetti Reservoir, Puerto Rico, where availability of CWD increased slightly at 1-2 m below full pool, but then disappeared entirely at lower water levels. Cerrillos Reservoir does not experience as great of change of CWD at depth, as this is a relatively new impoundment (*circa* 1996) with standing brush remaining in many inundated areas.

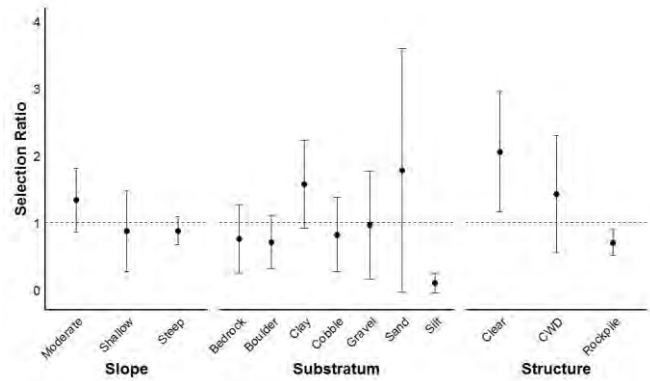


FIGURE 2. Selection ratios of littoral zone characteristics with 95% Bonferroni confidence intervals. Dashed line indicates division between selection (>1) and avoidance (<1).

Largemouth bass habitat selection within the littoral zone of Cerrillos Reservoir was positively correlated with moderate slopes, clay or sand substrata, and negatively correlated with rock piles and silt substrates. The observed preference for moderate slope supported previous findings from reservoirs in Puerto Rico. In Lucchetti Reservoir, Churchill et al. (1995) observed higher catch rates of largemouth bass at sites with moderate slope, and Waters and Noble (2004) reported this species avoided steep slopes and rocky substrates for spawning sites. Similar patterns have been observed for smallmouth bass *Micropterus dolomieu*, a closely related species (Hubert and Lackey 1980).

The avoidance of silt substrates contradicted the findings of Annett et al. (1996), who reported that non-nesting adults selected for silt substrates, particularly when associated with aquatic vegetation. Whereas aquatic vegetation grows well in silt substrates, their finding was likely an artifact of the correlation between substrate and structure, as aquatic vegetation was a highly desired habitat attribute in that study. Similarly, the correlation between substrate type and growth of terrestrial vegetation might help explain the selection for clay and sand substrates over rocky substrates in Cerrillos Reservoir. During extended periods of low water, terrestrial vegetation can grow on the softer clay and sand banks, which provides valuable habitat when water levels rise (Miranda et al. 1984; Waters and Noble 2004). Conversely, vegetation does not readily grow on rocky substrates, and thus does not provide additional structure. Our results support Annett et al. (1996), who reported that cobble and boulder habitat comprised about 9% of available habitat in their study, but only attracted 4-5% of non-nesting and nesting adults.

Although terrestrial vegetation may increase on clay banks following a drawdown, the benefits

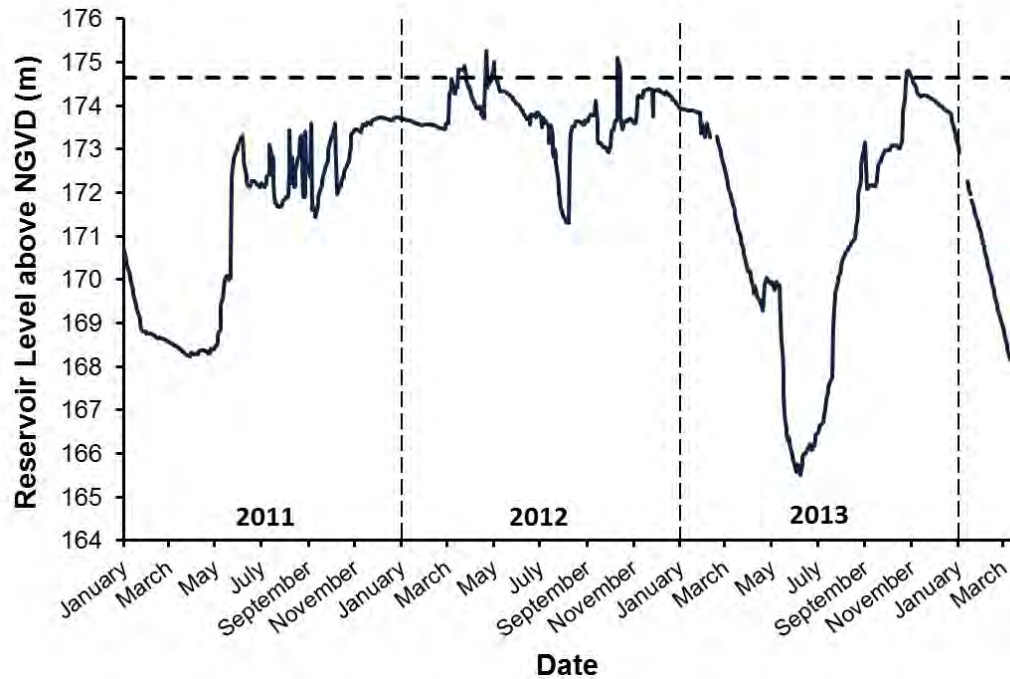


FIGURE 3. Cerrillos Reservoir hydrograph from January 2011 through March 2014. Horizontal line indicates normal operational pool (174.7 m).

following inundation are usually temporary (Kaczka and Miranda 2013). Furthermore, beneficial aquatic vegetation is unlikely to establish in this reservoir due to high water level variability, steep shorelines, and a dominance of rocky substrates. However, artificial structure has potential to attract fish and provide angling opportunities in areas devoid of natural structure. Installation of artificial habitats would be particularly beneficial for attracting fish to areas around fishing piers and where anglers can actively fish the habitats.

Largemouth bass in Cerrillos Reservoir have similar mean home range (9.65 ha) compared with kernel density estimated home ranges of other populations (e.g., Mesing and Wicker 1986; Ahrenstorff et al. 2009). Schleusner and Maughan (1999) classified four groups of movement patterns within a largemouth bass population, varying from fish that were always detected at the same site to fish that were never in the same place twice (roaming). Interestingly, the differences in behavior were not related to size or sex. In Cerrillos Reservoir, home range size was highly variable, with the largest estimated home range more than 260 times larger than the smallest. Two fish especially affected mean home range size, and removal of these individuals reduced mean home range size by nearly half.

Spatial distributions of piscivores may be tied to spatial distribution of prey (Savitz et al. 1983; Rogers

and Bergersen 1995). Threadfin shad *Dorosoma petenense* are the primary forage for largemouth bass in Cerrillos Reservoir and elsewhere on the island (Alicea et al. 1997; Neal et al. 2011). Prior research indicates that threadfin shad tend to be more abundant towards upper sections of reservoirs in Puerto Rico (Neal and Prchalová 2012). Given that habitats are selected in regards to biotic as well as abiotic factors, it is possible that largemouth bass are first selecting home ranges based on prey abundance, and then selecting habitats within home range based on abiotic factors (Johnson's second and third orders of selection; Johnson 1980). This hypothesis is supported by the preponderance of fish using areas closer to the river.

The differences in foraging strategy can affect movement patterns and home range sizes (Fish and Savitz 1983) as fish can either actively search for prey or employ an ambush strategy (Savino and Stein 1982). In Cerrillos Reservoir, large numbers of largemouth bass are consistently feeding on threadfin shad schools in limnetic habitats. Electrofishing is not effective in deep, offshore areas, so all telemetered fish in this study were collected near shore. During the course of this study, these fish were observed in open water habitat only 11% of the time, and rarely in deeper, down-lake areas where limnetic feeding was observed. This suggests that littoral fish and limnetic fish are not necessarily mixing across populations. Hence, there may be two subpopulations of largemouth bass in Cerrillos Reservoir; the typical littoral population, and

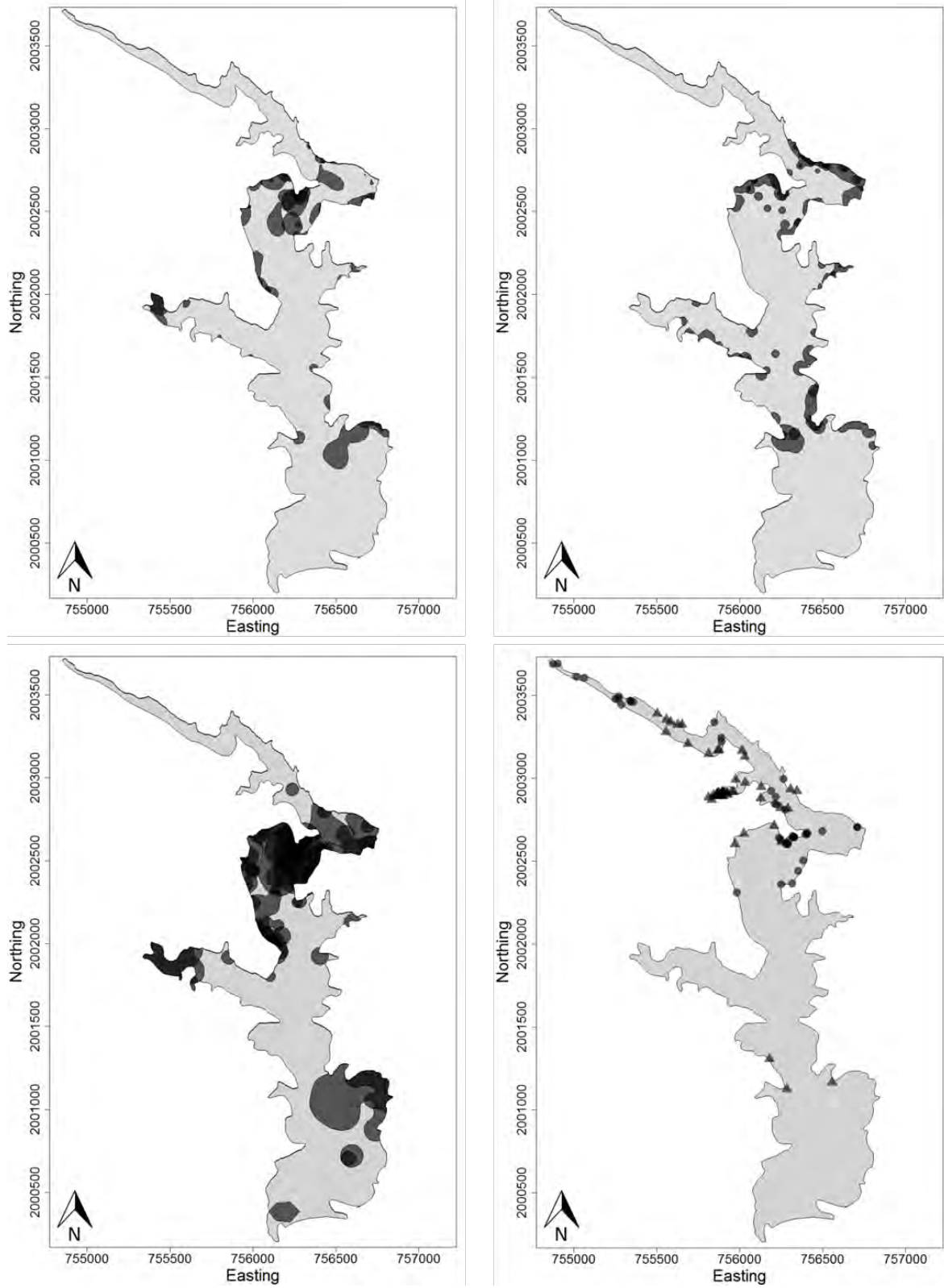


FIGURE 4. Kernel density estimates of core areas (top left), small home ranges (top right), and large home ranges (bottom left) of telemetered largemouth bass. Individual locations of two roaming fish in Cerrillos Reservoir (bottom right).

the active limnetic population. The implications, if this hypothesis is in fact true, are that sampling methods that exclusively sample shoreline habitat may be insufficient to fully characterize largemouth bass populations in this reservoir. Additional research is warranted to examine this hypothesis.

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## Chapter 8: Experimental Removal of Largemouth Bass Biomass from an Overpopulated Tropical Reservoir

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**Abstract:** Largemouth bass *Micropterus salmoides* were stocked in Cerrillos Reservoir in 1997. The population quickly expanded to carrying capacity, relative weight declined from above 100 to about 80, and the population displayed classic characteristics of overcrowding, with much of the population composed of fish  $\leq 350$  mm. A protected slot limit (356-508 mm) was implemented in 2003; however, limited effort and angler attitudes towards harvest of small bass maintained the bass crowded situation in the reservoir. To examine the effect of population reduction on growth and condition, experimental removals of fish from the crowded size classes (200-380 mm) were conducted in 2012 and 2013. In these two years, we removed and relocated 2,333 and 1,993 largemouth bass equaling 26% and 27% of the crowded biomass and 20% and 22% of the total biomass, respectively. Following the experimental removals, mean condition of largemouth bass increased from 81 to 87 ( $F = 294.7$ ,  $P < 0.0001$ ), and increases were observed in all largemouth bass size categories. Although mean growth in length did not increase, length distributions shifted towards larger size classes ( $D = 0.094$ ,  $P < 0.0001$ ) despite strong recruitment in 2014. Further, mean daily growth in weight increased markedly for fish stock size and larger. Despite removing about 20% of the population biomass over two consecutive years, the combination of increased growth in weight, improved condition, and strong recruitment yielded a population comprised of more largemouth bass and a greater biomass than prior to restructuring. We concluded that the removals were a success, but caution than continued harvest is required to maintain the observed improvements and to further restructure this population.

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Cerrillos Reservoir was stocked with largemouth bass *Micropterus salmoides* in the mid-1990s and the population quickly expanded to carrying capacity (Chapter 6; Neal et al. 2001). The reservoir experienced inconsistent and limited fishing pressure during the early years of its fishery and largemouth bass soon became overpopulated and crowded at a size range of about 250-350 mm TL. Because largemouth bass abundance was excessive in this size range, these fish quickly depleted prey populations, leading to slow largemouth bass growth, poor condition, and limited appeal to anglers. By the early 2000s, the most abundant size classes displayed relative weight values indicative of prey limitation (Neal et al. 2001).

In 2003, a protective slot limit was established to encourage harvest of small bass and to protect larger, faster growing bass. This regulation is the standard method to reduce crowding, increase prey availability, and improve growth and condition of remaining largemouth bass (Noble and Jones 1999; Aday and Graeb 2012). When anglers harvest subslot largemouth bass, protected slot limits can improve largemouth bass

size structure and condition (Gablehouse 1987; Novinger 1990; Neumann et al. 1994; Willis 2010). However, inadequate harvest of smaller largemouth bass due to limited access, limited effort, and angler unwillingness to harvest small bass prevented the success of the regulation. The regulation was removed early in 2011, and the only regulation on the fishery currently is a liberal harvest allowance of 10 bass/day with one bass  $>508$  mm TL.

In general, a reservoir system has a biomass carrying capacity that is relatively stable in the absence of habitat modification or excessive mortality. Within that carrying capacity, size structure can be flexible, as long as total biomass varies around carrying capacity. For example, if a reservoir can support 20 kg of largemouth bass per hectare, the relative biomass can be composed of 40 0.5-kg bass, 20 1.0-kg bass, 10 2-kg bass, or any combination of sizes that total 20 kg. Thus, the solution to largemouth bass crowding is to reduce numbers of fish, especially within the crowded size range (Willis 2010; Aday and Graeb 2012). Removal of fish biomass reduces overall demand for prey

resources, thus increasing prey resources available to the remaining population, and improving growth and condition.

In response to the continued crowded largemouth bass situation in Cerrillos Reservoir, we proposed to experimentally remove at least 25% of the crowded (200-380 mm) largemouth bass biomass. This size range was selected because it encompasses the primary mode of largemouth bass stockpiling and condition depression in Cerrillos Reservoir (Figure 1). In this chapter, we describe the response of the Cerrillos Reservoir largemouth bass population to a consecutive two-year reduction in crowded biomass.

## Methods

*Determination of Growth* – To estimate largemouth bass growth rates (length and weight) prior to and after removals, 1,000 largemouth bass  $\geq 150$  mm TL (2012 range 150-607 mm TL, 2014 range 150-596 mm TL) were collected using electrofishing and tagged externally with individually numbered T-bar tags (50 mm TL, Hallprint Manufacturing, Australia) in January 2012 and 2014. Tagged fish were recaptured using electrofishing 2-3 months later, and total length (mm), weight (g), and tag number were recorded for each bass. Daily growth rate in length (DGRL) was estimated as:

$$DGRL = (RTL - OTL)/DAL,$$

where OTL is the total length when tagged, RTL is the total length at recapture, and DAL is days at large since tagging. Daily growth rate in weight (DGRW) was estimated as:

$$DGRW = (RWT - OWT)/DAL,$$

where OWT is the weight when tagged, RWT is the weight at recapture, and DAL is days since tagging. Growth rates for largemouth bass prior to the removal were estimated using tagged bass that were recaptured in March and April 2012 and growth rates for largemouth bass after the removal were estimated using tagged bass that were recaptured in March and April 2014.

*Population Estimates and Removals* – A Peterson mark-recapture population estimate was conducted to determine largemouth bass population abundance, biomass, size structure, and condition (relative weight,  $W_r$ ) in spring each year from 2012 to 2014. Boat-mounted electrofishing standardized to an output of 3,000 W (Burkhardt and Gutreuter 1995) was used to collect largemouth bass. The entire shoreline and available off-shore habitats were systematically electrofished in March. Catch per unit effort (CPUE) was estimated as number of fish collected per hour of

electrofishing. All largemouth bass collected were measured (TL, mm) and weighed (g), and stock-size fish ( $>200$  mm) were marked by removing about 80% of one pelvic fin prior to release. Right and left fins were alternated between years. Abundance, size structure, biomass, and condition were used to characterize the largemouth bass population. Biomass was estimated by multiplying the mean weight of stock-sized largemouth bass by the estimated population size. Condition was determined using the relative weight index (Anderson 1980).

Marked fish were given 4 weeks to reintegrate into the population prior to recapture efforts. During the recapture sampling, the entire shoreline and available limnetic habitat was sampled using boat-mounted electrofishing and all largemouth bass were collected, measured, and examined for marks. The number of stock-size largemouth bass was estimated using the Petersen index, with a target 95% confidence interval of no more than  $\pm 25\%$  of  $N$  (Robson and Regier 1964).

Experimental removals of largemouth bass were conducted in 2012 and 2013 during and immediately following recapture efforts of the population estimates. Population estimates in 2010 and 2011 indicated that the most intense crowding was occurring among bass that ranged from 200-380 mm TL (Figure 1; Neal et al. 2010; Neal et al. 2011). Therefore, most largemouth bass within this size range collected during recapture and subsequent sampling efforts were removed from the population. Externally tagged fish were not removed. Biomass of fish removed was estimated by multiplying mean weight of fish 200-380 mm TL (during marking efforts) by number of fish removed. Because the crowded size range includes two of the length categories (stock and quality) used in largemouth bass size distribution assessment, we include it as a separate length category, termed “crowded” for analyses. Fish that were to be removed were held in cages at the Cerrillos Reservoir boat ramp until being loaded for transport. Largemouth bass were loaded on trucks in either a 1,014-L or 416-L aerated hauling tank and transported to Guajataca Reservoir for stocking.

*Statistical Analysis* – Analysis of variance (ANOVA) was used to compare mean length, weight, and condition of the total population and length categories between pre-removal (2012) and post-removal (2014) years. Length-frequency distributions were compared between pre- and post-removal years using a Kolmogorov-Smirnov two-sample (Zar 1996) and Chi-square ( $\chi^2$ ; Michaletz et al. 1995) tests. For these statistical analyses, we used  $\alpha = 0.05$  as our level of significance.

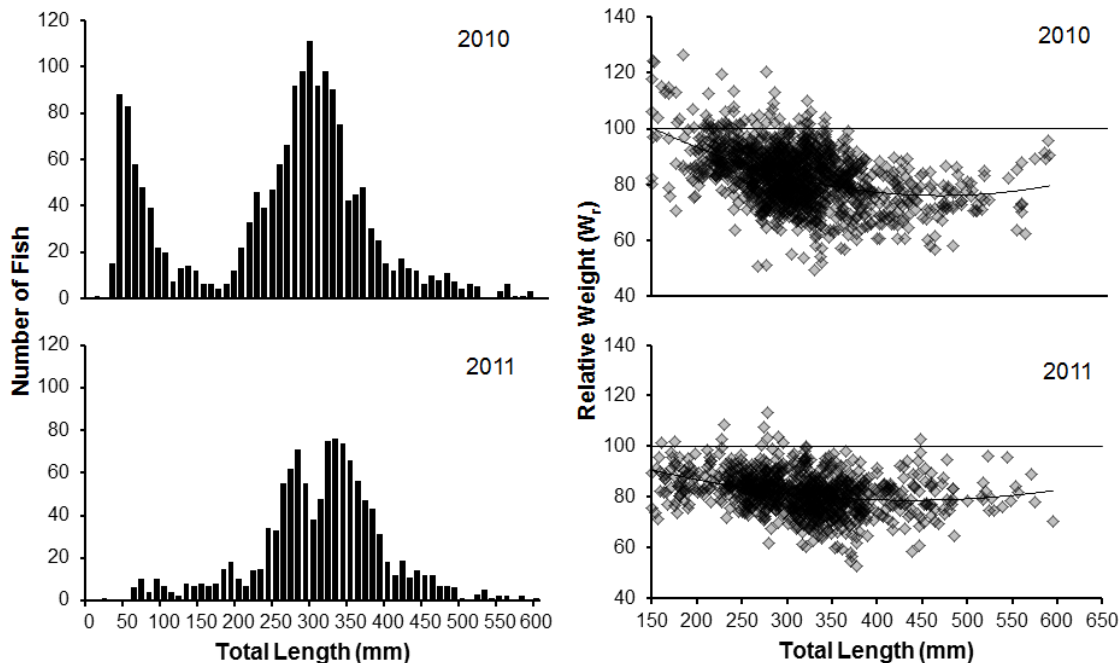


FIGURE 1. Length distribution and relative weight of largemouth bass in Cerrillos Reservoir during population sampling in spring 2010 and spring 2011. Line at 100 in relative weight figures indicates optimum relative weight for a balanced population.

## Results

The 2012 population estimate for Cerrillos Reservoir determined a stock-size population of 9,911 ( $\pm 936$ ) largemouth bass, with an associated biomass of 3,538.8 kg. Overall CPUE was high at 97.9 fish/h and CPUE of stock-size or greater largemouth bass was 95.2 fish/h. Mean DGRL for largemouth bass was low at 0.28 ( $\pm 0.04$ ) mm/day. This growth rate was heavily influenced by sub-stock (150-199 mm TL) fish. Average growth rate for stock-size and larger bass was only 0.18 ( $\pm 0.03$ ) mm/day (Figure 2).

In 2012, we removed 2,333 largemouth bass with a biomass of 725 kg (Figure 3). This represented 26% of the stock and quality size largemouth bass and 20% of total population by weight, and resulted in a reduction in amplitude of the primary length mode (Figure 4).

Transport of removed fish to Guajataca Reservoir was highly successful. A total of 2,148 largemouth bass were loaded for transport during the 13 hauling trips (Table 1). Only 69 mortalities occurred during transport, resulting in an overall transport survival rate of 96.8%. The final stocking density was 5.8 adult largemouth bass per hectare.

The 2013 population estimate determined a stock size population of 8,011 ( $\pm 1,372$ ) largemouth bass, with an associated biomass of 2,728.1 kg. This represented a 22.9% decline in biomass from the

previous year, in line with 2012 removal efforts. Overall CPUE decreased from 97.9 fish/h in 2012 to 73.6 fish/h, and catch rate of stock size largemouth bass declined from 95.2 fish/h to 57.1 fish/h. During recapture efforts of the 2013 population estimate, a second biomass removal was conducted in the same manner as in 2012. We removed 1,993 largemouth bass within the crowded size range, which accounted for 27% of the crowded biomass and 22% total biomass. These fish were transported to Guajataca and La Plata Reservoir with excellent survival reported by DNER.

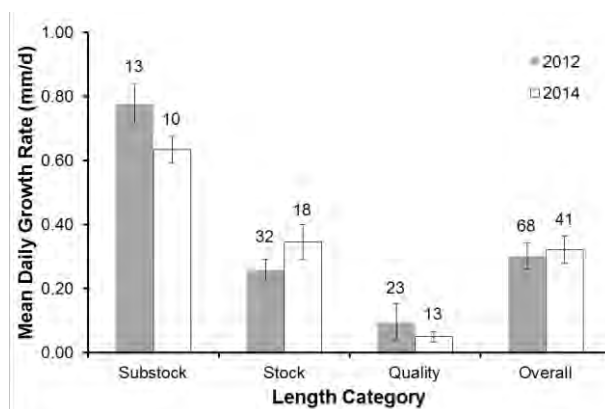


FIGURE 2. Mean ( $\pm$ SE) daily growth rates by length category for largemouth bass in Cerrillos Reservoir prior to (2012) and after (2014) experimental biomass removals in 2012 and 2013. Numbers above bars represent number of fish used in estimation.

The 2014 population estimate for Cerrillos Reservoir determined a stock size population of 11,038 ( $\pm 2,169$ ) largemouth bass with an associated biomass of 4,034.1 kg. Overall and stock size CPUE increased to 115.8 fish/h and 99.1 fish/hr, respectively. These increases are likely the result strong year class formation and recruitment to stock size. Although population size and biomass in 2014 was higher than prior to removals, condition of largemouth bass increased in all length categories (Figure 5). Overall mean relative weight increased for the first time since the reservoir initially reached crowded conditions, increasing from 81 in 2012 to 87 in 2014 ( $F = 294.7$ ,  $P < 0.0001$ ). Increases in all length categories were significant except for memorable-size fish ( $F = 0.15$ ,  $P = 0.708$ ). Overall mean DGRL for largemouth bass did not significantly increase; however, overall mean DGRW did increase after the removals (Figure 6) as was expected due to the increase in overall largemouth bass condition.

Length frequency distributions differed between years (2012 and 2014; Figure 7;  $D = 0.094$ ,  $P < 0.0001$ ).

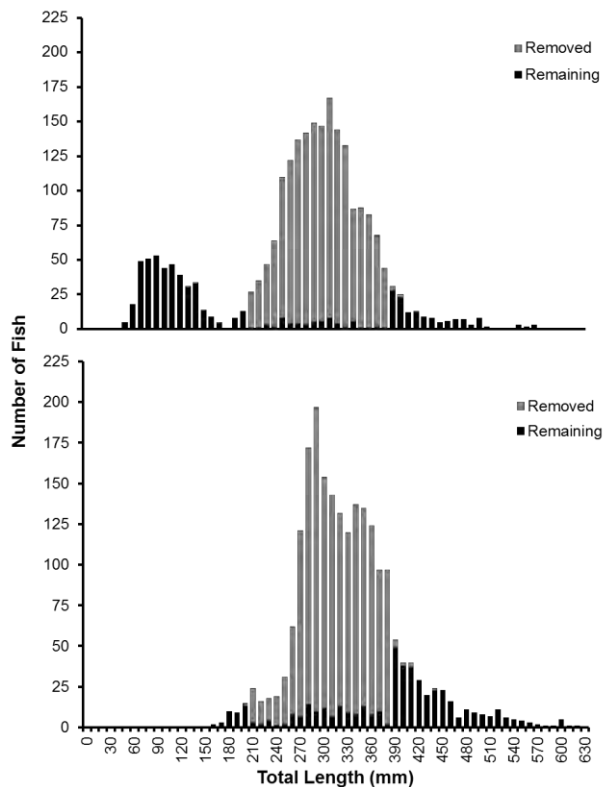


FIGURE 3. Length distribution of largemouth bass from April 2012 (top) and April 2013 (bottom) removal efforts in Cerrillos Reservoir. All fish 200-380 mm TL (grey bars) were removed from the population and fish outside that size range (black bars) were returned to the reservoir. Where grey and black bars are stacked, black bars represent externally tagged fish that were returned to the reservoir.

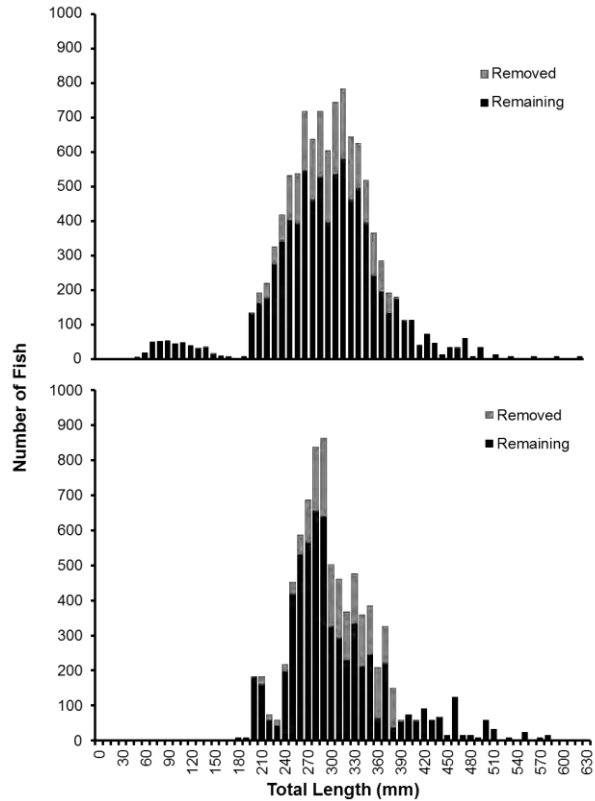


FIGURE 4. Estimated length distribution of largemouth bass in Cerrillos Reservoir during April 2012 (top) and April 2013 (bottom). The population prior to removal is represented by the stacked grey and black bars, and the population after the experimental removal is represented by the black bars only.

The lower end of the crowded size range shifted towards larger sizes, with a strong peak at 310-330 mm TL. A strong year class recruited in 2014, comprised of fish primarily 240-280 mm TL. Chi-square ( $\chi^2$ ) analysis indicated that the frequency of the memorable ( $P = 0.924$ ) length category was not different between years.

## Discussion

The experimental removal has begun to restructure the size distribution of largemouth bass in Cerrillos Reservoir. The combined effects of the two removals resulted in improvement of largemouth bass condition and growth rates, shifting the Cerrillos Reservoir population to one dominated by larger fish. Overall condition has increased above the target minimum of 85, and condition of larger size classes is improving. This is the first significant increase in condition in this reservoir since it was initially stocked with largemouth bass in 1997. Although there has been a great deal of debate among fisheries professionals about the use and interpretation of relative weight data due to the high potential for error and bias (Neumann et al. 2012; Schramm and Willis 2012), relative weight has served

TABLE 1. Relocation data for largemouth bass removed from Cerrillos Reservoir and released into Guajataca Reservoir during April 23-27, 2012. Data courtesy of DNER.

| Date          | Tank Size (L) | Release Site   | Loading Rate | Survivals   | Mortalities | Mortality Rate | Survival Rate |
|---------------|---------------|--|--------------|-------------|-------------|----------------|---------------|
| 23-Apr-12     | 1014          | DNER Ramp  | 200          | 188         | 12          | 6.0%           | 94.0%         |
| 23-Apr-12     | 416           | DNER Ramp  | 100          | 89          | 11          | 11.0%          | 89.0%         |
| 24-Apr-12     | 1014          | DNER Ramp  | 200          | 200         | 0           | 0.0%           | 100.0%        |
| 24-Apr-12     | 416           | DNER Ramp  | 91           | 86          | 5           | 5.5%           | 94.5%         |
| 24-Apr-12     | 1014          | Lobinero Bass Club Ramp                                    | 235          | 224         | 11          | 4.7%           | 95.3%         |
| 24-Apr-12     | 416           | DNER Ramp Children's Camp                                  | 71           | 70          | 1           | 1.4%           | 98.6%         |
| 25-Apr-12     | 1014          | DNER Ramp  | 209          | 208         | 1           | 0.5%           | 99.5%         |
| 25-Apr-12     | 416           | DNER Ramp Lobinero Bass Club Ramp Children's Camp          | 101          | 99          | 2           | 2.0%           | 98.0%         |
| 25-Apr-12     | 1014          | DNER Ramp  | 238          | 229         | 9           | 3.8%           | 96.2%         |
| 26-Apr-12     | 416           | DNER Ramp  | 207          | 204         | 3           | 1.4%           | 98.6%         |
| 26-Apr-12     | 1014          | DNER Ramp Lobinero Bass Club Ramp Children's Camp          | 98           | 93          | 5           | 5.1%           | 94.9%         |
| 26-Apr-12     | 1014          | DNER Ramp Lobinero Bass Club Ramp Soberao Point (Near Dam) | 194          | 193         | 1           | 0.5%           | 99.5%         |
| 27-Apr-12     | 1014          | DNER Ramp  | 204          | 196         | 8           | 3.9%           | 96.1%         |
| <b>Totals</b> |               |  | <b>2148</b>  | <b>2079</b> | <b>69</b>   | <b>3.2%</b>    | <b>96.8%</b>  |

as an alternate method for estimation of body composition and fish health (Rose 1989; Brown and Murphy 1991) and as a tool to assess prey abundance. Positive correlations between relative weight and prey abundance have previously been documented for largemouth bass (Wege and Anderson 1978), pumpkinseed *Lepomis gibbosus* (Liao et al. 1995), northern pike *Esox lucius* (Paukert and Willis 2003), and walleye *Sander vitreus* (Marwitz and Hubert 1997; Porath and Peters 1997).

Neal et al. (2001) suggested that the largemouth bass population in Cerrillos Reservoir had become prey

limited due to rapidly increasing catch rates and subsequent decreasing condition. That report recommended liberal harvest of small largemouth bass to reduce crowding and to increase growth rates and body condition of the fish that remained. After the experimental removals, mean condition of all length categories increased by an average of 5.2 (range 2.7-6.6) units. Thus, we feel confident that the improvement in condition observed 2 years after population reduction represent improvements in prey availability, not only for the crowded size class, but for all size classes.

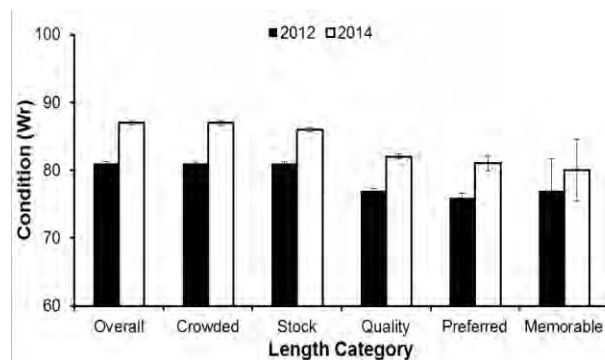


FIGURE 5. Mean (± SE) relative weight of largemouth bass for the total population (overall) and by length category in Cerrillos Reservoir for 2012 (pre-removal) and 2014 (post-removals).

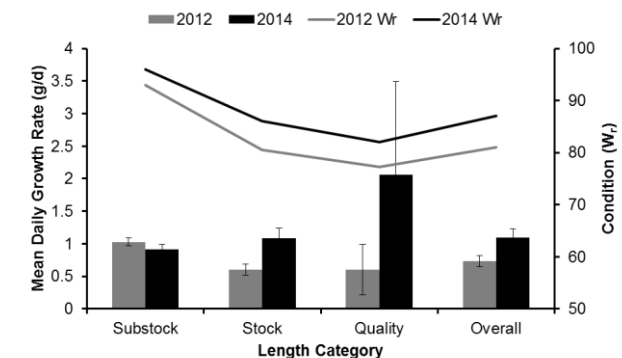


FIGURE 6. Mean (±SE) daily growth rates in weight (columns) and mean relative weight (lines) by length category for largemouth bass in Cerrillos Reservoir prior to (2012) and after (2014) experimental biomass removals in 2012 and 2013.

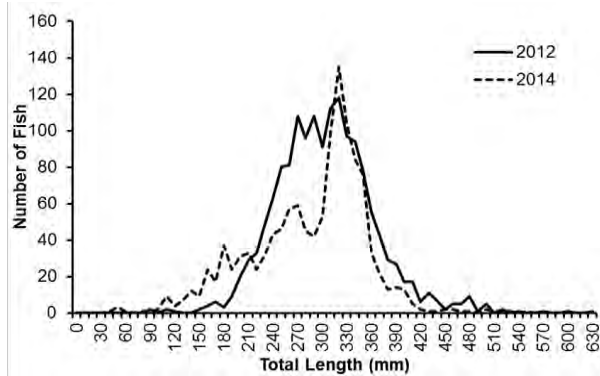


FIGURE 7. Length distribution of largemouth bass in Cerrillos Reservoir before (2012) and after (2014) the experimental biomass removal.

Positive relationships between relative weight and growth have been shown for a number of temperate species including largemouth bass (Wege and Anderson 1978; Neumann et al. 1994), palmetto bass *Morone chrysops x saxatilis* (Brown and Murphy 1991), white crappie *Pomoxis annularis* (Gablehouse 1991), and northern pike (Neumann and Willis 1996). Although size distributions shifted towards larger fish in Cerrillos Reservoir, growth rates in length of stock size fish in Cerrillos Reservoir did not increase during the study period. Unlike weight and condition that can change over relatively short periods of time (days, weeks; Blackwell et al. 2000), changes in length tend to present more slowly, specifically in mature fish (von Bertalanffy 1938). Because largemouth bass were in such poor condition prior to the removal, it is likely that they will need to invest much energy into improving condition before investment in growth in length will be possible. We observed increases in condition and growth in weight, so increases in growth in length may follow.

Previous research in northern temperate climates found that population reductions are necessary for several consecutive years to be effective. Willis (2010) conducted annual harvests of subslot largemouth bass (<300 mm TL) from a high density, slow growing population in South Dakota, and reported that it took 3 years of removals before the size structure of the population began to shift towards target PSD and condition. However, due to higher year-round temperatures, largemouth bass in Puerto Rico experience a much longer growing season than those in South Dakota ponds, and population changes as a result of the removal should occur more rapidly.

An important finding from this research is that, despite 20% of biomass being removed two consecutive years, population abundance and biomass actually increased following the second removal. This

is likely due to the expanded size structure, which opens up a wider breadth of potential prey to the population. When most of the population is crowded into a narrow size range, their potential population size and biomass is limited by the prey available to that size range. Further, few prey survive the predator gauntlet to grow to adult sizes, thus limiting reproduction and recruitment of prey. As the largemouth bass size structure becomes more protracted, the population can exploit a greater available prey base and the reservoir can support a higher overall carrying capacity.

It is likely that removals of this magnitude will need to occur periodically in Cerrillos Reservoir to maintain the observed improvements or to reach ultimate target levels in growth rate and condition. In the South Dakota study, all harvest of largemouth bass ceased once the population reached target size structure and condition, and when the population was sampled again seven years later, it had returned to its original state of high density, low condition, slow growing bass (Willis 2010). Thus, regular removals of small bass may be the best way to manage the Cerrillos Reservoir largemouth bass population to maintain a quality fishery. Conversely, concerted efforts to promote harvest of small bass with anglers could be successful, although it is unclear at this point whether angling pressure is sufficient to reach harvest goals even if anglers harvest all small bass caught. Ongoing analyses will address this issue in a doctoral dissertation that is in preparation.

The next step is to use bioenergetics models to explore population response to potential management and harvest regimes. These models will aid in the development of management strategies by providing information on actions that need to be taken to maintain the desired largemouth bass sport fishery. Chapter 9 provides preliminary modeling results for this reservoir, and Project F-69 will provide refined analyses over the next two years.

Several studies have shown that altering size structure and condition of fish populations requires multiple years of population reduction, and further demands continued assessment after goals are reached so that changes to management strategies can be implemented as necessary (Novinger 1990; Neumann et al. 1994; Noble and Jones 1999; Wilson and Dicenzo 2002; Willis 2010). At this time, we have begun to see the effects of the removals on the largemouth bass population in Cerrillos Reservoir and, while the results are positive, further examination is necessary to see the full potential of results and determine best management strategies for this important fishery.

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## Chapter 9: Current Mortality Vectors in Cerrillos Reservoir and Theoretical Models for Predicting Harvest Requirements

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**Abstract:** The largemouth bass population in Cerrillos Reservoir has been in a crowded, slow growing state since shortly after stocking in 1997. While management regulations designed to improve size structure have been attempted, limited angler access and low harvest rates have undercut these regulations. In this chapter, we examine the current population dynamics in this important fishery and provide a theoretical framework for determining removal requirements for restructuring the population. Natural and fishing (exploitation) mortality was determined using acoustic telemetry and harvest was verified using DNER creel data. Instantaneous mortality vectors were  $Z = 0.7274$ ,  $F = 0.1265$ , and  $M = 0.6009$ ; total annual mortality ( $A$ ) was 0.5169, with  $\mu = 0.0899$  and  $\nu = 0.4270$ . Our estimate of  $\mu$  was very close to the estimate using creel data (0.0895). Whereas these mortality vectors have proven insufficient to restructure the largemouth bass population, additional harvest is required to improve this fishery. We developed two theoretical models for predicting the exploitation required to improve largemouth bass growth and condition. Using pooled datasets from the largemouth bass native range to predict increases in prey consumption (measured as proportion of maximum consumption,  $P$ ) required to yield targeted increases in  $W_r$ , we determined the relationship  $\% \Delta P = 0.0098 \cdot \Delta W_r + 0.0081$ . This equation was used to estimate the increase in individual prey consumption required to increase  $W_r$  for largemouth bass in Cerrillos Reservoir. Whereas removing largemouth bass would release prey resources for remaining fish, a removal matrix was created to estimate removal requirements by desired  $W_r$  change. This matrix was empirically tested using data from the Cerrillos Reservoir population reduction (Chapter 8). The model suggested that an annual exploitation ( $\mu$ ) of 0.436 (including current  $\mu$ ) would be required to shift Cerrillos Reservoir largemouth bass to a balanced population. A second approach using a logistic growth function to model removal effects on  $W_r$  provided similar exploitation predictions ( $\mu = 0.390$  including current  $\mu$ ). However, due to synergistic relationships in predator and prey dynamics, combined with model inadequacies, we recommend a target annual exploitation of 0.32, as this was the  $\mu$  during the experimental removals of 2013 and 2014, which proved sufficient to gradually restructure the population. Cooperation of anglers in harvesting stock size fish is important to achieving this target, but management removals using electrofishing can also be used to restructure the population.

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The largemouth bass *Micropterus salmoides* population in Cerrillos Reservoir has shown classic signs of overcrowding beginning just a few years after stocking (Neal et al. 2001). The population is composed of mostly small largemouth bass ( $\leq 350$  mm TL), with few intermediate-sized and larger bass ( $>500$  mm TL; Fox and Neal 2011). Furthermore, condition of largemouth bass in Cerrillos Reservoir declines sharply as fish enter the crowded size classes (200 to

380 mm), which results from intense intraspecific competition for prey within this size range (Fox and Neal 2011). This suggests that prey populations are insufficient for intermediate size classes and, therefore, limit growth of largemouth bass.

Density dependence has received considerable attention as a factor leading to slow growth of sport fishes (e.g. Aday and Graeb 2012). Crowded predator

populations deplete food resources to the point where growth rates of individuals are substantially reduced. Interactions between predator and prey are generally predictable within simple trophic webs, such as most largemouth bass fisheries, where reduction of predator numbers results in an increase in prey abundance. In turn, increase prey abundance leads to improved growth rates and condition of remaining predators (Aday and Graeb 2012). This relationship forms the basis of small impoundment management in the continental USA, where largemouth bass harvest is the tool of choice for maintaining acceptable growth rates for this species (Willis 2010; Schramm and Willis 2012).

Harvest regulations have historically been a primary tool for fisheries management (Noble 2002). However, in the last two decades anglers have adopted a catch and release mentality when fishing for species such as largemouth bass (Quinn 1996; Myers et al. 2008), which has severely reduced the effectiveness of harvest regulations (Bonds et al. 2008). Failure to adequately harvest largemouth bass populations can lead to overcrowding when recruitment is high and stable (Willis et al. 2010), and several studies have all shown that lakes or impoundments with high numbers of small largemouth bass typically have low numbers of large bass (Surber 1949; Swingle 1950; Olive et al. 2005). In Cerrillos Reservoir, limited angling access combined with angler reluctance to harvest smaller largemouth bass has resulted in persistence of a slow growing population that is in poor condition (Chapter 6).

In the previous chapter, we hypothesized that a reduction of the crowded adult size classes would result in an increase in prey availability, thus increasing consumption and growth rates, potentially improving the size structure and condition of largemouth bass. To explore this hypothesis further, we examined current angler exploitation and natural mortality in Cerrillos Reservoir, and then used a unique approach with bioenergetics modeling to determine the increase in exploitation necessary to improve condition in this fishery. Finally, we used the experimental removal of largemouth bass biomass presented in Chapter 8 to empirically test the performance of our prediction model.

## Methods

*Estimation of Mortality Vectors.*—Mortality vectors were estimated using 100 adult largemouth bass (>300 mm TL) implanted using surgical techniques with ultrasonic transmitters (Sonotronics CT-05-36-I, length 63 mm, weight 10 g, 36 month battery life) in May

2011. Surgical procedures and tracking methods can be found in Chapter 7 of this report.

Mortality estimates in this chapter are from the first year of tracking (June 2011 – June 2012). Following the methods of Hightower et al. (2001) and Waters et al. (2005), if an individual fish was repeatedly located in the same position and did not move when approached, we assumed that the individual had died of natural causes, although we could not rule out catch-and-release delayed mortality. Transmitters that were not located during several consecutive tracking attempts were considered to have disappeared due to one of three causes: (1) unreported angler harvest, (2) transmitter failure, or (3) removal from the lake by predators. Waters (1999) found that avian predation could account for some lost transmitters; however, unreported harvest was the primary reason for disappearance. Because of the 3-year battery life and unlikely influence of avian predators, all tags that disappeared were considered unreported harvest. Regardless of mortality status, all tagged fish were sought out and recorded (if located) during each tracking event. To confirm that migration out of the reservoir (up river or downstream below dam) did not occur, we conducted telemetry sampling ~1.6 km above the river mouth and below the dam while the reservoir was at normal pool (174.7 m above NGVD). No largemouth bass were found in either location.

Annual instantaneous mortality rate ( $Z$ ) was estimated using

$$Z = -\log_e(1 - A),$$

where  $A$  is the annual interval mortality rate for tagged largemouth bass calculated as

$$A = ([N_t - N_{t+1}]/I_t)/N_t,$$

where  $N_t$  is the number of individuals present in the population at the start of an interval of length  $I_t$  and  $N_{t+1}$  is the number of individuals that survive to the end of the interval (Guy and Brown 2007). Interval fishing ( $\mu$ , hereafter exploitation) and natural ( $\nu$ ) mortality rates were estimated with the same equation as annual interval mortality rate using our perceived counts of harvest and natural mortality from the telemetry sampling. To determine the accuracy of our exploitation ( $\mu$ ), we calculated exploitation using creel data recorded by DNER biologists at the reservoir for the year of 2011. Instantaneous fishing ( $F$ ) and natural ( $M$ ) mortality rates were estimated based on their relationship to interval mortality rates (Guy and Brown 2007).

*Modeling Growth and Condition.*—Two approaches were used to model the effects of increased

mortality on largemouth bass growth and condition in Cerrillos Reservoir. In Model 1, we used up to 116 data sets from largemouth bass within the native range to examine the relationship between consumption and growth and condition (Brouder et al. 2009). This relationship was applied to Cerrillos Reservoir to provide estimates of removal targets. In Model 2, we used a modified logistic growth function at varying levels of initial growth rate ( $G$ ) to provide estimates of removal targets, and used the removal experiment in Cerrillos Reservoir (Chapter 8) to select the best prediction curve.

#### Model 1

Brouder et al. (2009) compiled largemouth bass length-at-age and relative weight data from all of North America and within defined ecoregions. These data provide backcalculated length at age data from many populations, and are divided by population growth characteristics into percentiles, with mean length-at-age and relative weights ( $W_r$ ) presented for 5%, 25%, 50%, 75%, and 95% percentiles (Figure 1). These percentiles represent poor, below average, average, above average, and exceptional growth parameters, respectively. The same categories were used to present mean  $W_r$  by size classification (e.g., stock, quality, or preferred). We used data from Ecoregion 8, the eastern temperate forests, which include the majority of the largemouth bass native range, to model the relationship between growth, condition, and individual consumption rates. The resulting model predicted the change in consumption rate ( $P$ ), which was the weight-specific proportion of maximum consumption ( $C_{max}$ ), required to shift between population percentiles. In other words, it predicted how much additional prey per individual is required to increase growth or condition by a predetermined amount.

Rice et al. (1983) previously determined largemouth bass physiological parameters for bioenergetics, which have been extensively used to model this species under a range of environmental

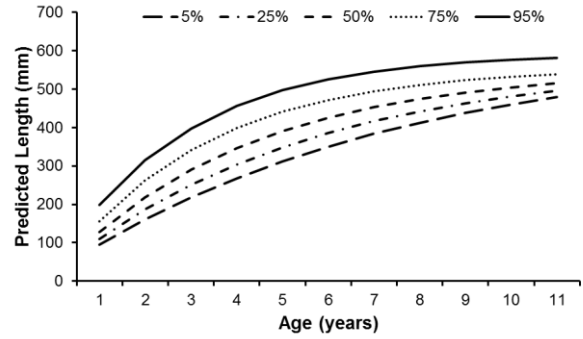


FIGURE 1. Von Bertalanffy growth forms for Region 8 largemouth bass populations with poor (5%), below average (25%), average (50%), above average (75%), and exceptional (95%) growth rates.

conditions (e.g., Rice and Cochran 1984; Perry et al. 1995; Neal and Noble 1996; Whitledge and Hayward 1997). In the present study, we used these physiological parameters in Fish Bioenergetics 3.0 (Hanson et al. 1997) to create our model by the following three step process: 1) determine rate of growth in weight for fish 1-6 years old in each percentile, 2) estimate  $P$  for each percentile based on observed growth rates, and 3) regress percent change in  $P$  ( $\% \Delta P$ ) against the corresponding change in ( $\Delta W_r$ ) for each percentile to provide a rate function. This function served as prediction model for determining the proportional change in  $P$  required to cause a corresponding change in relative weight.

In step 1, we converted the Ecoregion 8 mean length-at-age data to weight-at-age data by percentile using the standard weight equation proposed by Henson (1991) and relative weight data for stock-quality size largemouth bass from Brouder et al (2009). In step 2, mean daily growth rate was calculated across the 5 years and used to predict the length of time required for an individual in each percentile to grow from 50 g to 1,000 g. The data are presented in Table 1.

TABLE 1. Weight-at-age (g) values and mean daily growth (g/d) calculated using Ecoregion 8 average length-at-age data (Brouder et al. 2009) and mean relative weight ( $W_r$ ). Weights were calculated from length using the standard weight equation parameters from Henson (1991) and mean relative weight by percentile as listed below.

| Age (years)                           | Day equivalent (for model) | Estimated weight (g) by percentile and estimated relative weight ( $W_r$ ) |         |          |          |          |           |
|---------------------------------------|----------------------------|--|---------|----------|----------|----------|-----------|
|                                       |                            | Percentile $W_r$   | 5% 82.1 | 25% 86.2 | 50% 89.5 | 75% 96.5 | 95% 110.5 |
| 1                                     | 1                          |  | 5       | 10       | 19       | 48       | 124       |
| 2                                     | 366                        |  | 49      | 81       | 145      | 253      | 501       |
| 3                                     | 731                        |  | 127     | 202      | 304      | 501      | 371       |
| 4                                     | 1096                       |  | 212     | 358      | 489      | 815      | 1,436     |
| 5                                     | 1461                       |  | 334     | 507      | 706      | 1,193    | 1,975     |
| 6                                     | 1826                       |  | 492     | 656      | 1,096    | 1,730    | 2,477     |
| <b>Mean daily growth (g/d)</b>        |                            |  | 0.27    | 0.35     | 0.59     | 0.92     | 1.29      |
| <b>Day to grow from 50 to 1,000 g</b> |                            |  | 3,562   | 2,682    | 1,610    | 1,031    | 737       |

For the each model, temperature inputs were based on observed mean annual subsurface water temperatures (23-29°C; C. Fox, unpublished data) in Cerrillos Reservoir. Because adult largemouth bass diet in Cerrillos Reservoir is exclusively piscivorous, a single generic prey input of 4,500 J was used, which is an approximate mean of the energy density of primary prey species in this reservoir, which mainly includes threadfin shad *Dorosoma petenense*, sunfish *Lepomis* spp., and tilapia *Oreochromis* and *Tilapia* spp.

For each percentile, the model was run using the “fit to end weight” procedure, with run time equal to the time required to grow from a start weight of 50 g to an end weight of 1,000 g. This process provided the values of P by percentile. In step 3, simple linear regression was used to determine the relationship between %ΔP and Δ $W_r$ , to allow for prediction of the required change in P needed to yield a particular change in  $W_r$  (e.g., how much does P need to increase in order to increase  $W_r$  from 80 to 95?). We linearized each variable using natural log transformation. Hereafter, this model is referred to as the Generic Model.

Using the Generic Model derived above, we developed a prediction model for population dynamics of largemouth bass in Cerrillos Reservoir (Cerrillos Model). In this model, we used weight-at-age data from age 1 largemouth bass individually tagged in 2012 and recaptured in 2013 to calculate mean daily growth in weight. The initial model run used the observed mean starting weight of 69.5 g (SE=4.8 g) and an estimated end weight of 327.4 g after 365 days. Assuming a starting mean  $W_r$  of 81 per Brouder et al. (2009), we then increased P using the Generic Model by one  $W_r$  unit per iteration for 20 iterations ( $W_r = 81$  to 101) and ran the model using “fit to P.” This provided estimates of individual annual consumption required to yield target  $W_r$  values and an estimate of modified end weights.

In order to put consumption in terms of biomass for use in harvest predictions, we used the 2012 population estimate and biomass data for the largemouth bass population in Cerrillos Reservoir to model the foregone consumption of removed largemouth bass. In 2012, 2,333 largemouth bass with mean weight of 310.8 g were removed, representing a 20.5% biomass reduction. We used weight-at-age data from similar size (age 2) largemouth bass individually tagged in 2012 and recaptured in 2013 to calculate mean daily growth in weight and total prey consumption of these fish if they would have remained in the population. Assuming that consumption released by removed fish was available to remaining fish, we added the released consumption back to the remaining population and

compared the increase in P to the Cerrillos Model. Using this method, we created a matrix to predict biomass harvest requirements.

#### Model 2

We assumed that low levels of population reduction would cause little effect on growth and condition, but this effect would increase exponentially as more conspecifics were removed from the population. However, as growth and condition improved, biological limitations of the species would begin to moderate change in growth and condition towards some asymptotic maximum. Thus, we used a sigmoidal curved to represent this relationship.

To create this model, we used the minimum and maximum mean  $W_r$  of largemouth bass from the native range (Brouder et al. 2009) as the initial condition ( $C_i$ ) and maximum condition ( $C_\infty$ ), respectively. Because the initial growth rate ( $G$ ) of the curve is unknown, we modeled a range of  $G$  to provide multiple prediction equations. Thus, the prediction equation (Model 2) is:

$$C_t = C_i + (C_\infty - C_i) \times (1 / [(1 + G) \times e^{cP - d}]),$$

where  $C_t$  is the predicted  $W_r$  at some proportion ( $P$ ) of population reduction,  $C_i$  and  $C_\infty$  are observed  $W_r$  values for the 5% and 95% percentiles from Brouder et al. (2009), and  $G$  is the function growth constant which was varied from 0.002 to 0.5. The constants  $c$  and  $d$  were set at 20 and 10, respectively, as these values allowed the model to approach  $C_i$  and  $C_\infty$ .

*Empirical Verification.*—To test these models, we used an experimental removal of largemouth bass biomass from Cerrillos Reservoir. In short, we removed about 20% of the crowded biomass each year for two years (on top of current natural and exploitation rates) and assessed population characteristics from before the first removal (2012) and one year after the second removal. In Model 1, we compared model predictions to actual population response, using  $W_r$  as the response variable. For Model 2, we used the proportion removed during the experimental removal and the observed response in  $W_r$  to determine  $G$  that provided the best fit prediction curve.

#### Results

*Current Mortality Vectors.*— The 100 adult largemouth bass ranged from 304 to 574 mm TL (mean = 391 mm, SD = 45) and 344 to 2,622 g (mean 743 g, SD 350). Six fish were never located during the study and five fish never moved from the location where they were first recorded (three in open water, two ≤10 m from shore). These eleven fish were excluded from our analyses. Of the 89 remaining tagged fish, 46 were

determined to have died during the first year of tracking. Eight of those mortalities were labeled as angler harvest with two tags being returned by anglers (#2 returned 17 Dec 2011, #105 returned 04 Feb 2012). The other 38 were listed as natural mortalities because the tags did not move over multiple consecutive tracking events.

Total annual interval mortality rate ( $A$ ) for the first year was 0.5169 with exploitation ( $\mu$ ) and natural ( $\nu$ ) mortalities estimated as 0.0899 and 0.4270, respectively. Our estimate of  $\mu$  was very close to the estimate using creel data (0.0895). Total instantaneous mortality rate ( $Z$ ) was 0.7274 with instantaneous fishing ( $F$ ) and natural ( $M$ ) mortality rates being 0.1265 and 0.6009, respectively.

*Model 1 Development.*—Largemouth bass within their native range exhibited substantial variation in growth and condition (Table 1). By age 6, estimated weight was as low as 492 g within the lower 5%, and as high as 2,477g within the upper 95%. Similarly, relative weight averaged 81.1% for the lower 5%, and ranged up to 110.5% for the upper 95% of largemouth bass populations. This variability provided a perfect spectrum of population plasticity to model density dependent effects.

Basic bioenergetics modeling of Region 8 data demonstrated a strong relationship between the estimated value of  $P$  and the  $W_r$  of each percentile (Figure 2;  $F_{1,3} = 71.6$ ,  $P = 0.003$ ). Thus, the Generic Model:

$$\% \Delta P = 0.0098 \cdot \Delta W_r + 0.0081,$$

was developed using these datasets to predict increases in prey consumption required to yield targeted increases in  $W_r$  (Figure 3). In turn, the Generic Model was used to create a prediction model for Cerrillos Reservoir. This model estimates the increase in

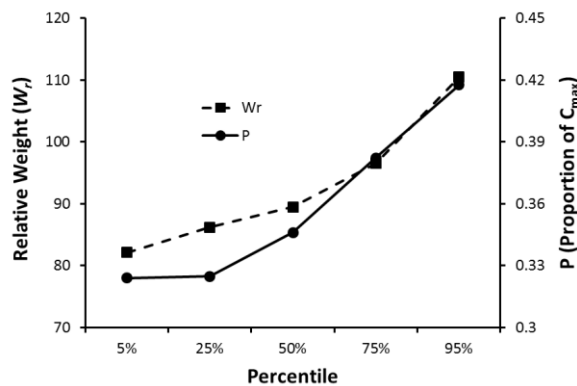


FIGURE 2. Relationship of relative weight ( $W_r$ ) and proportion of  $C_{max}$  by population percentile modeled using Bioenergetics Model 3.0 for fish growing from 50 g to 1,000 g at varying growth rates.

individual prey consumption required to increase  $W_r$  for largemouth bass in Cerrillos Reservoir (Cerrillos Model; Figure 4). For example, the Cerrillos Model predicted that, in order to increase  $W_r$  from the current value of 81 to a target value of 90 (9  $W_r$  units), a 35.3% increase in prey consumption would be required. This represents an annual increase in consumption of 483.6 g per individual fish remaining. Finally, using modeled growth and consumption of age 2 largemouth bass in Cerrillos Reservoir, we calculated the proportion of the population by biomass needed to be removed annually above current harvest regimes to produce desired changes in  $W_r$  (Table 2).

*An Empirical Test of Model Predictions.*—In 2012, we removed 2,333 stock and quality size largemouth bass, with a cumulative biomass removed of 725 kg (20% of total biomass; Chapter 8). In 2013, we removed 1,993 largemouth bass, accounting for 22% of total biomass. Thus, we maintained about a 20% reduction in biomass over two years. During that period,  $W_r$  increased from 81 to 87, or 6 units. Predictions from Model 1 suggested that a 20% reduction in population biomass should yield a 10 unit increase in  $W_r$ .

*Model 2 Development.*—An alternative approach to predict removal requirements was developed using a logistic growth function model (Model 2). This model provided a range of possible removal scenarios for improving population condition (Figure 5). Using data from the two consecutive years of population reduction in Cerrillos Reservoir, we selected  $G = 0.01$  as model best fit to the observed changes in  $W_r$  given a sustained 20% reduction in population biomass. Based on this model, a 30% sustained removal would increase mean  $W_r$  to 100.

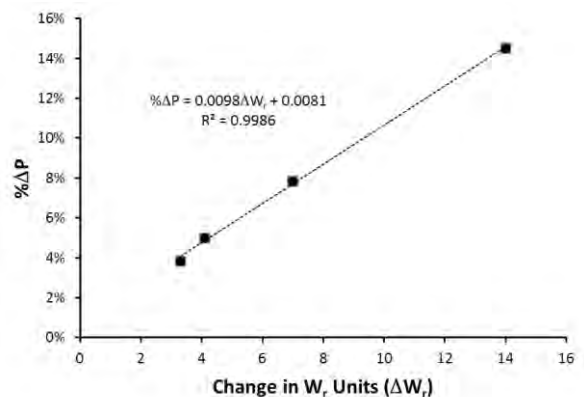


FIGURE 3. The Generic Model used to predict proportional change in  $P$  (proportion of  $C_{max}$ ) required to affect change in relative weight ( $W_r$ ).

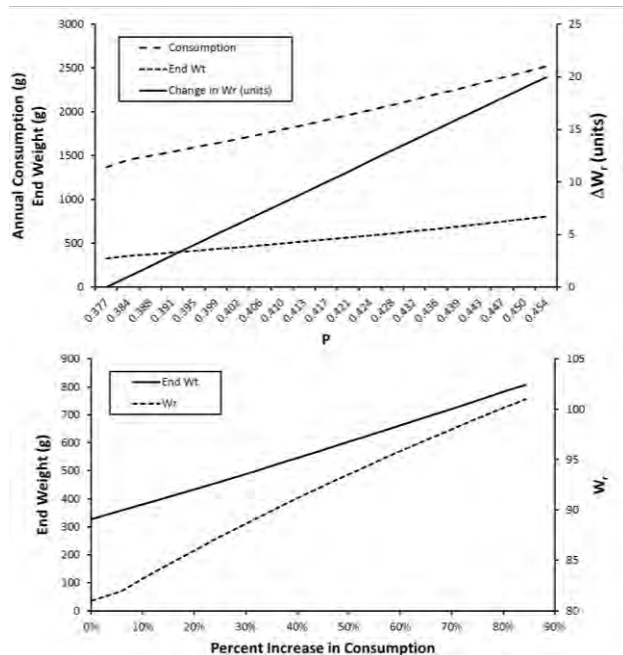


FIGURE 4. The Cerrillos Model used to predict theoretical response of largemouth bass size (end weight, g) and relative weight in Cerrillos Reservoir to proportional increases in individual consumption. Top: Total consumption, end weight, and change in  $W_r$  ( $\Delta W_r$ ) by proportion of maximum consumption (P). Bottom: End weight and  $W_r$  by percent increase in consumption. Fish were modeled with a starting weight of 69.5 g for 365 days.

## Discussion

All models are inherently wrong, and the models presented here should not be interpreted as accurate depictions of the population dynamics in Cerrillos Reservoir. However, models can serve as useful tools for enhancing overall understanding of this reservoir. In Model 1, the observed response was lower than the predicted response following a 20% biomass reduction in Cerrillos Reservoir. There are many possible explanations for these results. First, the model may simply be an inaccurate representation of this system. Many factors were not accounted for in the model, including recruitment processes, prey response to reduced predatory pressure, and changes in reproductive behavior with reduced density. Also, the reservoir has experienced two consecutive years of drastic water level declines, approaching 10 m in both 2013 and 2014. Another possible explanation is that 2 years was not long enough to observe maximum change in condition. Relative weight increased from 81 to 84 from 2012 to 2013 following the first experimental removal, and from 84 to 87 from 2013 to 2014 following the second removal. It is quite possible that sustained removals will continue to produce increases in condition. We contend that the latter explanation is the most probable.

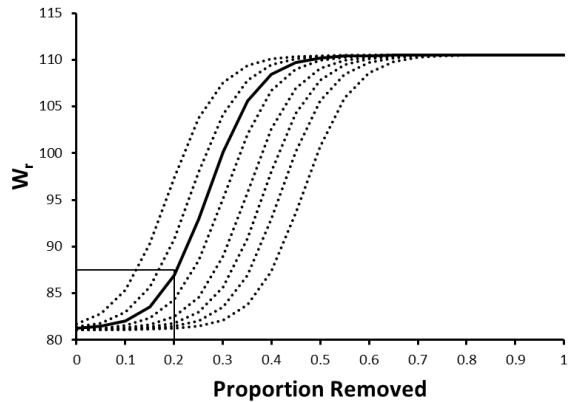


FIGURE 5. Sigmoidal response model used to conceptualize the response of largemouth bass condition ( $W_r$ ) to proportional reduction in population size at varying levels of the function growth constant (G). From left to right, G was 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, and 0.5. Based on the sustained population reduction of 20% and the observed increase in relative weight to 87,  $G = 0.01$  (solid line) appeared to be the best fit model for Cerrillos Reservoir.

Model 2 predicted that a sustained reduction of 30% of biomass would lead to  $W_r$  increasing to 100, the desired level for managed largemouth bass fisheries. This was similar to the 34% biomass reduction required in Model 1 for similar results. The close agreement of the two models suggests that these estimates are useful targets for the Cerrillos Reservoir fishery. In terms of numbers of largemouth bass, a 30% reduction of biomass is equivalent to about 35% of the population. This suggests that the current level of exploitation (0.09) is severely insufficient to utilize the surplus production of the fishery, and exploitation ( $\mu$ ) needs to be increased by about 0.35 to  $\mu = 0.44$  to improve population dynamics in this reservoir.

It is unknown if increases in exploitation would cause compensatory decreases in natural mortality. However, data from Chapter 8 suggest no compensatory response, as only a 20% biomass reduction on top of current harvest rates ( $\mu = 0.32$ ) demonstrated consistent improvement in condition over 2 years. Thus, our models assume no compensatory response. However, we suspect that the exploitation requirements predicted by the models might be excessive. This is because of the synergistic relationship between predators and prey. As predation declines due to increased exploitation (or target management removals), prey populations should respond with increased production. Thus, prey availability will increase not only because fewer largemouth bass are eating them but also because more prey survive and reproduce. In fact, preliminary results as part of a doctoral dissertation have demonstrated increases in threadfin shad, tilapia, and sunfish following the experimental removals. These analyses

TABLE 2. Estimated proportion of population needed to be removed above current harvest rates in order to increase  $W_r$ .

| Target<br>End $W_r$ | Starting $W_r$ |      |      |      |      |      |      |      |      |      |      |      |     |  |
|---------------------|----------------|------|------|------|------|------|------|------|------|------|------|------|-----|--|
|                     | 76             | 78   | 80   | 82   | 84   | 86   | 88   | 90   | 92   | 94   | 96   | 98   | 100 |  |
| 76                  | ---            |      |      |      |      |      |      |      |      |      |      |      |     |  |
| 78                  | 0.06           | ---  |      |      |      |      |      |      |      |      |      |      |     |  |
| 80                  | 0.10           | 0.06 | ---  |      |      |      |      |      |      |      |      |      |     |  |
| 82                  | 0.14           | 0.10 | 0.06 | ---  |      |      |      |      |      |      |      |      |     |  |
| 84                  | 0.17           | 0.14 | 0.10 | 0.06 | ---  |      |      |      |      |      |      |      |     |  |
| 86                  | 0.20           | 0.17 | 0.14 | 0.10 | 0.06 | ---  |      |      |      |      |      |      |     |  |
| 88                  | 0.24           | 0.20 | 0.17 | 0.14 | 0.10 | 0.06 | ---  |      |      |      |      |      |     |  |
| 90                  | 0.26           | 0.24 | 0.20 | 0.17 | 0.14 | 0.10 | 0.06 | ---  |      |      |      |      |     |  |
| 92                  | 0.29           | 0.26 | 0.24 | 0.20 | 0.17 | 0.14 | 0.10 | 0.06 | ---  |      |      |      |     |  |
| 94                  | 0.32           | 0.29 | 0.26 | 0.24 | 0.20 | 0.17 | 0.14 | 0.10 | 0.06 | ---  |      |      |     |  |
| 96                  | 0.34           | 0.32 | 0.29 | 0.26 | 0.24 | 0.20 | 0.17 | 0.14 | 0.10 | 0.06 | ---  |      |     |  |
| 98                  | 0.37           | 0.34 | 0.32 | 0.29 | 0.26 | 0.24 | 0.20 | 0.17 | 0.14 | 0.10 | 0.06 | ---  |     |  |
| 100                 | 0.39           | 0.37 | 0.34 | 0.32 | 0.29 | 0.26 | 0.24 | 0.20 | 0.17 | 0.14 | 0.10 | 0.06 | --- |  |

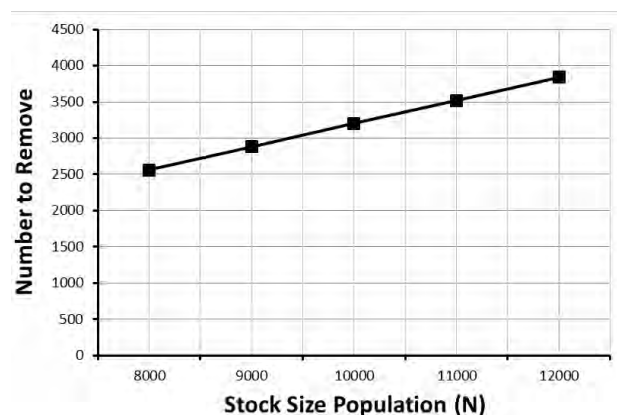


FIGURE 6. Number of stock size largemouth bass to remove annually by population size estimate to achieve a recommended annual exploitation ( $\mu$ ) of 0.32.

are ongoing and will be submitted with the completion of SFR Project F-69.

Because of synergistic responses such as this, we anticipate that lower level of sustained exploitation will be sufficient to continue restructuring the largemouth population. We recommend continuing the 20% supplemental biomass reduction performed during 2013 and 2014. In terms of exploitation by numbers of fish, this would mean increasing  $\mu$  by 0.23 to  $\mu = 0.32$ . Depending on standing population size, this represents and additional removal of 2,500 to 4,000 stock size fish annually (Figure 6).

Requiring harvest of stock and quality size bass (200-380 mm TL) during competitive tournaments alone may be sufficient to reach the target exploitation.

However, we recognize that changing angler behavior is difficult, and promoting a 350% increase in harvest among catch-and-release minded anglers would be a difficult task. Using educational approaches and the example of the current response of the fishery to experimental removals, many anglers may recognize the benefits to harvest. However, some anglers will undoubtedly resist. Regardless, any increase in angler exploitation would be helpful and, with accurate assessment of angler exploitation, additional removals using electrofishing could be used to meet harvest goals.

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## **Study 4:**

# **Alternative Sport Fish Species in Puerto Rico**



## Chapter 10: Report on Broodstock Management at the Maricao Fish Hatchery

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**Abstract:** Proper broodstock management is critical to successful hatchery operations, as the overall well-being of parental fish determines the quality and quantity of offspring produced. In this report, we suggest best management practices and recommendations to the Maricao Fish Hatchery for future broodstock management. The four most critical recommendations are 1) improve broodstock admission procedures to include a period of quarantine and use of passive integrated transponder (*PIT*) tags to improve record keeping, 2) use a commercially available feed with 35-40% protein content to ensure that the dietary requirements of broodfish are met, 3) improve feed storage conditions and reduce maximum storage time to improve nutritional content, and 4) maintain or improve genetic diversity of broodstock by importing unrelated individuals from within the native range of each species. Improvements in these four areas should improve fingerling production, which will benefit island reservoir fisheries.

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Continuous production of eggs, fry and fingerlings in a fish hatchery requires sufficient quantity and adequate quality of broodfish (Bromage and Ronald 2001; Piper et al. 2002). Broodstock management involves manipulating environmental factors surrounding broodfish to reduce stress levels, ensure maximum survival, enhance gonadal maturation (Bromage and Ronald 2001), and increase fecundity by providing the optimal conditions and diets (Izquierdo et al. 2001). Broodstock management can be used to increase number and quality of eggs and to control timing of maturation, mating, and spawning (Bromage et al. 1992). Control of mating allows for genetic improvement by selecting broodfish with traits such as fast growth rates, disease resistance, or higher fecundity over generations to produce more desirable fish (Bromage et al. 1992).

The quantity of broodfish needed is determined by the number of eggs required to produce the fry desired (Piper et al. 2002). Broodfish quality determines reproductive output as well as growth and survival of progeny. When broodfish are stressed or malnourished, egg production, hatching, and survival can be unsatisfactory. Difficulties in the supply of eggs and fry are among the major constraints to successful fish culture (Bromage and Ronald 2001).

Husbandry practices differ with species, and broodfish should be maintained under controlled conditions that match or improve those to which fish would have been exposed in their natural environment. It may not be practical to manage all of the rearing

conditions, but water quality, nutrition, feeding regimes, stocking density and other stressors may be optimized by appropriate broodstock management and husbandry practices. Such improvements may be difficult for species of fish that have only recently become cultured because the establishment of best husbandry practices requires years of development and experimentation (Bromage and Ronald 2001).

In this report, we discuss major factors that affect broodstock well-being and examine current broodstock management and husbandry practices at the Maricao Fish Hatchery (hereafter, the Hatchery). Furthermore, we identify possible issues/limitations and propose strategies that may improve broodstock management. These strategies could help improve broodfish general well-being, egg production in terms of quantity and quality, spawning frequency, and improve development and survival of fingerlings.

### **Primary Species Cultured**

Sport fish species that are currently reared at the Hatchery include largemouth bass *Micropterus salmoides*, channel catfish *Ictalurus punctatus*, bigmouth sleeper *Gobiomorus dormitor*, and peacock bass *Cichla ocellaris*. Both largemouth bass and channel catfish have a long history of production and extensive research, and thus standardized rearing and broodstock management protocols have been established. However, these protocols were developed principally for warmwater hatcheries in the temperate USA, and limited information on tropical culture of

these species has been published. Little is known about the appropriate husbandry and broodstock management practices of the bigmouth sleeper and peacock bass. For this reason, suggestions for bigmouth sleeper and peacock bass broodstock management have been drawn from ongoing spawning trials at the Hatchery.

Broodstock management protocols vary by species due to life history traits, water quality and husbandry requirements. Current Hatchery husbandry and management practices for bigmouth sleeper and channel catfish are to house and spawn in covered round tanks, while largemouth bass are held primarily in shallow outdoor cement tanks and moved to earthen ponds for spawning. Peacock bass are held in lined outdoor ponds and allowed to spawn naturally.

### **Collection, Admission, and Holding Protocols**

Currently, peacock bass, bigmouth sleeper, and channel catfish broodfish are wild caught, while largemouth bass broodfish may be hatchery raised, but are more commonly replenished with wild caught fish. Field collection of broodfish is conducted using mounted boom electrofishing along the shoreline. Broodstock are transported to the Hatchery in an aerated hauling tank at 2 ppm salinity to reduce stress. However, wild caught channel catfish do not usually make good broodstock (Dupree and Huner 1984), and it is suggested that, if channel catfish production becomes a priority, new hatchery-strain channel catfish should be imported from the southern USA.

Current admission practices vary by species. We recommend that all fish arriving to the hatchery undergo specific transportation, processing and quarantine procedures. During transportation, we suggest adding bactericides, such as oxytetracycline or sulfamerazine; this reduces the risk of pathogen introduction into the Hatchery (Piper et al. 2002; Timmons and Ebeling 2007). Each fish, regardless of species, should be sexed, measured and weighed, and implanted with a passive integrated transponder (*PIT*) tag for individual identification. This individual mark will facilitate record keeping (genetic inventory), broodstock selection and maintenance. Following processing, wild-caught fish should be placed into the Quarantine Facility for an observation period of at least 14 days before being placed with other resident fish. This relatively short quarantine period is primarily to allow hauling stress and injuries to run their course, as many secondary infections can be transferred to healthy fish. Longer quarantine periods, up to 30 days or more, may be warranted if particular pathogens are suspected (Timmons and Ebeling 2007). Use of mildly saline water in quarantine will help reduce mortalities and secondary infections. For largemouth bass,

peacock bass, and channel catfish, salinities of 3-7 ppt would be appropriate. For bigmouth sleeper, salinities up to 10 ppt have proven effective.

### **Broodstock Selection and Improvement**

Broodfish selection often involves factors such as size, color, shape, feed conversion, timing and duration of spawning, age at maturity, reproductive capacity, disease resistance, as well as hatching and survival of offspring. Depending on the Hatchery objectives, one or more of these traits can be maximized using strategic broodstock selection. However, a primary concern for broodstock selection should be to avoid breeding of related individuals (inbreeding). The use of individual marks such as PIT tags will help to prevent mating of related individuals.

Another important consideration to maintaining genetic integrity in the Hatchery is to periodically enrich bloodlines through the addition of unrelated individuals. The need to enrich bloodlines might be suspected if a high percentage of deformities, low hatch success, low survival of fry or poor growth become evident. This is particularly important for species that are non-native to Puerto Rico and were established with small, or often very small, founder populations. It is recommended that these lines be diversified with occasional importation of new fish, and that crossbreeding of unrelated stocks be used to ensure genetic diversity.

### **Stress**

Broodfish are far more tolerant to stress factors than fry and juveniles; however, their reproduction can be significantly impacted by stress. In some species, stress may delay ovulation and fertilized eggs may be smaller in size and of poorer quality (Campbell et al. 1992). Chronic stress can be imposed by modestly inappropriate rearing conditions, and as a consequence, many fish either fail to spawn or do not fully mature under culture conditions. Water flow rates, poor water quality, inadequate feeding regimes and diets, inappropriate photoperiod/light intensity, temperature, handling, and stocking density can increase stress in fish (Bromage and Ronald 2001; Timmons and Ebeling 2007). Stocking density is species dependent, and it is advisable to keep males and females separated until time for mating. Sex separation enables the broodstock males and females to be subjected to different conditions where necessary and reduce diseases and mortalities due to aggressive behaviors and courtship (Bromage and Ronald 2001). It also prevents unwanted release of gametes.

Furthermore, stressors experienced by the broodstock can be manifested in the resulting offspring. For example, Ostrand et al. (2004) reported

that largemouth bass fingerlings produced from broodfish exposed to capture and handling stress before spawning were smaller and weighed less than controls. Smaller fingerlings may be less competitive and less likely to survive compared to larger conspecifics.

### Water Quality

The impact of water quality on broodfish health, stress levels, food intake, and reproduction cannot be overstated. Carbon dioxide (CO<sub>2</sub>), pH, ammonia (NH<sub>3</sub>), nitrite (NO<sub>2</sub><sup>-</sup>), and hydrogen sulfide (H<sub>2</sub>S) can greatly affect broodfish well-being either individually or through their interactions. For example, pH values are typically higher when CO<sub>2</sub> concentrations are low (during periods of high primary production), and this situation increases the toxicity of ammonia to aquatic life. For these reasons, water quality parameters should be measured routinely so that dangerous situations can be averted or immediately corrected.

#### Water temperature

Water temperature is one of the main factors that significantly affect the development and well-being of a fish during its lifespan. It warrants proper monitoring in a hatchery system, as the activity, behavior, feeding, maturation of broodfish, spawning, development of fertilized ova, hatch success, growth, and survival of larvae are all directly affected by it. Each species has a temperature range that it can tolerate, and within that range it has optimal temperatures for reproduction and growth (Table 1). A large number of environmental factors are involved in controlling fish reproduction, but photoperiod and temperature are thought to be the main environmental cues (Bromage et al. 1993; Prat et al. 1999; Bacheleer et al. 2004).

Within a hatchery, extreme water temperature fluctuations may impart stresses that can dramatically affect production and render fish more susceptible to disease. Water temperature at the Maricao Fish

Hatchery typically ranges from 18°C (during colder nights) to 26°C (on warmer days), with average temperature around 23°C. Water temperature can be difficult to maintain during water changes, as standing water temperature is usually 3-4°C greater than the water source. Thus, water changes can cause rapid temperature drops that stress fish. This can easily be avoided by adding a heating system to main water supply, or use a 1,000 gallon black tank exposed to direct sunlight (to ensure maximum heat absorption) and adding a bypass to the main water supply pipe.

#### Dissolved oxygen and carbon dioxide

Temperature determines the saturation capacity of dissolved oxygen (DO) in the water; the higher the temperature, the lower the maximum amount of DO water can hold and vice versa. DO concentration in hatchery waters are depleted in several ways, mainly by fish and other organisms' respiration and by chemical reactions with organic matter such as feces, waste feed, decaying plant and animal remains. As temperature increases, the metabolic rates and respiration of fish increase, depleting oxygen concentrations in the water. As a consequence, stress or even death may follow. Broodfish should not be subjected to extended DO concentrations below 5 ppm. Levels of oxygen below 5 ppm have been implicated in fish deformities during embryonic development (Piper et al. 2002).

Generally, waters supporting good fish populations have carbon dioxide concentrations less than 5 ppm, and carbon dioxide in excess of 20 ppm may be harmful to fish. If the dissolved oxygen content drops to 3-5 ppm, even lower CO<sub>2</sub> concentrations may be detrimental. For most Hatchery systems, DO concentrations usually range from 6 to 10 ppm and mechanical aeration is provided to avoid lethal drops in DO or spikes in CO<sub>2</sub>.

TABLE 1. Reported temperature requirements for species currently cultured at the Maricao Fish Hatchery.

| Species                       | Survival range (°C) | Optimum range (°C) | Spawning range (°C) |
|-------------------------------|---------------------|--------------------|---------------------|
| Largemouth bass <sup>1</sup>  | 0.5 - 35            | 12.7 - 26.6        | 15.5 - 18.3         |
| Channel catfish <sup>1</sup>  | 0.5 - 35            | 21.1 - 29.4        | 22.2 - 27.7         |
| Bigmouth sleeper <sup>2</sup> | 13 - 29             | Not reported       | 28.1 - 32.1         |
| Peacock bass <sup>3</sup>     | 18 - 29             | 27.7 - 29.2        | 25.0 - 28.0         |

<sup>1</sup> Piper et al. (2002)

<sup>2</sup> Gilmore and Hastings (1983); Harris et al. (2011)

<sup>3</sup> Optimum: Sampaio (2000); Spawning: Maricao Fish Hatchery observations



## *pH*

Recommended pH for aquaculture systems is 6.5 to 9.0. Fish, especially young fish, may become stressed or die if pH drops below 6 or rises above 9. In warm environments where photosynthesis can be intense, pH can fluctuate from 5 to over 10 over a 24-h period. This fluctuation is related to CO<sub>2</sub> dynamics. During periods of elevated photosynthesis (mid-afternoon), CO<sub>2</sub> is removed from the water and pH increases. At night, photosynthesis ceases and respiration adds CO<sub>2</sub> to the water, which decreases pH.

Most cultured species prefer slightly alkaline conditions with pH between 7 and 9. Symptoms of inappropriate pH include rapid spinning near the water surface and attempting to leave the water, whitening of the eyes and blindness, as well as fraying of the fins and gills with the frayed portions turning white. Death usually follows soon after. Although more common, symptoms of lethal pH may go unnoticed in fish eggs and fry until it is too late. The best prevention is frequent examination of water quality.

## *Ammonia*

Ammonia gas dissolves in water, a portion reacts with water to produce ammonium ions (NH<sub>4</sub><sup>+</sup>), and the remainder is present as un-ionized toxic ammonia (NH<sub>3</sub>). The amount of toxic ammonia varies with salinity, dissolved oxygen, and temperature. However, un-ionized ammonia is primarily determined by pH, with a direct correlation between the two. Excessive levels of NH<sub>3</sub> may result in reduction in growth rate, damage to gill, kidney, and liver tissues, and blindness (Piper et al. 2002). Although a number of sources suggest that unionized ammonia becomes toxic to fish at levels between 0.6 and 2.0 ppm (EIFAC 1973; USEPA 1985), studies on channel catfish have reported fish health risks at 0.12 ppm (Piper et al. 2002). Ammonia can be removed either chemically or through adequate water change. At the Hatchery, water exchange is recommended as the most cost effective treatment for high ammonia.

## *Nitrite*

Ammonia (NH<sub>3</sub>) can be converted by bacteria into nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>). While NO<sub>3</sub><sup>-</sup> is relatively harmless to fish, NO<sub>2</sub><sup>-</sup> can be quite harmful. Nitrite enters the fish blood through the gills and combines with hemoglobin to form methemoglobin. This compound imparts a brown color to the blood and results in a toxic condition known as “brown blood.” This is especially common in channel catfish culture where high densities of fish are cultured. To prevent brown blood in fish ponds, at least three parts of chloride can be added for each part of nitrite in the water. Nitrite concentrations can also be reduced by

adding freshwater. Although nitrite poisoning is unlikely in Hatchery ponds, flushing with freshwater is the recommended treatment.

## **Nutrition and Feeding Regimes**

Important considerations for developing a feeding regime for a particular fish species include nutrient requirements, environmental conditions, stress factors, storage facilities, and production objectives (Piper et al. 2002). Nutrition is important not only for reproductive success of broodfish, but also for obtaining healthy larvae and fry, as broodstock nutrition directly influences hatchability and survival of larvae (Izquierdo et al. 2001; Pillay and Kutty 2001). Improvements in broodstock nutrition have been shown to greatly improve egg and sperm quality, as well as fecundity and hatching success (Duray et al. 1994).

A nutritionally balanced feed must contain required nutrients in proper proportion (Table 2). If a single essential nutrient is deficient, it will affect the efficient utilization of other nutrients (Piper et al. 2002). In severe cases, nutrient deficiencies can develop, affecting different physiological systems and produce a variety of deficiency signs. Essential fatty acids, vitamins A, C and E, trace minerals, beta-carotene, and other carotenoids can affect fecundity and egg quality (De Silva and Anderson 1995; Izquierdo et al. 2001). Embryo survival has been shown to be affected by the deficiency of vitamin C in broodstock diets (Izquierdo et al. 2001). Vitamin A is important for embryo and larval development due to its important role in bone development, retina formation and differentiation of immune cells (Izquierdo et al. 2001). These nutritional deficiencies may be caused by directly feeding broodstock with poor quality feeds, or indirectly by feeding broodstock live prey that were fed poor quality feeds.

Dietary essential fatty acid content is one of the major nutritional factors that affect broodfish reproductive performance and offspring survival (Watanabe et al. 1984). In some species, highly unsaturated fatty acids (HUFA) increase fecundity, fertilization, and egg quality (Watanabe et al. 1984; Fernández-Palacios et al. 1995). Increased *n*-3 HUFA (particularly docosahexaenoic acid) levels in broodstock diets can enhance the weight of larval fish and their resistance to osmotic shock (Izquierdo et al. 2001).

The importance of protein content in the diet of fishes has been well reported. Nutritional requirements have been determined for broodfish of largemouth bass (~40% crude protein; Pillay and Kutty 2001), channel catfish (35-40% crude protein; Pillay and Kutty 2001)

TABLE 2. Recommended amounts of vitamins in fish feeds. Values are amounts per kilogram of feed, and include total amounts from ingredients and vitamin premixes (Adapted from Piper et al. 2002).

| Vitamin                | Units | Warmwater fish feeds <sup>a</sup> |               |
|------------------------|-------|-----------------------------------|---------------|
|                        |       | Supplemental diet                 | Complete diet |
| Vitamin A              | IU    | 2,200                             | 5,500         |
| Vitamin D <sub>3</sub> | IU    | 220                               | 990           |
| Vitamin E <sup>b</sup> | IU    | 11                                | 50.6          |
| Vitamin K              | mg    | 5.06                              | 9.9           |
| Ascorbic acid          | mg    | 50.6                              | 99            |
| Biotin                 | mg    | 0                                 | 0.11          |
| B <sub>12</sub>        | mg    | 0.011                             | 0.022         |
| Choline                | mg    | 440                               | 550           |
| Folic acid             | mg    | 0                                 | 5.06          |
| Inositol               | mg    | 0                                 | 99            |
| Niacin                 | mg    | 28.6                              | 99            |
| Pantothenic acid       | mg    | 11                                | 110           |
| Pyridoxine             | mg    | 11                                | 19.8          |
| Riboflavin             | mg    | 6.6                               | 19.8          |
| Thiamine               | mg    | 0                                 | 19.8          |

<sup>a</sup> These amounts do not allow for processing or storage losses.

<sup>b</sup> Requirement is affected directly by the amount and type of unsaturated fat.

and peacock bass (~37-41% crude protein; Sampaio et al. 2000), but nutritional requirements for bigmouth sleeper have not been reported. At the time of this report, the pelletized prepared feed being used at the Hatchery contained 24% crude protein – considerably less than recommended levels.

Feeding rate and ration can affect spawning output. Reduction in feeding rate can cause an inhibition of gonadal maturation (Cerdá et al. 1949; Berglund 1955; Sasayama and Takahashi 1972; Izquierdo et al. 2001), and optimal feeding rates and rations depend greatly on temperature, species, body size, age and standing crop. Temperature is an important factor to determine feeding requirements of fish. Caloric needs increase with rising water temperatures, resulting in increased appetite of fish (Piper et al. 2002).

Broodfish must have a nutritionally balanced feed and be fed 2 to 3% of their body weight daily during spawning season and on alternate days during cooler periods. Broodfish tend to lose 10-20% of their body weight during spawning season, and this weight must be regained to ensure broodfish health. A quality feed is critical to recovery. For predator species such as largemouth bass, peacock bass, and bigmouth sleeper, a suitable forage fish is critical to guarantee optimal growth and reproduction. Forage species must be fed with high-quality, nutritionally-appropriate feed to ensure proper nutritional transfer to piscivorous broodfish. In cases where personnel may be limited or

disturbance of broodstock is undesirable, feeding may occur every other day at double the daily rate.

It is not enough to purchase high quality commercial feed that meets broodstock nutritional requirements. Quality must be maintained using proper handling and storage procedures. Improper storage will cause rapid spoilage and loss of nutritional value (Piper et al. 2002). During storage, physical conditions (moisture, heat, light), oxidation, micro-organisms (molds, bacteria, yeast), and enzymatic action can all cause spoilage of feed. Feed in bags or bulk should be stored in a cool dry area. Low humidity must be maintained because moisture enhances mold growth and attracts insects (Piper et al. 2002). Insects not only consume the feed, they also accelerate spoilage by secretion of enzymes such as lipase (Pillay and Kutty 2001). Molds, which grow when storage conditions are too humid, will cause spoilage and may produce toxins. High temperatures may cause rancidity of oils and deterioration of vitamins. The storage area should be kept clean and adequately ventilated, and the stored feed should be protected from rodents, insects and contamination (Piper et al. 2002).

Ideal conditions for storing bagged dry feed include stacking the bags not over ten high on pallets so the bags are 3 to 4 inches off the floor. Space should be provided between stacks for air circulation and rodent control. The recommended maximum storage time for dry pelleted feeds is 90-100 days. If less than optimal

storage conditions exist, storage time should be shortened (Piper et al. 2002).

Nutritionally deficient food and inadequate food handling and storage procedures appears to be the primary limitation to broodstock health at the Hatchery. Improvements in storage practices may improve fish health and reduce abnormalities in offspring due to nutrient deficiencies. A great number of *Tilapia rendalli* and largemouth bass offspring have been observed to be affected by vitamin C deficiencies, including symptoms such as abnormal opercula (Figure 1), deformed vertebrae and scoliosis, and reduced larval survival. Channel catfish broodfish appear affected by amino acid deficiencies, as many fish have lens cataracts, a common indicator of this deficiency.

The nutritional deficiencies observed at the Hatchery stem from three sources. First, the use of a 24% minimum protein content feed is below the recommended protein content for the species cultured. This directly affects channel catfish, which are fed the prepared feeds, and it indirectly affects largemouth bass, peacock bass, and bigmouth sleeper, which are fed tilapia produced from these inferior feeds. Secondly, and perhaps more seriously, storage conditions and storage time of these feeds is resulting in reductions in feed quality and nutritional content (Figure 2). The effect of poor storage conditions is apparent in both prey fish and target sport fish, with both showing symptoms of nutritional deficiencies.

Lastly, it is possible that piscivorous species are not being fed live prey with sufficient frequency or quantity when compared to recommended rations. For example, a 1,000-g largemouth bass that is fed five 45-mm tilapia (~2.0 g each) twice per week is only averaging about 0.28% body weight per day – nowhere near the recommended ration of 2-3% body weight per



FIGURE 1. *Tilapia rendalli* displaying common symptom of vitamin C deficiency – the characteristic opercular deformity resulting in exposed gill arches.

day. Largemouth bass broodfish appear to be healthy and reproductive, so this theoretical ration may not be a fair representation of actual feeding rates. However, it is recommended that the Hatchery conduct a thorough review of piscivore feeding protocols to ensure that optimum rations are employed.

### Recommended Broodstock Care by Species

#### *Bigmouth sleeper*

Bigmouth sleeper should be held in covered 1,665-L round polyethylene tanks. Cover/spawning tubes are placed on the bottom of each holding tank to provide shelter and removable spawning surfaces (Figure 3). In holding tanks, fish should be separated by gender and size to reduce aggressive behaviors and cannibalism. For spawning tanks, similar sizes should be grouped using a 2:1 female to male ratio. Four females and two males per tank has been an effective combination. Do not feed for 48-72 h following stocking. Each tank should have a waterproof notepad hanging from the side to record stocking dates, broodfish tag number, total biomass in tanks, feeding regimes and rations. Broodstock should be replenished from holding tanks when mortalities occur.

Bigmouth sleeper should be fed 2-3% their body weight with live *Tilapia rendalli* fingerlings on alternate days (approximately 30 – 60 mm standard length (SL)). Four to five fingerlings (2.0- 4.0 g) per bigmouth sleeper is usually sufficient. Maintaining adequate prey is especially important immediately prior to and during the spawning season. During the non-spawning period, two feedings per week at the same ration is sufficient. For a 500-g bigmouth sleeper, five 2.0-g tilapia fed every 2 days represents only about 1% body weight per day. Although this is considerably lower than recommended, these sedentary fish appear to be healthy and growing on this ration.

Water quality parameters should be monitored daily. See *Water Quality* for specific parameters. Dead fish should be promptly removed and discarded and these mortalities recorded. Cleaning tanks containing fish should be limited to periodic siphoning, only removing detritus accumulated on the bottom of the tanks. It is not necessary, and can be detrimental, to clean periphyton and biofilms from the tank surfaces. When water changes are required, incoming water must be the same temperature as tank water. Do not exceed 50% volume change per 24 h period.

#### *Channel catfish*

Channel catfish do well when held in 1,665-L round polyethylene tanks with cover tubes placed on the bottom of each holding tank to provide shelter (Figure 3). Fish should be separated by species and

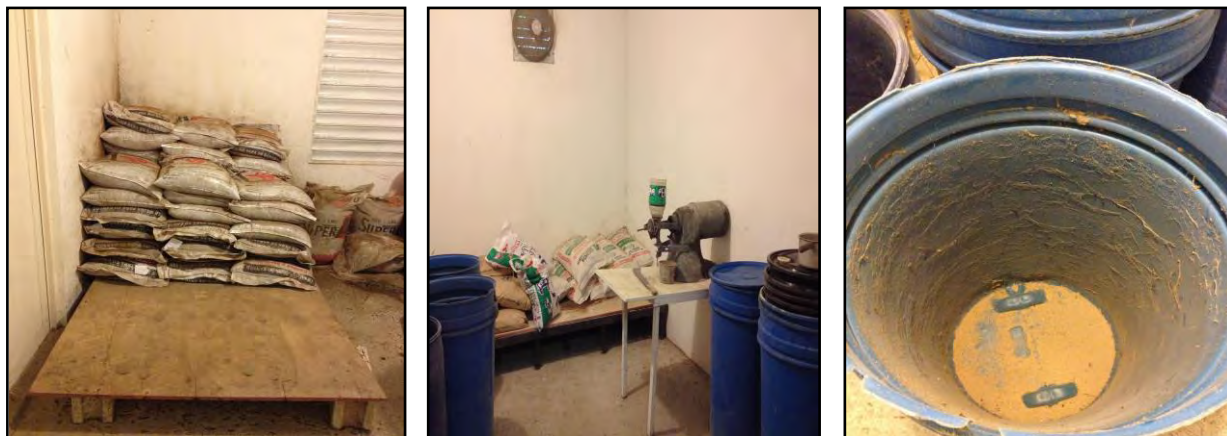


FIGURE 2. *Left:* Feed stacked on floor and on non-ventilated pallet; *Center:* Inadequate ventilation in storage facility; and *Right:* Mold growth in storage bin resulting from elevated moisture levels.

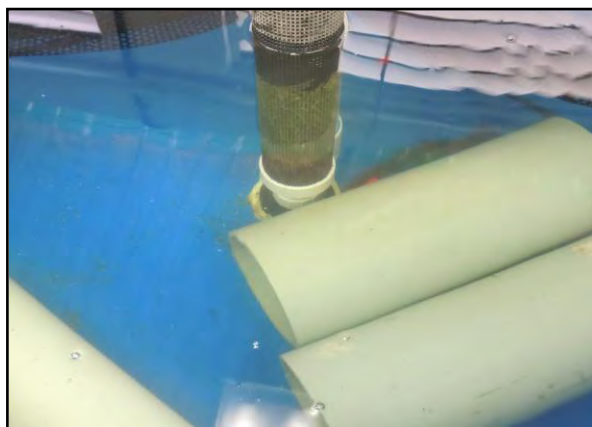


FIGURE 3. Use of PVC pipes as an ambush, loafing, and spawning cover for bigmouth sleepers and channel catfish.

size to reduce aggressive behaviors. Tank spawning has not proven effective for this species in Puerto Rico, so spawning should be conducted in earthen ponds. Broodstock should be replenished from holding tanks when mortalities occur.

Channel catfish should be fed a prepared feed at approximately 2-3% body weight daily during spawning season. This feed needs to be of sufficient protein content and freshness (See *Nutrition and Feeding Regimes*). Outside of the spawning season, feeding frequency can be reduced to two times per week at the same ration. Although this is considerably lower than the recommended ration, channel catfish at the Hatchery appear to be healthy and growing on this ration, likely due to the lower water temperatures present at the Hatchery.

Water quality monitoring should be done daily while fish are held in holding tanks. It is important to not overfeed pelleted feeds in order to maintain optimal water quality and reduce feed waste. Weekly water

quality monitoring is recommended for earthen ponds, or whenever fish behavior changes or other problems are observed. See *Water Quality* for specific parameters. Dead fish should be removed promptly, properly discarded, and these mortalities recorded. Cleaning holding tanks containing fish should be limited to periodic siphoning, only removing detritus accumulated on the bottom of the tanks. It is not necessary, and can be detrimental, to clean periphyton and biofilms from the tank surfaces. When water changes are required, incoming water must be the same temperature as tank water. Do not exceed 50% volume change per 24 h period.

#### *Largemouth bass*

Largemouth bass are generally held outside in concrete raceways. Whereas these raceways are shallow and exposed, fabric cover panels should be placed over half of the tank to provide shade and escapement from terrestrial and areal predators. Fish should be separated by size to reduce aggressive behaviors and cannibalism. Males and females should be separated to facilitate broodfish selection. Do not feed for 48-72 h following stocking.

Largemouth bass should be fed on alternate days with live *Tilapia rendalli* fingerlings (approximately 30-60 mm standard length (SL)) prior to and during spawning season. After spawning is complete, feeding can be reduced to two times per week. Feed five to six tilapia fingerlings (2.0-4.0 g) per largemouth bass per feeding. Maintaining adequate prey is especially important immediately prior to, during, and immediately following the spawning season. During the non-spawning period, one to two feedings per week at the same ration is sufficient. This increased ration post-spawning helps broodstock recover weight lost to reproduction quickly, maintaining healthy broodstock.

To select largemouth bass for spawning, use a visual inspection and discard fish that have lesions and appear unhealthy, and select for larger and healthier fish. Stock selected broodfish into reproduction ponds (150 x 5 m, about 1 m maximum depth) at 9:8 male to female ratio. Place spawning mats at regular intervals around the pond in locations that are easily accessible by hatchery personnel. Spawning will occur voluntarily on the mats.

Water quality monitoring is recommended at least weekly, or whenever fish behavior changes or other problems are observed. See *Water Quality* for specific parameters. Dead fish should be promptly removed, properly discarded, and these mortalities recorded. Cleaning raceways should be limited to periodic removal of debris, and light brushing when tanks are emptied.

#### *Peacock bass*

Peacock bass should be stocked directly into a grow-out pond filled to a maximum depth of 1.5 m.



FIGURE 4. *Top*: Peacock bass spawning set-up using a weighted tire and ceramic tiles. *Bottom*: Peacock bass spawn on ceramic tile removed from pond and placed in hatching aquarium.

Simple spawning structures can be constructed using a submerged tire filled with soil for ballast, with ceramic tile placed coarse side out, leaning against the tire at an angle of about 60-70 degrees from horizontal. Additional horizontal tiles can be placed on top of tires and anywhere within the pond where peacock bass are observed fanning potential nest sites. Using these tiles, each peacock bass spawn can be easily removed from the pond and transferred to the nursery (Figure 4).

Peacock bass should be fed the equivalent of 5-6 live *Tilapia rendalli* fingerlings (approximately 30 – 60 mm SL) per peacock bass on alternate days prior to and during spawning season. Once spawning is complete, the same ration should be fed two times per week. Peacock bass will quickly learn the feeding schedule, and will gather at the feeding location. In addition, many forms of natural prey will supplement peacock bass diets in the earthen ponds.

Water quality monitoring is recommended at least weekly, or whenever fish behavior changes or other problems are observed. See *Water Quality* for specific parameters. Dead fish should be promptly removed, properly discarded, and these mortalities recorded.

#### *Tilapia rendalli*

Although not a primary sport fish species, *Tilapia rendalli* are cultured at the hatchery as a live prey for piscivorous broodstock, particularly largemouth bass, peacock bass, and bigmouth sleeper. They can tolerate temperatures in excess of 38°C, and are tolerant of low dissolved oxygen and high ammonia concentrations. Under optimal conditions (water temperature  $\geq 24^{\circ}\text{C}$ ) and nutrition, broodfish tilapia can spawn and rear new brood every 10-14 days. Tilapia should be held in the deep raceways until spawning, which is conducted in the shallow earthen spawning ponds and stocked at a 1:4 male to female ratio. Tilapia broodstock and offspring should be fed 1- 2% their body weight daily with a high-protein diet to ensure adequate production and nutrient content. It is important to not overfeed pelleted feeds in order to maintain optimal water quality and reduce feed waste. Selection and storage of feeds is critical to nutrient retention, and no feed should be retained for more than 3 months, although it is recommended that all feed be used by 2 months following acquisition.

Weekly water quality monitoring is recommended for concrete tanks and daily in earthen ponds, or whenever fish behavior changes or other problems are observed. See *Water Quality* for specific parameters. Dead fish should be promptly removed, properly discarded, and these mortalities recorded.

## Summary

Proper broodstock management is critical to successful hatchery operations, as the overall well-being of parental fish determines the quality and quantity of offspring produced. We have suggested specific recommendations to the Maricao Fish Hatchery for future broodstock management: The four most critical recommendations are 1) improve broodstock admission procedures to include a period of quarantine and use of passive integrated transponder (PIT) tags to improve record keeping, 2) use a commercially available feed with 35-40% protein content to ensure that the dietary requirements of broodfish are met, 3) improve feed storage conditions and reduce maximum storage time to improve nutritional content, and 4) maintain or improve genetic diversity of broodstock by importing unrelated individuals from within the native range of each species. Improvements in these four areas should improve fingerling production, which will benefit island reservoir fisheries.

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**Chapter 11:**  
**Notes on Propagation of Butterfly Peacock Bass *Cichla ocellaris***  
**at the Maricao Fish Hatchery**

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**Abstract:** Sport fish management of butterfly peacock bass *Cichla ocellaris* can be enhanced using supplemental stocking of hatchery reared fingerlings. In this short communication, we describe techniques we have used to successfully reproduce this species at the Maricao Fish Hatchery in Puerto Rico. Wild-caught broodstock were stocked into a 0.1-ha lined pond at a density of about 250 fish/ha. Removable spawning substrates were created using weighted automotive tires with ceramic flooring tiles, placed rough-side out, leaning against the tire at a 60-70° angle, and lying horizontally on top of the tire. Spawning occurred on tiles, which were removed and placed in heated aquaria 48 h after the spawning event. Allowing for parental care during the first 48 h increased hatching success. No preference was apparent between horizontal and near-vertical tile placement, and evidence of monogamy and spawning site fidelity was observed. Hatching occurred in 3-5 d, and swim-up/first feeding occurred 3-6 d post-hatch. Development rates were temperature dependent. Swim-up larvae readily consumed *Artemia* spp. nauplii, and we postulate that larvae could be stocked into prepared grow-out ponds within 1-week post-hatch.

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Butterfly peacock bass *Cichla ocellaris* have been introduced outside of their native range where subtropical and tropical climates are appropriate for their survival (Shafland 1999a; Neal et al. 2006). Reasons for these introductions generally include control of prey species and diversification of sport fisheries (Shafland 1995). Introductions of *Cichla ocellaris* often have resulted in fisheries that provide significant economic stimulus to the surrounding region (Shafland 1999a). Although introduced populations normally establish reproductive populations, management capabilities can be enhanced using supplemental stocking of fingerlings when necessary (Neal et al. 2004). In this short communication, we describe techniques we have used to successfully reproduce *C. ocellaris* at the Maricao Fish Hatchery in Puerto Rico.

Constructed in 1937 on the Maricao River, the initial purpose of the Maricao Fish Hatchery was primarily for research, propagation, and introduction of coldwater fish species, including rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta*. In 1946, the hatchery shifted emphasis to warmwater species, which initiated the development of recreational fisheries in Puerto Rico's reservoirs. The majority of current production is directed towards largemouth bass

*Micropterus salmoides* and sunfish *Lepomis* spp. for reservoir stocking, and redbreast tilapia *Tilapia rendalli* as a hatchery forage, although *C. ocellaris* and several other species are cultured for research purposes.

We collected adult *C. ocellaris* from Carraizo Reservoir using electrofishing and transported selected broodstock in a 560-L hauling tank with constant aeration. Broodstock were tempered for 30 min and then stocked directly into a 0.1-ha lined pond with a maximum depth of 1 m. These fish were fed live *Tilapia rendalli* two to three times per week. At feeding, 5-6 individual tilapia (30-60 mm TL) per broodfish were tossed toward the center of the pond. *C. ocellaris* quickly trained to this process, and gathered at the surface awaiting prey, which normally were consumed within a few seconds. In addition, adult tilapia were stocked in the pond and provided natural prey via periodic spawning events. At the start of spawning season in late-April 2011, there were about 25 broodfish in the pond.

*Cichla* spp. have been reported to spawn primarily on horizontal substrates (Zaret 1980), including wood such as inundated tree trunks (Rutledge and Lyons 1976; Zaret 1980). However, anecdotal evidence at the Maricao Fish Hatchery suggested that *C. ocellaris*



spawned voluntarily on nearly vertical surfaces. Therefore we developed a simple spawning structure composed of an automotive tire, cut to allow air escapement, and laid on the pond bottom on its side and filled with soil for ballast. We positioned 6 or 7 ceramic floor tiles, rough surface out, leaning against the tire at about a 60 ° to 70° angle (Figure 1). Additional tiles were placed on top of the tire's horizontal surface and flat on the pond liner when broodstock pairs displayed nesting tendencies in those areas (cleaned area of liner). This design provided both substrate orientations to spawning fish.

During the two years of this study, more than 60 spawning events were detected. A number of spawns appeared to be infertile, while many more were not detected until hatching had already begun. Fecundity, hatching success, and time to swim up were not determined for these spawns. Thirteen (13) successful spawns were evaluated, with an estimated 500-2,500 eggs/spawn produced during the spawning season (late-April through September). Although observed egg production per spawn was lower than fecundities of 6,500 eggs per female reported by Shafland (1999), our fish spawned multiple times during an individual spawning season, and may have similar fecundities as Shafland (1999) across cumulative spawning events.

There did not seem to be a preference between horizontal and vertical substrates, but there was an apparent fidelity to spawning locations by broodstock pairs. After initial spawning, paired fish appeared to return to the same location for subsequent spawning events, suggesting spawning fidelity as well as potential monogamy in broodstock pairings.

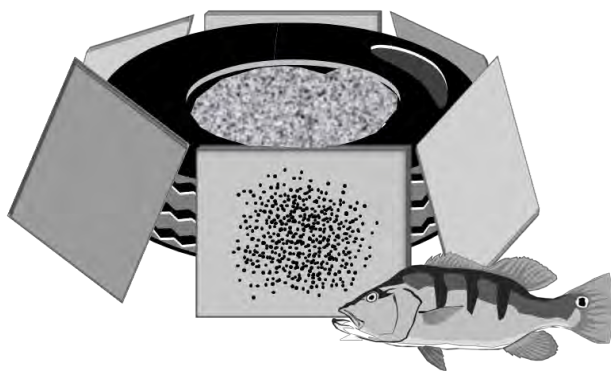


FIGURE 1. Effective spawning substrates for *C. ocellaris* broodstock. An automotive tire is cut to allow air escapement, filled with soil, sand, cement or other ballast material, and place on its side in water about 1 m deep. Flooring tiles are placed upright, roughest side out at about 60-70°, as a spawning media. Additional tiles may be laid horizontally on top of the tire or on the pond bottom. When spawning is complete, egg-covered tiles can be removed for incubation in a hatchery setting.

A few early spawns that were removed promptly upon detection appeared to not be fertilized properly and eggs quickly deteriorated. To avoid this during subsequent spawns, tiles containing eggs were left in the pond for up to 48 h to allow for fertilization and parental care of the eggs. In the event that a portion the eggs were not fertilized, the adults would remove dead eggs prior to decomposition or fungal development. This resulted in a more sanitary environment when the tiles were moved to aquaria for hatching. Allowing for some parental care also appeared to improve re-nesting when complete nest failure occurred.

Water temperature affected hatch rate and larval development. Tiles containing eggs were placed in 38-L aquaria where temperatures ranged from 22.6-25.6°C (Table 1). At these low temperatures, hatch rate was below expectations and larval development appeared slow. In mid-2012, submersible heaters were placed in each aquarium to maintain target temperatures of 28°C per Fontenelle (1950) and Sampaio (2000). Heating aquaria improved hatch rates (percent hatch  $\pm$  SE at  $< 26^\circ\text{C} = 42.5 \pm 0.1$ ; percent hatch  $\pm$  SE at  $> 28^\circ\text{C} = 93.6 \pm 0.0$ ) and visually appeared to improve larval development.

Methylene blue was added at a dosage of 3 ppm to reduce fungal growth, and low to moderate aeration was provided. Hatching generally occurred within 3-5 d of spawning, although precise time of fertilization was not known. This finding is similar to Fontenelle (1950), who reported *C. temensis* hatched in 68 h at water temperatures ranging from 27.2 to 28.9°C. When adults were allowed to provide care for 48-72 h prior of nest removal, most unfertilized eggs were removed and hatching success was high (up to 94%). Swim-up and first feeding occurred at 3-6 d post-hatch (Table 1), and was related to water temperature. Our experience was similar to that reported for *C. temensis*, which had hatch rates of 90% and swim-up at 4 d post-hatch (Rutledge et al. 1976).

First feeding occurred shortly after swim-up. We fed brine shrimp *Artemia* spp. nauplii, although other small invertebrates may also be appropriate. We used *C. ocellaris* fry produced during this study for age and growth experiments and did not stock them into prepared grow-out ponds. However, we believe that standard pond preparation procedures as used for other species would be appropriate, and that *C. ocellaris* fry could be stocked into grow-out ponds as early as 1 week post-hatch. Additional research is required to determine optimum stocking densities. Because *Cichla* species appear to switch to piscivory at approximately 50 mm (Rutledge et al. 1976), husbandry of this species to larger sizes will require protocols to reduce excessive cannibalism within grow-out ponds.

TABLE 1. Summary of *C. ocellaris* larval rearing attempts April-September 2011-2012

| Date hatched | Number of eggs<br>(approx. $\pm$ 100) | Temperature ( $^{\circ}$ C) | Days to first<br>swim/feeding | Number larvae<br>collected |
|--------------|---------------------------------------|-----------------------------|-------------------------------|----------------------------|
| 2011         |                                       |                             |                               |                            |
| 5/22/2011    | 500                                   | 22.6–25.1                   | 6                             | 265                        |
| 5/23/2011    | 2,100                                 | 23.8–24.2                   | 6                             | 189                        |
| 6/14/2011    | 2,000                                 | 24.1–25.2                   | 5                             | 482                        |
| 7/15/2011    | 900                                   | 23.9–24.2                   | 6                             | 577                        |
| 8/10/2011    | 1,500                                 | 23.8–24.1                   | 6                             | 446                        |
| 8/12/2011    | 600                                   | 22.6–23.8                   | 5                             | 387                        |
| 2012         |                                       |                             |                               |                            |
| 5/02/2012    | 900                                   | 24.1–25.2                   | 4                             | 400                        |
| 5/28/2012    | 1,000                                 | 23.8–24.1                   | 5                             | 350                        |
| 6/07/2012    | 1,600                                 | 23.9–24.5                   | 5                             | 623                        |
| 7/01/2012    | 1,350                                 | 26.0                        | 3                             | 841                        |
| 8/07/2012    | 750                                   | 28.2                        | 3                             | 696                        |
| 6/27/2012*   | 1,400                                 | 28.1                        | 4                             | 3,400**                    |
| 6/27/2012*   | 2,200                                 | 28.2                        | 4                             |                            |

\* Spawns were collected together

\*\* Total from two spawns

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## **Study 5:**

### **Threadfin shad prey production in tropical reservoirs**



**Chapter 12:**  
**Comparison of Gill Nets and Fixed-Frame Trawls  
for Sampling Threadfin Shad in Tropical Reservoirs**

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**Abstract:** Threadfin shad *Dorosoma petenense* populations were sampled quarterly from 2010 to 2011 to determine appropriate sampling techniques for this species in tropical reservoirs of Puerto Rico. Offshore gill netting and night trawling were compared in terms of catch per unit of effort, size distribution, sampling precision, and bycatch. In total, 90 gill net–trawl pairs of catches were compared, which collected more than 80,000 threadfin shad. Gill-net and trawl catches were not correlated in either number or biomass. Coefficients of variation were greater in gill-net sampling (0.761 for numbers, 0.747 for biomass) than in trawl sampling (0.433 and 0.465, respectively) and were not dependent on reservoir, sampling season, reservoir section, or any combination of these factors for any gear. There was no correspondence in size distributions between gill-net and trawl catches. Gill nets collected threadfin shad in the range of 23–169 mm total length (TL) and displayed strong mesh size selectivity, causing distinct unrealistic peaks in size distribution. Gill nets underestimated threadfin shad smaller than 35 mm TL and overestimated shad bigger than 90 mm TL. The size range of threadfin shad collected via trawl was 10–108 mm TL, and trawls did not appear to be as size selective for fish up to 80 mm TL. Trawling was found to be the superior sampling technique for shad populations in pelagic habitats of Puerto Rico reservoirs, which consisted mainly of fish up to 80 mm TL. Trawling provided a more realistic picture of

size distributions, collected far less bycatch, was less affected by the schooling of shad, and was less laborious and more cost-effective than gill nets.

*Previously Published:*

Prchalová, M., J. W. Neal, M. Muñoz-Hincapié, T. Jůza, M. Říha, J. Peterka, and J. Kubečka. 2012. Comparison of gillnets versus fixed-frame trawl for sampling threadfin shad in tropical reservoirs. *Transactions of the American Fisheries Society* 141:1151-1160.

The historical approach of single-species fisheries management is gradually being replaced by more holistic consideration of prey production as it relates to predator dynamics (Noble 1986). This is because single-species management schemes often fail to consider trophic relationships within the ecosystem (Larkin 1979; May et al. 1979). The development of bioenergetics models in recent decades has further established that effective predator management requires accurate knowledge of prey characteristics (Jenkins and Morais 1978). For example, managers often require precise estimates of prey population variables such as abundance, age and size structure, recruitment, growth, mortality, and production to predict the effects of management actions on predator populations. The precision of these population estimates is determined primarily by the biologist's ability to collect unbiased, representative samples of the prey population under study.

Threadfin shad *Dorosoma petenense* is a primary prey species in many reservoirs located in temperate, subtropical, and tropical environments (Noble 1981; Johnson et al. 1988; DeVries and Stein 1990; Neal et al. 2009). This species is less tolerant of cold temperatures than larger gizzard shad *D. cepedianum* (Strawn 1965), which limits its northern distribution. The southern distribution has been expanded well beyond the native range of threadfin shad in the lower Mississippi River basin to include peninsular Florida and the area down to Guatemala and Belize (Miller 1964; Carlander 1969). Threadfin shad have also been introduced as a forage fish into tropical reservoirs of the Caribbean, where they serve as the primary prey species for important sport fish such as largemouth bass *Micropterus salmoides* and butterfly peacock bass *Cichla ocellaris*.

Although the life history characteristics of threadfin shad in temperate waters are well described, little is known about populations in tropical reservoirs. Stancil et al. (1999) reported that threadfin shad spawning occurs nearly year-round in Lucchetti Reservoir, Puerto Rico, with the exception of a short period from mid-August until mid-September. The same study reported that the maximum length ( $n = 2,002$ ) was only 86 mm (TL) and that the maximum age ( $n = 124$ ) was only 141

d. These data suggested that threadfin shad in tropical systems are a prolific and short-lived species. However, Neal et al. (1999) observed threadfin shad in Cidra Reservoir, Puerto Rico, that were up to 175 mm TL, which is about the maximum size reported for this species (Jenkins and Burkhead 1993). Almost nothing is known about the abundance, biomass, or production of this species in tropical waters, largely because targeted threadfin shad sampling has not occurred and effective sampling protocols have not been developed.

Six sampling methods, including hydroacoustics, electrofishing, gill nets, rotenone, seines, and midwater trawls, were used concurrently to obtain data on threadfin shad in Lake Texoma, Oklahoma–Texas (Boxrucker et al. 1995). This study concluded that surface-set gill nets, trawls, and hydroacoustics were the most appropriate gears for sampling threadfin shad in southern reservoirs. Trawls and hydroacoustics were capable of providing biomass estimates, while gill nets were limited to catch per unit of effort (CPUE) trends and size structure. Neal et al. (2001) reported that hydroacoustic data were difficult to interpret for Puerto Rico reservoirs, likely due to the presence of many other species in the open water. Frouzová et al. (2008) also found that the size overlap of multiple species, particularly small invertebrates, complicates the use of hydroacoustics in studies of tropical freshwater ecology. Therefore, hydroacoustics were not considered in this comparison.

Because it is unclear which of these gears is most appropriate for sampling threadfin shad in tropical systems, this paper compares the catch, bycatch, and size selectivity of an active gear (fixed-frame trawl) with those of a passive gear (experimental gill nets) in steep-sided tropical reservoirs.

## Methods

*Study sites* – This research was conducted at four reservoirs located on the island of Puerto Rico. Puerto Rico is exclusively tropical habitat and is primarily of volcanic origin. It is 175 km long and approximately 62 km wide and has a central mountain range that runs east to west. Reservoirs in Puerto Rico are generally mesotrophic to eutrophic and are anoxic below about 3 m depth except following infrequent mixing events.

Surface water temperatures average around 27°C, though this varies somewhat with altitude and season (Neal et al. 2009). The four study reservoirs were Lucchetti, Guajataca, Cerrillos, and Dos Bocas, which range from 108 to 360 ha in surface area. Turbidity in these reservoirs changes seasonally, being relatively low in winter and spring (up to 50 NTU) and much higher during the rainy season (> 200 NTU). These reservoirs contain a mixture of fish species, with largemouth bass, threadfin shad, tilapia *Oreochromis* spp., sunfishes *Lepomis* spp., ictalurids, and Amazon sailfin catfish *Pterygoplichthys pardalis* being common to all four. Guajataca and Dos Bocas reservoirs also contain the butterfly peacock bass and red devil cichlid *Amphilophus* as additional predators.

*Gear specifications* – Experimental gill nets were 1.5 m × 20 m with 8 monofilament panels (2.5 m long) of 19.5-, 6.25-, 10-, 8-, 12.5-, 15.5-, 5-, and 24-mm-bar mesh (mesh size order as in the gill net). Mesh sizes were selected based on those identified as most effective for sampling threadfin shad (6.25–19.5 mm; Van Den Avyle et al. 1995b), with one smaller and one larger mesh added. Fully floating epipelagic gill nets were used to sample the surface water layer (0–1.5 m), and slowly sinking mesopelagic gill nets were suspended to sample the deeper water layer (1.5–3.0 m). To make installation of gill nets easier, epipelagic and mesopelagic gill nets were connected end to end and set together (Figure 1). Along with open-water gill nets (set approximately on the center axes of the reservoirs), inshore, fully floating gill nets were set close to the shore above the depth of 6 m maximum. The inshore catches were included only in portraying the catches of individual mesh sizes; they could not be used in the gear comparison analysis due to their sampling different habitat (Figure 1).

A custom frame fry trawl was designed with frame aperture dimensions of 3 × 3 m, 6-mm mesh in the body of the trawl, 4-mm mesh in the cod end, and a total length of 10.5 m (3.5 m of cod end). Trawls of these dimensions have been reported to be an effective tool for sampling fish up to 90 mm TL (Jůza and Kubečka 2007). The cod end of the trawl was equipped with a funnel, which prevented fish from escaping. The towing rope between the trawler boat and the trawl was 100 m long, and during the tow the boat was kept on a slightly curved trajectory so that the trawl never sampled exactly the area disturbed by the trawler boat. Both gill nets and the trawl were constructed by Pokorný-Site Co., Brloh, Czech Republic ([www.pokorny-site.cz/en/](http://www.pokorny-site.cz/en/)).

*Study design* – All four reservoirs were sampled during the first quarterly sampling in May 2010. After the first quarterly sampling, recreational activities

prevented trawling in Cerrillos Reservoir. Hence, Dos Bocas, Lucchetti, and Guajataca reservoirs were sampled for all four quarters and Cerrillos Reservoir was sampled for one quarter, providing 13 reservoir–quarter data sets for comparison. Each reservoir was divided into upper and lower (near dam) sections (Dos Bocas has two upper sections, as it has two primary arms). At each section, three sites suitable for both gill netting and trawling were selected randomly prior to sampling; these sites were generally located in the middle of the reservoir, with water depths of 10–20 m.

Combined gill nets (epipelagic and mesopelagic sets) were installed before sunset by 1700 hours and lifted after sunrise (0800 h) the following day. This time frame was chosen because fish activity shows predictable peaks that are usually around sunset and sunrise (Prchalová et al. 2010). After landing, all fish were removed from the gill nets. Sport fish (largemouth bass, butterfly peacock bass, tilapia) were released alive after being measured and weighed during retrieval. Other species, excluding threadfin shad, were measured, weighed, and released directly if possible, depending on the degree of entanglement. Catches were separated according to mesh size of capture and processed immediately. Each fish was measured for total length (TL, mm) and weighed to the nearest 0.01 g (fish <100 mm TL) or the nearest gram (fish ≥100 mm TL). When large numbers (>50) of threadfin shad were captured per mesh size, subsampling was used.

Trawling was conducted using a two-boat system, with the first boat being used as a trawler and the second boat to retrieve the cod end, empty the catch, and process the samples. Trawling was performed at night starting 2 h after sunset near sites used for open-water gill nets the previous night. The trawl was rigged using buoys to sample the surface layers (0–3 m), thus allowing comparison between gear types. Because of the small size of these reservoirs, the large mouth of the trawl, and excessively high catch rates, the duration of each trawl tow was held at 2 min, which resulted in a trawled distance of about 120 m with average speed of 3.6 km/h and a sampled volume around 1,080 m<sup>3</sup>. Further, 2-min tows were justified by the time necessary for a fish to reach the cod end of a trawl, which is 7 and 17 s for slow and fast swimmers, respectively (Winger et al. 2010). At the end of each tow, the cod end of the trawl was lifted and emptied. The catch was stored in a labeled ziplock bag and placed on ice in the cooler for processing in the laboratory. Occasionally, species other than threadfin shad were caught as bycatch; these species were identified, measured, and released alive back to the water. The iced trawl catches were processed the next day using the same procedure as in the gill-net catches. Threadfin shad smaller than 30 mm TL were



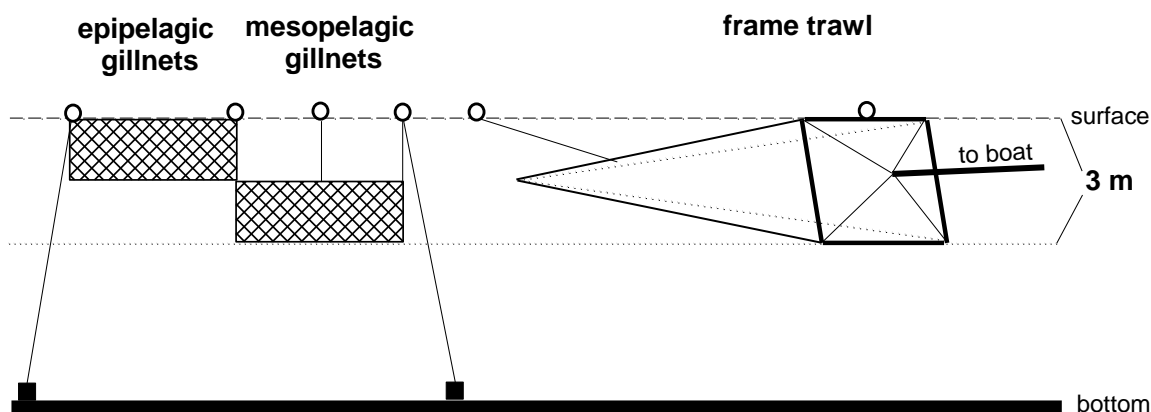


FIGURE 1. Generalized gear rigging for comparison of surface (0–3-m) sampling of threadfin shad using offshore gill nets and a frame trawl.

considered larvae and counted only. Only the smallest individuals were measured to obtain information about the smallest catchable size of threadfin shad.

*Data processing and analysis* – Fish records were stored in the database software Pasgear (J. Kolding, University of Bergen, Norway; [www.imr.no/forskning/bistandsarbeid/nansis/pasgear2/en](http://www.imr.no/forskning/bistandsarbeid/nansis/pasgear2/en)). Results (e.g., relative abundance, biomass, deviation, proportion) were calculated within the Pasgear database. Abundance and biomass are expressed as number per unit effort (NPUE) and biomass per unit effort (BPUE), respectively, reported as the number of individuals or grams per 1,000 m<sup>2</sup> of gill nets or 1,000 m<sup>3</sup> of open water sampled by the trawl. Statistical analyses were performed in the Statistica software. The study design resulted in 90 gill net–trawl pairs of NPUEs and BPUEs to be compared using the linear regression. Two pairs were removed from the set as obvious outliers (the uppermost sample in Lucchetti Reservoir in January 2011; and the middle dam sample in Dos Bocas Reservoir in January 2011, which had an extremely high [4 times] trawl catch and a subnormal [one-tenth] gill-net catch in comparison with the other catches in the section). Coefficients of variation (CVs [SD/mean]) were compared using the *t*-test for independent samples between gears. Factorial analysis of variance (ANOVA) was used to assess the effects of reservoir, sampling season, reservoir section, and their interactions on the CVs. As the size ranges of gill-net and trawl catches did not correspond to each other, a size range 35–80 mm was selected in order to compare NPUEs, BPUEs, CVs, and size distributions between the two gears (Table 1; Figure 2). The range was set as the most frequent minimum and maximum sizes (5-mm intervals) that contained reasonable amounts of shad in both gears across all campaigns. In setting the thresholds, we took into account the size limitations of

the gill nets used (minimum, 40 mm TL; Prchalová et al. 2009) and trawl (maximum, 90 mm TL; Jůza and Kubečka 2007). On average, 87% and 32% of shad were within the comparable size range in gill net and trawl catches, respectively (Table 1). Size distributions were compared using the two-sample Kolmogorov–Smirnov test.

## Results

A total of 6,631 and 73,459 threadfin shad were captured in offshore gill-net and trawl sampling, respectively, over the duration of the study. Trawl and gill-net NPUEs were not significantly correlated ( $t = 1.124$ ,  $df = 78$ ,  $P = 0.265$ ), with the coefficient of determination being only 0.016 (Figure 3). However, the intercept of the NPUE regression was significant ( $t = 4.515$ ,  $df = 78$ ,  $P < 0.001$ ), so that the curve did not go through zero. The comparison of biomasses provided similar, nonsignificant correlation ( $t = 0.980$ ,  $df = 78$ ,  $P = 0.330$ ,  $r^2 = 0.012$ ; intercept:  $t = 3.824$ ,  $df = 78$ ,  $P < 0.001$ ). The trawl and gill-net catches significantly differed in sampling precision in both the NPUE and BPUE comparisons within comparable size ranges (NPUE:  $t = -3.645$ ,  $df = 56$ , not differ between reservoirs, sampling seasons, sections, or any combination of these three factors ( $P > 0.050$  in every comparison).

Ten of the 13 comparisons of size distributions within the comparable size ranges of gill-net and trawl catches showed significant differences (Table 2). Only in 3 cases did the size distributions correspond between gill-net and trawl samples. In 12 cases, the mean total length of the threadfin shad caught in gill nets was greater than fish caught by trawl; the range for the gill nets was 23–169 mm, that for trawls 10–108 mm. Contrary to the gill-net samples, a majority of the trawl

TABLE 1. Catches of threadfin shad in four Puerto Rico reservoirs sampled by both gill nets and trawls, in the aggregate and by size-class. The values in parentheses are percentages.

| Reservoir | Gear     | Total  | <35 mm        | 35–80 mm     | >80 mm     |
|-----------|----------|--------|---------------|--------------|------------|
| Dos Bocas | Gill net | 3,486  | 5 (0.1)       | 2,948 (84.6) | 533 (15.3) |
|           | Trawl    | 27,883 | 17,899 (64.2) | 9,965 (35.7) | 19 (0.1)   |
| Lucchetti | Gill net | 1,847  | 1 (0.1)       | 1,238 (67.0) | 608 (32.9) |
|           | Trawl    | 34,340 | 25,003 (72.8) | 9,152 (26.7) | 185 (0.5)  |
| Guajataca | Gill net | 652    | 4 (0.6)       | 641 (98.3)   | 7 (1.1)    |
|           | Trawl    | 7,012  | 3,968 (56.6)  | 3,044 (43.4) | 0 (0)      |
| Cerrillos | Gill net | 646    | 1 (0.2)       | 627 (97.0)   | 18 (2.8)   |
|           | Trawl    | 4,224  | 3,364 (79.6)  | 856 (20.3)   | 4 (0.1)    |

catch was represented by larvae (< 30 mm TL), with little evidence of length-cohorts (Table 1, Figure 2).

In contrast, the gill-net size distributions showed distinct peaks of size-groups corresponding to the catches of individual mesh sizes. The relative proportions of these peaks did not correspond to the size distribution obtained by trawling. All mesh sizes but 24 mm caught threadfin shad, and most shad were captured by 8-mm mesh and adjacent mesh sizes (6.25 and 10 mm). Other mesh sizes represented small (<5%) or negligible proportions of the total catch. The mean total lengths of the threadfin shad collected displayed a positive relationship with mesh size, as did the standard deviations and ranges (Figure 4). Mesh sizes 5–12.5 mm displayed unimodal distributions of catch. The distribution for the 15.5-mm mesh showed two distinct peaks, with that for the smaller fish being generated by shad with higher body height (probably females) than those of similar sizes caught in the 12.5-mm mesh.

Gill-nets and trawl catches differed markedly in their by-catches ( $t = 3.558$ ,  $df = 24$ ,  $P = 0.002$ ). Bycatch comprised 15.4% (1,208 fish) of the gill-net catch, whereas it accounted for only 0.7% (537) of the trawl catch (Table 3). Thirteen species were recorded in the gill-net bycatch. Trawl bycatch consisted of 8 species, all of which also were collected by gill nets. The principal bycaught species in the gill-net catches were red devil cichlids, channel catfish *Ictalurus punctatus*, white catfish *Ameiurus catus*, and tilapias. The most important bycatch species in the trawl sampling were tilapias and Amazon sailfin catfish (both mainly as larvae up to 20 mm TL) and red devil cichlids (Table 3).

Gill netting and trawling further differed in the labor needed per standardized unit of catch, including sampling and catch processing. Gill nets required 3.5 h of labor per 100 processed shad, excluding exposure and repair time. Trawling was almost nine-fold more effective, with 0.4 h of labor per 100 processed shad.

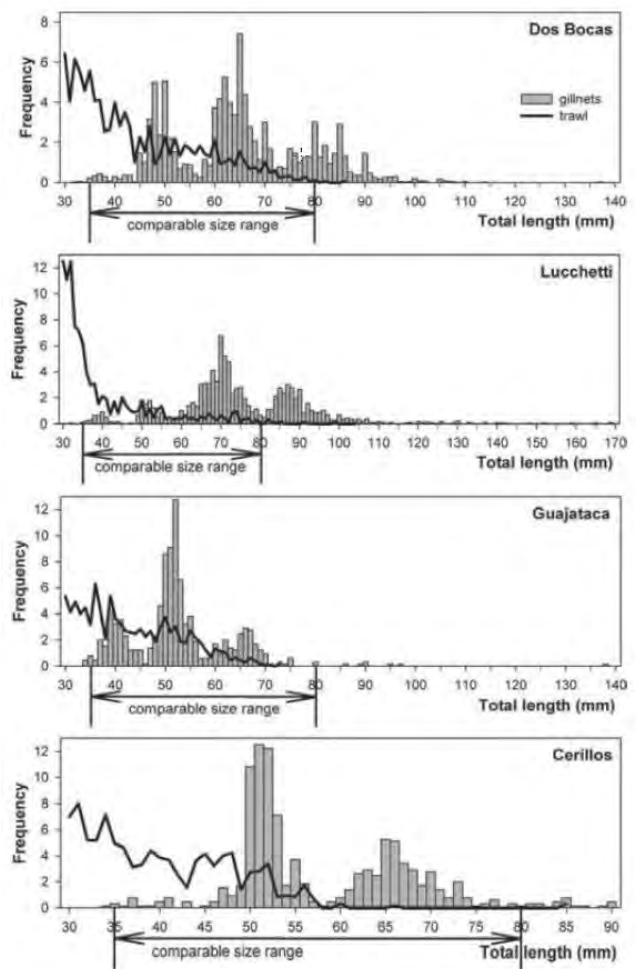


FIGURE 2. Size distributions and ranges of gill-net and trawl-caught threadfin shad above 30 mm TL in four Puerto Rico reservoirs used to compare NPUE, BPUE, CV, and size distributions.

## Discussion

Comparison of gill-net and trawl sampling in this study suggests that trawling is better for sampling threadfin shad in the tropical reservoirs of Puerto Rico.

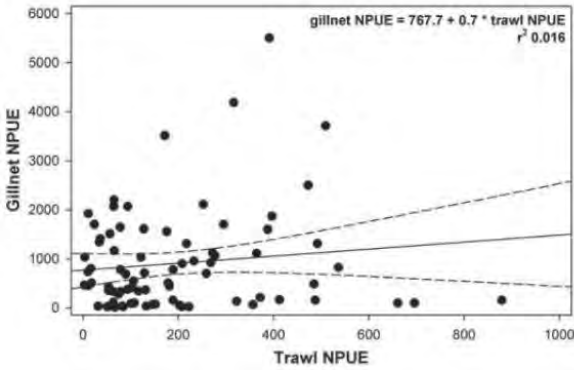


FIGURE 3. Relationship between trawl and gill-net NPUEs within the comparable size range 35–80 mm, with the regression line (solid) and its 95% confidence band (dashed). The units are individuals/1,000 m<sup>3</sup> for trawls and individuals/1,000 m<sup>2</sup> for gill nets.

The majority of shad populations consisted of fish up to 80 mm TL, and in this size range trawling provided a more realistic picture of the size distribution than gill nets. Further, as an active sampling, trawling was less affected by schooling, thus providing less variable results. Trawling also produced negligible bycatch and was less laborious and more cost-effective than gill nets. These factors support the recommendation of trawling as the method of choice for threadfin shad management and research in Puerto Rico and other tropical reservoirs with similar characteristics. In some reservoirs, shad larger than 80 mm TL were also present in the population (Figure 2). To obtain representative samples of this part of the size spectrum

by trawling, larger trawl openings, higher towing speeds, and/or longer tows have to be used.

Comparison of the results from active and passive gears is difficult. Passive gill nets provide a picture of the fish community that is active during the exposure time of the nets (Prchalová et al. 2010). It is generally accepted that the gill-net catch is dependent on a given fish density (e.g., Mehner and Schulz 2002; Bonar et al. 2009; Olin et al. 2009); however, some authors pointed out that elevated gill-net catches were related to increased fish activity rather than to the density recorded by trawling (Olin and Malinen 2003). Furthermore, gill-net catchability does not necessarily vary linearly with fish density, as factors such as net saturation, fish escapement, and avoidance of/attraction to a gill net with already enmeshed fish play a significant role during every gill-net sample (Borgström 1992; Olin et al. 2004; Prchalová et al. 2011). Thus, the passive nature of gill nets results in sensitivity to several types of serious selectivities. This is further support for the selection of active trawl sampling over passive gill-net sampling.

Active trawling offer an immediate picture of a fish community, biased only by selectivity dependent on gear characteristics and sampling design and not on fish community characteristics (Kubečka et al. 2012). In general, it is important to select sampling protocols like the diel period of trawling, towing speed, mesh sizes, and mouth opening area based on the community to be sampled. Usually, night is the most efficient period for trawling due to the lower

TABLE 2. Results of two-sample Kolmogorov–Smirnov tests of the size distributions of threadfin shad caught in gill nets and trawls in each reservoir–quarter comparison. Mean total lengths (mm) and SDs are presented for each gear. *P*-values for the size distributions that corresponded to each other are given in bold italics.

| Campaign       | <i>P</i> -value | Gill net |        | Trawl  |        |
|----------------|-----------------|----------|--------|--------|--------|
|                |                 | Mean     | SD     | Mean   | SD     |
| Cerrillos, Apr | <0.001          | 56.632   | 9.832  | 44.090 | 6.592  |
| Dos Bocas, Apr | <0.001          | 55.861   | 9.949  | 44.588 | 9.257  |
| Dos Bocas, Jun | <0.001          | 63.881   | 10.037 | 58.226 | 11.298 |
| Dos Bocas, Oct | > <b>0.100</b>  | 66.445   | 9.309  | 65.750 | 9.014  |
| Dos Bocas, Jan | <0.010          | 49.673   | 8.564  | 48.362 | 10.764 |
| Guajataca, Apr | <0.001          | 51.648   | 7.246  | 42.165 | 6.648  |
| Guajataca, Jun | < <b>0.100</b>  | 50.981   | 8.006  | 49.192 | 6.661  |
| Guajataca, Oct | <0.001          | 47.427   | 7.734  | 41.788 | 6.792  |
| Guajataca, Jan | <0.001          | 61.437   | 9.165  | 55.137 | 6.875  |
| Lucchetti, Apr | <0.001          | 59.184   | 10.183 | 45.558 | 7.892  |
| Lucchetti, Jun | > <b>0.100</b>  | 53.104   | 11.368 | 53.327 | 11.104 |
| Lucchetti, Oct | <0.001          | 66.505   | 4.816  | 62.286 | 10.514 |
| Lucchetti, Jan | <0.001          | 67.170   | 9.936  | 46.570 | 13.277 |

TABLE 3. Species caught by offshore gill nets (G) and trawls (T) in each reservoir. The absolute numbers (*N*) and percentages of the total catch (%) are presented.

| Reservoir (gear) | Measure  | Threadfin shad | Red devil cichlid | Channel catfish | Tilapia | White catfish | Largemouth bass | Amazon sailfin catfish |
|------------------|----------|----------------|-------------------|-----------------|---------|---------------|-----------------|------------------------|
| Cerrillos (G)    | <i>N</i> | 646            |                   |                 |         | 7             | 1               |                        |
|                  | %        | 98.0           |                   |                 |         | 1.1           | 0.2             |                        |
| Cerrillos (T)    | <i>N</i> | 4,224          |                   |                 |         |               | 1               |                        |
|                  | %        | 99.06          |                   |                 |         |               | 0.02            |                        |
| Dos Bocas (G)    | <i>N</i> | 3,486          | 559               | 279             | 115     | 56            |                 |                        |
|                  | %        | 77.5           | 12.4              | 6.2             | 2.6     | 1.2           |                 |                        |
| Dos Bocas (T)    | <i>N</i> | 27,883         | 42                | 7               | 268     | 7             |                 | 136                    |
|                  | %        | 98.4           | 0.1               | 0.02            | 0.9     | 0.02          |                 | 0.5                    |
| Guajataca (G)    | <i>N</i> | 652            | 3                 |                 | 6       | 6             | 1               | 23                     |
|                  | %        | 91.7           | 0.4               |                 | 0.8     | 0.8           | 0.1             | 3.2                    |
| Guajataca (T)    | <i>N</i> | 7,012          |                   |                 |         |               |                 | 2                      |
|                  | %        | 99.07          |                   |                 |         |               |                 | 0.03                   |
| Lucchetti (G)    | <i>N</i> | 1,847          |                   | 54              | 33      |               | 26              | 1                      |
|                  | %        | 93.7           |                   | 2.7             | 1.7     |               | 1.3             | 0.1                    |
| Lucchetti (T)    | <i>N</i> | 34,340         |                   | 3               | 48      |               | 2               | 20                     |
|                  | %        | 99.8           |                   | 0.01            | 0.1     |               | 0.01            | 0.1                    |
| Total G          | <i>N</i> | 6,631          | 562               | 333             | 154     | 69            | 28              | 24                     |
|                  | %        | 84.6           | 7.2               | 4.2             | 2.0     | 0.9           | 0.4             | 0.3                    |
| Total T          | <i>N</i> | 73,459         | 42                | 10              | 316     | 7             | 3               | 158                    |
|                  | %        | 99.3           | 0.1               | 0.01            | 0.4     | 0.01          | 0.004           | 0.2                    |

TABLE 3. Extended.

| Reservoir (gear) | Butterfly peacock bass | Redear sunfish <i>Lepomis microlophus</i> | Bluegill <i>Lepomis macrochirus</i> | Green swordtail <i>Xiphophorus helleri</i> | Redhead cichlid <i>Cichlasoma synspilum</i> | Brown bullhead <i>Ameiurus nebulosus</i> | Marbled bullhead <i>Ameiurus nebulosus marmoratus</i> | Total bycatch |
|------------------|------------------------|---|-------------------------------------|--|---|--|---|---------------|
| Cerrillos (G)    |                        | 5   |                                     |  |   |  |   | 13            |
|                  |                        | 0.8                                       |                                     |  |   |  |   | 2.0           |
| Cerrillos (T)    |                        | 1   |                                     |  |   |  |   | 2             |
|                  |                        | 0.02                                      |                                     |  |   |  |   | 0.04          |
| Dos Bocas (G)    |                        |   |                                     | 3  |   |  |   | 1,012         |
|                  |                        |   |                                     | 0.1  |   |  |   | 22.5          |
| Dos Bocas (T)    |                        |   |                                     |  |   |  |   | 460           |
|                  |                        |   |                                     |  |   |  |   | 1.6           |
| Guajataca (G)    | 19                     |   |                                     |  | 1   |  |   | 59            |
|                  | 2.7                    |   |                                     |  | 0.1   |  |   | 8.3           |
| Guajataca (T)    |                        |   |                                     |  |   |  |   | 2             |
|                  |                        |   |                                     |  |   |  |   | 0.03          |
| Lucchetti (G)    |                        | 3   | 5                                   |  |   | 1  | 1   | 124           |
|                  |                        | 0.2                                       | 0.3                                 |  |   | 0.1                                      | 0.1   | 6.3           |
| Lucchetti (T)    |                        |   |                                     |  |   |  |   | 73            |
|                  |                        |   |                                     |  |   |  |   | 0.2           |
| Total G          | 19                     | 8   | 5                                   | 3  | 1   | 1  | 1   | 1,208         |
|                  | 0.2                    | 0.1                                       | 0.1                                 | 0.04                                       | 0.01  | 0.01                                     | 0.01  | 15.4          |
| Total T          |                        | 1   |                                     |  |   |  |   | 537           |
|                  |                        | 0.001                                     |                                     |  |   |  |   | 0.7           |

visibility of the net (Glass and Wardle 1989), lower activity of most fish species (Prchalová et al. 2010; Rakowitz et al. 2012), and more homogeneous spatial distribution of fish species that tend to school or shoal during the day (Vondracek et al. 1989). The 3- × 3-m fixed-frame trawl used in this study was found to be a quantitative tool for fry abundance and size estimates in artificial temperate reservoirs, showing that fish up to 90 mm TL are mostly passive without important avoidance behavior at night (Jůza and Kubečka 2007). Larger individuals or species are less vulnerable to capture as they detect the trawl sooner (Zhang and Arimoto 1993), and they are more able to avoid trawls at slower towing speeds, with finer mesh sizes in the trawl netting, and/or with smaller mouth openings (Wardle 1993; Jůza and Kubečka 2007). Only a few studies have correlated gill-net and trawl catches, usually with a positive correlation in numbers or biomass assessments. For example, Van Den Avyle et al. (1995a) compared six gears for sampling threadfin shad in Lake Texoma and reported correlations between trawl and gill-net catches with relatively strong correlation coefficients. Significant positive correlations of trawl and gill-net estimates were found for four species in the study of Olin et al. (2009) when they removed the smallest fish from the comparison. The same approach was used by Olin and Malinen (2003), who reported a positive relationship but were not able to detect a significant correlation.

The size distributions of the threadfin shad captured in gill nets did not correspond to those of the fish captured by trawls and showed distinct size groupings. Multimodal size distributions are common in temperate waters, where populations consist of several cohorts that are different in size due to fixed period annual spawning, and peaks in the size distributions in these cases can be detected with both active and passive gears (Prchalová et al. 2008, 2009; Vašek et al. 2009). In Puerto Rico reservoirs, threadfin shad tend to spawn nearly year-round (Stancil et al. 1999), creating almost continuous size distributions, as was evidenced by the trawl samples in this study. Similar differences between gill-net and trawl size distributions have also been reported by Van Den Avyle et al. (1995a) in Lake Texoma. These discrepancies between gill-net and trawl size distributions indicate the strong mesh size selectivity of gill nets, with biased proportions of adjacent-size peaks. Gill-net size selectivity could be responsible for the lack of correlations between gill-net and trawl NPUEs and BPUEs.

There are several potential reasons for the observed mesh size selectivity of threadfin shad in Puerto Rico reservoirs. First, it is possible that the mesh sizes, which were selected according to a geometric series with a factor 1.25 between adjacent meshes, were not

appropriate for the continuous size distributions. The factor 1.25 was found to be the best one for covering the whole size spectrum of coarse species in temperate European waters and, as such, it has been accepted as a norm for standardized gill netting (CEN 2005). In the case of threadfin shad in Puerto Rico, this factor may need to be reduced to better sample the range of fish sizes. This topic would require specialized research involving calibration with representative active gear or experiments with known populations.

The mesh size selectivity could also be caused by different behavior or activity of threadfin shad of different sizes. It is generally known that fish of bigger sizes swim with higher speeds, thus crossing larger distances, which may increase their probability of encountering the gill net (Rudstam et al. 1984; Anderson 1998; Čech and Kubečka 2002). This is well documented by overestimation of catches of larger fish in larger meshes (Mattson 1994; Jensen 1995; Kurkilahti et al. 1998; Huse et al. 2000; Irwin et al. 2008). However, Borgström (1989) proposed that higher catches of larger fish cannot be explained solely by the higher probability of encounter due to swimming speed, as it would require swimming speeds around 100 m/s to account for some observed differences. Thus, underestimation of smaller fish due to the mechanical parameters of gill nets as well as the biological characteristics of smaller fish should be considered too (for an overview, see Prchalová et al. 2009). In the case of Puerto Rico threadfin shad, the smallest mesh size (5 mm) was still too big to cover the high proportion of shad smaller than 40 mm TL in the population. It is not feasible to construct gill nets with smaller mesh and finer thread, so it is not possible to reduce the underestimation of small fish in this way. Another feature that may differ during shad ontogeny is the use of space during the 24-h cycle. Larger fish sometimes migrate inshore from open water at dusk and back to open water at dawn (Kubečka 1993; Říha et al. 2011), which may result in offshore gill-net catches but no catches in solely night offshore trawl samples.

The lack of correlation between gill-net and trawl catches could be also explained by catch variability, which was much greater with gill nets. The coefficients of variation for gill-net samples were greater than 0.5, which has been proposed as the target maximum CV for comparative studies (Cyr et al. 1992). The variability of gill-net catches within a single section was substantial, and we assumed this variability was caused by the schooling of threadfin shad and by predators attacking shad enmeshed in gill nets. Threadfin shad have been reported to aggregate intensively, with dense, smaller schools during the day and larger, looser schools during the night (Vondracek

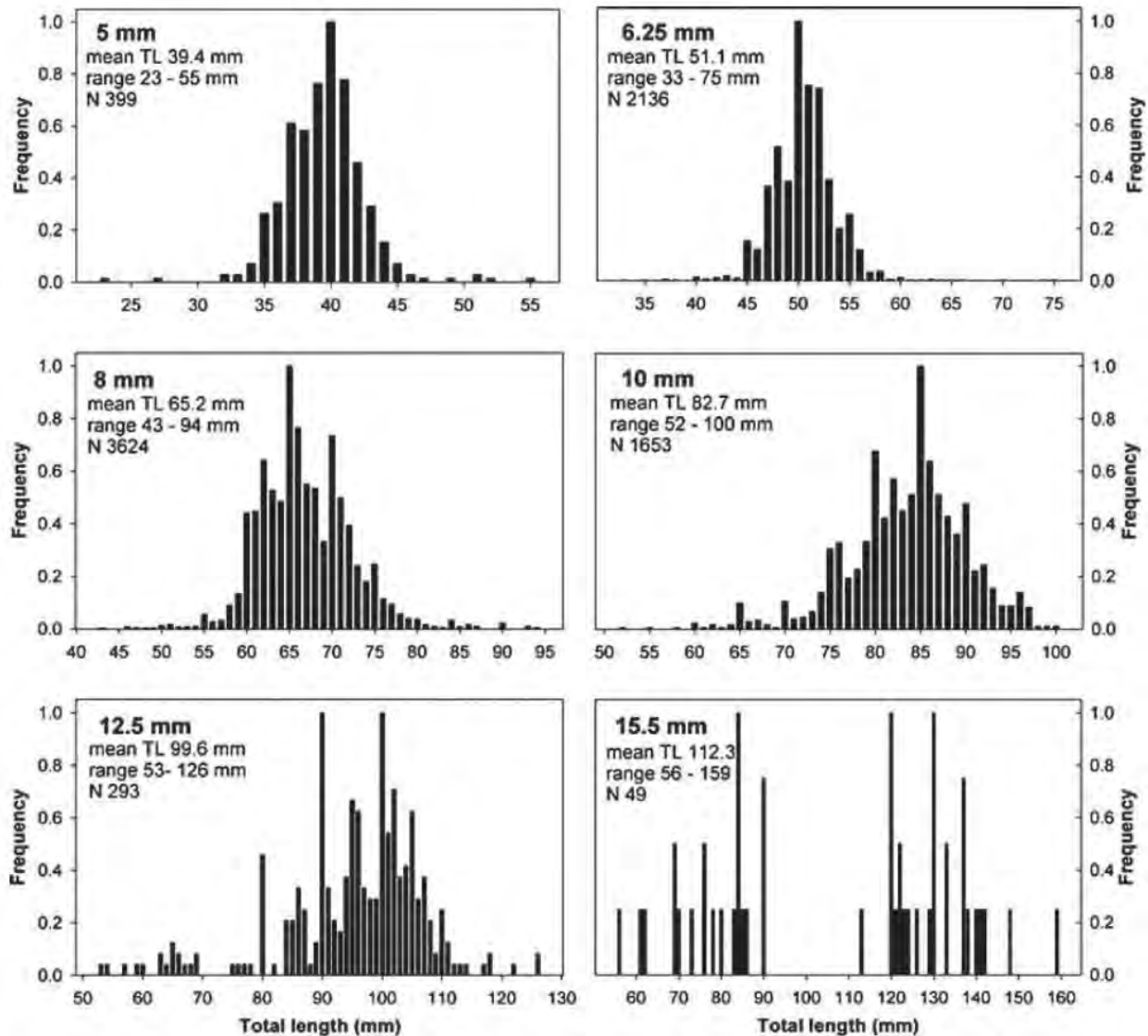


FIGURE 4. Size distributions of threadfin shad caught in gill nets with different mesh sizes. The mean sizes of captured shad (mean TL), the size range, and the total number of shad caught ( $N$ ) are listed as well.

and Degan 1995). Therefore, the distribution of threadfin shad is decidedly uneven, which introduces variability into any passive sampling. In contrast, active sampling with a frame trawl covers representative volumes of water and has the potential to reduce the natural variability caused by uneven distribution, which was evident in the small values of CV obtained. Considerable gill-net damage occurred during each deployment, and the finer mesh sizes displayed multiple holes that significantly reduced the effective netting area. Thus, gill nets had to be repaired or replaced by new ones very frequently (after each quarterly sampling of approximately three exposures). It appeared that threadfin shad enmeshed in gill nets represented an irresistible bait for predators, and many

shad showed signs of being attacked. On the other hand, we did not observe any damage to or significant wear of the single trawl net used throughout the study, and due to this fact it kept the same efficiency. This also favors using trawling rather than gill netting for monitoring shad in tropical reservoirs.

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## Chapter 13: Spatiotemporal Distributions of Threadfin Shad in Tropical Reservoirs

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**Abstract:** Effective predator management requires accurate knowledge of prey availability and dynamics, yet prey species are rarely studied as intensely as their predatory counterparts. Almost nothing is known about the biology or behavior of threadfin shad *Dorosoma petenense* in tropical environments, despite that this species is the primary prey in many systems. The goals of this study were to examine spatial and temporal patterns in threadfin shad abundance in tropical reservoirs and to refine protocols for sampling this species. A custom, 3 × 3-m frame trawl was used to sample four reservoirs in Puerto Rico every 3 months for 1 year. Samples were stratified by longitudinal section (upper and lower lake) and by depth strata (0–3, 3–6, 6–9 m). Threadfin shad densities varied markedly among seasons, reservoirs, longitudinal sections, and depth (overall model:  $F_{36, 188} = 32.0$ ,  $P < 0.001$ , coefficient of variation [CV] = 22.1%,  $r^2 = 0.86$ ). Density estimates from samples were highest in spring and winter when there was substantially more larval production, and density fluctuated as much as 42-fold among seasons within reservoirs. Differences in mean density were as much as 23 times greater in productive reservoirs than in the least productive reservoir during the same season. Longitudinal variability was substantial between upper and lower reservoir sections (mean percent difference = 70%), but patterns were not consistent among reservoirs or seasons. Threadfin shad showed clear preference for shallow depth strata where 75.4% of fish were collected in the 0–3-m stratum, and only 2.4% collected in the 6–9-m stratum. Oxygen profiles suggested that threadfin shad selected shallow depths even when dissolved oxygen levels were suitable in deeper depth strata. Understanding spatiotemporal patterns in threadfin shad distributions will improve sampling programs in terms of timing and frequency of sampling, longitudinal and vertical sampling design, and sample size requirements.

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All biological communities vary greatly in space and time (e.g., Wiens 1986; Southwood 1988; Schael et al. 1995), and the pattern and degree of patchiness of this variation is of fundamental interest to ecologists and managers because of its importance in biological interactions (Margalef 1985; Carlile et al. 1989; Levin 1992). Understanding spatiotemporal variability is critical to development of principles in ecosystem management (Wiens 1989), as this variability affects,

and is affected by, the distribution of resources, predators, habitat, and physiochemical variables such as temperature or dissolved oxygen in aquatic environments (Neill and Magnuson 1974; Schael et al. 1995). The importance of spatial and temporal dimensions is certainly evident for aquatic communities (e.g., Minshall 1988; Baussant et al. 1993; Mérigoux et al. 1999) and there is increasing

interest in incorporating this variability into landscape-scale management plans for aquatic resources.

Knowledge of the distribution of a population as it varies with space and time can lead to insights regarding important processes such as feeding, predator avoidance, habitat variability, and reproduction (Wiebe 1970; Frost et al. 1988; Rose and Leggett 1990; Brandt et al. 1991). Furthermore, variability across both space and time affects the ability of scientists to sample organisms, and thus their ability to understand these processes (Schael et al. 1995; Jůza et al. 2009). This is particularly true for small-bodied pelagic prey fish species, which are known to exhibit strong diel and seasonal variability in distribution on a broad scale, and fine-scale patchiness associated with schooling behavior. For example, Schael et al. (1995) reported that patch size of threadfin shad *Dorosoma petenense* in Lake Norman, North Carolina, was relatively small (<100 m<sup>2</sup>), and about 50% of the fish were located in only 10% of the reservoir surface area. For these reasons, sampling regimes should be designed to be sufficiently robust to collect representative samples across both broad and fine scales (Kuběcka et al. 2009, 2012).

Threadfin shad are the primary prey species for sport fish in many temperate (Noble 1981; Mosher 1984; Guest et al. 1990) and tropical (Alicea et al. 1997; Neal 2003; Neal et al. 2009) reservoirs. As such, this species is important to the management of the sport fishes that depend upon them (DeVries and Stein 1990). Almost nothing is known about the biology or behavior of threadfin shad in tropical environments, and no previous research has addressed spatial or temporal patterns in their abundance or distribution. Effective predator management requires accurate knowledge of prey availability and dynamics, particularly recruitment and size-structure (Jenkins and Morais 1978; Larkin 1979); thus, targeted sampling programs for prey species are critical to sport fish management.

The goal of this study was to examine spatial and temporal patterns in threadfin shad abundance in tropical reservoirs. The objectives were to (1) determine seasonal patterns in threadfin shad density and larval production, (2) determine longitudinal differences in threadfin shad density between up-lake transitional zones and down-lake lacustrine zones, (3) examine threadfin shad density patterns with depth, and (4) explore linkages of environmental parameters with threadfin shad distributions. These results are incorporated into sampling recommendations for tropical reservoirs.

## Methods

*Study Sites* – This research was conducted on four reservoirs located on the island of Puerto Rico. Puerto Rico is an exclusively tropical habitat and is primarily of volcanic origin. It is 175 km long by approximately 62 km wide, and has a central mountain range that runs east to west. More than 50 rivers originate in this mountainous terrain, many of which have been impounded. Reservoirs in Puerto Rico are generally mesotrophic to eutrophic, and anoxic conditions usually occur at depths greater than 3–9 m depending on the severity of and time since mixing events. Surface water temperatures average around 27°C, though this varies somewhat with altitude and season (Neal et al. 2009).

The four study reservoirs were Lucchetti, Guajataca, Dos Bocas, and Carite, which range from 108 to 360 ha in surface area (Figure 1). These reservoirs contain a mixture of fish species, and largemouth bass *Micropterus salmoides*, threadfin shad, tilapia *Oreochromis* and *Tilapia* spp., sunfishes *Lepomis* spp., ictalurids, and Amazon sailfin catfish *Pterygoplichthys pardalis* are common to all four. Red devil cichlid *Amphilophus labiatus* has been collected in all four systems but is abundant in Dos Bocas and Guajataca reservoirs. Guajataca, Dos Bocas, and Carite reservoirs also contain butterfly peacock bass *Cichla ocellaris*. Carite Reservoir was added to the study after the first sampling period, so no data were available for spring 2010.

*Gear Specifications and Study Design* – Boxrucker et al. (1995) reported that the preferred gears for sampling threadfin shad in temperate systems were surface-set gill nets, trawls, and hydroacoustic sampling, although only trawls and hydroacoustic sampling were useful for estimating biomass. In tropical reservoirs, hydroacoustic sampling has been attempted, but the results were difficult to interpret and to ground truth, particularly because of the presence of several cichlid species that are abundant in pelagic areas and difficult to sample quantitatively (Neal et al. 2001; Prchalová et al. 2003; Frouzová et al. 2008). Prchalová et al. (2012) determined that the preferred sampling gear for threadfin shad in tropical reservoirs was a fixed-frame trawl, as this gear yields much higher catch rates, much lower bycatch, and substantially less size-selectivity than gill nets. Therefore, for this study a custom-frame fry trawl was designed with frame aperture dimensions of 3 × 3 m, 6-mm mesh in the body of the trawl, and 4-mm mesh in the cod end. The total length of the trawl was 10.5 m, and the towing rope between the tow boat and the trawl was 100 m long.

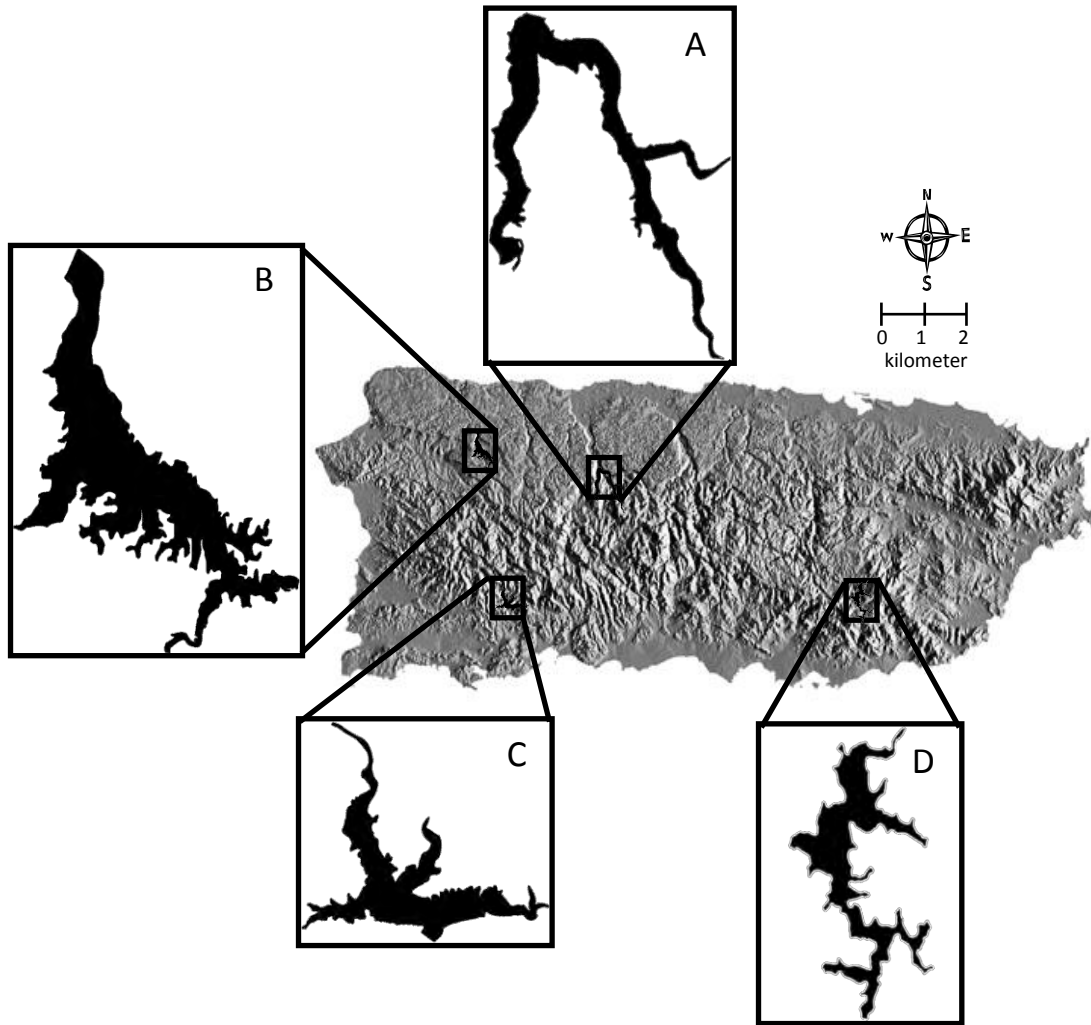


FIGURE 1. Location of (A) Dos Bocas, (B) Guajataca, (C) Lucchetti, and (D) Carite reservoirs in Puerto Rico.

Each reservoir was divided into upper (river arm) and lower (near dam) sections. Dos Bocas Reservoir had two upper sections that were sampled independently (Figure 2), but the data from the two arms were averaged for analyses to simplify statistical design. Prior to trawling, three preset transects were selected per reservoir section to provide three replicates each. At each transect location, three depths were sampled. These were upper open water (0–3 m), middle open water (3–6 m), and lower open water (6–9 m). Depths deeper than 9 m were not sampled because most reservoirs in Puerto Rico are oligomictic, and anoxia is usually present below depths of 3–9 m depending on time since the last mixing event. The depth of towing was determined by the length of the rope between the buoy on the surface and the upper rim of the trawl frame. Following sample completion at each depth stratum, the trawl was lowered to the next depth to be sampled and was flushed by towing with

the cod end open in order to wash out fish that may have remained in the trawl from the previous depth layer. In the shallow upper sections of each reservoir, only 0–3-m and 3–6-m depth strata were sampled due to insufficient depth for trawling. In the dam sections of reservoirs, all three depth strata were sampled.

All trawling was conducted at night using a two-boat system; the first boat was used as a trawler and the second boat used to retrieve the cod end, empty the catch, and process the samples. Using two boats made trawl sampling more efficient as it was not necessary to fully retrieve the entire trawl after each tow. Because of the small size of these reservoirs and large mouth of the trawl, duration of each trawl tow was held at 2 min, which resulted in a trawled distance of about 120 m with average speed of 3.6 km/h and a sampled volume around 1,080 m<sup>3</sup>. At the end of each tow, the funnel section of the trawl was retrieved using the surface

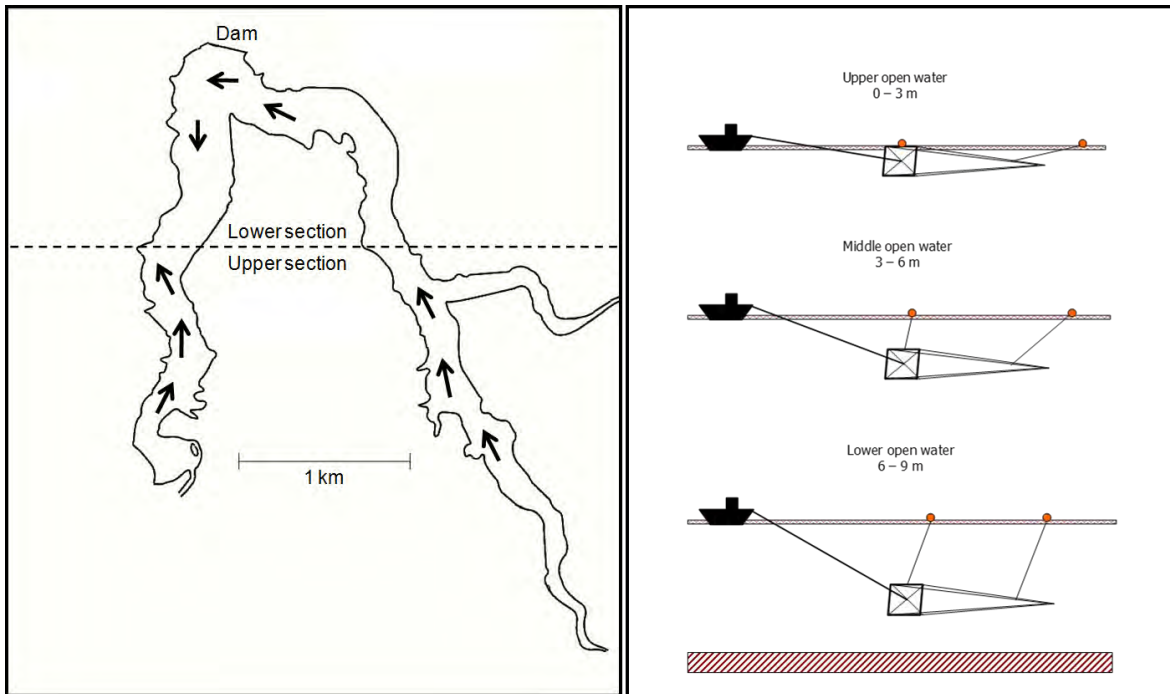


FIGURE 2. Left panel: Example of trawling locations (arrows) for Dos Bocas Reservoir. Right panel: Sampling design for depth strata.

buoy, and the catch was moved down the funnel section through the open cod end to a collection bucket on board the retrieval boat. The catch was stored in a labeled Ziploc plastic bag and placed on ice in the cooler for processing in the laboratory. Occasional species other than threadfin shad caught as bycatch were identified, measured, and released alive back into the water. The iced trawl catches were processed the next day. Each fish was measured for total length (TL, mm) and threadfin shad smaller than 30 mm TL were recorded as larvae. The characterization of fish less than 30 mm as larvae was primarily for practical concerns, as these smaller fish were often considerably more abundant than larger fish. Stancil et al. (1997) reported that the minimum size at maturity for female threadfin shad in Puerto Rico was 45 mm, so this designation probably did not include any adult shad.

*Assessment of water quality* – Hydraulic retention time for each reservoir was calculated using mean annual discharge and reservoir storage capacity data from the U.S. Geological Survey’s National Water Information System website (USGS 2011). Vertical profiles of temperature (°C), dissolved oxygen (mg/L), and turbidity (NTU) were collected at the first trawling location in each reservoir section during every sampling event. Water quality parameters were recorded using a Manta II Water Quality Multiprobe at 1-m increments beginning at the surface (0 m) and extending downward until dissolved oxygen levels

dropped below 2 mg/L (typically by 15 m), all of the cable was extended, or the probe had reached the bottom sediments.

*Data processing and analysis* – Fish records were stored in the database software Pasgear (J. Kolding, University of Downloaded by Bergen, Norway). Results (e.g., relative abundance, biomass, proportion) were calculated within the Pasgear database and manipulated using other software including Microsoft Excel and Sigma Plot.

Densities (fish/1,000 m<sup>3</sup>) of all threadfin shad (total), larval threadfin shad (< 30 mm TL, larval), and nonlarval threadfin shad (≥ 30 mm TL, nonlarval) were determined for each trawl tow. Density estimates were transformed using  $\log_e(X + 1)$  where  $X$  = density to normalize data and stabilize variances. Total, larval, and nonlarval threadfin shad density estimates (fish/1,000 m<sup>3</sup>) were compared among season, reservoir, reservoir section, and depth strata using ANOVA (PROC GLM, SAS version 9.2). For significant comparisons, differences among variables were determined using the least-squares means procedure (LSMEANS, SAS version 9.2). The model used the class variables of season, reservoir, section, and depth, and interaction terms. The model was constructed using backward selection, with all class variables and interaction terms included initially, and then an iterative process was used to remove the least significant variable per run until all model variables

were significant at  $P \leq 0.05$ . This is a common statistical approach for use with exploratory analyses when specific hypotheses are not warranted.

To visually examine differences in threadfin shad density between upper and lower reservoir sections, density ratios were calculated as:

$$\text{Density ratio} = [X_u / (X_u + X_l)] - 0.5,$$

where  $X_u$  is the mean density of the upper section and  $X_l$  is the mean density of the lower section. The proportion was adjusted by subtracting 0.5 so that sampling events with a greater proportion of threadfin shad in the upper section would yield positive density ratios, and sampling events with a greater proportion of threadfin shad in the lower reach would yield negative density ratios.

To determine the proportion of the threadfin shad population composed of larval shad (< 30 mm TL), the number of larvae collected in each tow was divided by the total number of threadfin shad collected in each tow. This removed much of the variability associated with fluctuating density and allowed easier graphical and statistical comparison. These proportions were transformed using arcsine-root transformation [Arcsine ( $X^{0.5}$ )] to normalize data and stabilize variances, and were compared among season, reservoir, reservoir section, and depth strata using ANOVA (PROC GLM, SAS version 9.2). For significant comparisons, differences among variables were determined using the least squares means procedure (LSMEANS, SAS version 9.2). The model included the class variables reservoir, season, section, and depth, and interaction terms. The model was constructed using backward selection, with all class variables and interaction terms included initially, and then an iterative process was used to remove the least significant variable per run until all model variables were significant at  $P \leq 0.05$ .

Multiple regression analysis (PROC REG, SAS version 9.2) was used to determine the effects of environmental variables on threadfin shad density for total, larval, and nonlarval threadfin shad discretely. For each size range analyzed, replicate transformed density estimates were averaged for each depth strata within each section. Temperature, dissolved oxygen, and turbidity readings were averaged across corresponding depth strata and served as independent variables used to predict threadfin shad density.

## Results

### *Overall Spatiotemporal Models*

Analyses of overall spatiotemporal influence on threadfin shad density evaluated all shad together (total), all shad < 30 mm TL (larval), and all shad  $\geq 30$  mm TL (nonlarval) to account for differences related to

recruitment. Overall results of each model are presented below and are followed by individual analyses of each class variable.

Total threadfin shad density displayed statistical relationships with the spatial and temporal parameters of interest. The general linear model for total threadfin shad density explained the majority of variability in the data ( $F_{36, 188} = 32.0$ ,  $P < 0.001$ , coefficient of variation [CV; SD/mean  $\times 100$ ] = 22.1%,  $r^2 = 0.86$ ), and the class variables season ( $F_3 = 70.1$ ,  $P < 0.001$ ), reservoir ( $F_3 = 37.6$ ,  $P = 0.001$ ), section ( $F_1 = 4.6$ ,  $P = 0.033$ ), and depth ( $F_2 = 162.8$ ,  $P < 0.001$ ) each displayed influence over density. Depth ( $r^2 = 0.40$ ) explained most of the variation in total threadfin shad density, followed by season ( $r^2 = 0.21$ ) and reservoir ( $r^2 = 0.14$ ). Although significant, reservoir section accounted for < 1% of the variability.

Similar to total threadfin shad density, larval threadfin shad density exhibited statistical relationships with spatial and temporal parameters of interest. The general linear model for larval threadfin shad density explained the majority of variability in the data ( $F_{36, 188} = 32.0$ ,  $P < 0.001$ , CV = 22.1%,  $r^2 = 0.86$ ), and the class variables season ( $F_3 = 70.1$ ,  $P < 0.001$ ), reservoir ( $F_3 = 37.6$ ,  $P < 0.001$ ), section ( $F_1 = 4.6$ ,  $P = 0.033$ ), and depth ( $F_2 = 162.8$ ,  $P < 0.001$ ) each displayed influence over larval threadfin shad density. Season ( $r^2 = 0.40$ ) explained most of the variation in larval threadfin shad density, followed by depth ( $r^2 = 0.22$ ), reservoir ( $r^2 = 0.07$ ), and reservoir section ( $r^2 = 0.02$ ).

Density of nonlarval shad displayed statistical relationships with the independent variables of interest ( $F_{36, 188} = 32.0$ ,  $P < 0.001$ , CV = 19.4%,  $r^2 = 0.85$ ). Season ( $F_3 = 48.3$ ,  $P < 0.001$ ), reservoir ( $F_3 = 36.4$ ,  $P = 0.001$ ), and depth ( $F_2 = 160.5$ ,  $P < 0.001$ ) displayed significant influence over nonlarval density, but section was not significant in this model ( $F_1 = 0.6$ ,  $P = 0.433$ ). Depth ( $r^2 = 0.42$ ) explained most of the variation in nonlarval threadfin shad density, followed by reservoir ( $r^2 = 0.15$ ) and season ( $r^2 = 0.14$ ).

### *Seasonal Effects*

Threadfin shad densities displayed considerable variability seasonally, with estimates fluctuating as much as 42-fold within a 3-month period. Spring and winter exhibited greater threadfin shad densities than summer or fall (all  $P < 0.001$ ), but spring and winter were not different from each other ( $P = 0.974$ ; Figure 3). Similarly, summer and fall were not statistically different from each other in terms of total threadfin shad density ( $P = 0.360$ ). Although not sampled during spring, Carite Reservoir did not follow the same seasonal pattern and exhibited peak density in fall

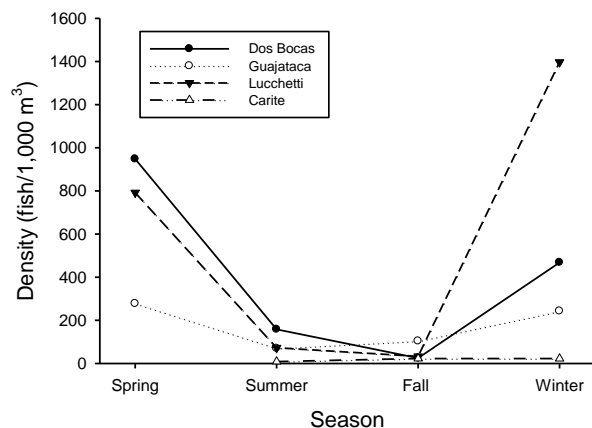


FIGURE 3. Seasonal density (fish/1,000 m<sup>3</sup>) estimates for threadfin shad (all sizes) in four Puerto Rico reservoirs collected 2010–2011 (SE values omitted for readability).

2010, although the magnitude of change was much less than in other reservoirs.

Larval production was clearly influenced by season, with all seasons statistically different from one another. Larval abundance was greatest in spring 2010, dropped markedly by summer 2010, continued to decline into fall 2010, and then increased sharply in winter 2011. All differences were statistically significant with  $P < 0.001$ . An alternative means of examining spatiotemporal patterns in larval threadfin shad density was to compare the proportion of the observed shad composed of larval fish across spatial and temporal scales (Figure 4). This analysis revealed that more than half of the threadfin shad collected in the spring were larvae, and this ratio generally declined through fall before increasing in winter ( $P < 0.001$ ).

#### Reservoir Effects

Considerable variability was detected among reservoirs. Carite Reservoir consistently produced much lower threadfin shad densities than other reservoirs, and seasonal mean densities ranged from 8.4 to 22.3 fish/1,000 m<sup>3</sup> (all sizes, Table 1). Lucchetti Reservoir (range, 32.9–1,397 fish/1,000m<sup>3</sup>) presented the greatest variability across seasons and the greatest observed density in winter 2011, but Dos Bocas Reservoir (range, 24.9–948 fish/1,000 m<sup>3</sup>) produced the greatest densities in two out of four seasons. Guajataca Reservoir was intermediate, ranging from 69.6 to 277 fish/1,000 m<sup>3</sup>. Based on LSMEAN results, each reservoir differed from the others in observed density estimates with the exception of the Guajataca and Dos Bocas comparison ( $P = 0.056$ ), which slightly exceeded the pre-established alpha level.

#### Longitudinal Effects

Although substantial variability in density was observed among reservoir sections, this variability was not consistent across seasons or reservoirs (all sizes, Figure 5). For example, the lower section of Dos Bocas Reservoir exhibited the greatest densities in each season except spring, whereas in Guajataca Reservoir the upper section always produced greater densities. In some cases, densities were up to 240% greater in one section versus the other, but in general the difference in density averaged 70% (SE = 18%).

There was a clear longitudinal difference in larval abundance, and greater larval densities were observed in upper transition areas than in lower lacustrine zones ( $P = 0.002$ ). Larval threadfin shad comprised a greater proportion of the catch in upper lake sections ( $P = 0.046$ ) in all reservoirs.

#### Depth Effects

Depth strata consistently influenced density estimates, and 75.4% (SE = 3.5%) of the total threadfin shad catch occurred the 0–3-m stratum, and only 2.4% (SE = 0.8%) of the total threadfin shad were collected at depths of 6–9 m (Table 1). However, there were several notable exceptions. During winter sampling, upper sections of three of the four reservoirs displayed greater threadfin shad densities in the 3–6-m stratum (9–34% more fish), but this pattern was not repeated in the lower sections. Conversely, the upper section of Lucchetti Reservoir had nearly nine times more threadfin shad in the 0–3-m stratum than in the 3–6-m stratum during the same time period. The only other instance of greater density of fish in the 3–6-m layer was in Carite Reservoir during the fall sampling (58% greater density). Lower reservoir sampling always observed more threadfin shad at 0–3 m than at 3–6 m. There was one instance in Guajataca Reservoir during the spring sampling where density in the 6–9-m depth stratum was 176% greater than in the 3–6-m stratum, but this stratum comprised only 23% of the total threadfin shad collected in the lower reservoir during this sampling period.

Larval threadfin shad also displayed greater densities at shallower depth, and 88% of the larval catch occurred in the 0–3-m depth stratum. Less than 1% of larval shad came from the 6–9-m stratum. Larval shad composed a greater proportion of the catch in the upper 3 m of the water column ( $P < 0.001$ ) in all reservoirs, except Dos Bocas Reservoir.

#### Water Quality Patterns

The estimated hydraulic retention times for the four study reservoirs were extremely variable. Guajataca Reservoir exhibited the greatest retention time at 453 d,

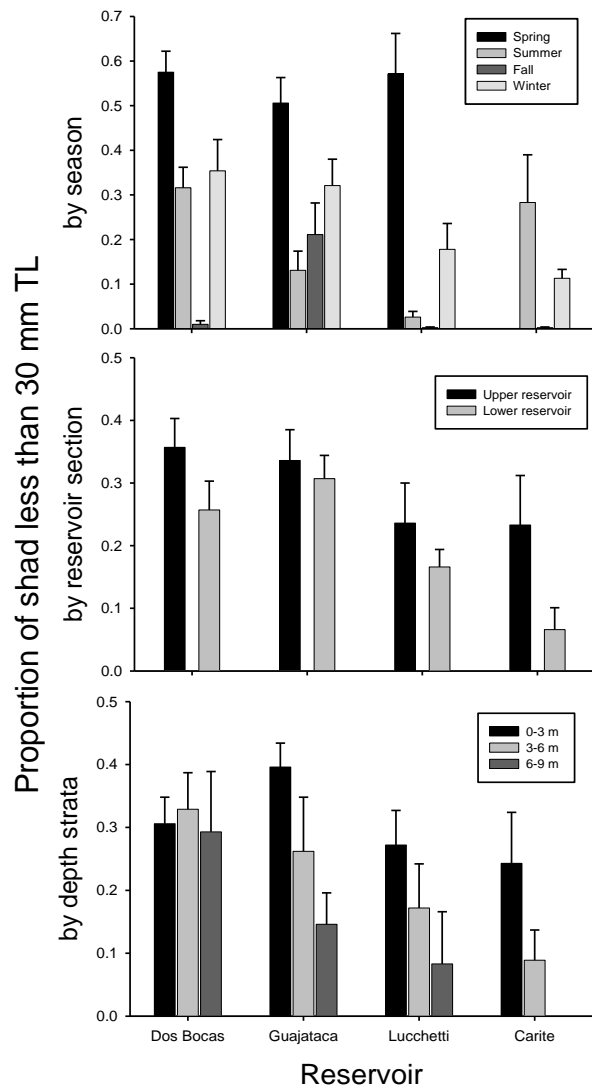


FIGURE 4. Relative proportion of larval threadfin shad (< 30 mm TL) by season (top panel), reservoir section (middle panel), and depth stratum (bottom panel). Error bars are +SE.

followed by Carite Reservoir (312 d) and Lucchetti Reservoir (231 d). Dos Bocas Reservoir exhibited a very limited retention time of only 16 d.

Temperature stratification was present in all reservoirs to a limited extent. All reservoirs varied less than 5.4°C from the surface to the deepest depth sampled, although this difference was typically 1–3°C. Heavy rain events prior to the onset of this study and periodically during the study resulted in somewhat unpredictable oxygen stratification. Guajataca Reservoir was the most stable in terms of oxycline, and dissolved oxygen concentrations declined to 2 mg/L at or above 9 m depth in every season (Figure 6). On the

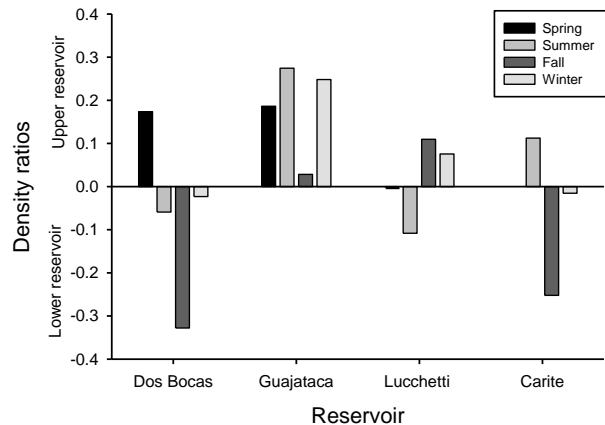


FIGURE 5. Density ratios of threadfin shad (all sizes) during seasonal sampling events in four reservoirs in Puerto Rico. Each reservoir was divided into upper transitional and lower lacustrine sections. Positive ratios indicate greater densities in the upper reservoir, whereas negative values indicate greater densities near the dam.

other end of the spectrum, Dos Bocas Reservoir was much more variable, and often exhibited dissolved oxygen levels exceeding 5 mg/L down to at least 16 m depth. Lucchetti Reservoir and Carite Reservoir were intermediate, but generally displayed reduced levels of dissolved oxygen below 9 m, which was the maximum depth sampled by trawl.

Likewise, turbidity varied widely among reservoirs and across seasons (Figure 7). Dos Bocas Reservoir was consistently the most turbid system, and the western arm was generally more turbid than the eastern arm or the lower lake confluence. Lucchetti Reservoir exhibited the second greatest turbidity readings, and a peak in turbidity occurred during the fall sampling, which coincided with especially heavy rains. Carite Reservoir exhibited low turbidity throughout the study period, as did Guajataca Reservoir, which was the most transparent reservoir studied.

Total threadfin shad densities were influenced by water quality ( $F_{3, 71} = 23.5, P < 0.001, CV = 38.1\%, r^2 = 0.50$ ). However, only dissolved oxygen was a significant factor ( $t^1 = 7.4, P < 0.001$ ), and temperature ( $t_1 = 0.1, P = 0.941$ ) and turbidity ( $t_1 = -0.44, P = 0.658$ ) showed no effect. Similar results were observed for larval threadfin shad ( $F_{3, 71} = 17.7, P < 0.001, CV = 40.6\%, r^2 = 0.43$ ), with dissolved oxygen as the only significant variable ( $t_1 = 5.9, P < 0.001$ ). Conversely, nonlarval threadfin shad densities ( $F_{3, 71} = 18.4, P < 0.001, CV = 34.1\%, r^2 = 0.44$ ) were influenced by dissolved oxygen ( $t_1 = 5.8, P < 0.001$ ) and turbidity ( $t_1 = -5.9, P = 0.032$ ). Increased turbidity exerted a



TABLE 1. Mean density  $\pm$  SE (fish/1,000 m<sup>3</sup>) of threadfin shad (all sizes) for Puerto Rico reservoirs by season, reservoir, section, and depth strata. Carite Reservoir was added to the sampling efforts in summer 2010. Bold values indicate seasonal and annual mean density across habitat categories.

| Season                          | Longitudinal section | Depth strata (m) | Mean density (fish/1,000 m <sup>3</sup> ) |                                     |                                       |                                  |
|---------------------------------|----------------------|------------------|---|-------------------------------------|---------------------------------------|----------------------------------|
|                                 |                      |                  | Dos Bocas <sup>1</sup>                    | Guajataca                           | Lucchetti                             | Carite                           |
| Spring                          | Upper                | 0-3              | 2,310.1 $\pm$ 809.4                       | 732.8 $\pm$ 131.5                   | 1,751.9 $\pm$ 221.3                   |                                  |
|                                 |                      | 3-6              | 532.1 $\pm$ 112.6                         | 60.6 $\pm$ 40.7                     | 147.3 $\pm$ 40.7                      |                                  |
|                                 | Lower                | 0-3              | 931.6 $\pm$ 291.2                         | 322.9 $\pm$ 113.4                   | 1,550.6 $\pm$ 331.5                   |                                  |
|                                 |                      | 3-6              | 445.1 $\pm$ 213.3                         | 39.9 $\pm$ 23.6                     | 351.2 $\pm$ 253.2                     |                                  |
|                                 |                      | 6-9              | 46.1 $\pm$ 16.7                           | 110.0 $\pm$ 34.5                    | 1.8 $\pm$ 0.9                         |                                  |
| <b>Spring estimated density</b> |                      |                  | <b>947.7 <math>\pm</math> 325.3</b>       | <b>277.2 <math>\pm</math> 116.8</b> | <b>792.1 <math>\pm</math> 291.1</b>   |                                  |
| Summer                          | Upper                | 0-3              | 281.9 $\pm$ 98.5                          | 141.2 $\pm$ 30.3                    | 137.6 $\pm$ 48.8                      | 22.3 $\pm$ 9.4                   |
|                                 |                      | 3-6              | 46.5 $\pm$ 10.7                           | 90.7 $\pm$ 9.2                      | 4.4 $\pm$ 1.2                         | 1.4 $\pm$ 1.4                    |
|                                 | Lower                | 0-3              | 357.6 $\pm$ 9.9                           | 65.4 $\pm$ 12.8                     | 202.1 $\pm$ 6.6                       | 15.0 $\pm$ 14.8                  |
|                                 |                      | 3-6              | 58.8 $\pm$ 10.2                           | 2.2 $\pm$ 0.8                       | 18.4 $\pm$ 11.9                       | 0.0 $\pm$ 0.0                    |
|                                 |                      | 6-9              | 38.0 $\pm$ 9.3                            | 2.2 $\pm$ 0.9                       | 0.3 $\pm$ 0.3                         | 0.0 $\pm$ 0.0                    |
| <b>Summer estimated density</b> |                      |                  | <b>157.8 <math>\pm</math> 32.4</b>        | <b>69.6 <math>\pm</math> 17.9</b>   | <b>72.3 <math>\pm</math> 33.4</b>     | <b>8.4 <math>\pm</math> 4.1</b>  |
| Fall                            | Upper                | 0-3              | 12.7 $\pm$ 3.1                            | 176.5 $\pm$ 15.1                    | 87.7 $\pm$ 30.0                       | 10.9 $\pm$ 2.8                   |
|                                 |                      | 3-6              | 9.3 $\pm$ 2.2                             | 82.5 $\pm$ 7.8                      | 4.1 $\pm$ 0.9                         | 17.2 $\pm$ 10.4                  |
|                                 | Lower                | 0-3              | 84.2 $\pm$ 27.5                           | 220.4 $\pm$ 68.7                    | 47.0 $\pm$ 17.9                       | 48.2 $\pm$ 17.1                  |
|                                 |                      | 3-6              | 21.7 $\pm$ 4.2                            | 10.9 $\pm$ 2.5                      | 11.8 $\pm$ 1.0                        | 37.1 $\pm$ 16.5                  |
|                                 |                      | 6-9              | 10.5 $\pm$ 4.1                            | 0.3 $\pm$ 0.3                       | 0.9 $\pm$ 0.6                         | 8.2 $\pm$ 0.5                    |
| <b>Fall estimated density</b>   |                      |                  | <b>24.9 <math>\pm</math> 6.9</b>          | <b>103.4 <math>\pm</math> 35.6</b>  | <b>32.9 <math>\pm</math> 16.9</b>     | <b>22.6 <math>\pm</math> 7.8</b> |
| Winter                          | Upper                | 0-3              | 461.5 $\pm$ 50.4                          | 371.6 $\pm$ 15.6                    | 3,386.7 $\pm$ 1,287.1                 | 23.0 $\pm$ 2.7                   |
|                                 |                      | 3-6              | 617.1 $\pm$ 261.7                         | 404.5 $\pm$ 81.4                    | 340.8 $\pm$ 67.6                      | 28.7 $\pm$ 4.5                   |
|                                 | Lower                | 0-3              | 1147.2 $\pm$ 454.8                        | 165.2 $\pm$ 41.7                    | 2,343.9 $\pm$ 313.4                   | 33.3 $\pm$ 11.7                  |
|                                 |                      | 3-6              | 36.1 $\pm$ 7.6                            | 96.2 $\pm$ 43.3                     | 404.5 $\pm$ 92.9                      | 21.7 $\pm$ 0.9                   |
|                                 |                      | 6-9              | 5.9 $\pm$ 0.6                             | 30.1 $\pm$ 4.6                      | 42.6 $\pm$ 9.5                        | 1.3 $\pm$ 0.6                    |
| <b>Winter estimated density</b> |                      |                  | <b>467.9 <math>\pm</math> 116.2</b>       | <b>242.6 <math>\pm</math> 53.3</b>  | <b>1,397.0 <math>\pm</math> 325.4</b> | <b>22.3 <math>\pm</math> 5.0</b> |
| <b>Annual mean density</b>      |                      |                  | <b>399.6 <math>\pm</math> 204.9</b>       | <b>173.2 <math>\pm</math> 51.0</b>  | <b>573.5 <math>\pm</math> 325.3</b>   | <b>25.1 <math>\pm</math> 9.0</b> |

<sup>1</sup>The two upper arms of Dos Bocas Reservoir were sampled independently, but the average for each depth stratum is presented.

negative influence on nonlarval threadfin shad densities, and density increased as dissolved oxygen increased for both larval and nonlarval shad.

## Discussion

Threadfin shad population dynamics in the tropical reservoirs of Puerto Rico clearly vary on spatial and temporal scales with generally consistent patterns. There was considerable variability among reservoirs both seasonally and overall, with a 23:1 ratio in annual mean density between the most dense and least dense reservoirs. This was not surprising as these reservoirs are known to differ sharply in geographic and physiochemical characteristics. Carite Reservoir, which was by far the least productive in terms of threadfin shad density, drained a relatively pristine, primarily forested watershed, which reduced nutrient availability in this system. Harris (2007) reported that primary production in Carite Reservoir was low compared with other island reservoirs, and this report was supported by Carvajal-Zamora (1979). Fish production has repeatedly been shown to be highly correlated with

total phosphorus concentration and annual phytoplankton production (McConnell et al. 1977; Oglesby 1977; Downing et al. 1990), and this correlation appears to hold true of planktivore populations in the tropics.

Seasonal patterns in threadfin shad density within reservoirs displayed unexpectedly great variability considering that conditions are appropriate for year-round reproduction and foraging. Stancil et al. (1997) reported that threadfin shad in Lucchetti Reservoir reproduced in every month of the year with a reduction of spawning in late summer to early fall. Seasonal patterns in total density, larval density, and the ratio of larval to nonlarval threadfin shad in this study supported this report, as densities and larval shad ratios were high in the spring, dropped markedly in summer, reached a low point in fall, then spiked again during the winter. However, in some cases density estimates increased as much as 42-fold from one season to the next, suggesting that the vast majority of spawning occurred during winter and spring and that mortality during summer and fall substantially outweighed

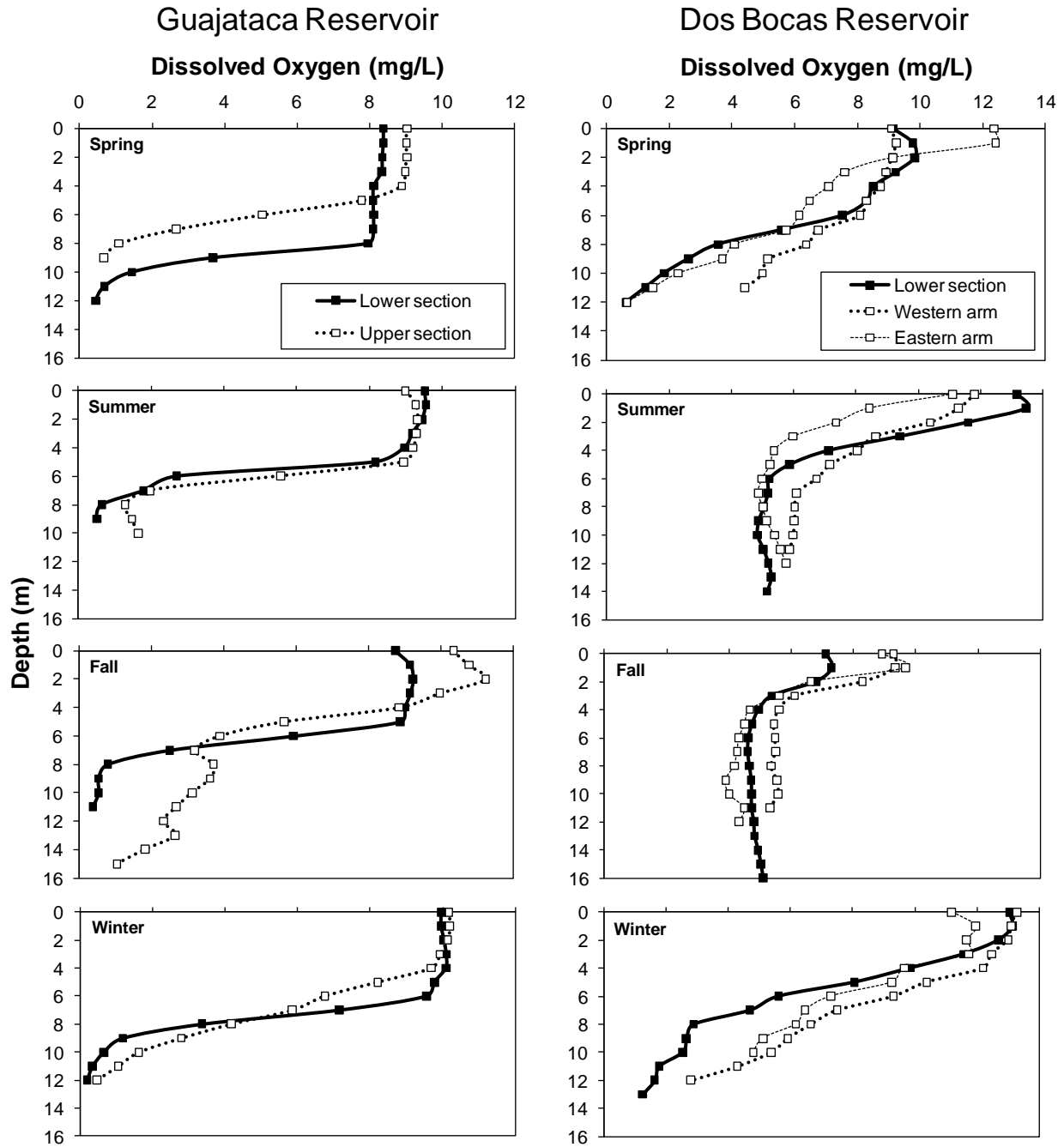


FIGURE 6. Seasonal dissolved oxygen profiles for Guajataca Reservoir (left panels) and Dos Bocas Reservoir (right panels).

recruitment. We are unaware of any other study that has examined patterns in threadfin shad density seasonally.

This study found consistent differences between up-lake transitional zones and down-lake lacustrine zones for larval threadfin shad, but consistent patterns across reservoirs were not apparent for nonlarval shad. Hence, the longitudinal influence on threadfin shad

density in total threadfin shad abundance was driven by larval abundance. This suggests that larval production within these reservoirs varies spatially, and understanding spatial variability is important to managing prey production in these reservoirs. The data presented in this study suggest that threadfin shad primarily spawn in transitional reservoir reaches or near river inputs, or less likely, larvae migrate to these

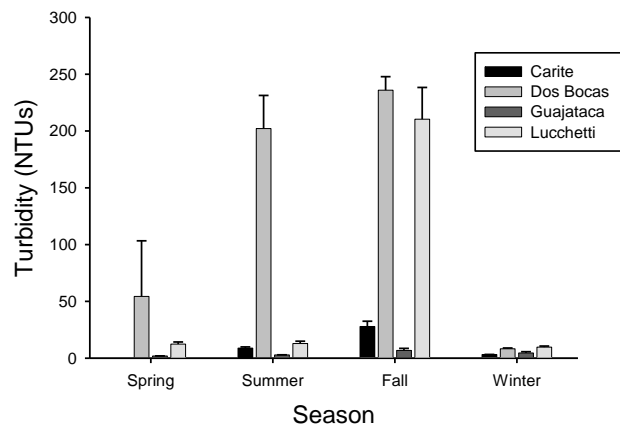


FIGURE 7. Mean seasonal turbidity across the sampled depths (0–9 m in lower lake, 0–6 m in upper arms) in four Puerto Rico reservoirs. Error bars are +SE.

areas shortly after hatch. It is not believed that threadfin shad migrate up river reaches to reproduce.

Another possible explanation for the longitudinal variability may be hydrology, which affects the distribution of primary productivity in each system. Guajataca Reservoir always displayed higher threadfin shad densities in the upper reservoir section than in the lower reservoir section, and hydraulic retention time for Guajataca Reservoir was estimated to be 453 d, which is very high for these relatively small systems. Given the primarily run-of-the-river shape of this reservoir and the long period of storage, it is likely that Guajataca Reservoir displays the classic longitudinal zonation of larger storage reservoirs. This zonation is characterized by decreasing nutrient availability, primary productivity, and consequently fish density as water moves longitudinally from riverine inputs towards the dam (e.g., Kennedy and Walker 1990; Prchalová et al. 2008, 2009; Jůza et al. 2009). Thus, up-lake reaches generally support greater prey resources for threadfin shad, and population density in Guajataca Reservoir reflected this.

Conversely, Dos Bocas Reservoir has a very short hydraulic retention time (16 d) because of its small size and substantially greater mean annual discharge. This reservoir has very high sedimentation rates (Soler-López 2001) and elevated turbidity in uplake reaches, which would reduce primary productivity by limiting light availability (Kimmel et al. 1990). Because of the short storage time, there should be little decrease in nutrient availability as water flows toward the lower reach, and increased sunlight transmission associated with reduced turbidity would lead to greater primary productivity nearer to the dam. Consequently, greater threadfin shad densities in the lower, more lacustrine

portions of the reservoir might indicate that shad were seeking better water quality, especially greater transparency, and potentially better prey resources. Lucchetti Reservoir and Carite Reservoir had intermediate hydraulic retention times (231 and 312 d, respectively) and were generally intermediate in terms of longitudinal threadfin shad distributions. This hypothesis was supported by the regression model, which determined that turbidity has an inverse relationship on nonlarval threadfin shad density.

Vertical distributions of threadfin shad displayed relatively consistent patterns, and most threadfin shad were collected in shallow surface layers. Overall, 94% of the fish were collected from 0 to 6 m depth, and the majority was from the 0–3-m stratum. This pattern persisted despite occasional mixing events that created oxygenated environments at greater depths. Dos Bocas Reservoir, for example, often displayed dissolved oxygen levels greater than 5 mg/L as deep as 16 m, yet most threadfin shad were collected in the 0–3-m stratum. This finding suggests that threadfin shad in tropical reservoirs prefer shallower depths even when greater depth strata are supportive in terms of physiochemical environment. A likely explanation is that most primary and secondary productivity, which produces the prey resources for threadfin shad, will be confined to these upper, well-sunlit depth strata. Thus, even though dissolved oxygen was identified as a factor that can influence fish densities, it is likely that other factors, especially resource availability, were the driving factors behind threadfin shad depth selection.

#### Management Implications

The spatiotemporal patterns observed in these tropical reservoirs have important implications for sampling programs. Understanding these patterns will improve sampling designs for threadfin shad, success of sport fish supplemental stocking activities, and management of sport fish populations in Puerto Rico and similar tropical systems. Based on the findings of this study, the authors offer the following recommendations for future threadfin shad sampling in tropical reservoirs.

*Timing and frequency of sampling* – Sampling season should be determined based on sampling objectives. For basic monitoring studies, annual winter or spring sampling would provide information on threadfin shad recruitment as well as an approximation of maximum density, whereas summer or fall sampling would be useful for determining potential prey limitations. For comprehensive studies of production, biomass, or reproduction, sampling should be conducted at least quarterly in order to account for rapid changes in threadfin shad density and size distributions throughout the year. A limitation of this

study was that it examined only 1 year and thus assumed that the observed patterns during the year were representative. The consistency of patterns among reservoirs supports this assumption; however, multiyear sampling would be useful to confirm seasonal patterns and elucidate annual variability.

*Longitudinal design* – Clear yet unpredictable longitudinal differences were observed in this study, suggesting that a robust sampling design is needed to account for spatial variability in these reservoirs. A systematic sampling design that places sampling stations at equal distances along a longitudinal transect would help account for longitudinal variability. More sampling stations will be needed in larger reservoirs or if detection of finer scale changes is required (discussed below).

*Sampling depth strata* – Assuming that the low catch rates observed in the 6–9-m stratum are indicative of few threadfin shad at even greater depths, we suggest that sampling designs include at least the 0–3-m and 3–6-m strata, and at most all strata from 0 to 9 m. Sampling only the strata from 0 to 6 m would have collected 94% of threadfin shad in this study, and no differences in statistical results would have been observed. Furthermore, sampling only from 0 to 6 m would have allowed for less complicated statistical designs, as upper and lower reservoir sampling stations would have had the same depth strata to compare. Because most reservoirs in most seasons were anoxic below 9 m, and because few threadfin shad were collected at 6–9 m even when the water was oxygenated, there is no reason to concentrate sampling efforts in deeper strata.

*Sample size requirements* – This study used three trawl tows per depth stratum in each reservoir section, providing an overall sample size of 18 (27 for Dos Bocas Reservoir). The mean CV, which was calculated as the average of individual CVs from the three replicate tows across all depths, sections, and reservoirs, was 0.54 (SE = 0.04), or 54%. While this value was slightly greater than the target of  $CV \leq 0.5$  (Cyr et al. 1992), it was sufficient to detect differences among seasons, sections, and depths given the magnitudes of the observed differences. However, when detecting changes on a finer scale, these sample sizes may not be sufficient. Using standard sample size calculation based on a power of 0.80, it appears that the 18 tows used in this study were capable of detecting changes in threadfin shad density of 45% or greater among sampling periods with the observed variability. To detect a change in threadfin shad density of only 25%, 70 total tows would be required (i.e., 14 tows per depth stratum in each reservoir section). Whereas observed changes among seasons were typically much

greater, three to five replicate tows per depth stratum per reservoir section should be adequate for most research and management applications. If a systematic sampling design as recommended previously is adopted, 6 to 10 longitudinal stations with one tow per depth stratum would be sufficient for most sampling applications in similar-size reservoirs.

*Broader implications* – Although this study focused on tropical reservoirs, these analyses have illustrated a much broader issue for fisheries managers; that is, we know almost nothing about threadfin shad populations. The authors found only four instances in the literature where threadfin shad biomass or density was estimated for aquatic systems in the USA, and only a handful of gizzard shad *D. cepedianum* estimates. Furthermore, many of these estimates were based on rotenone sampling, which can be considerably biased (Hayne et al. 1967; Summers and Axon 1980; Davies and Shelton 1983). Although more recent studies have used gear types that are less biased, particularly trawling, sampling in those studies has always been conducted in a single season (e.g., Siler 1986; Michaletz et al. 1995). If the variability observed across seasons in Puerto Rico is also present in temperate reservoirs, the single instantaneous measure of threadfin shad biomass or density would have limited utility in management decisions, and these estimates would not be comparable with samples from different times of the year or from different points along the threadfin shad population cycle. Accordingly, we recommend that fisheries researchers and managers consider incorporating prey fish sampling schemes that are stratified across seasons to better understand the dynamics of prey populations as they relate to predator management. Data collection that is systematically stratified across the year will allow for greater confidence in determining patterns in prey availability and production.

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## Chapter 14: Comparison of Threadfin Shad Density and Biomass between Temperate and Tropical Reservoirs

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**Abstract:** Threadfin shad *Dorosoma petenense* are the primary prey species for sport fish in many southern and tropical reservoirs. However, quantification of density or biomass for threadfin shad is rare in the literature and limited in geographic scope. The objectives of this paper were to report seasonal threadfin shad biomass estimates from tropical systems and to compare tropical threadfin shad populations between temperate and tropical reservoirs. Previously reported density estimates of threadfin shad in four Puerto Rico reservoirs were converted to biomass estimates and used for comparison. Considerable variability was observed between Puerto Rico reservoirs for biomass ( $F_{3,210} = 14.8$ ,  $P < 0.0001$ ), with estimates ranging from 2.0 to 32.2 kg/ha. Within reservoirs, biomass fluctuated as much as 24-fold between seasonal sampling events (CV ranged 51 – 126%). Comparison to data from temperate reservoirs suggested that Puerto Rico reservoirs may have threadfin shad populations that are generally less dense with less biomass, calling into question the previous assumption that prey limitation was not an issue in these tropical systems. This research also indicates that threadfin shad populations are under-evaluated in most temperate systems, and that current threadfin shad sampling strategies of a single sampling event per year may not be adequate to provide accurate characterization of threadfin shad populations.

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Holistic fisheries management schemes based on trophic relationships within ecosystems are generally more effective than single-species approaches (Larkin 1979; May et al. 1979). This is because sport fish, which are usually predators in freshwater systems, are dependent on bottom-up influences affecting forage abundance and availability (Noble 1986). Whereas effective predator management requires accurate knowledge of prey availability and dynamics (Jenkins and Morais 1978), targeted sampling programs for prey species are critical to support sport fisheries management.

For most reservoirs in the southern U.S., gizzard shad *Dorosoma cepedianum* and threadfin shad *D. petenense* are the principal prey species (Noble 1981). Despite the importance of these species, Neal and Prchalová (2012) concluded that abundance and biomass data on clupeid prey was lacking in the peer-reviewed literature. Furthermore, the few studies that addressed density or biomass largely relied on

sampling strategies and methods that were biased or incomplete. Rotenone was the primary sampling method used in most pre-1980s studies, though this toxicant has been largely discredited in recent years for sampling pelagic prey (e.g., Hayne et al. 1967; Summers and Axon 1980; Davies and Shelton 1983). Trawling has proven much less biased (Boxrucker et al. 1995), but the handful of studies to use this method have ignored seasonal variability by sampling prey species only once per year, usually during the summer or fall (e.g., Siler 1986; Michaletz et al. 1995). Furthermore, many studies that address clupeid populations fail to distinguish between clupeid species in their reporting (e.g., Taylor et al. 2005).

Threadfin shad are the primary prey species for sport fish in many tropical reservoirs, particularly for largemouth bass *Micropterus salmoides* and butterfly peacock bass *Cichla ocellaris* (Alicea et al 1997; Neal 2003). However, directed research has rarely quantified threadfin shad abundance or biomass in



tropical systems (Neal and Prchalová 2012). Much of the research and management decisions for the sport fish in Puerto Rico have assumed that prey availability was not limiting (e.g., Alicea et al. 1997; Stancil et al. 1997; Neal et al. 2008), despite that conclusive data to this end have not been available. Furthermore, temporal dynamics of prey fish communities are poorly understood and complicated by the fact that tropical reservoirs do not experience the same magnitude of seasonal cues in physicochemical environment as temperate reservoirs (e.g., Dadzie and Aloo 1990; Gran 1995). Whereas effective sport fish management requires consideration of both predator and prey, improved understanding of threadfin shad population dynamics would enhance management for reservoirs in Puerto Rico and other tropical locations.

Recently, Prchalová et al. (2012) concluded that trawling was the most preferred method for sampling threadfin shad in steep-sided tropical reservoirs, and Neal and Prchalová (2012) recommended threadfin shad sampling protocols using this gear. Moreover, the latter study presented the first quantification of threadfin shad density in four Puerto Rico reservoirs. Building on those findings, the goal of this manuscript is to 1) provide much needed analysis of threadfin shad biomass in Puerto Rico, and 2) compare Puerto Rico threadfin shad populations to those in temperate U.S. reservoirs. These data will improve biological understanding of trophic interactions and holistic sport fish management in tropical reservoirs, and provide a benchmark for comparisons of this species across its geographic range.

## Methods

In this paper, the available literature on threadfin shad density and biomass from Puerto Rico and the temperate USA was reviewed. The authors previously published estimates of threadfin shad density from four Puerto Rico reservoirs (Neal and Prchalová 2012), and use the raw data collected from that study to estimate biomass for comparisons in the current paper.

*Study Sites* – This research was conducted on four reservoirs located on the island of Puerto Rico. Reservoirs in Puerto Rico are generally mesotrophic to eutrophic, and typically anoxic below 3 – 9 m depth, depending on infrequent mixing events (Kröger et al. 2010). Surface water temperatures average around 27 °C, though this varies somewhat with altitude and season (Neal et al. 2009). The four study reservoirs were Lucchetti, Guajataca, Dos Bocas, and Carite, which range 108 – 360 ha in surface area. These reservoirs contain a mixture of fish species, with largemouth bass, threadfin shad, tilapia (*Oreochromis* and *Tilapia* spp.), sunfishes *Lepomis* spp., ictalurids, and Amazon sailfin catfish *Pterygoplichthys pardalis*

common to all four. Guajataca, Dos Bocas, and Carite Reservoir also contain butterfly peacock bass, and red devil cichlid *Amphilophus labiatus* has been collected in all four systems, but is abundant only in Dos Bocas and Guajataca Reservoir.

*Gear Specifications and Study Design* – A custom frame fry trawl was designed with frame aperture dimensions of 3 × 3 m, 6-mm mesh in the body of the trawl, 4-mm in the cod-end, and total length of the trawl of 10.5 m. The towing rope between the trawler boat and the trawl was 100 m long. Each reservoir was divided into lower (near dam) and upper sections (Dos Bocas had two upper sections as it has two primary arms). Prior to trawling, three fixed transects were selected per reservoir section to provide three replicates each. At each transect location, up to three depth strata were sampled (0 – 3, 3 – 6, and 6 – 9 m). Depths deeper than 9 m were not sampled because most reservoirs in Puerto Rico are oligomictic, and anoxia is usually present at depths greater than 9 m. In the upper sections of each reservoir, only upper (0 – 3 m) and middle (3 – 6 m) depth strata were sampled due to insufficient depth for trawling. The trawl was flushed between depth samples to avoid cross contamination of biota.

Sampling occurred within each reservoir at 3-month intervals for 1 year. The corresponding seasonal data were spring (April), summer (June), fall (October), and winter (January). All trawling was conducted at night. Duration of each trawl tow was held at 2 min, which resulted in trawled distance of about 120 m with average speed of 3.6 km/h and a sampled volume around 1,080 m<sup>3</sup>. All fishes collected were measured for total length (TL) with the precision of 1 mm and weighed to the nearest 0.01 g. When excessive numbers of threadfin shad were captured, appropriate sub-sampling was done.

*Data Processing and Analysis* – Threadfin shad biomass estimates (g/1,000 m<sup>3</sup>) were calculated for individual tows and then averaged across replicates to obtain section-depth averages. All depth strata from each section were averaged to produce section means, and sections were averaged assuming a 1:1 areal ratio between upper and lower reservoir sections to produce the seasonal reservoir mean. Threadfin shad biomass estimates for Puerto Rico were compared between season and reservoir using analysis of variance (ANOVA; PROC GLM, SAS Version 9.2). Estimates were transformed using  $\log_e(X + 1)$ , where X = biomass, to normalize data and stabilize variances. For significant comparisons, differences between variables were determined using the least squares means procedure (LSMEANS; SAS Version 9.2), with an alpha level 0.05 used for all comparisons.

Seasonal reservoir biomass estimates were converted from g/1,000 m<sup>3</sup> to kg/ha for comparison to other studies by summing depth strata estimates and expanding area. Seasonal biomass and density estimates were averaged across the year to provide the annual mean biomass. Statistical comparisons between Puerto Rico and temperate reservoirs were not feasible because of many differences in sampling gears and designs, insufficient data availability in the literature, and limitations in the format used for data reporting. Instead, a descriptive approach was used to compare temperate and tropical reservoirs and highlight limitations in threadfin shad sampling in all habitats.

## Results

*Puerto Rico Population Dynamics* – Biomass estimates differed considerably between Puerto Rico reservoirs ( $F_{3,210} = 14.8$ ,  $P < 0.0001$ ). Lucchetti Reservoir displayed the greatest annual mean biomass ( $32.2 \pm 18.7$  kg/ha), but this value was heavily influenced by an unusually large winter estimate ( $86.9 \pm 11.4$  kg/ha). Dos Bocas Reservoir exhibited the greatest biomass in 3 out of 4 seasons. Carite Reservoir consistently displayed the least threadfin shad biomass

across seasons (ranged 0.2 – 4.7 kg/ha), with an annual mean of  $2.0 \pm 1.2$  kg/ha.

Within individual reservoirs in Puerto Rico, threadfin shad biomass displayed marked variability between seasons (Table 1;  $F_{3,210} = 25.7$ ,  $P < 0.0001$ ). Whole lake mean biomass estimates varied as much as 24-fold between samples, and coefficients of variation ranged 51 – 122%. Dos Bocas Reservoir had the least seasonal variability in mean biomass (51%) followed by Guajataca Reservoir (CV = 68%). Conversely, biomass was most variable in Carite Reservoir (122%) followed by Lucchetti Reservoir (CV = 116%).

*Comparison of Puerto Rico to U.S. Reservoirs* – A review of the literature confirmed that data on threadfin shad populations are limited, as only five studies on four reservoirs were found to have quantified adult threadfin shad biomass or density. These studies examined populations in Arkansas (Rainwater and Houser 1982; Fourt et al. 2002), North Carolina (Siler 1986), Tennessee (Sammons et al. 1998), and Texas (Michaletz et al. 1995) (Table 2). Biomass estimates in Beaver Lake, Arkansas ranged 0.0 – 81.5 kg/ha, with median value of 13.1 kg/ha and mean of 19.8 kg/ha.

TABLE 1. Threadfin shad biomass estimates for four Puerto Rico reservoirs from spring 2010 until winter 2011. Mean standing biomass (g/1,000m<sup>3</sup>) ± one SE for each depth stratum by reservoir section, and overall biomass estimates (kg/ha) by season and year are presented. Carite Reservoir was added to the sampling efforts in summer 2010.

| Season                     | Longitudinal section            | Depth strata (m) | Mean biomass by depth strata (g/1,000 m <sup>3</sup> ) |                   |                    |                    |
|----------------------------|---------------------------------|------------------|--|-------------------|--------------------|--------------------|
|                            |                                 |                  | Dos Bocas <sup>a</sup>                                 | Guajataca         | Lucchetti          | Carite             |
| Spring                     | Upper                           | 0-3              | 825.5 ± 205.7  | 371 ± 71.5        | 673.9 ± 76.8       | n/a                |
|                            |                                 | 3-6              | 326.6 ± 107.8  | 18.8 ± 4.8        | 32.8 ± 13.1        | n/a                |
|                            | Lower                           | 0-3              | 567.2 ± 249.6  | 139.1 ± 51.0      | 573.5 ± 114.1      | n/a                |
|                            |                                 | 3-6              | 206.1 ± 139.6  | 11 ± 5.6          | 110.3 ± 21.2       | n/a                |
|                            |                                 | 6-9              | 21.9 ± 4.3   | 77.9 ± 30.0       | 1.5 ± 0.3          | n/a                |
|                            | <b>Spring estimated biomass</b> |                  | <b>kg/ha</b>   | <b>35.1 ± 5.0</b> | <b>11.1 ± 2.4</b>  | <b>25.1 ± 5.2</b>  |
| Summer                     | Upper                           | 0-3              | 428.2 ± 156.7  | 129.1 ± 38.6      | 113.3 ± 41.5       | 6.4 ± 2.6          |
|                            |                                 | 3-6              | 62.8 ± 10.5  | 142.8 ± 23.5      | 4.8 ± 2.3          | 0.4 ± 0.4          |
|                            | Lower                           | 0-3              | 624.5 ± 46   | 43.6 ± 13.3       | 375.3 ± 24.9       | 1.6 ± 1.5          |
|                            |                                 | 3-6              | 80.5 ± 25.9  | 2.2 ± 0.7         | 24.5 ± 16.4        | 0.0 ± 0.0          |
|                            |                                 | 6-9              | 40.2 ± 16.5  | 2.4 ± 1.0         | 1.1 ± 1.1          | 0.0 ± 0.1          |
|                            | <b>Summer estimated biomass</b> |                  | <b>kg/ha</b>   | <b>22.3 ± 4.2</b> | <b>5.8 ± 1.1</b>   | <b>9.3 ± 2.5</b>   |
| Fall                       | Upper                           | 0-3              | 36.6 ± 11.2  | 85.8 ± 8.3        | 254.3 ± 96.6       | 19.9 ± 5.4         |
|                            |                                 | 3-6              | 31.1 ± 7.5   | 82.4 ± 13.6       | 7.6 ± 2.9          | 30.2 ± 18.8        |
|                            | Lower                           | 0-3              | 262.7 ± 118.7  | 75.9 ± 23.8       | 140.4 ± 3.2        | 96.3 ± 34.9        |
|                            |                                 | 3-6              | 55.8 ± 13.7  | 4.5 ± 3.1         | 11.1 ± 4.7         | 96.1 ± 0.1         |
|                            |                                 | 6-9              | 30.1 ± 11.5  | 0.1 ± 0.1         | 2.2 ± 1.4          | 18 ± 2.6           |
|                            | <b>Fall estimated biomass</b>   |                  | <b>kg/ha</b>   | <b>7.5 ± 1.7</b>  | <b>4.5 ± 0.7</b>   | <b>7.5 ± 1.8</b>   |
| Winter                     | Upper                           | 0-3              | 366.9 ± 55.8   | 296 ± 40.4        | 2,053.8 ± 837.4    | 14.7 ± 2.9         |
|                            |                                 | 3-6              | 103.1 ± 30.5   | 527.8 ± 100.5     | 694.2 ± 125.7      | 14.9 ± 1.1         |
|                            | Lower                           | 0-3              | 1,204.5 ± 398.4  | 195.8 ± 56.0      | 1,020.4 ± 181.6    | 11.7 ± 3.7         |
|                            |                                 | 3-6              | 67.1 ± 11.4  | 83.5 ± 62.4       | 961.9 ± 307.8      | 13.6 ± 1.2         |
|                            |                                 | 6-9              | 13.4 ± 1.2   | 14.3 ± 3.8        | 97.3 ± 20.5        | 1.3 ± 0.6          |
|                            | <b>Winter estimated biomass</b> |                  | <b>kg/ha</b>   | <b>31.6 ± 8.0</b> | <b>20.1 ± 3.2</b>  | <b>86.9 ± 11.4</b> |
| <b>Annual mean biomass</b> |                                 | <b>kg/ha</b>     | <b>24.1 ± 6.2</b>                                      | <b>10.4 ± 3.6</b> | <b>32.2 ± 18.7</b> | <b>2.0 ± 1.2</b>   |

<sup>a</sup> The two upper arms of Dos Bocas Reservoir were sampled independently, but the average for each depth stratum is presented.

TABLE 2. Comparison of threadfin shad biomass (kg/ha) and density (fish/1,000 m<sup>3</sup>) estimates between temperate and tropical reservoirs. Unless otherwise noted, values are displayed with  $\pm$  one *SE*. Sampling methods used were cove rotenone (CR), frame trawl (FT), and Tucker trawl (TT).

| Reservoir/Site (Year)                                    | Seasonal estimate |                      |                             |                     | Annual mean                    | Gear | Source                      |
|--|-------------------|----------------------|-----------------------------|---------------------|--------------------------------|------|-----------------------------|
|  | Spring            | Summer               | Fall                        | Winter              |                                |      |                             |
| <b>Mean biomass estimates (kg/ha)</b>                    |                   |                      |                             |                     |                                |      |                             |
| <b>Puerto Rico</b>                                       |                   |                      |                             |                     |                                |      |                             |
| Dos Bocas Reservoir (2010-2011)                          | 35.1 $\pm$ 5.0    | 22.3 $\pm$ 4.2       | 7.5 $\pm$ 1.7               | 31.6 $\pm$ 8.0      | 24.1 $\pm$ 6.2 <sup>a</sup>    | FT   | This study                  |
| Guajataca Reservoir (2010-2011)                          | 11.1 $\pm$ 2.4    | 5.8 $\pm$ 1.1        | 4.5 $\pm$ 0.7               | 20.1 $\pm$ 3.2      | 10.4 $\pm$ 3.6 <sup>a</sup>    | FT   | This study                  |
| Lucchetti Reservoir (2010-2011)                          | 25.1 $\pm$ 5.2    | 9.3 $\pm$ 2.5        | 7.5 $\pm$ 1.8               | 86.9 $\pm$ 11.4     | 32.2 $\pm$ 18.7 <sup>a</sup>   | FT   | This study                  |
| Carite Reservoir (2010-2011)                             |                   | 0.2 $\pm$ 0.0        | 4.7 $\pm$ 0.6               | 1.0 $\pm$ 0.1       | 2.0 $\pm$ 1.2 <sup>a</sup>     | FT   | This study                  |
| <b>North Carolina</b>                                    |                   |                      |                             |                     |                                |      |                             |
| Lake Norman (1979)                                       |                   |                      | 16.5 $\pm$ 3.5 <sup>b</sup> |                     |                                | TT   | Siler (1986)                |
| Lake Norman (1980)                                       |                   |                      | 26.3 $\pm$ 5.8 <sup>b</sup> |                     |                                | TT   | Siler (1986)                |
| Lake Norman (1981)                                       |                   |                      | 23.3 $\pm$ 4.9 <sup>b</sup> |                     |                                | TT   | Siler (1986)                |
| <b>Arkansas</b>  |                   |                      |                             |                     |                                |      |                             |
| Beaver Lake (1966)                                       |                   | 5.3 <sup>c</sup>     |                             |                     |                                | CR   | Rainwater and Houser (1982) |
| Beaver Lake (1967)                                       |                   | 0.0 <sup>c</sup>     |                             |                     |                                | CR   | Rainwater and Houser (1982) |
| Beaver Lake (1968)                                       |                   | 23.1 <sup>c</sup>    |                             |                     |                                | CR   | Rainwater and Houser (1982) |
| Beaver Lake (1969)                                       |                   | 48.3 <sup>c</sup>    |                             |                     |                                | CR   | Rainwater and Houser (1982) |
| Beaver Lake (1970)                                       |                   | 0.6 <sup>c</sup>     |                             |                     |                                | CR   | Rainwater and Houser (1982) |
| Beaver Lake (1971)                                       |                   | 20.7 <sup>c</sup>    |                             |                     |                                | CR   | Rainwater and Houser (1982) |
| Beaver Lake (1972)                                       |                   | 30.4 <sup>c</sup>    |                             |                     |                                | CR   | Rainwater and Houser (1982) |
| Beaver Lake (1973)                                       |                   | 7.5 <sup>c</sup>     |                             |                     |                                | CR   | Rainwater and Houser (1982) |
| Beaver Lake (1974)                                       |                   | 81.5 <sup>c</sup>    |                             |                     |                                | CR   | Rainwater and Houser (1982) |
| Beaver Lake (1975)                                       |                   | 58.3 <sup>c</sup>    |                             |                     |                                | CR   | Rainwater and Houser (1982) |
| Beaver Lake (1976)                                       |                   | 14.2 <sup>c</sup>    |                             |                     |                                | CR   | Rainwater and Houser (1982) |
| Beaver Lake (1979)                                       |                   | 0.1 <sup>c</sup>     |                             |                     |                                | CR   | Rainwater and Houser (1982) |
| Beaver Lake (1980)                                       |                   | 0.2 <sup>c</sup>     |                             |                     |                                | CR   | Rainwater and Houser (1982) |
| Beaver Lake (1995)                                       |                   | 13.1 <sup>c</sup>    |                             |                     |                                | CR   | Fourt et al. (2002)         |
| Beaver Lake (1996)                                       |                   | 4.2 <sup>c</sup>     |                             |                     |                                | CR   | Fourt et al. (2002)         |
| Beaver Lake (1997)                                       |                   | 16.8 <sup>c</sup>    |                             |                     |                                | CR   | Fourt et al. (2002)         |
| Beaver Lake (1998)                                       |                   | 29.2 <sup>c</sup>    |                             |                     |                                | CR   | Fourt et al. (2002)         |
| Beaver Lake (1999)                                       |                   | 4.0 <sup>c</sup>     |                             |                     |                                | CR   | Fourt et al. (2002)         |
| Beaver Lake (2000)                                       |                   | 12.5 <sup>c</sup>    |                             |                     |                                | CR   | Fourt et al. (2002)         |
| <b>Mean density estimates (fish/1,000 m<sup>3</sup>)</b> |                   |                      |                             |                     |                                |      |                             |
| <b>Puerto Rico</b>                                       |                   |                      |                             |                     |                                |      |                             |
| Dos Bocas Reservoir (2010-2011)                          | 947.7 $\pm$ 325.3 | 157.8 $\pm$ 32.4     | 24.9 $\pm$ 6.9              | 467.9 $\pm$ 116.2   | 399.6 $\pm$ 204.9 <sup>a</sup> | FT   | This study                  |
| Guajataca Reservoir (2010-2011)                          | 277.2 $\pm$ 116.8 | 69.9 $\pm$ 17.9      | 103.4 $\pm$ 35.6            | 242.6 $\pm$ 53.3    | 173.2 $\pm$ 51.0 <sup>a</sup>  | FT   | This study                  |
| Lucchetti Reservoir (2010-2011)                          | 792.1 $\pm$ 291.1 | 72.3 $\pm$ 33.4      | 32.9 $\pm$ 16.9             | 1,397.0 $\pm$ 325.4 | 573.5 $\pm$ 325.3 <sup>a</sup> | FT   | This study                  |
| Carite Reservoir (2010-2011)                             |                   | 8.4 $\pm$ 4.1        | 22.6 $\pm$ 7.8              | 22.3 $\pm$ 5.0      | 25.1 $\pm$ 9.0 <sup>a</sup>    | FT   | This study                  |
| <b>Tennessee</b>   |                   |                      |                             |                     |                                |      |                             |
| Normandy Reservoir (1992)                                |                   | 237 <sup>c,d</sup>   |                             |                     |                                | CR   | Sammons et al. (1998)       |
| Normandy Reservoir (1993)                                |                   | 634 <sup>c,d</sup>   |                             |                     |                                | CR   | Sammons et al. (1998)       |
| Normandy Reservoir (1994)                                |                   | 3,296 <sup>c,d</sup> |                             |                     |                                | CR   | Sammons et al. (1998)       |
| Normandy Reservoir (1995)                                |                   | 178 <sup>c,d</sup>   |                             |                     |                                | CR   | Sammons et al. (1998)       |
| Normandy Reservoir (1996)                                |                   | 1,052 <sup>c,d</sup> |                             |                     |                                | CR   | Sammons et al. (1998)       |
| <b>Texas/Oklahoma</b>                                    |                   |                      |                             |                     |                                |      |                             |
| Lake Texoma/Central Pool (1991)                          |                   | 50.4 $\pm$ 4.0       |                             |                     |                                | FT   | Michaletz et al. (1995)     |
| Lake Texoma/Little Mineral (1991)                        |                   | 148.8 $\pm$ 26.9     |                             |                     |                                | FT   | Michaletz et al. (1995)     |
| Lake Texoma/Big Mineral (1991)                           |                   | 548.1 $\pm$ 83.5     |                             |                     |                                | FT   | Michaletz et al. (1995)     |
| Lake Texoma/Central Pool (1991)                          |                   | 44.6 $\pm$ 5.5       |                             |                     |                                | TT   | Michaletz et al. (1995)     |
| Lake Texoma/Little Mineral (1991)                        |                   | 116.5 $\pm$ 24.2     |                             |                     |                                | TT   | Michaletz et al. (1995)     |
| Lake Texoma/Big Mineral (1991)                           |                   | 565 $\pm$ 206.2      |                             |                     |                                | TT   | Michaletz et al. (1995)     |

<sup>a</sup> *SE* presented for annual means in Puerto Rico represents variability across seasons.

<sup>b</sup> These data were presented with 95% *CI*, not with *SE*.

<sup>c</sup> No measure of variability was provided in the source publication.

<sup>d</sup> These data were collected from a reservoir that was subject to frequent winterkill of threadfin shad.

Biomass estimates in Lake Norman, North Carolina ranged 16.3 – 26.3 kg/ha, with median value of 23.3 kg/ha and mean of 22.0 kg/ha. Density estimates in Normandy Reservoir, Tennessee ranged 178 – 3,296 fish/1,000 m<sup>3</sup>, with median value of 634 fish/1,000 m<sup>3</sup> and mean of 1,079 fish/1,000 m<sup>3</sup>. Density estimates in Lake Texoma, Texas-Oklahoma ranged 44.6 – 565.0 fish/1,000 m<sup>3</sup>, with median value of 132.7 fish/1,000 m<sup>3</sup> and mean of 245.6 fish/1,000 m<sup>3</sup>.

## Discussion

Neal and Prchalová (2012) reported that threadfin shad density varied across spatiotemporal gradients in tropical reservoirs, with high between-reservoir variability. Not surprisingly, threadfin shad biomass varied similarly across reservoirs in the current study. The observed variability was likely due to the relationship of primary productivity and hydraulic retention time. Carvajal-Zamora (1979) noted that Carite was one of the least productive reservoirs in Puerto Rico, which was reflected by threadfin shad biomass reported here. Furthermore, when analyzed for phosphate (PO<sub>4</sub>) and nitrate (NO<sub>3</sub>) content (Kröger et al. 2010), Dos Bocas exhibited the highest nutrient levels (PO<sub>4</sub> = 0.057 ± 0.012 ppm; NO<sub>3</sub> = 0.378 ± 0.105 ppm), followed by Lucchetti Reservoir (PO<sub>4</sub> = 0.017 ± 0.000 ppm; NO<sub>3</sub> = 0.080 ± 0.004 ppm), and Guajataca Reservoir (PO<sub>4</sub> = 0.014 ± 0.002; NO<sub>3</sub> = 0.050 ± 0.002 ppm). Water quality data were not available for Carite Reservoir. Neal and Prchalová (2012) reported hydraulic retention times for these reservoirs to vary considerably, ranging from unusually long in Guajataca Reservoir (453 d), to extremely short in Dos Bocas Reservoir (16 d). This suggests that although Dos Bocas Reservoir is considerably more nutrient-rich than the other reservoirs, these nutrients may be flushing out of the reservoir before they are fully utilized by biota (Kennedy and Walker 1990). Carite Reservoir and Lucchetti Reservoir were intermediate (312 d and 231 d, respectively), but would likely be more similar to Guajataca Reservoir.

Threadfin shad reproduction and recruitment have been shown to vary widely across the annual cycle in Puerto Rico (Stancil et al. 1997; Neal and Prchalová 2012), and this episodic production combined with rapid growth and high mortality commonly produces peaks and troughs in estimated biomass across seasons. This variability has both biological and management consequences. Biologically, seasonal variation in threadfin shad biomass implies that prey availability to sport fish also will vary seasonally, with potential effects on sport fish growth, survival, and recruitment. In terms of management, seasonal variability of key prey species can affect many factors, including the

success of supplemental stocking of sport fishes and the utility of fish sampling designs.

All threadfin shad biomass or density estimates found in the literature for temperate reservoirs were based on a single sampling event per year. All estimates also were generated during summer months except for Lake Norman, North Carolina, which used fall sampling. If temperate reservoirs are subject to the same extreme seasonal variability as observed in tropical reservoirs, these instantaneous estimates of biomass will not provide a complete picture of threadfin shad populations. For the purposes of comparison, it was assumed that seasonal patterns in temperate reservoirs were similar in pattern and magnitude to tropical reservoirs, and that seasonal estimates from the continental U.S. can be compared to the appropriate seasonal data from Puerto Rico.

Comparisons of biomass estimates from Puerto Rico were possible with two temperate reservoirs (Figure 1). Beaver Lake, Arkansas, exhibited numerically greater mean biomass across its sampling history than all Puerto Rico reservoirs except Dos Bocas Reservoir during summer. The Dos Bocas Reservoir summer mean (22.3 kg/ha) was slightly greater than the Beaver Lake mean (19.5 kg/ha), but was almost one quarter of the maximum observed biomass in Beaver Lake (81.5 kg/ha). The other Puerto Rico reservoirs displayed summer biomass estimates that were, at most, only about half of the Beaver Lake mean biomass. The Beaver Lake samples were collected using cove rotenone, while Puerto Rico estimates were derived from open water trawls. Several studies have shown the difficulties of extrapolating cove rotenone data to open water (Hayne et al. 1967; Summers and Axon 1980; Davies and Shelton 1983). Furthermore, threadfin shad have been observed to be denser in open-water habitats than in littoral-zone habitats, which suggested that the Beaver Lake cove rotenone data may have underestimated reservoir-wide biomass. Perhaps a more valid comparison can be made between the Puerto Rico frame trawl estimates and the Tucker trawl estimates from Lake Norman, North Carolina (Siler 1986). These samples were collected in the fall and averaged 22.0 kg/ha across three years. When compared to the fall Puerto Rico data, the Lake Norman biomass estimates were 290 – 490% greater than each tropical reservoir.

Two temperate reservoirs reported seasonal threadfin shad density estimates that could be compared to season densities in Puerto Rico. Normandy Reservoir, Tennessee, reported a mean density of 1,079 fish/1,000 m<sup>3</sup> during summer across 5 sampling years. This mean density was considerably greater than the summer densities found in Puerto Rico

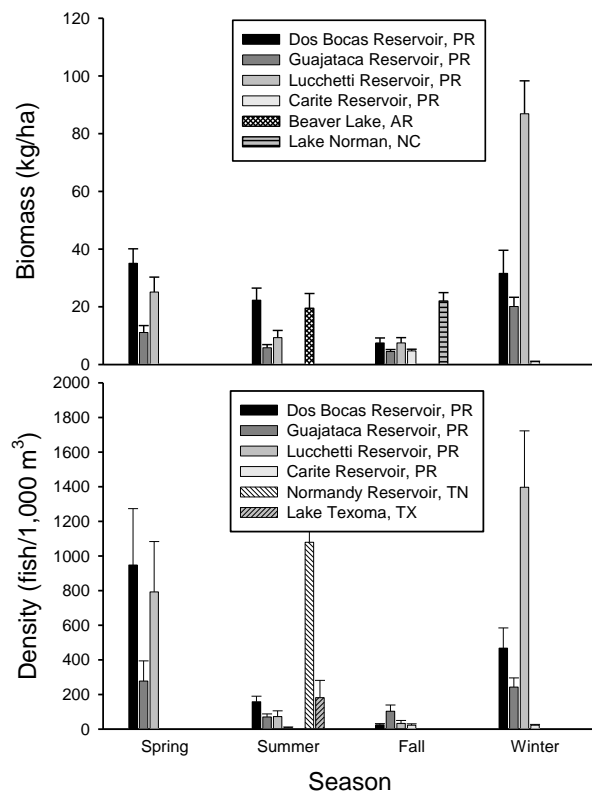


FIGURE 1. Comparison of mean seasonal biomass (Top; kg/ha) and density (Bottom; fish/1,000 m<sup>3</sup>) of threadfin shad between four Puerto Rico reservoirs and four temperate reservoirs. Puerto Rico systems from darkest to lightest are Dos Bocas, Guajataca, Lucchetti, and Carite (this study); temperate reservoirs are Lake Norman, NC (biomass; Siler 1986), Beaver Lake, AR (biomass; Rainwater and Houser 1982, Fourt et al. 2002), Normandy Reservoir, TN (density; Sammons et al. 1998), and Lake Texoma, TX/OK (density; Michaletz et al. 1995). Error bars represent one SE.

reservoirs, which ranged 8.4 – 157.8 fish/1,000 m<sup>3</sup>. The Normandy Reservoir data also were collected using cove rotenone sampling and subject to the same biases previously described. Furthermore, this reservoir was subject to frequent episodes of threadfin shad winterkill. For these reasons, the Normandy Reservoir data also may have underestimated reservoir-wide biomass carrying capacity.

Conversely, data from Lake Texoma, Texas-Oklahoma, were collected using similar sampling gear as this Puerto Rico study, so direct within-season comparison should be valid. Threadfin shad density estimates from Lake Texoma averaged 245.6 fish/1,000 m<sup>3</sup> during summer 1991 across three different reservoir arms using two types of trawls (frame trawl and Tucker trawl; Michaletz et al. 1995). Lake Texoma displayed considerable spatial heterogeneity, with spatially explicit estimates ranging 44.6 – 565 fish/1,000 m<sup>3</sup> depending on the embayment

sampled. All Puerto Rico systems displayed summer threadfin shad densities less than the mean Lake Texoma threadfin shad density, but three reservoirs were within the range of estimates reported for Lake Texoma. Carite Reservoir, however, had considerably lower threadfin shad density than all Lake Texoma mean estimates.

The comparisons presented have potential limitations. One confounding issue is that the sampling gears varied, and similar gears were deployed in a variety of ways. This consideration is especially true for sampling depth. In the Puerto Rico study, all depths between the surface and 9 m were sampled systematically and each depth was accounted for in the calculation of threadfin shad densities and biomasses. The Lake Norman study indicated that multiple depths were sampled, but did not provide detailed descriptions on the actual depths sampled or how depth was factored into estimates. The Lake Texoma trawling study similarly sampled from the surface to depths of 7 m, but did not provide a description of shad densities by depth.

The comparison of Puerto Rico and temperate reservoir estimates is further confounded by the fact that threadfin shad are the sole pelagic herring (Clupeidae) species in Puerto Rico, but are found in conjunction with other herring species in the comparison lakes. This is often why threadfin shad density or biomass is not estimated independently in temperate reservoirs (e.g., Taylor et al. 2005). Instead, species of the family Clupeidae are often estimated jointly, yielding estimates of “shad” or “forage fishes” that are not directly comparable. It is unknown if the co-existence of threadfin shad with other species such as gizzard shad and alewife *Alosa pseudoharengus* will result in niche partitioning and compensatory decrease in density and biomass. A number of studies have suggested that the presence of threadfin shad can cause displacement of gizzard shad in reservoir systems (Noble 1981; Shelton et al. 1982; Stiefvater and Malvestuto 1987). Sammons et al. (1998) reported that catch of gizzard shad larvae was inversely related to biomass and density of adult threadfin shad in Normandy Reservoir, suggesting that the carrying capacities of these two species were not mutually exclusive. Furthermore, Armstrong et al. (1998) concluded that larval gizzard shad and threadfin shad are similar relative to gape morphology and feeding ecology, and thus, direct competition is likely.

If the presence of other Clupeids does indeed reduce a reservoir’s carrying capacity for threadfin shad, it is arguable that the threadfin shad biomass in Puerto Rico and other reservoirs that do not contain additional Clupeid species should be greater than in

reservoirs containing multiple Clupeid species. This was not the case, as most Puerto Rico reservoirs displayed comparatively less biomass or density than temperate reservoirs. However, Guest et al. (1990) did not find a decrease in threadfin shad biomass when gizzard shad were introduced in small ponds, which suggested that the ecological displacement may be one-way.

All four reservoirs in Puerto Rico are generally considered mesotrophic to eutrophic (Carvajal-Zamora 1979), and should be similar to the temperate reservoirs in trophic status. For example, Lake Norman, North Carolina, has been classified as slightly eutrophic (EPA 1975) and Normandy Reservoir, Tennessee, is classified as eutrophic (Sammons et al. 1998). Lake Texoma has been classified as eutrophic overall (EPA 1977; Franks et al. 2001), with individual reaches ranging mesotrophic to hypereutrophic (Sager et al. 2011). Beaver Lake, Arkansas, is generally less productive, with the eutrophic conditions in the headwaters (Haggard et al. 1999) quickly declining to oligotrophic conditions near the dam (ADPCE 1992). Thus, Puerto Rico reservoirs were within the range of trophic states of the temperate reservoirs, and should be capable of producing comparable threadfin shad populations.

In general, most of the Puerto Rico reservoirs sampled in this study displayed threadfin shad populations that were fewer by number and weight than the temperate reservoirs used for comparison. Though the comparisons between these systems must be approached cautiously, the implications of potentially reduced threadfin shad availability in Puerto Rico are significant, particularly in terms of sport fish management. Managers of these reservoirs have historically followed management schemes created for temperate reservoirs (e.g., Neal et al. 2008), yet these systems may behave less like temperate reservoirs than previously thought. If threadfin shad populations in Puerto Rico are not comparable to those in temperate systems, then it is unreasonable to expect sport fish populations to mirror reservoirs in the southern U.S.

Neal and Prchalová (2012) first reported that threadfin shad populations in the U.S. and elsewhere may be understudied and more poorly understood than previously thought. The authors found only five instances in the literature where threadfin shad biomass or density was estimated for aquatic systems, and only a handful of gizzard shad estimates (e.g., Michaletz et al. 1995; Schramm and Pugh 2005; Schaus et al. 1997 2002). Many of these estimates were based on rotenone sampling, which can be considerably biased (Hayne et al. 1967; Summers and Axon 1980; Davies and Shelton 1983). The Lake Texoma series of studies, popularly

known as the Shad-a-thon and overviewed by Boxrucker et al. (1995), was a major step forward in sampling shad in southern U.S. impoundments. However, although sampling during that effort and the few subsequent studies have used gears that are less biased, sampling in these studies has always been conducted in a single season. Data from Puerto Rico reservoirs revealed that tropical threadfin shad populations display marked changes seasonally, with changes in biomass of up to 2,400% within a 3-month sampling window. If similar patterns are present in temperate reservoirs, the single instantaneous measures of shad biomass or density would have limited utility in supporting fisheries management decisions.

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## Chapter 15: Spatial and Temporal Changes in Nutrients and Water Quality Parameters in Four Puerto Rico Reservoirs: Implications for Reservoir Productivity and Sport Fisheries Restoration

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**Abstract:** Water quality of reservoirs is the foundation of the ecological cascade that results in productive fisheries. The current study evaluated four tropical reservoirs (Dos Bocas, Cerrillos, Guajataca, and Lucchetti) in Puerto Rico for spatial and temporal dynamics in water quality parameters to better understand effects on bait fish and subsequently largemouth bass sport fisheries. Surface mapping, and depth profiles of in situ parameters of dissolved oxygen, pH, temperature and turbidity using an automated flow through Eureka Manta datason yielded distinct differences between reservoirs in space and time. Several limnological phenomenon were observed within this dataset including distinct influence of river inputs into reservoirs, the prevalence of irradiance avoidance, and substantial and significant oxyclines with depth at varying times of the year. These spatial variations in water quality variables result in direct implications for resource availability. Nutrient concentration ranges were significantly different between reservoirs ( $F = 6.45$ ;  $P < 0.05$ ) and were attributed to varying degrees of land use in the respective upland catchments (Dos Bocas  $\text{NO}_3\text{-N}$ : 0.8 mg/L; Guajataca  $\text{NO}_3\text{-N}$ : 0.04 mg/L). Nutrient concentrations were low in all reservoirs, with certain reservoirs (Cerrillos and Guajataca) being classified as oligotrophic. Although no direct correlations can be made to fish production, it is important to understand limits to resource production within these systems. Dissolved oxygen, pH, water temperature and nutrient concentrations all work in unison to provide a bottom-up controlled aquatic system that sustains phytoplankton production, baitfish and subsequently sports fisheries.

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Heterogeneity of water quality in reservoirs affects abundance, growth, and distribution of fishes. Reservoir productivity directly influences prey production, and is largely determined by availability and retention of nutrients and water chemistry parameters (Kimmel et al. 1990). This creates a bottom-up cascade to higher trophic levels, eventually resulting in increased biomass of sport fish (Carpenter and Kitchell 1993). Likewise, within reservoir variability of physiochemical parameters can influence predator and prey distributions and interactions (Coutant 1985; Neal et al. 2005), which can have considerable effects on biological function and management in these systems. In Puerto Rico, largemouth bass *Micropterus salmoides* are the primary sport fish, and much of the research and management activity is directed at this species. Management decisions for largemouth bass in Puerto

Rico have been based on the assumption that primary productivity and prey availability are not limiting, yet conclusive data to this end are not available (Neal et al. 2009).

Spatial and temporal changes in concentrations of dissolved compounds (nutrients, sediments and agrochemicals) will influence basic autotrophic dynamics with a cascading effect through the food web (Wetzel 2001). Likewise, nutrient availability and contaminant concentrations are a product of land-use patterns within the watershed (Cech 2010; Pennington and Cech 2010), necessitating a watershed-scale approach to reservoir management. Good water quality is necessary for fisheries, with increased non-point source pollution beyond an inflection point on a productivity scale, being in direct conflict for production (Thomas et al. 1992). In the tropics with conducive temperatures and conditions for production, oligotrophic systems would

limit the production of higher trophic levels (Wetzel, 2001).

Studies to date have illustrated the relationships between water quality and freshwater biotic integrity (Miltner and Rankin 1998; Rask et al., 2010; Weigel and Robertson 2007; Weijters et al. 2009). Typically, most studies agree that there is a positive linear or curvilinear relationship between nutrients (total phosphorus) and chlorophyll *a* for most freshwater bodies (Miltner and Rankin 1998; Weigel and Robertson 2007). Weijters et al. (2009) highlighted, from a review of 240 studies, that land-use was the variable that explained the greatest variation in freshwater biodiversity. Miltner and Rankin (1998) described how increases in nutrient concentrations in Ohio freshwater systems shifted carrying capacity away from smaller, sensitive fish species and subsequent effects on top piscivores, towards more tolerant omnivorous fish species. Similarly, in Finnish lakes, Rask et al. (2010) showed that high nutrient loadings resulted in eutrophic conditions and high algal biomass, which subsequently resulted in high biomasses of low value cyprinid fishes. This is not surprising, as many cyprinid fish species take advantage of eutrophic conditions because of their effective planktivory and ability to consume plant material. Weigel and Robertson (2007) stressed that nutrients, dissolved oxygen, pH, and water temperature accounted for the majority of variance on fish assemblage structure in Wisconsin non-wadeable rivers. These studies suggest that biotic integrity of freshwater systems, which includes sport fish production, is a product of nutrient delivery as influenced by upper catchment land use, delivered system nutrient concentrations, and water chemistry variables.

This study evaluated four sub-tropical reservoirs in Puerto Rico (Cerrillos, Dos Bocas, Guajataca and Lucchetti) for spatial and temporal variations of surface and profile water quality parameters (dissolved oxygen, pH, turbidity, temperature) and nutrient concentrations.

## Methods

Water quality sampling of four reservoirs in Puerto Rico was undertaken in quarterly sampling periods. The sampling events occurred in February, June, August and December 2010. The temporal component of quarterly sampling allowed for monitoring changes through seasons and reservoir management cycles. The four reservoirs (Figure 1) monitored were selected for their popularity as sport fisheries (primarily largemouth bass and peacock bass *Cichla ocellaris*), and because

each has a substantial research database available on these fisheries

All reservoirs were sampled for *in situ* water quality parameters of dissolved oxygen (mg/L), pH, specific conductance ( $\mu$ S), temperature ( $^{\circ}$ C), turbidity (NTU) and oxidation reduction potential (ORP; mV). Surface mapping of each reservoir took place to understand spatial patterns in parameters. An automated, flow through Eureka MANTA2 datason was setup on the bow of a 4.3 m aluminum boat. Flow through setup consisted of a 12V bilge pump with a intake tube positioned 0.3 m below the water surface, connected with high density polyethylene tubing to the flow through chamber of the data-son. Flow through occurred from the bottom of the chamber to the top and discharge occurred over the side of the boat through high density polyethylene tubing. Water samples were analyzed every 10 seconds for the duration of the surface mapping exercise. Each water quality data point consisted of all the above mentioned water quality parameters as well as GPS coordinate of each sample recorded. The boat travelled at an average of 5 km/hr, with reservoir size determining time spent surface water mapping. Water quality parameters through depth were recorded with 25 depth profiles, evenly spaced within each reservoir. Depth profiles started at the surface, and were recorded at 1 m intervals until the oxycline was reached ( $<1$  mg/L), whereby samples were taken every 2 m. The lowest recorded depth reading (cable restriction) was 28 m, which was well below the oxycline in all reservoirs. Recorded turbidity plumes at maximum depth in all reservoirs as a result of the datason hitting the substrate bottom were removed from the respective data sets. The datason was calibrated for respective water quality parameters prior to each quarterly sampling period, according to calibration instructions, to within quality assured, quality controlled specifications.

Within each reservoir a random number of surface water samples based on surface area of each reservoir were taken to analyze for select nutrients: nitrate-N, ammonia-N, soluble reactive phosphate and nitrite-N. Nitrite-N was initially analyzed, but all water samples from February and June sampling events were non-detects for nitrite-N, and thus analysis was discontinued. Nutrients were collected in 250 ml polyethylene containers (Fisher Scientific), transported on ice and refrigerated. Samples were subsequently transported on ice to the Water Quality Analysis Laboratory at the University of Puerto Rico, San Juan, Puerto Rico. Ammonia was analyzed using the QuickChem<sup>®</sup> Method 10-107-06-1-J, with lower

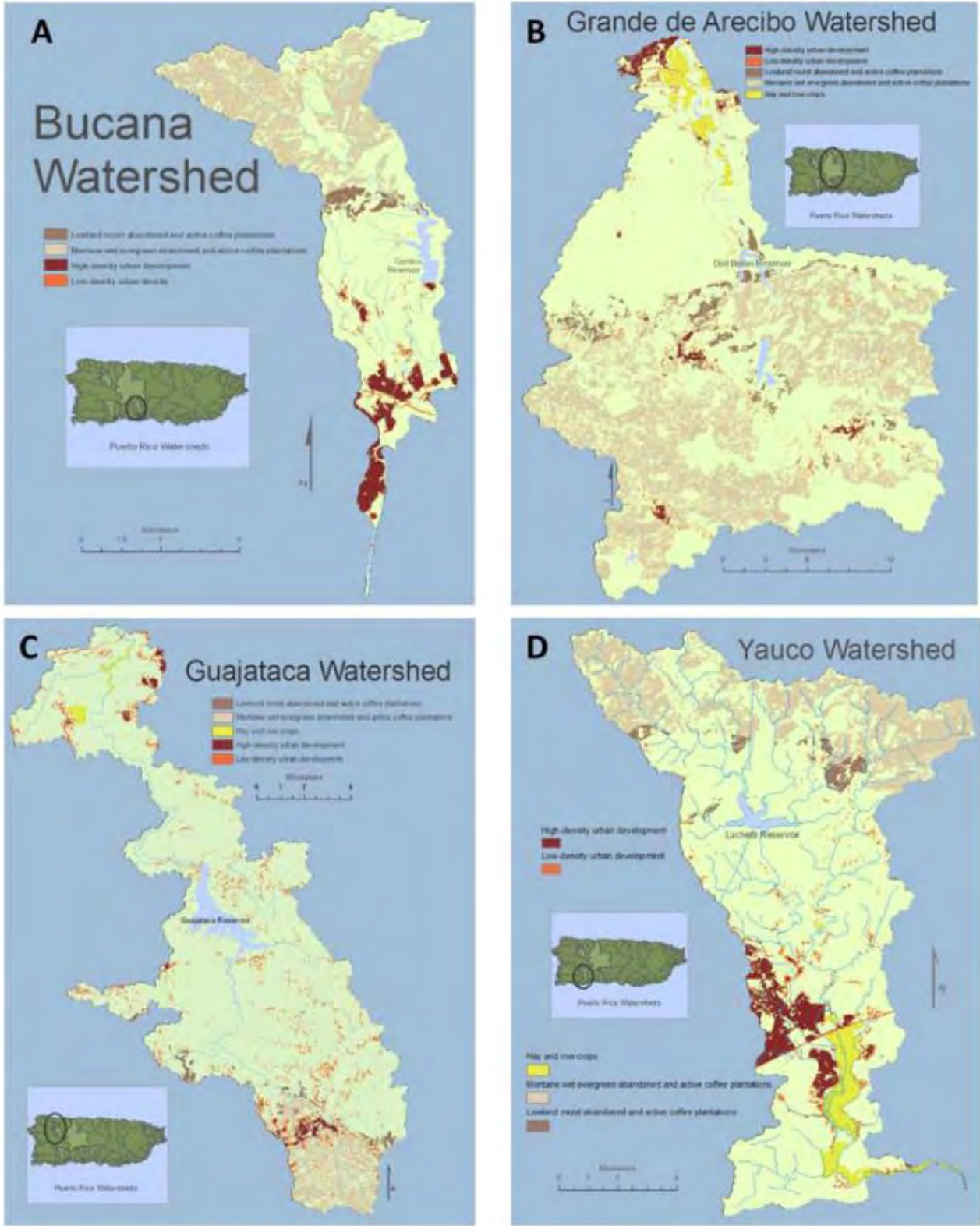


FIGURE 1. Watershed and reservoir locations of all four reservoirs, Cerrillos (A), Dos Bocas (B), Guajataca (C), and Lucchetti (D). Note the positioning of each reservoir in relation to one another and the variation in watershed size and upper catchment areas for each reservoir.

detection limits (MDL) of 0.01-2.0 NH<sub>3</sub>-N mg/L.

Nitrate and nitrate + nitrite were analyzed using QuickChem® Method 10-107-04-1-B, by flow injection analysis, with MDL's between 0.002-0.10 NO<sub>3</sub>, NO<sub>2</sub>-N mg/L. Reactive P was analyzed using QuickChem® Method 10-115-01-1-A, by flow injection analysis colorimetry. Nutrient samples were incorporated in ARCMAP to create spatial distributions of nutrient concentrations throughout each reservoir. Similarly spatial distributions of select water quality parameters including dissolved oxygen, turbidity, pH and temperature were created through ARCMAP. Spatial mapping in ARCMAP used inter-distance weighting to interpolate selected measures between sampling points. Inter-distance weighting was used over spatial kriging to avoid value manipulation and change from statistical interpolation through kriging. The low number of surface water samples was a limitation to the IDW, but the cost of additional sample processing was prohibitive. Two-dimensional maps of water parameters by depth were analyzed using Surfer™ Software. Latitudinal sites, with depth and selected water quality parameter were spatially arranged using inter-weighted distance to view changes in depth and latitude for each water quality parameter. Nutrient concentrations between reservoirs were compared using a one-way ANOVA, with an alpha of 0.05.

## Results

### *In Situ Water Quality*

Dissolved oxygen, temperature and pH are all measures that are directly affected by phytoplankton productivity. Higher temperatures (general increase from February to June) correlated tightly with higher dissolved oxygen concentrations (February range: 5.73 – 10.65 mg/L; August range: 8.88 – 12.11 mg/L), and subsequently higher pH values (February range: 7.89 – 8.80; August range: 8.80 – 9.04) (Table 1). Using profile data, three important limnological processes were observed that occurred in all reservoirs: oxycline development, riverine influences with water quality and depth, and irradiance avoidance by photosynthesizing phytoplankton.

Distinctive oxycline development as result of temperature, density and oxygen concentration decreasing with depth was observed in all four systems (Figure 2). The oxycline varied in depth and severity based on the season in all reservoirs, but the trends were similar. There was distinct oxycline development for June and August sampling events in deeper waters of Cerrillos Reservoir (Figure 2). Shallower waters (< 17 m) in August were oxic environments throughout the water column. A significant proportion of the water

column was oxic in February (Figure 2), with similar results occurring for the December sampling event (not graphed). Figure 3 highlights the spatial distributions (latitude × depth) of dissolved oxygen concentrations in Lucchetti, Guajataca and Dos Bocas Reservoir, respectively, for the month of June. Similar trends of strong oxycline development between 6 – 8 m in depth, with a significant proportion of the water column being anoxic were evident among all reservoirs.

The influence of river inflows were evident when water levels were high in Cerrillos Reservoir. The river influence was distinctly observed in a plot of specific conductance, temperature and dissolved oxygen with latitude for Cerrillos in February (Figure 4). When the water levels were lower (June, August and December ~ 12 m decrease), the river influence was negligible. Other reservoirs showed variable river influences with tributaries, but Cerrillos is the only reservoir with a distinct main river arm of the catchment that contributes the majority of water to the reservoir.

Irradiance avoidance was observed within all reservoirs (Figure 3 and 5). June and August sampling had the highest recorded surface water temperatures across the quarterly sampling periods. Often, the highest dissolved oxygen concentrations occurred at 2 – 4 m below the water surface. Oxygen concentrations were similar between the surface and 4 – 5 m with ranges of oxygen typically between 11 – 14 mg/L at 2 – 4 m below the surface. Irradiance avoidance by phytoplankton was common among all four reservoirs for June and August.

Turbidity was low in most reservoir surface waters (Lucchetti June mean: 138 NTU; Range all other months and reservoirs: 3 – 79 NTU) (Figure 6). There was a distinct trend of increasing surface turbidity from February to August in all reservoirs except Lucchetti. Lucchetti had the highest mean turbidity value in June of 138 ± 18 NTU. Most often, mean surface turbidity values were very low (< 80 NTU). Higher values were encountered in shallow tributary arms for each reservoir.

### *Nutrient Distributions*

Comparing mean nutrient concentrations between sampling periods highlights certain temporal trends (Figure 7). Cerrillos had overall very low nutrient concentrations. Ammonia concentrations were less than 0.1 mg/L throughout the year. Nitrate, and soluble P concentrations were also low (> 0.3 mg/L and >0.04 mg/L, respectively). Guajataca, similar to Cerrillos, had very low spatial and temporal concentrations of nitrate, ammonia and soluble P. Guajataca's catchment has some urban development in the upper reaches of the catchment (Figure 1), but for the majority, the

TABLE 1. *In situ* surface water quality parameters for Luchetti, Cerrillos, Guajataca and Dos Bocas Reservoirs in Puerto Rico. Quarterly sampling occurred in February (1st), June (2nd), August/September (3rd) and November/December (4th).

| Water Quality Parameter    | Lucchetti  |            |            |            | Cerrillos  |            |            |            | Guajataca   |             |            |             | Dos Bocas  |            |            |            |
|----------------------------|------------|------------|------------|------------|------------|------------|------------|------------|-------------|-------------|------------|-------------|------------|------------|------------|------------|
|                            | 1st        | 2nd        | 3rd        | 4th        | 1st        | 2nd        | 3rd        | 4th        | 1st         | 2nd         | 3rd        | 4th         | 1st        | 2nd        | 3rd        | 4th        |
| Temperature Mean           | 25.08      | 29.5       | 30.47      | 26.1       | 25.80      | 29.4       | 31.05      | 26.4       | 27.14       | 29.7        | 30.9       | 26.3        | 26.80      | 30.3       | 30.69      | 26.4       |
| S.E.                       | 0.06       | 0.01       | 0.01       | 0.01       | 0.01       | 0.01       | 0.03       | 0.01       | 0.01        | 0.013       | 0.01       | 0.004       | 0.02       | 0.02       | 0.01       | 0.01       |
| Median                     | 25.1       | 29.7       | 30.42      | 26.1       | 25.8       | 29.3       | 31.11      | 26.5       | 27.1        | 29.5        | 30.93      | 26.3        | 26.7       | 30.3       | 30.63      | 26.4       |
| Dissolved Oxygen Mean      | 8.31       | 13.7       | 10.27      | 9          | 5.73       | 10.1       | 8.88       | 9.73       | 9.28        | 10.03       | 10.56      | 7.2         | 10.6       | 13.1       | 12.11      | 13.1       |
| S.E.                       | 0.02       | 0.03       | 0.04       | 0.01       | 0.02       | 0.07       | 0.03       | 0.04       | 0.09        | 0.03        | 0.06       | 0.01        | 0.05       | 0.06       | 0.05       | 0.08       |
| Median                     | 8.19       | 14.0       | 10.22      | 8.99       | 5.6        | 9.5        | 8.75       | 9.3        | 9.29        | 9.7         | 9.97       | 7.14        | 10.3       | 13.2       | 12.05      | 13.9       |
| Turbidity Mean             | 8.1        | 139.4      | 42.94      | 28.9       | 17.2       | 26.6       | 29.77      | 23.3       | 10.4        | 26.8        | 39.85      | 11          | 12.5       | 60.5       | 67.38      | 50.5       |
| S.E.                       | 0.16       | 15.9       | 0.79       | 0.27       | 3.1        | 0.47       | 1.03       | 0.2        | 0.88        | 1.1         | 0.51       | 0.09        | 0.15       | 2.1        | 11.63      | 0.3        |
| Median                     | 6.3        | 48.4       | 42.2       | 28.4       | 0.9        | 23.5       | 28.8       | 22         | 7.5         | 16.1        | 37.5       | 10.7        | 11.5       | 53.3       | 44.75      | 50.5       |
| Specific Conductance Mean  | 284        | 210.1      | 233        | 234        | 217        | 197.5      | 185.9      | 191        | 289         | 221.0       | 202        | 253         | 193        | 156.9      | 168.9      | 150        |
| S.E.                       | 0.06       | 0.05       | 0.14       | 0.04       | 0.08       | 0.09       | 0.05       | 0.07       | 0.02        | 0.1         | 0.36       | 0.04        | 0.37       | 0.15       | 0.33       | 0.4        |
| Median                     | 284        | 210.2      | 232.7      | 234        | 218        | 198.4      | 185.9      | 191        | 289         | 221.5       | 205.8      | 253         | 193        | 158        | 170.3      | 146        |
| pH Mean                    | 8.33       | 9.04       | 8.80       | 8.57       | 7.89       | 8.70       | 8.91       | 8.65       | 8.51        | 8.51        | 8.82       | 8.1         | 8.80       | 9.06       | 9.04       | 9.1        |
| S.E.                       | 0.03       | 0.003      | 0.004      | 0.002      | 0.03       | 0.008      | 0.003      | 0.008      | 0.01        | 0.003       | 0.004      | 0.004       | 0.01       | 0.009      | 0.007      | 0.01       |
| Median                     | 8.32       | 9.08       | 8.82       | 8.58       | 7.88       | 8.66       | 8.9        | 8.61       | 8.53        | 8.5         | 8.78       | 8.13        | 8.80       | 9.1        | 9.08       | 9.3        |
| <b>Total N / reservoir</b> | <b>549</b> | <b>741</b> | <b>159</b> | <b>947</b> | <b>884</b> | <b>697</b> | <b>309</b> | <b>611</b> | <b>1163</b> | <b>1116</b> | <b>484</b> | <b>1187</b> | <b>724</b> | <b>771</b> | <b>372</b> | <b>961</b> |

Guajataca watershed is rural with low-density urban development reservoirs, including nitrate-N, ammonia-N and soluble phosphate. Lucchetti Reservoir is the highest-located reservoir in its respective catchment (Figure 1). The upper reaches of the catchment are dominated with rural areas with high densities of abandoned and active coffee plantations. Lucchetti also has multiple tributaries that could influence water quality in terms of nutrients and sediments. Lucchetti had the second highest mean nitrate-N values for all the reservoirs when averaged over the three sampling periods. Ammonia and soluble P were low through time and had very low ranges (Ammonia: 0.02 – 0.08 mg/L; SRP: 0.01 – 0.2 mg/L).

## Discussion

Nutrients, as resources, play an important role in bottom-up regulation of aquatic ecosystems, promoting primary productivity, increasing food availability and energy transfer through trophic levels to higher trophic states such as piscivores (e.g., largemouth bass). This is an important concept when exploring relationships that

determine the productivity of sport fisheries in freshwater systems such as reservoirs. Limnologists have been investigating reasons for and improvements to reservoir or lake productivity for the last 80 years. Schindler's (1978) classic examples of productivity limits as a result of N and P additions highlighted succinctly how systems are typically N and P limited. Water quality and reservoir productivity are synonymous with the growth of good fisheries. Management and regulation of reservoirs for water quality is prevalent in the temperate regions of the U.S. through the implementation of best management practices for the abatement of nutrients and sediments, as well as several reservoir management strategies including drawdown, discharge and habitat management. Often, nutrients maybe added to oligotrophic systems to increase primary productivity and enhance the sport fishery. Very little is known and has been attempted for tropical sport fisheries management from a water quality perspective. Understanding spatial and temporal changes to *in situ* water quality parameters, such as dissolved oxygen,

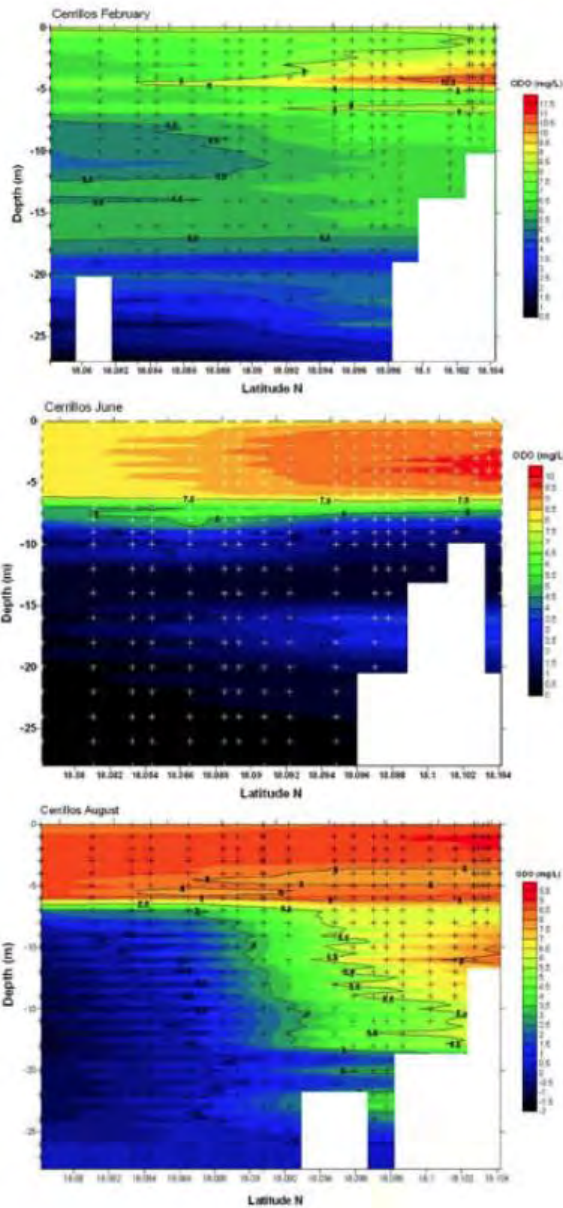


FIGURE 2. Surface and profile changes in dissolved oxygen concentrations (mg/L) for Cerrillos Reservoir for February, June and August. Note distinct oxycline development for summer (June), and August in deeper waters. Shallow waters (<17 m) in August were oxic environments throughout the water column. Significant proportion of the water column was oxic in February, with similar results for December sampling event.

pH, and temperature, along the surface as well as with depth is vitally important to baitfish production and occurrence. Elucidating first order trophic level interactions between water quality parameters and baitfish will provide some insights in sport fisheries management in terms of species locations, food resource availability and habitat suitability within the entire reservoir. Changes in spatial and temporal nutrient concentrations will affect trophic level

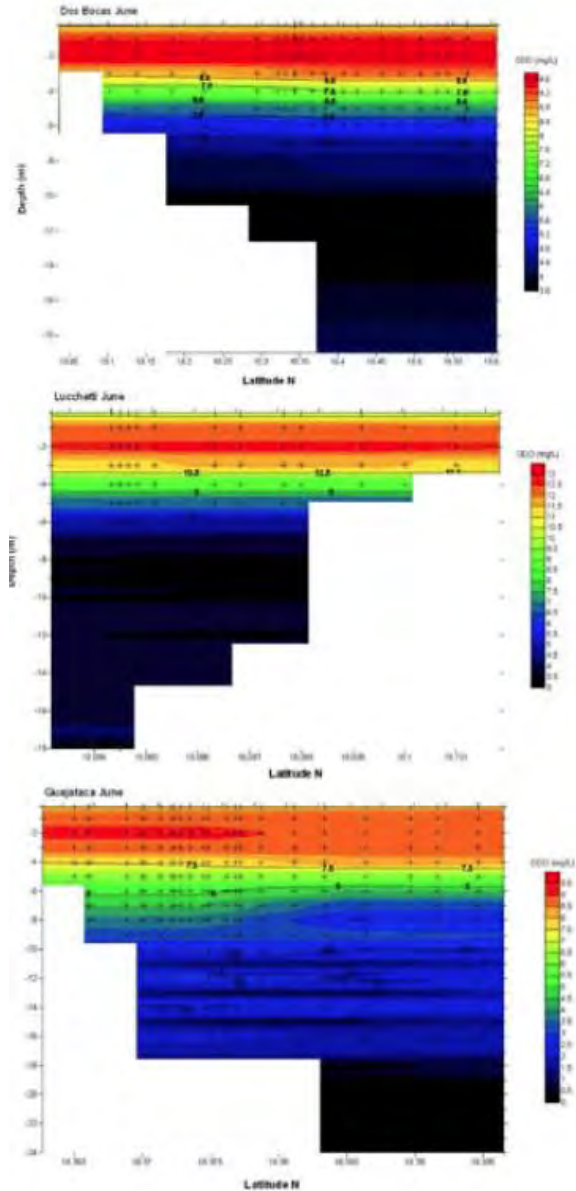


FIGURE 3. Spatial and depth distributions of dissolved oxygen concentrations (mg/L) for Dos Bocas, Luchetti and Guajataca Reservoir.

productivity within the reservoir, with extremes of excess and limitation affecting productivity. Manny et al. (1994) highlighted how phosphorus contributions from waterfowl were close to 70% of total P inputs into Wintergreen Lake, raising nutrient concentrations in the water column, increasing primary productivity and chlorophyll *a*, and decreasing Secchi disk transparency. Relative aquatic ecosystem productivity can be described using nutrient concentration data as well as

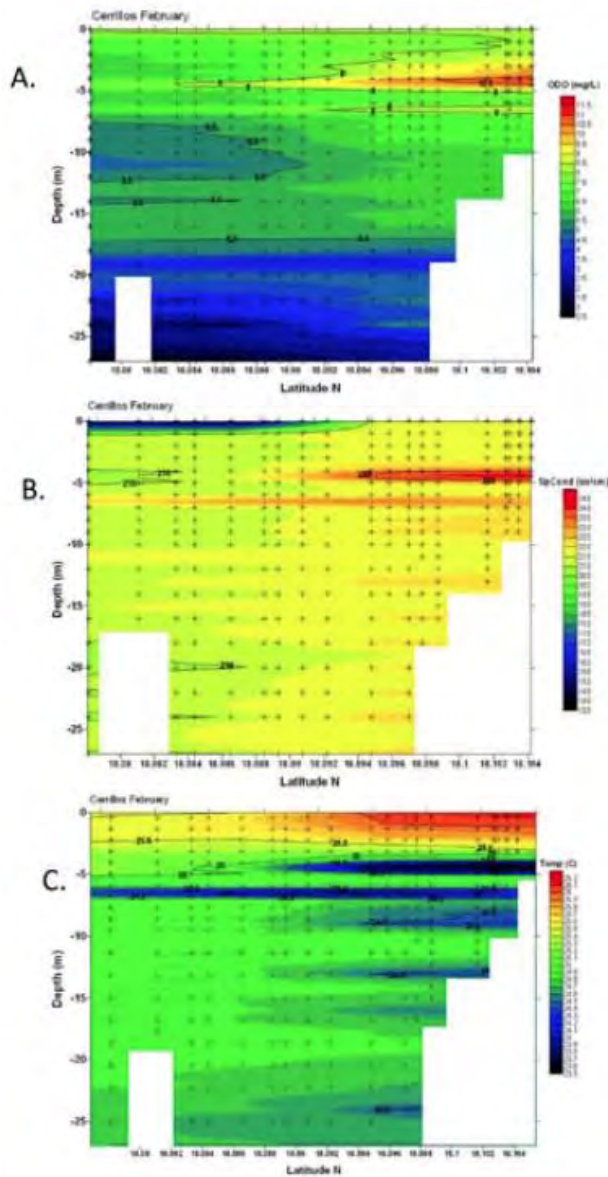


FIGURE 4. Dissolved oxygen (A), specific conductance (B) and temperature (C) spatial and depth distributions in Cerrillos Reservoir in February. Note distinct riverine signature in all three parameters as reservoir water levels were high.

*in situ* parameters of dissolved oxygen, pH and temperature.

Spatiotemporal dissolved oxygen concentrations in surface waters provide the basic ecological link to reservoir productivity. Dissolved oxygen is a response variable through bottom up controls such as phytoplankton productivity. Ideal conditions of DO, pH and temperature create conducive conditions for reservoir productivity – i.e., algal photosynthesis. Higher algal productivity in summer months (June/August) resulted in increased dissolved oxygen

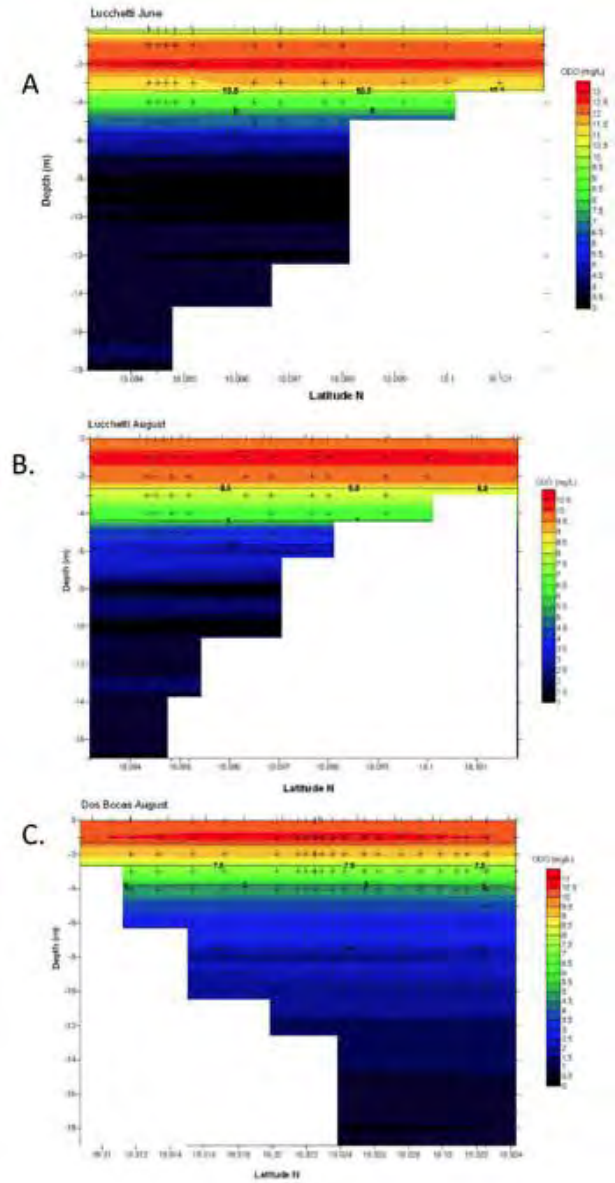


FIGURE 5. Dissolved oxygen profiles illustrating irradiance avoidance in Lucchetti in June (A), and August (B), and Dos Bocas in August (C).

production, increased carbon dioxide consumption, and an increase in the pH of the water column. Often though, high irradiance at the surface resulted in photosensitivity of phytoplankton, and photo irradiance avoidance was often prevalent in these reservoirs. Dissolved oxygen concentrations at the surface typically increased during daylight hours as a result of phytoplankton production. Conversely, dissolved oxygen concentrations will also decrease with depth, as DO is removed through phytoplankton respiration and is not replenished without mixing. Decreases in water column temperature results in density changes



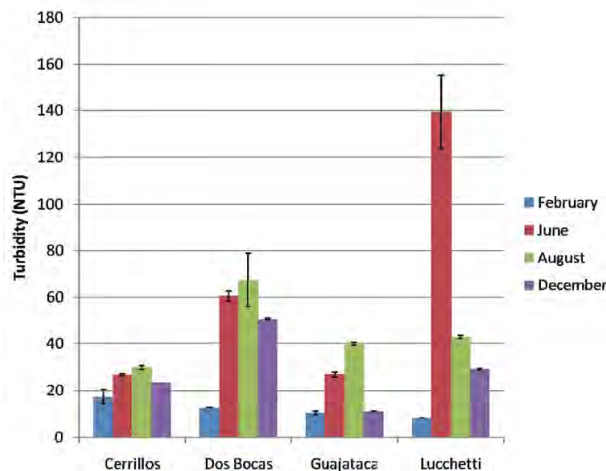


FIGURE 6. Mean turbidity (NTU ± S.E.) for each reservoir by quarterly sampling period.

with depth and further hinders mixing. From February through August, depth profiles of all four reservoirs showed similar patterns. There were strong stratifications of temperature and oxygen in June for all four reservoirs. Oxyclines typically fell between 4 – 8 m in June. Below 8 m, oxygen concentrations were usually below 2 mg/L. Warm water fish generally require minimum oxygen concentrations of at least 3 – 4 mg/L. Weakly anoxic (< 2 mg/L), or anoxic conditions would limit the habitat selection of baitfish and sport fish species.

Algal photosynthesis and reservoir production will be limited by nutrients. Nutrients are the basic elements required for algal growth. Nutrient concentrations in excess, will lead to a decrease in reservoir productivity, eutrophication and a decline in aquatic ecosystem health. A balance is required that enhances basic food web productivity through photosynthesis, but doesn't lead to an alteration of habitat suitability through changes to *in situ* water quality parameters. Total inorganic nitrogen concentrations were varied between reservoirs. Dos Bocas Reservoir displayed inter-reservoir heterogeneity in nutrient concentration between the two arms. Increased total inorganic N and P in the western arm of Dos Bocas is hypothesized to be a result of runoff and delivery from high density urban developments in the upper reaches of the western arm catchment. The Grande de Arecibo watershed, within which Dos Bocas is situated, has a high amount of abandoned and active coffee plantations, but is also significantly larger than all other reservoir catchments. The eastern arm's water quality is better than the western arm for two reasons: 1) no urban development in the upper reaches of the sub-catchment, 2) a reservoir in the upper reaches that has the opportunity

to trap and transform sediments and nutrients prior to effluent reaching Dos Bocas. From field observations, the western arm also receives debris (garbage, refuse, organic materials) during rainfall events that accumulate on the dam wall, while over the same period there is very little debris in the eastern arm. Soluble reactive P concentrations were very low across all reservoirs < 0.2 mg/L. Median SRP concentrations between quarterly sampling events and across all reservoirs was 0.01 mg/L. This low concentration between reservoirs and the lack of phytoplankton turbidity associated with algal production might suggest that all these systems are phosphorus limited. Schindler (1978) revealed that both annual phytoplankton production and mean annual chlorophyll were tightly correlated with P loadings into freshwater systems. Schindler (1978) also highlighted that stratification, whether by temperature or oxygen, had no effect on the distribution of phosphorus and its effect on productivity. Low SRP concentrations in the current study across reservoirs and through time support this finding.

High surface turbidity (> 60 NTU) occurred in only three sampling events across all reservoirs (Lucchetti-June; Dos Bocas-June and August). Lucchetti Reservoir had very high surface measured turbidity in June as a result of strong winds causing significant turbulence, while high turbidity was encountered in Dos Bocas in June and August as a result of storm events bringing increased river inflows. Dos Bocas had significant differences in surface turbidity concentrations (60 NTU west/urban arm vs. 30 NTU east/reservoir arm) between the two river arms. Surface turbidity levels across all reservoirs were typically very low (Median: 28 NTU), often with large Secchi depths (> 1.8 m). Low turbidity in all reservoirs coupled with high irradiance leads to photoinhibition. Light is required by all phytoplankton species for photosynthesis. Excess light, however, can inhibit photosynthesis through the photooxidative destruction of the photosynthetic apparatus (Long et al. 1994). High irradiance and high levels of UV-A and B result in phytoplankton migrating through the water column for photosynthesis. Dissolved oxygen concentrations at the surface and are comparable to concentrations at 3 – 4 m. This is interesting as dissolved oxygen concentrations should be the highest at the surface and decrease with depth. In all reservoirs at all time intervals, except for the time where turbidity in the surface averaged over 60 NTU, dissolved oxygen concentrations increased from surface to 2 – 3 m and then returned to surface conditions at 4 – 5 m. Depending on the time of the year (December, February and August) dissolved oxygen concentrations slowly decreased with depth, whilst in June, strong

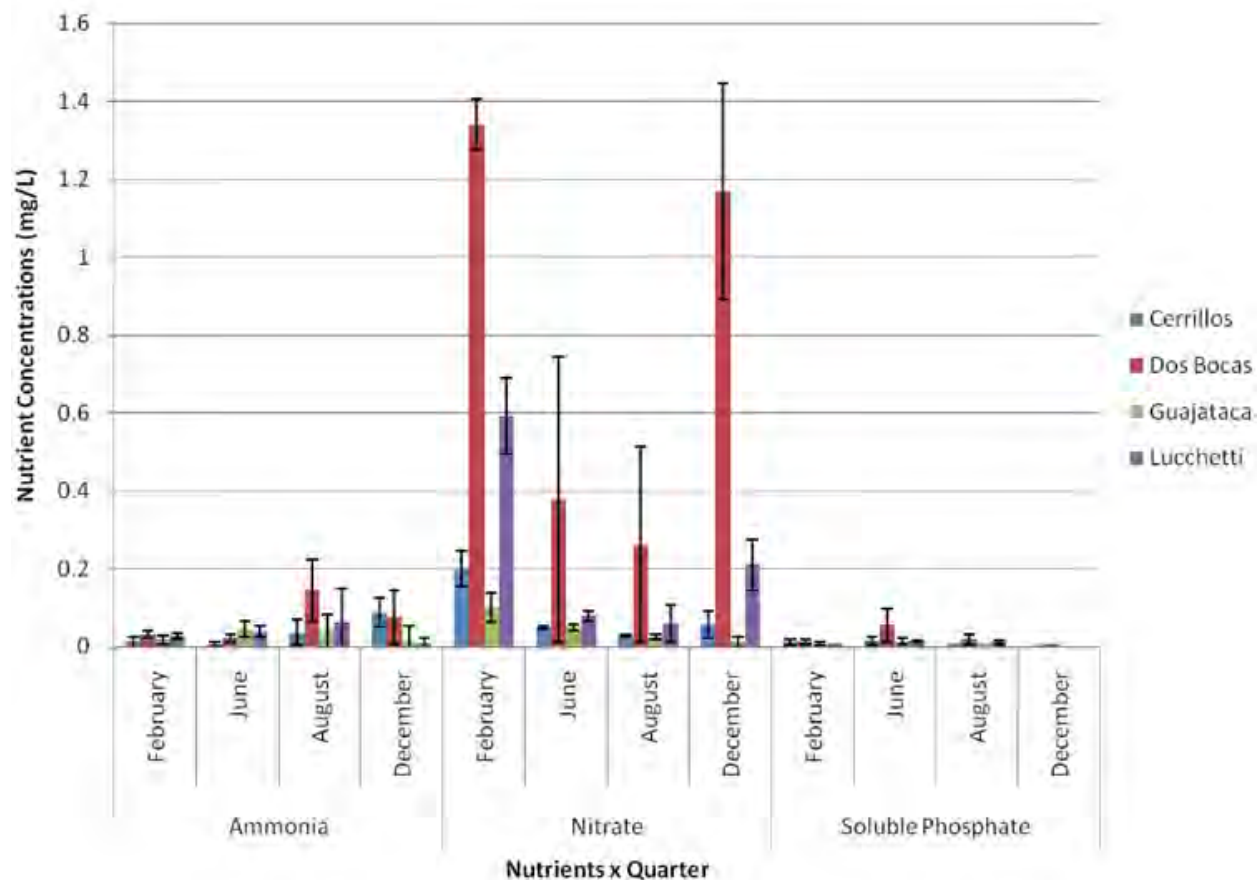


FIGURE 7. Mean surface nutrient concentrations for all four reservoirs (Dos Bocas, Cerrillos, Guajataca and Lucchetti) over the four quarterly sampling events in 2010.

oxycines had formed, below which oxygen concentration declined to less than 2 mg/L within 2 – 4 m. Typically these oxycines were associated with thermoclines, effectively stratifying the water column into areas of adequate food resource availability and habitat suitability (shallow strata), and areas of poor habitat quality (deeper strata). This strong thermal and oxygen stratification that occurs in June could limit the diel migration of phytoplankton, and thus alter reservoir dynamics of resource availability for baitfish and sport fisheries.

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## **Study 6:**

# **Response of Toa Vaca Reservoir to Destratification**



## Chapter 16: Response of Toa Vaca Reservoir to the Installation and Operation of a Destratification System

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**Abstract:** Due to chronic issues with water quality in Toa Vaca Reservoir, an oxygen recovery system (ORS) was installed during winter/spring 2013. Installation of the ORS provided a unique opportunity to examine the effects of destratification on water quality and fish assemblages in a tropical reservoir. Pre-destratification sampling indicated that all fish (seven species) utilize the entire reservoir longitudinally, with greatest catch rates composed of sport fish (largemouth bass) and locally targeted food fish (redear sunfish and tilapia). Additionally, threadfin shad, the primary prey species in Puerto Rico reservoirs, utilized more of the water column than was previously shown in studies of other reservoirs. Water quality and nutrient analysis indicated that the system is stratified and nutrient limited; however, our findings did not corroborate the initial reports of poor water quality, which were the basis for the ORS installation. However, our sampling only occurred once per year and did not examine seasonal changes in water quality, and therefore should not be used to make water quality improvement suggestions. Because the ORS system was not installed in 2012 as planned, our data only represent pre-installation parameters. Whereas we have collected these data on the system prior to ORS implementation, it is recommended that this research continue in the future to determine the effects of ORS implementation on water quality and fish communities in Toa Vaca Reservoir.

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Artificial aeration and destratification are common methods used to alleviate dissolved oxygen depletion at depth and have been used in lakes and reservoirs for many years (Smith et al. 1975). The effects of artificial aeration can be variable in terms of water chemistry (Fast 1971; Haynes 1971; McNall 1971), but are generally positive in improving water quality (Fast 1973a; Leslie et al 1993). Destratification of a reservoir can lead to increased oxygen levels throughout the water column, thereby improving overall water quality and expanding the habitat of either warm or coldwater fish populations (Summerfelt 1972; Fast 1973a; Dunst et al. 1974; Leslie et al. 1993).

Toa Vaca Reservoir was initially constructed with the intention of providing water to irrigate croplands in southern Puerto Rico. However, soon after construction was completed in 1972, its primary use was altered to provide water for public use and its management was transferred from the Puerto Rico Electric Power Authority (PREPA) to the Puerto Rico Aqueduct and Sewer Authority (PRASA). Due to chronic issues with water quality in this reservoir, an oxygen recovery system (ORS) was planned for installation in spring 2012 that consisted of 8 compressors in 2 compressor

houses, with 14 diffusers placed in the deepest areas of the reservoir (Figure 1). The primary purpose of the system was to destratify the reservoir thereby improving the water quality; however, fish populations often respond to lake destratification by expanding their habitat into the newly oxygenated areas of the water column (Fast 1973a,b). This can lead to increased abundance of fish as larger adults may utilize more offshore habitats (Leslie et al. 1993).

Although the primary purpose for destratifying Toa Vaca Reservoir was for municipal purposes, this manipulation provided an unprecedented opportunity to examine the response of fish populations to destratification in a tropical reservoir. Assessment of fish communities and available habitat prior to and following the installation of the ORS will provide insight into how fish population dynamics in tropical reservoirs change in response to mechanical aeration. In this study, the objectives were to 1) describe the effects of an ORS on fish assemblages in Toa Vaca Reservoir, 2) determine the effects of an ORS on threadfin shad abundance and distribution throughout the water column, and 3) Measure changes in available physiochemical habitat following destratification.



FIGURE 1. Final design of ORS installed in Toa Vaca Reservoir. Note that 10 diffusers were installed, not the original 14 proposed. Image credit unknown; provided by: Craig Lilyestrom and Miguel A. Garcia Bermudez, DNER.

## Methods

Actual installation of the ORS system did not occur until winter/spring 2013. Therefore, sampling occurred prior to (January 2012) and during (January 2013) implementation of the ORS system. Sampling included fish community and water quality assessment. Post-installation sampling is planned beginning in 2014 to assess changes to the reservoir following full ORS implementation, pending a new funding request submitted to PR DNER.

All sampling occurred annually in January at pre-established sites (Table 1). We sampled the sport and prey fish community using boom-mounted electrofishing at fifteen sites. The reservoir was divided into three (upper, middle, lower) sections. Five sites in each section were electrofished with a standardized output of 3,000 W (Burkhardt and Gutreuter 1995) for 600 seconds of pedal down time. The sites were chosen *a priori* and were used throughout the study. All fish encountered were collected, identified, measured (TL; mm) and weighed (g) then released. Catch per unit of effort (CPUE) was calculated for every species at each site, and relative weight was calculated for sport fish. These data will be compared between years to detect initial spatial and temporal differences in the fish community. For the purposes of this report, only qualitative comparisons are presented.

Threadfin shad *Dorosoma petenense* sampling was conducted to determine abundance and distribution throughout the water column. A custom frame fry trawl consisting of frame aperture dimensions of 3 x 3 m with 6 mm mesh in the body of the trawl, 4 mm mesh in the cod end, and a total length of 10.5 m was used to sample threadfin shad. Prior to trawling, three transects (T1, T2, T3) were chosen in the portion of the reservoir

where the diffusers are located. At each site, four depths (0-3, 3-6, 6-9, and 9-12 m) were sampled using 2 minute tows. Threadfin shad densities will be compared among depths and years to determine if destratification increases use of available habitat or overall shad density.

Vertical profiles of dissolved oxygen (DO; mg/L), pH, specific conductivity ( $\mu\text{S cm}^{-1}$ ), temperature ( $^{\circ}\text{C}$ ), and turbidity (NTU) were collected at five locations per section to determine the effects of the ORS on available fish habitat throughout the reservoir. The sampling locations were chosen *a priori* and used for the duration of our study. Water quality parameters were recorded using a Manta™ Water Quality Multiprobe at one meter increments beginning at the surface (0 meters) and extending downward until the probe reached the bottom or 30 m in depth. These vertical profiles will be compared between years to determine changes in available fish habitat.

For nutrient analysis, a Kemmerer bottle was used to collect water samples at the surface (<1 m depth) and one meter above the reservoir bottom. Samples were emptied into labeled plastic cups and fixed with sulfuric acid (2 mL/L) then kept refrigerated until being processed for ammonia ( $\text{NH}_3$ ), nitrate ( $\text{NO}_3$ ), nitrite ( $\text{NO}_2$ ), and phosphorus ( $\text{PO}_4$ ) to determine changes in nutrient availability.

## Results

A total of seven species were collected during annual sampling in Toa Vaca Reservoir in 2012 and 2013. Overall mean reservoir CPUE was lower in 2013 than in 2012; however, there was no difference in CPUE among sections during or between years. In both years, largemouth bass *Micropterus salmoides* was the most frequently collected species throughout the reservoir, followed by tilapia *Tilapia* and *Oreochromis* spp. and redear sunfish *Lepomis microlophus* (Table 2). CPUE for largemouth bass was different among sections, but not between years. More redear sunfish and white catfish *Ameiurus catus* were collected in the upper section than in the middle and lower sections, but catch rates for these two species were not different between 2012 and 2013 (Table 3). There were no differences in catch rates among sections or between years for the remaining four species. The majority (90%) of largemouth bass collected were stock-size (>200 mm TL; Figure 2) and the overall mean relative weight was 101. Relative weight was highest for smaller fish (250-350 mm TL), but dropped precipitously upon reaching larger sizes (~380 mm TL; Figure 3).

In 2012, approximately 3,400 threadfin shad were collected during trawling. As expected the greatest

TABLE 1. Locations for water quality (WQ), electrofishing (EF) and trawl (T) sample collections in Toa Vaca Reservoir. Number of replicates in Lower (L), Middle (M), and Upper (U) reservoir sections varied by sampling gear. All trawl samples were collected in the lower reservoir section within the habitats affected by the ORS.

| Sample Type | Site | Latitude   | Longitude   |
|-------------|------|------------|-------------|
| WQ          | L1   | 18°6'16.4" | 66°29'16.4" |
| WQ          | L2   | 18°6'5.9"  | 66°29'8.9"  |
| WQ          | L3   | 18°6'5.4"  | 66°28'50.9" |
| WQ          | L4   | 18°6'16.7" | 66°28'57.1" |
| WQ          | L5   | 18°6'24.7" | 66°28'46.1" |
| WQ          | M1   | 18°6'58.1" | 66°28'40"   |
| WQ          | M2   | 18°6'5.5"  | 66°28'28.9" |
| WQ          | M3   | 18°6'54.8" | 66°28'14.8" |
| WQ          | M4   | 18°6'6"    | 66°28'11.9" |
| WQ          | M5   | 18°6'2.4"  | 66°28'4.9"  |
| WQ          | U1   | 18°6'2.1"  | 66°27'43"   |
| WQ          | U2   | 18°6'15"   | 66°27'42.7" |
| WQ          | U3   | 18°6'13.6" | 66°27'27.6" |
| WQ          | U4   | 18°6'24.2" | 66°27'17.4" |
| WQ          | U5   | 18°6'45.4" | 66°27'18.7" |
| EF          | L1   | 18°6'4.4"  | 66°28'51.3" |
| EF          | L2   | 18°6'0.3"  | 66°29'10.9" |
| EF          | L3   | 18°6'14.8" | 66°29'20.4" |
| EF          | L4   | 18°6'19.4" | 66°29'4.4"  |
| EF          | L5   | 18°6'25.9" | 66°28'43.6" |
| EF          | M1   | 18°6'59.9" | 66°28'43.8" |
| EF          | M2   | 18°6'58.4" | 66°28'31.3" |
| EF          | M3   | 18°6'54.5" | 66°28'19.9" |
| EF          | M4   | 18°6'9.7"  | 66°28'9.2"  |
| EF          | M5   | 18°6'2.4"  | 66°28'4.9"  |
| EF          | U1   | 18°6'4.6"  | 66°27'55.9" |
| EF          | U2   | 18°6'59.3" | 66°27'50.2" |
| EF          | U3   | 18°6'11.3" | 66°27'42.5" |
| EF          | U4   | 18°6'23.6" | 66°27'27.4" |
| EF          | U5   | 18°6'30.5" | 66°27'13.6" |
| T           | 1    | 18°6'3.8"  | 66°28'22.6" |
| T           | 2    | 18°6'17.8" | 66°28'46.2" |
| T           | 3    | 18°6'4.9"  | 66°29'2.8"  |

TABLE 2. Total catch of all species collected during electrofishing in Toa Vaca Reservoir, Puerto Rico. NC = Not collected.

| Species                 |                                       | Total Catch |      |       |
|-------------------------|---------------------------------------|-------------|------|-------|
|                         |                                       | 2012        | 2013 | Total |
| Sailfin armored catfish | <i>Pterygoplichthys spp.</i>          | 10          | 24   | 34    |
| Tilapia                 | <i>Oreochromis &amp; Tilapia spp.</i> | 201         | 28   | 229   |
| Largemouth bass         | <i>Micropterus salmoides</i>          | 325         | 438  | 763   |
| Bluegill                | <i>Lepomis macrochirus</i>            | NC          | 3    | 3     |
| White catfish           | <i>Ameiurus catus</i>                 | NC          | 7    | 7     |
| Channel catfish         | <i>Ictalurus punctatus</i>            | NC          | 7    | 7     |
| Redear sunfish          | <i>Lepomis microlophus</i>            | 126         | 84   | 210   |



TABLE 3. CPUE of species collected during electrofishing in Toa Vaca Reservoir, Puerto Rico. NC = Not collected.

| Species                        |                                       | CPUE  |        |       |       |       |        |       |       |
|--------------------------------|---------------------------------------|-------|--------|-------|-------|-------|--------|-------|-------|
|                                |                                       | 2012  |        |       |       | 2013  |        |       |       |
|                                |                                       | Lower | Middle | Upper | Mean  | Lower | Middle | Upper | Mean  |
| <b>Sailfin armored catfish</b> | <i>Pterygoplichthys</i> spp.          | 2.4   | 2.4    | 7.2   | 4     | 16.6  | 5.8    | 7.1   | 9.8   |
| <b>Tilapia</b>                 | <i>Oreochromis &amp; Tilapia</i> spp. | 49.2  | 93.6   | 88.8  | 78    | 5.9   | 15     | 14.3  | 11.7  |
| <b>Large mouth bass</b>        | <i>Micropterus salmoides</i>          | 181.2 | 92.4   | 115.2 | 128.8 | 254.4 | 177    | 100.1 | 177.2 |
| <b>Bluegill</b>                | <i>Lepomis macrochirus</i>            | NC    | NC     | NC    | NC    | 3.6   | NC     | NC    | 1.2   |
| <b>White catfish</b>           | <i>Ameiurus catus</i>                 | NC    | NC     | NC    | NC    | NC    | 1.2    | 8.6   | 3.3   |
| <b>Channel catfish</b>         | <i>Ictalurus punctatus</i>            | NC    | NC     | NC    | NC    | 7.1   | NC     | 1.4   | 2.8   |
| <b>Redear sunfish</b>          | <i>Lepomis microlophus</i>            | 56.4  | 54     | 40.8  | 50.4  | 20.1  | 24.3   | 65.8  | 36.7  |

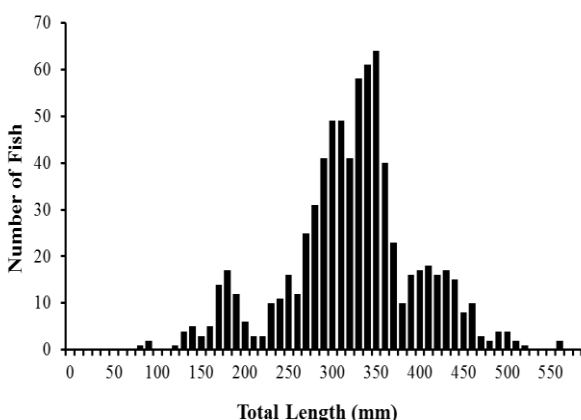


FIGURE 2. Length distributions of largemouth bass collected during annual sampling in Toa Vaca Reservoir, Puerto Rico

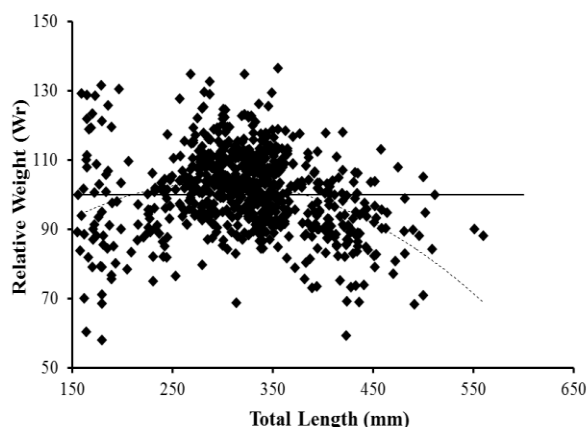


FIGURE 3. Condition of largemouth bass collected during annual sampling in Toa Vaca Reservoir, Puerto Rico. Dotted line is a fitted trendline, and solid line at 100 indicates optimal relative weight.

percentage (71.0%) of the total catch was collected at the 0-3 m depth. Surprisingly, however, the second highest percentage (19.1%) of shad was collected at the 9-12 m depth with very low numbers of shad collected in the 3-6 m or 6-9 m depths (Figure 4). In 2013, only 110 threadfin shad were collected during trawling efforts. As expected the highest percentage of total catch was collected at the 0-3 m depth, and catch decreased with deeper depths.

Temperature and dissolved oxygen measurements were similar throughout the reservoir in both years (Figure 5). However, in 2013 the thermocline and oxycline were much less prevalent than in 2012. Temperature remained fairly high and dissolved oxygen never fell below 2 mg/L. Additionally, turbidity was similar throughout the water column and higher at most depths in 2013 than in 2012 (Figure 5). Nitrogen and phosphorus levels remained low throughout the reservoir (Table 4), suggesting this system is nutrient limited.

## Discussion

This research was designed to be a two year study to examine the before and after effects of

implementation of an oxygen recovery system (ORS) in a tropical reservoir. Delays in installation of the system prevented post-implementation sampling during the period of this report. The system was installed in winter 2012/2013 and became operational some time later; however, our second annual sampling in January 2013 occurred before this system became operational. Therefore, results of this report principally reflect pre-installation data, and further study over subsequent years is warranted. Furthermore, water level had been reduced for installation, and was still very low (~8 m below normal pool; Figure 6) at the time of sampling, which appeared to substantially affect water quality and fish community samples.

Pre-ORS installation sampling of sport and prey fish species in Toa Vaca Reservoir indicates that all species were distributed throughout the reservoir prior to ORS implementation. Catch rates were moderate for the commonly targeted sport fish species of the island's reservoirs (largemouth bass, redear sunfish and tilapia). Overall catch rate was lower in 2013 than in 2012; however, that is likely due to lower than normal water level. Additional sampling is necessary to determine how fish assemblages will be affected by the ORS.

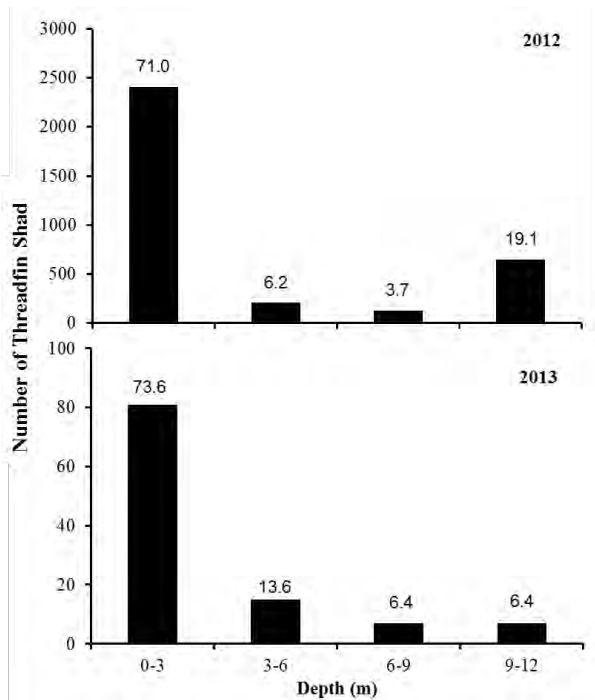


FIGURE 4. Number of threadfin shad (TFS) collected 10 January 2012 and 12 January 2013 in Toa Vaca Reservoir, Puerto Rico. Numbers above columns indicate the percentage of total TFS catch collected at each depth.

Extremely low catches of threadfin shad in 2013 also most likely were related to low water level. The 2012 threadfin shad catch was similar to the winter shad catches in other Puerto Rico reservoirs (Neal et al. 2011), suggesting that the loss of reservoir volume and concurrent concentration of predators affected threadfin shad recruitment and survival. Our initial hypothesis was that we would not collect many shad at the 9-12 m depth prior to the ORS installation and shad populations would expand into deeper depths following installation. As expected, the highest numbers of threadfin shad were collected at the 0-3 m depth. However, in 2012 the second highest numbers of shad were collected at the 9-12 m depth. Although this depth was not sampled in the other reservoirs, we did not expect to collect many threadfin shad at this depth due to poor habitat quality.

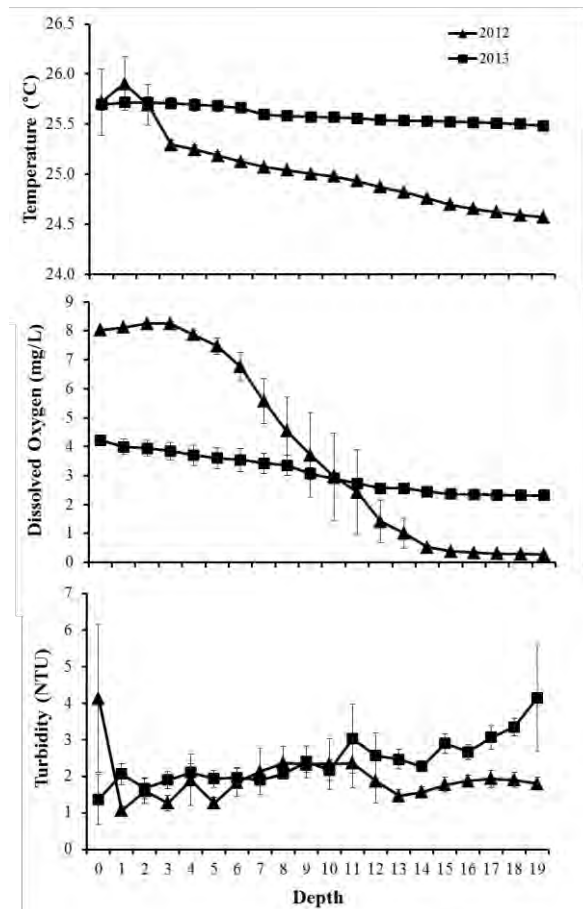


FIGURE 5. Mean ( $\pm$  SE) temperature (top), dissolved oxygen (middle), and turbidity (bottom) by depth in Toa Vaca Reservoir for 2012 and 2013

Threadfin shad have been collected in other reservoirs in the upper 9 m of the water column, but usually the numbers of shad decreased as depth increased. Although habitat utilization post-ORS implementation remains to be seen, it is clear that threadfin shad were using deeper habitats in this reservoir prior to ORS installation.

Water quality and nutrient analysis indicated that the overall quality of the water in the reservoir is adequate. Although the system is stratified and nutrient limited, our

TABLE 4. Mean ( $\pm$ SE) nutrient concentrations (ppm) in Toa Vaca Reservoir, Puerto Rico. ND = Not detected.

| Nutrient   | Nutrient Concentration |                   |                   |                   |                    |                    |                   |                   |
|------------|------------------------|-------------------|-------------------|-------------------|--------------------|--------------------|-------------------|-------------------|
|            | 2012                   |                   |                   |                   | 2013               |                    |                   |                   |
|            | Lower                  | Middle            | Upper             | Mean              | Lower              | Middle             | Upper             | Mean              |
| Ammonia    | 0.254 $\pm$ 0.094      | 0.309 $\pm$ 0.113 | 0.163 $\pm$ 0.099 | 0.454 $\pm$ 0.286 | 0.015 $\pm$ 0.001  | 0.010 $\pm$ 0.002  | 0.053 $\pm$ 0.013 | 0.027 $\pm$ 0.006 |
| Nitrate    | ND                     | ND                | ND                | ND                | 0.915 $\pm$ 0.006  | 0.838 $\pm$ 0.020  | 0.222 $\pm$ 0.067 | 0.658 $\pm$ 0.019 |
| Nitrite    | ND                     | ND                | ND                | ND                | 0.012 $\pm$ 0.001  | 0.020 $\pm$ 0.002  | 0.047 $\pm$ 0.004 | 0.026 $\pm$ 0.004 |
| Phosphorus | 0.053 $\pm$ 0.013      | 0.054 $\pm$ 0.012 | 0.031 $\pm$ 0.008 | 0.046 $\pm$ 0.035 | 0.040 $\pm$ 0.0003 | 0.040 $\pm$ 0.0005 | 0.035 $\pm$ 0.002 | 0.038 $\pm$ 0.002 |

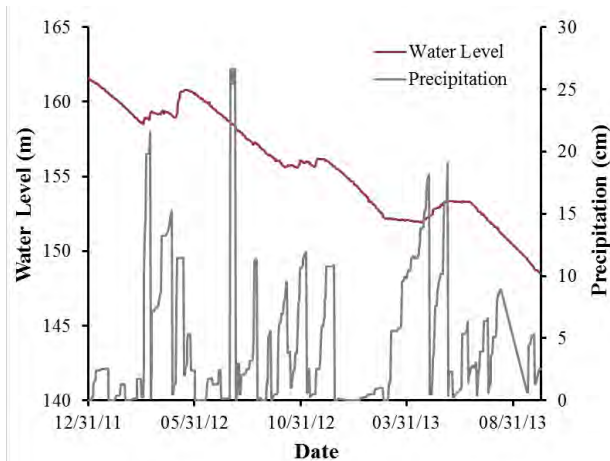


FIGURE 6. Water level above (NVGD, m) and precipitation (cm) for Toa Vaca Reservoir from 21 December 2011 through 31 August 2013 (USGS 2008).

data did not encounter the water quality issues that were the basis for the installation of the ORS. In 2012, the oxycline occurred throughout the reservoir at approximately 15 meters, and even in the more shallow areas of the reservoir we saw high concentrations of dissolved oxygen. The following year, the oxycline was not as prevalent, but did occur at approximately 9 meters. Moreover, mean temperature and turbidity throughout the reservoir were higher in 2013 than in 2012, but like CPUE, these parameters could have been affected by the lower than normal water level and therefore our results could be skewed and should not be used to make any major assumptions about the fish community until additional data are available.

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