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DETERMINATION OF THE SPAWNING SEASON OF BIGMOUTH SLEEPER IN PUERTO RICO BY EXAMINATION OF GONAD MATURATION AND REPRODUCTIVE HORMONE CYCLES

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ABSTRACT: Bigmouth sleepers, *Gobiomorus dormitor,* are diadromous fish that have potential for hatchery production as both food and sport fish and for conservation purposes. Understanding of bigmouth sleeper maturation and seasonal hormone cycling are necessary in order to realize hatchery production. Therefore, seasonal trends in gonadosomatic index (GSI) and plasma vitellogenin, estradiol, progesterone and total testosterone concentrations were examined in wild and captive populations in Puerto Rico during the presumed spawning season. The spawning season for wild river populations of bigmouth sleepers was protracted over several months, but peaks in male testosterone (6.5 ng/mL) and female vitellogenin (11.3 ng/mL), estradiol (3.3 ng/mL), and GSI (9.5–12.0%) demonstrated that the bigmouth sleeper spawning season occurred primarily in July and August in southwestern Puerto Rico. Captive female broodstock held in shallow hatchery ponds demonstrated accelerated maturation, presumably due to warmer water temperatures in the ponds. Therefore, induced and natural spawning attempts using captive female bigmouth sleeper broodstock should be conducted from June through July. However, advanced gonadal maturation and increased testosterone production in captive males was minimal. Hence, induced spawning for captive rearing purposes should use wild broodstock captured during the peak of the natural spawning season in July and August.

KEY WORDS: *Gobiomorus dormitor,* estradiol, progesterone, reproduction, vitellogenin

INTRODUCTION

The bigmouth sleeper, *Gobiomorus dormitor*, is distributed in coastal areas of the Caribbean, northern parts of South America, and the southernmost subtropical United States (Lindquist 1980, Gilmore 1992). Bigmouth sleepers are believed to be amphidromous (Holmquist et al. 1998), with spawning presumably occurring in freshwater streams, followed by larval migration to marine environments, and return to fresh water as juveniles. Because of this life history strategy, construction of impassable dams on rivers impedes bigmouth sleeper movement between freshwater and marine environments. Thus, their life cycle is effectively interrupted, resulting in the extirpation of the species upstream of these impoundments in many river systems (Holmquist et al. 1998). In response to these events, exotic fish species have been introduced to create sport fishing opportunities in the absence of bigmouth sleeper populations (Erdman 1984).

Bigmouth sleepers can survive and grow well in reservoirs and upstream river reaches where juveniles can colonize. In recent decades, three reservoirs in Puerto Rico have periodically supported limited bigmouth sleeper populations, presumably due to dam passage during high flow events (Churchill et al. 1995, Neal et al. 1999). At least one impoundment, Carite Reservoir, supports an abundant, self–sustaining, landlocked population (Neal et al. 2001, Bacheler et al. 2004a). Self–sustaining, landlocked populations also have been reported in Lago de Yojoa, Honduras (Darnell 1962) and Lago Jiloa and Lago Apoyo, Nicaragua (McKaye et al. 1979, Bedarf et al. 2001). 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 under certain environmental conditions.

The spawning season, defined as the period in which the majority of reproduction for a population occurs, can be variable for bigmouth sleepers and depends on the water body (lotic vs. lentic) and latitude of the population in question. Previously, the gonadosomatic index (GSI) has been used as a biological indicator of sexual maturation in bigmouth sleepers (Bedarf et al. 2001, Bacheler et al. 2004a). However, assessing gonadal development using classification criteria such as "ripe", "mature", or "developed" gonads is vague and not well defined within the literature for bigmouth sleepers. Therefore, these terms are considered to refer to ovaries with oocytes in advanced developmental stages or testes in which spermiation has occurred and the testes have become hydrated.

Bacheler et al. (2004a) found GSI values for bigmouth sleepers in Carite Reservoir increased in April, peaked in May and June, and then declined to base levels by October, although no samples were taken from July through September. Bedarf et al. (2001) assessed the duration of bigmouth sleeper breeding in two lakes in Nicaragua, Lake Jiloa and Lake Apoyo. Using GSI values, they reported that mature gonads were found in bigmouth sleeper year–round to varying degrees, and GSI peaked between March and June. In Tortuguero Lagoon, Costa Rica, Kelso (1965) encountered "gravid" females in May. However, "developed" gonads

were reported from March to December in another Costa Rica study (Winemiller and Ponwith 1998), and from April through November in Tecolutla estuary in Veracruz, Mexico (Hernández–Saavedra et al. 2004). Collectively, these findings suggest near year–round or extended spawning periods in some bigmouth sleeper populations.

In addition to examination of GSI values, plasma or serum sex steroid concentrations also can be effective in assessing reproductive development. Hormones such as 17β–estradiol (estradiol) and testosterone have been used to assess reproductive development in multiple fish species (Zohar and Billard 1984, Foster et al. 1993, Holcombe et al. 2000, Davis et al. 2005, Gross et al. 2006), including members of the family Eleotridae (Wang et al. 2001). Vitellogenin has been effective for determining onset of sexual maturation and its continued development in female teleosts, specifically by using an enzyme–linked immunosorbent assay (ELISA) (Jackson and Sullivan 1995, Heppell et al. 1999, Gross et al. 2006). Progesterone also has been used with some success to indicate reproductive maturation in fish (Kagawa et al. 1981, Nagahama et al. 1991, Foster et al. 1993, Gross et al. 2006).

Evidence of natural reproduction in reservoirs, recent management preferences for native species (Clarkson et al. 2005), and the fact that anglers currently target bigmouth sleepers for sport and food (Bacheler et al. 2004b) suggest high potential for this species to serve both sport and food interests in Puerto Rico and elsewhere. Likewise, declines in bigmouth sleeper abundance within its range (Holmquist et al. 1998, Warren et al. 2000) warrant directed conservation efforts for this species. If appropriate hatchery propagation techniques can be developed, supplemental and restoration stocking could become a viable fisheries management and conservation tool for bigmouth sleepers. However, spawning attempts using captive and wild–caught bigmouth sleepers in Puerto Rico have demonstrated minimal success. At present, the major obstacle preventing the development of appropriate bigmouth sleeper spawning protocols is the apparent lack of seasonal reproductive development in captive specimens of reported breeding size (Harris 2007). Thus, to improve bigmouth sleeper spawning techniques, this study examined reproductive hormone cycling and reproductive development for both wild river populations and captive pond populations in Puerto Rico. This information will be used to ascertain the peak times at which induced spawning should be attempted.

Materials and Methods

Study area and fish collections

Three free–flowing rivers in Puerto Rico without significant instream barriers to fish migration were used as sources for wild bigmouth sleeper (Figure 1, Table 1). The Rosario River, northeast of the city of Hormigueros, and the Nueve Pasos River, northwest of the city of San Germán, flow into the Guanajibo River before draining into the Mona Passage south of the city of Mayagüez. The Cañas River drains into the Caribbean Sea on the south central portion of the island near the city of Ponce. Fish were collected using a backpack electrofishing unit using up to 400 volts DC and a 60–Hz pulse cycle. Due to the close proximity, similar physiochemical and habitat characteristics, and stable tropical water temperatures (e.g., summer temperatures 24.8–25.9°C; Kwak et al. 2007) of these rivers, they were considered as one sample population for this manuscript. The amphidromous life history of bigmouth sleepers (Holmquist et al. 1998), proximity of these river systems, absence of significant in stream barriers to fish migration, and similar physical and water quality characteristics of these river systems, suggests that population mixing among these rivers systems is likely to occur.

Captive bigmouth sleepers were originally collected at least one year prior to this study from the Cañas, Rosario, and Nueve Pasos Rivers, and from Carite Reservoir. These fish were transported to the Agricultural Research Station of the University of Puerto Rico–Mayagüez located near Lajas, Puerto Rico, and held in four 100 m^2 ponds with a maximum depth of 1.0 m (summer temperatures 28.1–32.1°C). At least 2 male and 4 female bigmouth sleepers were stocked in each pond. Ponds were stocked with a self–sustaining prey base of mollies *Poecilia* spp., swordtails *Xiphophorus* spp., tilapia *Oreochromis* and *Tilapia* spp., and threadfin shad *Dorosoma petenense*. Sampling was conducted monthly in conjunction with sampling of wild populations using a standard seine. Whereas physiological parameters may change with fish size, bigmouth sleeper total lengths (TL) were compared using general linear models (PROC GLM, SAS Version 9.2) with the class variable month, source (wild or hatchery), and sex.

Gonadosomatic index, plasma vitellogenin, and steroid hormones

Wild bigmouth sleeper specimens collected from the Nueve Pasos, Rosario, and Cañas Rivers between March and October 2007 were used to determine GSI and hormone concentrations before, during, and after the presumed breeding season (May–June; Bacheler et al. 2004a). Each month, 5–7 females and 3–5 males of breeding sizes were collected. All specimens exceeded the reported minimum size at maturity (159 mm and 179 mm TL for male and female bigmouth sleepers, respectively; Bacheler et al. 2004a), and most exceeded 200 mm TL. Fish were measured, weighed (nearest 2.0 g), and euthanized with 150 mg/L of tricaine methansulfonate (MS–222; Argent Chemical Laboratories, Inc., Redmond, WA) buffered to pH 7 with sodium bicarbonate. Blood (1.0–1.5 mL) was taken by insertion of a sodium heparin coated syringe (21 gauge, 38 mm needle) into the caudal vasculature. In cases where blood collection was insufficient, the tail was severed behind the posterior dorsal fin and blood was collected using 470 µL heparinized Caraway tubes. Blood samples were immediately placed on ice in 1.5 mL

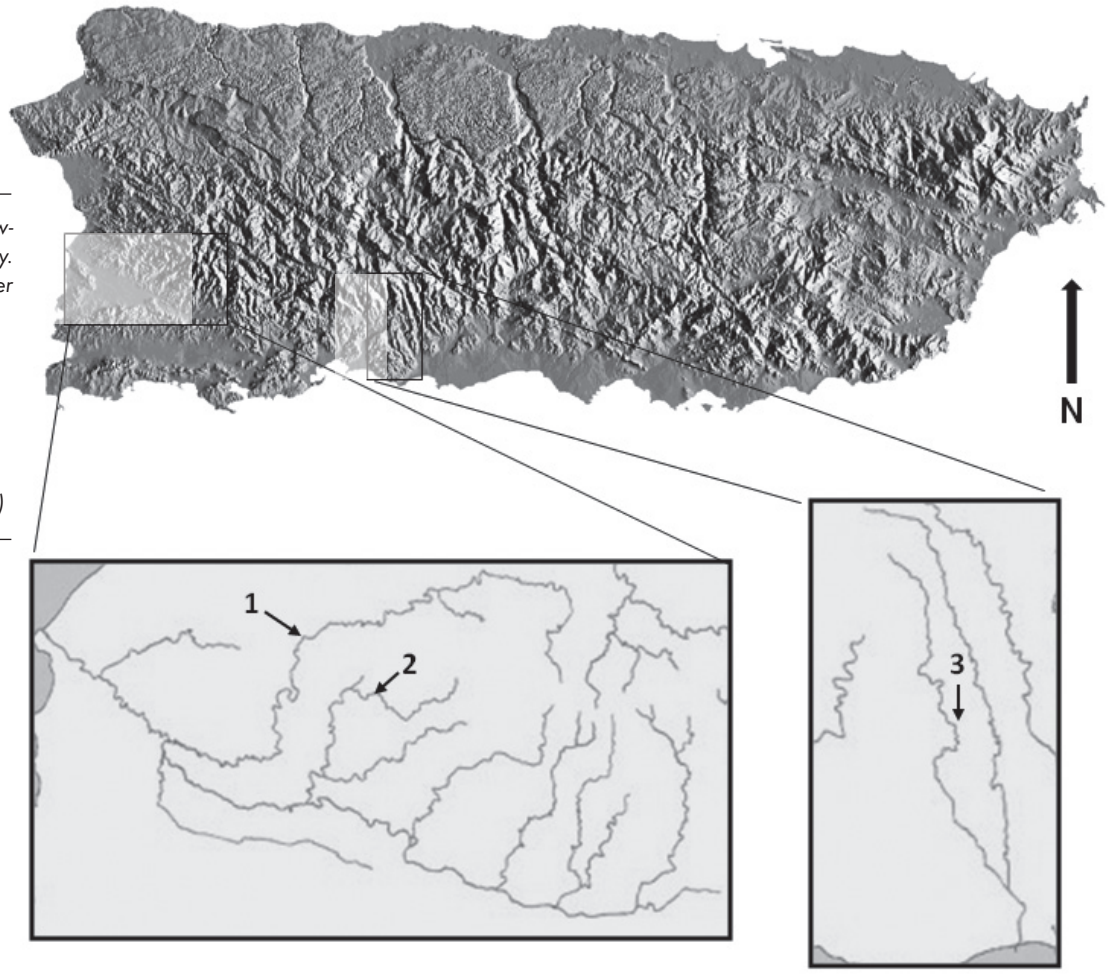

Figure 1. *Map of Puerto Rico showing rivers sampled in this study. The rivers (GPS coordinates of river mouth) were:*

1) Río Rosario (18°7′48.76″N, 67°7′59.74″W)

2) Río Nueve Pasos (18°7′1.47″N, 67°4′27.13″W)

3) Río Cañas (17°58′53.34″N,66°38′.16.63″W)

cryotubes coated with ammonium heparin to prevent clotting. Fish specimens were then dissected to remove testis or ovaries, weighed, and GSI calculated according to the equation: GSI = (gonad weight/body weight) × 100, and is expressed as percent body weight (Murphy and Willis 1996). Due to limitations of the electronic field balance, all gonads weighing less than 2 g resulted in a GSI of less than 0.4, the minimum calculated GSI threshold. Sample collection from captive bigmouth sleepers held in 100 m^2 ponds at the University of Puerto Rico – Mayagüez's Aquaculture Research Station was repeated as described above for 3–4 male and 3–4 females from March through July. No samples were taken from captive fish after July because no fish were available.

Blood samples were centrifuged at the laboratory to separate the plasma. Plasma was transferred into new cryotubes and frozen at –20°C until transportation on dry ice to the University of Arkansas at Pine Bluff (UAPB) for determination of vitellogenin and hormone concentrations. The samples were immediately frozen at –70°C upon arrival at UAPB. Enzyme–linked immunosorbent assays (ELISA) were partially validated for use in bigmouth sleepers using methods presented in Sink et al. (2008), and then utilized for qualitative determination of plasma 17β–estradiol (Bio-Quant, San Diego, CA), progesterone (Assay Designs, Inc., Ann Arbor, MI), and vitellogenin (Biosense Laboratories, Bergen, Norway) concentrations. An ELISA kit was also used for the qualitative determination of total testosterone (Assay Designs, Ann Arbor, MI), though no validation procedures were conducted due to monetary constraints. Males were tested for total plasma testosterone concentrations only, while females were tested for plasma estradiol, progesterone, and vitellogenin concentrations.

Vitellogenin and hormone assays

Each assay was conducted as per the instructions provided with the kits except for the vitellogenin assay. The vitellogenin ELISA kit (V01003402) was specific for detection of carp *Cyprinus carpio* vitellogenin, although it has been used for detection of vitellogenin from fathead minnow *Pimephales promelas*, zebrafish *Danio rerio*, goldfish *Carassius auratus*, mullet *Mugil* sp., pinfish *Lagodon rhomboides*, sucker Catostomidae sp., and other cyprinids. The monoclonal mouse anti–carp vitellogenin antibody does not react with vitellogenin from striped bass *Morone saxatilis* or brown bullhead *Ameiurus nebulosus* (Biosense kit insert). The monoclonal mouse anti–striped bass vitellogenin antibodies bind vitellogenin from striped bass and rainbow trout *Oncorhynchus mykiss*, and also cross–reacts with vitellogenin from largemouth bass *Micropterus salmoides*, Atlantic cod *Gadus morhua*, Nile tilapia *Oreochromis niloticus*, mummichog *Fundulus heteroclitus*, and sheepshead minnow *Cyprinodon variegates* (Biosense kit

TABLE 1. *Source and sample size of bigmouth sleepers sampled for GSI, plasma vitellogenin and steroid hormone concentrations. No fish remained in the captive population after the July sample.*

	Number in sample				
	Wild		Captive		
Month	Male	Female	Male	Female	Source population(s) of wild fish
March	3	7	4	3	Nueve Pasos River and Rosario River
April	3	5	3	3	Cañas River
May	4	7	3	3	Nueve Pasos River
June	3	5	4	4	Rosario River
July	3	5	4	3	Cañas River
August	5	5	–	–	Nueve Pasos River
September	5	6	–	–	Nueve Pasos River
October	5	5	–	–	Nueve Pasos River
Totals	**31**	**45**	**18**	**16**	

insert). To ensure the greatest probability that vitellogenin from bigmouth sleepers was bound during the assay, monoclonal mouse anti–striped bass vitellogenin antibodies were diluted 1:100 with phosphate buffered saline and 10 µL of the antibody solution was added to each well of the microplates coated with monoclonal mouse anti–carp vitellogenin antibody. The aqueous solution that contained the antibodies was then evaporated under a gentle stream of nitrogen.

The assay was then run using carp and rainbow trout (V01004301–001) vitellogenin standards and pooled bigmouth sleeper controls. The greatest bigmouth sleeper vitellogenin binding affinity occurred when compared to the carp vitellogenin standard. Since the carp vitellogenin assay is not known to detect rainbow trout vitellogenin, does not bind striped bass vitellogenin, and there is a high probability that a portion of the monoclonal mouse anti–striped bass vitellogenin antibodies were removed during the washing phase, only the unaltered carp vitellogenin ELISA was used for determination of vitellogenin concentrations from bigmouth sleepers during the study.

Because hormone trends were of interest, and not absolute concentrations, only partial validations were conducted for the vitellogenin, estradiol, and progesterone assays to determine the suitability of the kits for detecting hormonal shifts in bigmouth sleepers. The samples were thawed and 150 µL of plasma from 3 males were pooled and used for the male standard and 5 females were pooled and used for the female standard to validate each kit. All samples were run in duplicate for the validation procedures and sample hormone determination. Validation tests followed the Validation of Analytical Procedures: Methodology (FDA CVM 1999) and were similar to validation tests used in Barry et al. (1993) for a cortisol ELISA. Accuracy was tested by calculation of recoveries from samples spiked with known amounts of hormones (3 increments of the hormone standards provided with the kits). Precision was tested by repeated assays of samples (8 times in duplicate) on the same plate and by calculation of coefficient of variation (% CV). Mean sample recovery percentage limits of within 90–110% were defined as meeting validation criteria. A percent coefficient of variation of ≤ 20.0 was set as the acceptable limit for the intra–assay % CV.

Statistical analyses

Plasma vitellogenin, steroid hormone concentrations, and GSI values were plotted over time to illustrate monthly trends in reproductive development, and to examine relationships between gonad maturation and hormone levels. To determine significant monthly trends, one–way analysis of variance (ANOVA) was used unless data failed normality, in which case Kruskal–Wallis non–parametric test of ranks was employed. In either test, month served as the main effect. The Kolmogorov–Smirnov test (with Lilliefors' correction) was used to test data for normality. Pair–wise multiple comparisons were used to separate months. Differences in GSI, hormone and vitellogenin concentrations of wild and captive populations were analyzed using a Student's t–test conducted by month. In cases where normality tests failed, analyses were conducted using a Mann–Whitney Rank Sum Test. These tests were chosen over paired data tests because periodicity of peaks was of interest, and may not have been discerned by a paired–t test or ANOVA on repeated measures. A Pearson product–moment correlation coefficient was used to analyze correlation of hormones and GSI for individual fish. Data for GSI were transformed by taking ArcSine of the square root as per Osborne (2002). Statistical significance for all tests was set at an alpha level of 0.05 (Zar 1999).

Results

Fish characteristics

A total of 57 wild female bigmouth sleepers and 31 wild male bigmouth sleepers were collected for this study. Mean ± se size of wild–caught females was 226 ± 3 mm TL and 100 ± 4 g.; wild–caught males averaged 271 ± 10 mm TL and 185 ± 23 g in size. Sixteen captive females and 18 captive males were sacrificed for this research. Captive females averaged 253 ± 11 mm TL and 134 ± 16 g, and captive males averaged 270 ± 13 mm TL and 156 ± 22 g. The general linear model indicated that differences in fish TL occurred (Overall model: $F_{12, 51} = 5.71$, $p < 0.0001$, $r^2 = 0.57$), with month ($F_3 = 6.02$, $p = 0.0014$, $r^2 = 0.10$) and sex ($F_1 = 20.84$, $p < 0.0001$, $r^2 = 0.34$) contributing to the observed variability. Total length

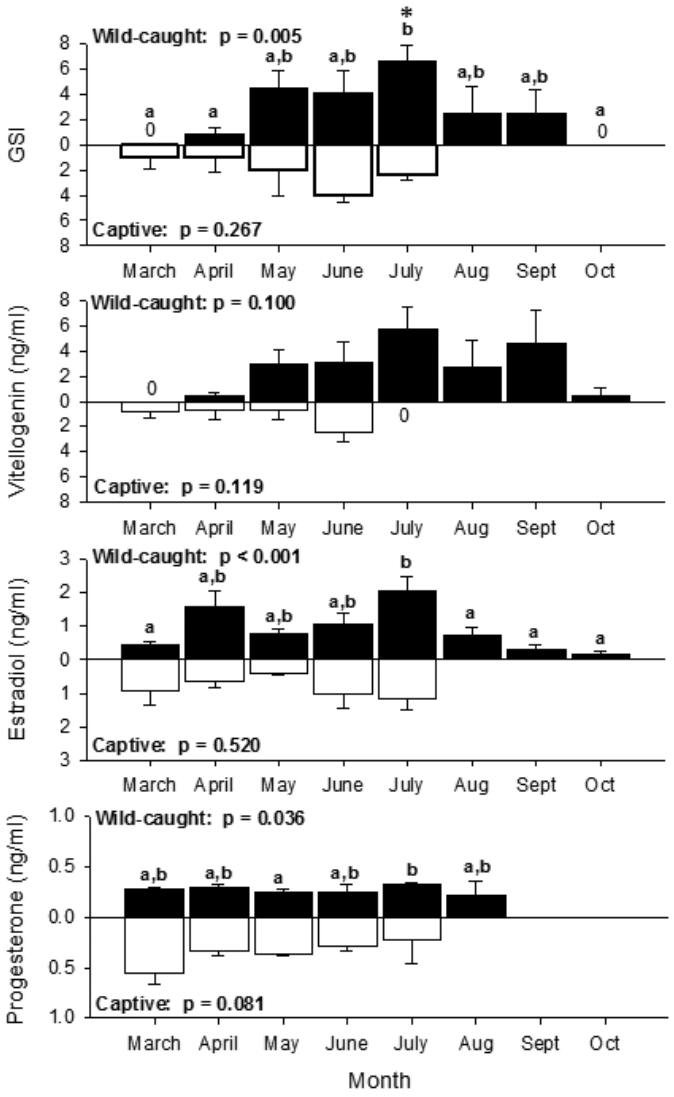

Figure 2. *Comparison of gonadosomatic index (GSI), plasma vitellogenin and steroid hormone concentrations in female bigmouth sleepers from wild–caught (solid bars) and captive (open bars) populations. GSI (gonad weight/body weight x 100), vitellogenin (ng/mL), estradiol (ng/mL), and progesterone (ng/mL) are presented. Progesterone was not measured in wild–caught fish in September and October. No captive fish remained after July samples. Error bars represent standard errors (se). The p–values reflect analysis of variance among months for each population, with pair–wise comparisons indicated with lowercase letters. Individual months that differed between wild–caught and captive fish are indicated with an asterisk (*).*

did not differ significantly between wild versus hatchery fish (F_1 = 3.03, p = 0.0880, r^2 = 0.04).

GSI

Wild–caught females showed clear temporal trends in GSI during spring and summer ($F_{7,37}$ = 6.36, p < 0.001). Values for GSI were below the minimum calculated GSI threshold of 0.4 in March samples, but increased from April through June, and peaked in July before declining in August and September to baseline levels in October (Figure 2). Minimum individual values for each month were below the minimum calculated GSI threshold of 0.4, and maximum individual values reached peaks in May through September of 9.5–12.0. Conversely, captive females had greater GSI values in March than wild females, which moderately increased in April and May before peaking in June, then dropped to nearly half the peak value in the final samples of captive fish in July (Figure 2). However, this trend was not statistically significant ($F_{4,11}$ = 1.837, p = 0.192). Minimum individual values of captive fish in every month were below the minimum calculated GSI threshold of 0.4, and maximum individual values peaked at nearly 6.0 for May and June. Monthly comparisons of wild–caught versus captive females found only one significant difference in GSI that occurred during July (t_6 = 2.58, p = 0.042; Table 2).

Wild–caught males had baseline GSI values below the minimum calculated GSI threshold of 0.4 for both March and April samples, followed by slight increases from May through June, peaking in July before decreasing to baseline values again in August ($F_{7,23}$ = 16.94, p < 0.001; Figure 3). A slight increase of GSI values was observed in September with a final decrease in gonadal development in October. Minimum individual values were below the minimum calculated GSI threshold of 0.4 for every month. Peak measurements for individuals were observed in June and July at 2.0 and 2.7, respectively. Captive males showed no discernable gonad development throughout the study, and no significant difference between wild and captive male GSI was found in any month, although differences approached significant levels in March, June, and July (Table 2).

Vitellogenin

The ability of the carp vitellogenin kit to accurately detect bigmouth sleeper vitellogenin was low in the range of samples tested (mean recovery of samples spiked with 5, 10, or 15 ng/mL = 84.4%). The precision of sample detection and ability to reproduce results from the same sample were within the established limits (%CV < 20%) for the validation (mean inter–assay %CV = 18.9%; mean intra–assay %CV = 11.3%; range 2.3–25.8%).

Plasma samples from wild–caught female bigmouth sleeper contained no detectable vitellogenin concentrations in March, followed by a steady increase in mean vitellogenin from April through June before peaking in July (Figure 2). Vitellogenin concentrations decreased from August to near initial concentrations by October. Minimum individual female vitellogenin concentrations were 0.0 for every month, and maximum concentrations for individual females reached 10.4, 11.3, and 17.0 ng/mL for July, August and September, respectively. There was no apparent relationship between fish size and maximum vitellogenin measurements. Statistical analysis did not indicate differences between months for vitellogenin ($F_{5,26}$ = 2.05, p = 0.100) despite an obvious trend graphically. This anomaly was likely the result of the low sample sizes in conjunction with the high variability in the data.

Plasma samples from captive females contained greater

concentrations of vitellogenin in March compared to wild fish, maintaining averages just below 1.0 ng/mL every month until June, when it peaked before dropping to 0.0 in July (Figure 2). Minimum individual female vitellogenin concentrations were 0.0 for every month except June, which had a minimum sample concentration of 1.4 ng/mL. Maximum concentrations for individual females ranged from 1.7 to 3.7 ng/mL from March through June. Monthly comparisons suggested no significant differences in vitellogenin concentrations between wild and captive populations (Table 2). However, wild–caught bigmouth sleeper in July had much greater concentrations than captive fish, and approached statistical significance (t_6 = –2.35, p = 0.057).

TABLE 2. Statistical results for comparisons of reproductive development indicators between wild and captive bigmouth sleeper populations. Test statistic *(T)* represents a Mann–Whitney Rank Sum Test used when normality or equal variance tests failed. A Student's t–test statistic *(t)* was used when normality and equal variance tests passed. **Bold type** indicates statistical significance at alpha level of 0.05. There were no captive fish remaining after July, so further comparisons were not possible.

Month	Female	Male	Vitellogenin	Estradiol	Progesterone	Testosterone
	GSI	**GSI**				
March	*T* = 20.000 p = 0.517	*T* = 18.000 p = 0.057	*T* = 18.500 p = 0.143	*t* = 1.500 p = 0.133	*T* = 11.000 p = 0.133	*t* = 3.742 **p = 0.013**
April	*T* = 14.000 p = 1.000	*T* = 6.000 p = 0.100	*T* = 14.000 p = 1.000	*t* = 1.309 p = 0.238	*t* = 0.953 p = 0.384	*t* = 0.267 p = 0.802
May	*t* = 1.267 p = 0.241	*T* = 7.500 p = 0.114	*t* = 1.166 p = 0.277	*t* = 1.511 p = 0.169	*T* = 24.000 p = 0.117	*t* = 0.260 p = 0.808
June	*t* = 0.582 p = 0.579	*T* = 18.000 p = 0.057	*t* = 0.309 p = 0.768	*t* = 0.106 p = 0.919	*t* = 0.550 p = 0.611	*t* = 1.025 p = 0.363
July	*t* = 2.584 **p = 0.042**	*T* = 18.000 p = 0.057	*t* = 2.351 p = 0.204	*t* = 1.425 p = 0.204	—	*t* = 4.033 **p = 0.010**

Estradiol

The ability of the estradiol kit to accurately detect bigmouth sleeper estradiol was acceptable in the range of samples tested (mean recovery of samples spiked with 1, 3, or 10 ng/mL = 90.0%). However, the precision and ability to reproduce results from the same sample did not fall within the established limits (%CV < 20%) for the validation (mean inter–assay %CV = 21.7%; mean intra–assay %CV = 16.8%, range 6.4–22.8%).

Plasma samples from wild–caught females contained low concentrations of estradiol in March and exhibited a slight increase in April (Figure 2). This trend was followed by a decline in plasma estradiol concentrations in May before concentrations peaked in July (Figure 2). Another decline in estradiol concentrations began in August, which led to low concentrations in September and October. Minimum individual estradiol concentrations never dropped below 0.05 ng/mL, which was found in October. Maximum readings for individual fish were 3.5 and 3.3 ng/mL, respectively; minimum and maximum values were recorded in April and July, respectively.

Captive female bigmouth sleeper contained greater plasma estradiol concentrations in March than wild fish, and exhibited a decline in concentrations through April and May before increasing in June and peaking in July (Figure 2). The minimum individual plasma estradiol concentration was 0.3 ng/mL from a female sampled in May. Maximum plasma estradiol concentrations for individual fish were 1.8 and 1.9 ng/mL for March and June, respectively. Monthly comparisons between wild and captive populations found no significant differences in estradiol concentrations (p > 0.05) (Table 2).

Progesterone

The ability of the progesterone kit to accurately detect bigmouth sleeper progesterone was acceptable (mean recovery of samples spiked with 1, 5, or 10 ng/mL = 94.4%). The precision and ability to reproduce results from the same sample was within the established limits for the validation (mean inter–assay %CV = 12.9%; mean intra–assay %CV = 9.8%, range 1.2–13.7%).

Wild–caught female bigmouth sleeper showed no discernible trends in plasma progesterone concentrations, averaging between 0.2 and 0.3 ng/mL throughout the study (Figure 2). The minimum and maximum progesterone concentrations for individual bigmouth sleeper were 0.1 and 0.5 ng/mL, respectively. Both minimum and maximum values were recorded in August. Captive female bigmouth sleeper showed slightly more variation in progesterone concentrations, ranging from 0.3 to 0.6 ng/mL, with a steady decline from March to July, though the trend was not statistically significant (H_4 = 8.31, p = 0.081) (Figure 2). The minimum and maximum plasma progesterone concentrations for individual captive fish were 0.2 and 0.7 ng/mL, respectively; minimum and maximum values were recorded in June and March, respectively. Monthly comparisons of wild and captive populations found no significant differences in progesterone concentrations (Table 2). Due to no discernable trends in progesterone data, no testing was conducted for progesterone in September and October.

Testosterone

Plasma testosterone concentrations (mean intra–assay %CV = 8.4%, range 0.6–14.2%) in wild–caught male big-

mouth sleeper steadily increased from March to June before increasing rapidly in July (Figure 3). Mean testosterone concentrations peaked in August and September before declining in October. Minimum and maximum testosterone concentrations for individual fish were 0.3 and 6.5 ng/mL, respectively; minimum and maximum values were recorded in March and August, respectively.

Captive male bigmouth sleeper showed little variation in plasma testosterone concentrations for the duration of the study, with monthly averages ranging from 0.6 to 0.8 ng/mL. Minimum and maximum values for individual fish were 0.3 and 0.9 ng/mL, respectively; minimum and maximum values were recorded in April and March, respectively. Comparisons of testosterone concentrations in wild–caught versus captive bigmouth sleeper indicated significant differences in both March and July (Table 2). In March, the average testosterone concentration for captive fish was twice that of wild fish (t_5 = 3.74, p = 0.013). In July, testosterone concentrations in wild fish peaked and averaged more than four times that of captive male testosterone concentrations (t_5 = –4.03, p = 0.010).

Pearson product–moment correlation coefficient

For wild–caught bigmouth sleeper, significant positive correlations over time were detected between female GSI and vitellogenin (r = 0.90, p < 0.001) and estradiol (r = 0.47, p < 0.001), but not for progesterone (r = –0.24, p = 0.151). Vitellogenin production was positively correlated with estradiol concentration (r = 0.28, p = 0.033), but not progesterone concentration (r = –0.25, p = 0.123). Estradiol concentration was not correlated with progesterone concentration (r = –0.13, p = 0.445).

Discussion

Seasonal trends in GSI and plasma vitellogenin, estradiol, and total testosterone concentrations indicated that the spawning season of bigmouth sleepers is protracted over several months, but occurs primarily in July and August in wild river populations of southwestern Puerto Rico. For female bigmouth sleepers, vitellogenin concentrations and GSI values peaked in July, while estradiol concentrations showed a bimodal distribution. Estradiol peaked in April and again in July coincident with the peak of GSI values. The bimodal pattern of estradiol secretion found in this study corresponded to an initial increase prior to vitellogenesis and gonadal development (as indicated by GSI) and a second increase prior to presumed spawning. This bimodal pattern of estradiol secretion has been documented in other fish species (Jackson and Sullivan 1995). The correlation between estradiol, vitellogenin, and GSI were anticipated with ovarian development preceding spawning, and is attributed to vitellogenesis induced by secretion of estrogen. The secretion of estradiol during ovarian development stimulates vitellogenin synthesis in the liver. Vitellogenins are integrated into developing eggs as phosvitin and lipovitellin, resulting in increased ovarian size and GSI (Davis et al. 2005). Data from this study similarly indicate the same progression with an increase in estradiol secretion followed by simultaneous increases in vitellogenin production and GSI.

The lack of any noticeable increase in production of total progesterone during this study was enigmatic, as Zohar and Billard (1984) reported several periods of increased progesterone–derived 17α–hydroxy–20β–dihydroprogesterone during the maturation cycles of fish. While the assay kit used to detect progesterone was only 3.46% cross–reactive with 17α–hydroxy–20β–dihydroprogesterone, increased progesterone secretion would be likely in response to elevated 17α–hydroxy–20β–dihydroprogesterone, as it is the primary precursor to this hormone. However, no such increases in progesterone production were noted in this study. Perhaps although concentrations of 17α–hydroxy–20β–dihydroprogesterone undergo several changes during fish gonad matura-

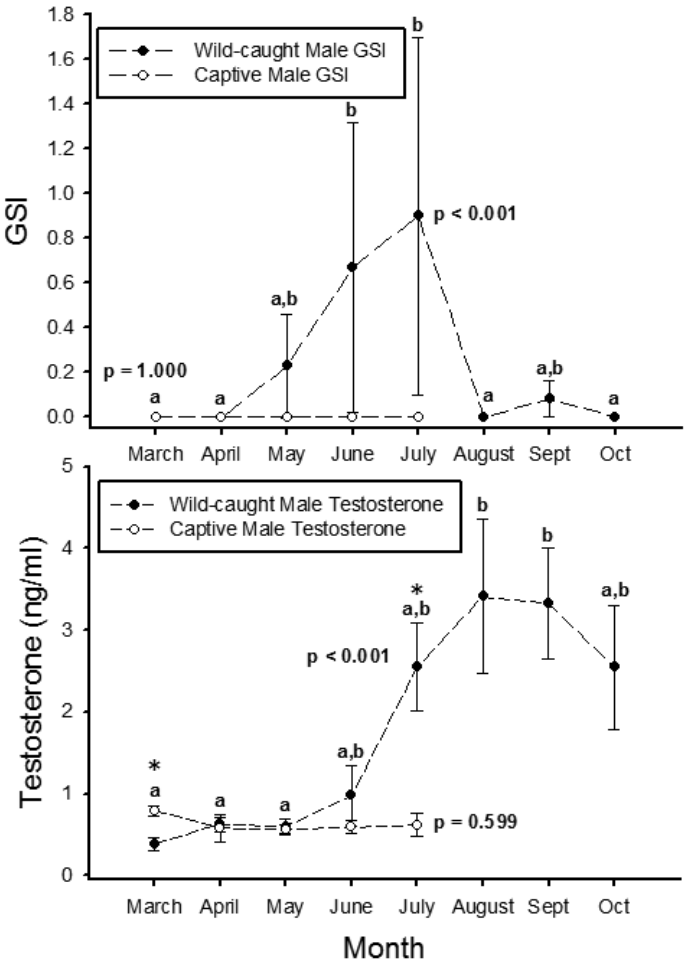

Figure 3. *Comparison of gonadosomatic index (Top; GSI = gonad weight/body weight x 100) and plasma testosterone concentrations (Bottom) in wild–caught and captive male bigmouth sleepers. Error bars represent standard errors (se). The p–values reflect analysis of variance among months for each population, with pair–wise comparisons indicated with lowercase letters. Individual months that differed between wild–caught and captive fish are indicated with an asterisk (*).*

tion, concentrations of its precursor progesterone remain relatively unchanged in bigmouth sleepers. Significant increases in progesterone concentrations just prior to final maturation may have been missed, as we were unable to collect any females that were in the final stages of maturation or about to undergo ovulation. 17α—hydroxy—20β—dihydroprogesterone is often only apparent for short durations during maturation and easily could have been missed. Very little is known about maturation or hormone cycling in bigmouth sleepers, so it may be possible that progesterone is not a maturation hormone in this species.

Wild male bigmouth sleeper GSI peaked in July and dropped to below the minimum calculated GSI threshold of 0.4 in August, but testosterone concentrations continued to increase in August before gradually decreasing through September and October. One explanation for this was that total testosterone production was actually measured, not specifically 11—ketotestosterone. 11—ketotestosterone is the primary sexual steroid responsible for gonadal maturation in male teleost fish and the greatest concentrations of 11—ketotestosterone have been recorded just prior to the start of spermiation (Zohar and Billard 1984). Production of 11—ketotestosterone may have declined following the GSI peak in July, though total testosterone continued to increase. Male bigmouth sleepers are nest guarders (McKaye et al. 1979) and the increased production of total testosterone may be a physiological response to territorial defense and nest guarding activities.

The primary environmental stimulus for reproductive development in females appeared to be water temperature. Captive fish held in warm ponds (March temperature = 27.8°C) demonstrated earlier development than wild fish in cooler rivers (March temperature = 23°C). In Carite Reservoir, peak reproductive development was intermediate to ponds and rivers, which would be expected based on the surface temperature of 26°C recorded by Bacheler (2002) on 6 April 2001, about two weeks later in the year than the March data from this study. Bacheler et al. (2004a) also reported peak GSI values in May and June, indicating that spawning occurred during these months.

Lack of male development in ponds, relative to wild fish, as indicated by lower GSI and testosterone concentrations suggests that males may require another stimulus for reproductive development not present in ponds, such as increased water discharge. The importance of elevated discharge to spawning behavior has been documented for Hawaiian gobies and eleotrids (Fitzsimons et al. 2002). This hypothesis is further supported by data showing greater numbers of bigmouth sleeper larvae were collected in the wet season than in the dry season during sampling in the Tecolutla River in Veracruz, Mexico (Hernández—Saavedra et al. 2004). It may be possible to artificially provide this stimulus in a hatchery situation, and further research on this concept is warranted.

The sample sizes used during this study were relatively small due to the remote location, decline in native populations, and difficulty in collection of mature specimens. Similarly, captive fish sample sizes were limited because of low fish availability, as all captive broodstock at the Aquaculture Research Station were sacrificed for this research. Despite low sample sizes, the statistical design maintained enough power (> 0.8) to determine statistically significant differences in hormone concentrations at monthly sampling intervals. While more samples may be needed to truly characterize hormonal changes during the bigmouth sleeper spawning season, the data gathered nonetheless allow for determination of when spawning cycles were initiated and when peak spawning periods for bigmouth sleepers occurred on the island of Puerto Rico.

The spawning season for wild river populations of bigmouth sleepers is protracted over several months, but appears to peak in July and August in southwestern Puerto Rico. Captive female bigmouth sleeper broodstock held in shallow hatchery ponds matured earlier in the season likely because of accelerated environmental stimuli, increased water temperatures in particular. Therefore, induced and natural spawning attempts for captive bigmouth sleeper broodstock should be conducted in June through July, while wild broodstock should be collected and spawned in July and August. However, as gonad development of captive male bigmouth sleeper requires an additional stimulus that is presently unknown, collection and spawning of wild broodstock is currently the only viable option for bigmouth sleeper propagation.

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