

Notes on hatchery spawning methods for bigmouth sleeper *Gobiomorus dormitor*

Nathan J Harris, Jason Wesley Neal*, Peter W Perschbacher, Christopher E Mace[†] & Milton Muñoz-Hincapié*

Aquaculture/Fisheries Center of Excellence, University of Arkansas at Pine Bluff, Pine Bluff, AR, USA

Correspondence: N J Harris, PO Box 1554, Willow Creek, CA 95573, USA. E-mail: njharris30@gmail.com

***Present address:** J W Neal and M Muñoz-Hincapié, Department of Wildlife and Fisheries, Thompson Hall, Box 9690, Mississippi State, MS 39762-9690, USA.

[†]**Present address:** C E Mace, Texas Parks and Wildlife, PO Box 1844, Rockport, TX 78381, USA.

Abstract

The bigmouth sleeper *Gobiomorus dormitor*, a popular sport fish within its native range, offers a management alternative to exotic species currently managed for recreational fishing. Hatchery production and stocking are needed to create reservoir sport fisheries because this species usually requires access to marine environments for recruitment. Bigmouth sleepers have not been spawned previously in a hatchery, and hence we used natural, artificial and semi-natural spawning techniques. No egg deposition or propagation resulted from natural pond spawning. Artificial spawning techniques using Ovaprim[®] and Chorulon[®] injections followed by hand stripping were more successful. Semi-natural (hormone injections plus volitional spawning) spawning trials using Ovaprim[®] and Chorulon[®] displayed the greatest potential, and three consecutive evening injections (1.0 mL kg^{-1}) of Chorulon[®] appeared most conducive. High-fecundity spawning was achieved by injecting bigmouth sleeper pairs and allowing volitional spawning in aquaria with spawning cavities. Initial success in 2007–2008 was isolated to wild-caught fish, but captive broodstock were spawned using these techniques in 2009. Eggs hatched in < 20 h at 23–24 °C. The resulting larvae, 1.0–1.5 mm in length, displayed large yolk sacs, unpigmented eyes and no apparent mouth. These hatchery propagation efforts suggest that hormone-induced semi-natural spawning has the greatest potential for bigmouth sleeper propagation.

Keywords: bigmouth sleeper, *Gobiomorus dormitor*, Puerto Rico, hatchery spawning, native species, reservoir stocking

Introduction

The bigmouth sleeper *Gobiomorus dormitor* (Lacepède) is distributed in coastal areas of the Caribbean, northern parts of South America and southern Florida and Texas (Lindquist 1980). Studies suggest that this species is diadromous, requiring unimpeded access between freshwater and marine environments (Holmquist, Schmidt-Gengenbach & Yoshioka 1998). Consequently, impoundment of river systems disrupts the migration patterns of bigmouth sleeper and other diadromous species, thus eliminating reproduction and extirpating native fish from most reservoirs and upstream reaches. To fill the void, non-native species are introduced to create sport fishing opportunities (Neal, Noble, Olmeda & Lilyestrom 2004).

In Puerto Rico, there are no truly freshwater native species, and only a handful of euryhaline species that require open access to the marine environment to complete their life cycles (Neal *et al.* 2004). Bigmouth sleepers grow well in lentic freshwater systems when they are present, and anglers often target them for sport and food (Neal, Noble, Bachelier, McGee & Lilyestrom 2001; Bachelier 2002). 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, Noble, Gran & Alicea 1995; Neal, Noble, Lilyestrom, Churchill, Alicea, Ashe, Holliman & Waters 1999). In rare situations, self-sustaining landlocked populations have been reported (Darnell 1962; Mckaye, Weiland & Lim 1979; Bedarf, Mckaye, Van Den Berghe, Perez & Secor 2001), including one reservoir in Puerto Rico (Bachelier, Neal & Noble 2004). 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.

Because of their popularity with anglers and their adaptability to reservoir conditions (Bacheler 2002), bigmouth sleepers may offer an opportunity to use a native species as an alternative to non-native sport fish species in tropical reservoirs. However, because of their limited reproductive capacity in reservoirs, introductory and maintenance stocking would be required to create and maintain these fisheries. This necessitates the development of spawning and rearing protocols for this species.

There have been few observations on the natural spawning of bigmouth sleepers (Harris 2007; Adelsberger 2009), and no attempts to propagate them in a hatchery setting have been reported. Other eleotrid species have garnered more spawning research. The marble (or sand) goby *Oxyeleotris marmoratus*, a high-value fish also known as the soon hock (Herbert & Graham 2004), has been cultured for decades in ponds and in cages in lakes and rivers (Luong, Yi & Lin 2005). Spawning is usually natural, in that broodstock are allowed to spawn volitionally without hand stripping. Sleepy cod *Oxyeleotris lineolatus*, an endemic Australian fish closely related to the marble goby, has also been reproduced in captivity. Both species are cavity spawners, and substrates successfully used include polyvinyl chloride (PVC) pipe cut into two halves, corrugated piping and asbestos egg collectors formed into triangles (Tavarutmaneegul & Lin 1988; Cheah, Senoo, Lam & Ang 1994; Herbert & Graham 2004). The bigmouth sleeper is also believed to be a cavity spawner, and similar spawning structures and propagation techniques may have efficacy in the hatchery production of bigmouth sleepers.

The goal of this study was to determine methods for spawning bigmouth sleepers in a hatchery environment. Voluntary pond spawning (natural), hand-stripping following hormone induction (artificial) and tank spawning following hormone induction (semi-natural) were attempted from 2005 to 2009. Two hormone types and multiple injection regimens were tested in a multifaceted approach to identify protocols that could be successful.

Methods

Study area

Natural pond spawning was attempted in 100 m² ponds with a depth up to 1.0 m at the Agricultural Research Station of the University of Puerto Rico-Mayagüez (UPRM) located in Lajas, Puerto Rico. Semi-

natural and artificial propagation research were conducted at the research nursery of the Maricao Fish Hatchery (MFH) located in Maricao, Puerto Rico. The MFH research nursery contains a new 6660 L recirculation research nursery, composed of four 1665 L circular tanks with aeration, filtration and gravity flow-thru options; 16 multipurpose aquaria (76 L); and a flow-thru McDonald-type hatching jar/raceway egg incubation system for larval hatching and rearing with recirculation capabilities.

Broodstock for the trials were collected from two reservoirs and three river systems. The reservoir sources were Carite Reservoir, a 124 ha impoundment located at 18°04'N by 66°05'W in the mountains of south-central Puerto Rico, and Patillas Reservoir, a 126 ha impoundment located southeast of Carite Reservoir at 18°02'N by 66°02'W. Carite Reservoir contains the only known reproducing landlocked population of bigmouth sleepers in Puerto Rico, while Patillas Reservoir contains a relic population of large individuals that enter the reservoir during infrequent high-flow events (Bacheler 2002). Riverine broodstock sources were the Rosario River, located on the west side of the island northeast of the city of Hormigueros; the Nueve Pasos River on the west side of the island, northwest of the city of San Germán; and the Cañas River, which drains southwards near the city of Ponce. All three rivers contain native diadromous bigmouth sleeper populations.

Reservoir fish collection was conducted using boat-mounted electrofishing at 7–8 amps and 60 Hz along the shoreline, and river fish were collected using a backpack electrofishing unit set at up to 400 V DC and 60 Hz. Captured fish were transported from water bodies to holding locations in a divided 560 L hauling tank with constant aeration. Gender determination was made using genital papillae. Male papillae are long and thin with a terminal pore and female papilla are circular with bristles on the posterior and a flap partially covering the genital pore (Bacheler 2002).

Natural pond spawning

Natural bigmouth sleeper spawning in Puerto Rico occurs in late spring through the end of summer (Bacheler 2002; Harris 2007). Broodstock for natural pond spawning were collected from wild riverine and reservoir populations before the spawning season in early March, and transported to the UPRM Research Station.

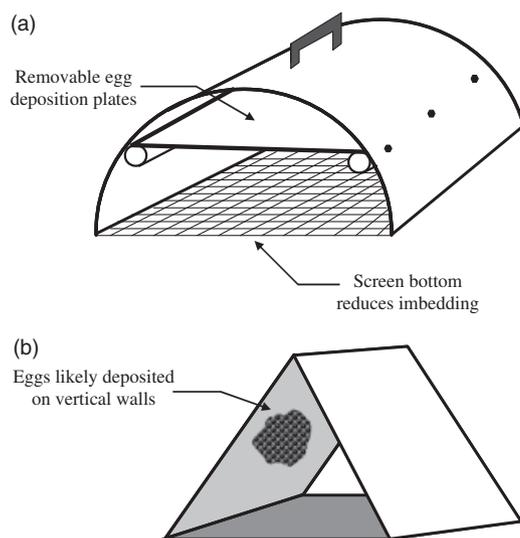


Figure 1 Two of the spawning structure designs available to bigmouth sleepers in this study. (a) Spawning traps modelled after structures used by Herbert and Graham (2004) for sleepy cod. The egg deposition board rested on polyvinyl chloride (PVC) guides and was removable. (b) Spawning triangles modelled after Cheah *et al.* (1994). Segments (45 cm) of 30 cm diameter PVC piping were also provided.

Before the study, four 100 m² ponds were stocked with a self-sustaining prey base of mollies *Poecilia* spp., platies *Xiphophorus* spp., tilapia *Oreochromis* and *Tilapia* spp. and threadfin shad *Dorosoma petenense*. Spawning structures, based on successful designs used with sleepy cod and marble goby, were placed in ponds before the introduction of broodstock. Three spawning structure designs were used in this study: (1) whole PVC pipes of 30 cm diameter and 45 cm length, (2) plastic 30 × 30 × 30 cm triangular structures based on Cheah *et al.* (1994) and (3) spawning traps with removable egg deposition plates based on Herbert and Graham (2004) (Fig. 1). Two of each type of structure were placed in every pond so that males had a free choice of substrate and were not influenced by competition with other males (Masson & Stone 2005). Ponds were partitioned into six equivalent sections and each was assigned a structure randomly. All structures were in place before fish stocking.

Each pond was stocked with six sexually mature bigmouth sleepers, two male and four female, at the end of March. Only broodstocks exceeding 200 mm total length were selected whenever possible, as Bachelier (2002) reported that the minimum length at maturity was 159 and 179 mm total length for males

and females respectively. Following stocking, spawning structures were checked weekly between 08:00 and 12:00 hours for the presence of eggs, and ponds were seined monthly to check for offspring from April to October. Temperature and dissolved oxygen were recorded weekly. Ponds were drained in late October to check for juvenile production.

Artificial spawning

Broodstock for the artificial spawning trials were collected from the wild and transported to MFH for delayed hormone induction in 2006 (up to 14 days), and for immediate hormone induction in 2007 and 2008. For each trial, an experimental group of sexually mature pairs were injected in the dorsal musculature. Spawning agents were Ovaprim[®] (Syndel Laboratories, BC, Canada) and Chorulon[®] (Intervet/Schering-Plough Animal Health, Millsboro, DE, USA), and several different dosages and regimens were tested (Table 1). Syringes were sterilized with 70% ethyl alcohol before injection, and treated fish were placed in a 2 mg L⁻¹ NaCl bath for 5–10 min post injection before being returned to holding tanks.

Broodstock were examined for up to 3 days post injection. When free-flowing eggs were observed, they were collected on a sheet of fine-meshed fabric and mixed with milt for approximately 1 min before being placed in hatching jars with moderate water flow. Milt was collected by euthanizing males with tricaine methanesulphonate (MS-222) and removing and macerating the testes. Milt was diluted with a small amount of water and poured over the eggs. Hatching jars were checked regularly for 48 h following attempted fertilization to check for hatching larvae.

Semi-natural spawning

In 2008, two female bigmouth sleepers injected with Chorulon[®] as part of the artificial spawning trials spawned overnight on the vertical wall of a glass aquarium. This 'semi-natural' spawning activity prompted new trials to attempt hormone-induced passive spawning. Over the next 2 years, broodstock were collected from the Cañas, Rosario and Nueve Pasos Rivers and Carite and Patillas Reservoirs. These fish were transported to the MFH for immediate hormone induction. Fish collection and injection were timed according to the natural breeding season (Bachelier 2002).

Table 1 Summary of bigmouth sleeper spawning trials in Puerto Rico from 2005 to 2009

Date	Trial type	Number of females	Number of males	Spawning agent	Dosage/regimen [mL kg ⁻¹ (h)]	Spawning notes	Number hatched larvae
2005							
Summer	Natural	16	8	None	None	No evidence of spawning	0
2006							
Summer	Natural	16	8	None	None	No evidence of spawning	0
4/7/2006	Artificial	5	3	Ovaprim [®]	0.1/0.4 (8)	No eggs	0
4/20/2006	Artificial	5	4	Ovaprim [®]	0.1/0.4 (8)	No eggs	0
5/18/2006	Artificial	5	5	Ovaprim [®]	0.1/0.4 (8)	Increased milt	0
6/15/2006	Artificial	6	6	Ovaprim [®]	0.5	Increased milt	0
6/21/2006	Artificial	10	6	Ovaprim [®]	0.5	No eggs	0
6/28/2006	Artificial	3	3	Ovaprim [®]	0.5	No eggs	0
7/26/2006	Artificial	7	6	Ovaprim [®]	0.1/0.4 (8)	No eggs	0
2007							
5/18/2007	Artificial	3	3	Chorulon [®]	1.0/1.0/1.0/1.0 (24)	No eggs	0
5/29/2007	Artificial	16	13	Chorulon [®]	1.0/1.0/1.0/1.0 (24)	Seven females spawned	10 000
6/6/2007	Artificial	8	10	Chorulon [®] /Ovaprim [®]	1.0/1.0/1.0/1.0 (24)	Three females spawned	0
6/12/2007	Artificial	8	12	Chorulon [®] /Ovaprim [®]	1.0/1.0/1.0/1.0 (24)	One female spawned	200
6/26/2007	Artificial	11	5	Chorulon [®] /Ovaprim [®]	1.0/1.0/1.0/1.0 (24)	One female spawned	0
2008							
7/16/2008	Artificial	3	3	Chorulon [®]	1.0/1.0/1.0/1.0 (24)	At least one female spawned	4000
7/16/2008	Semi-natural	3	3	Chorulon [®]	1.0/1.0/1.0/1.0 (24)	At least one female spawned	12 000
7/22/2008	Semi-natural	24	20	Chorulon [®]	1.0/1.0/1.0/1.0 (24)	At least one female spawned	10 000
8/19/2008	Semi-natural	20	17	Chorulon [®]	1.0/1.0/1.0/1.0 (24)	At least two females spawned	40 000
9/14/2008	Semi-natural	7	6	Chorulon [®]	1.0/1.0/1.0/1.0 (24)	At least one female spawned	0
2009							
7/6/2009	Semi-natural	17	10	Chorulon [®]	1.0/1.0/1.0/1.0 (24)	At least one female spawned	10 000
8/3/2009	Semi-natural	8	10	Chorulon [®]	1.0/1.0/1.0/1.0 (24)	At least three females spawned	50 000
8/24/2009	Semi-natural	12	7	Chorulon [®]	1.0/1.0/1.0 (24)	No evidence of spawning	0
8/31/2009	Semi-natural	11	10	Chorulon [®]	1.0/1.0 (24)	At least three females spawned	107 000
9/11/2009	Semi-natural	2	2	Chorulon [®]	1.0/1.0/1.0 (24)	At least one female spawned	50 000
9/18/2009	Semi-natural	2	2	Chorulon [®]	From dosage applied 9/11/2009	At least one female spawned	10 000

Natural pond spawning (natural), artificial hormone spawning with hand stripping (artificial) and natural spawning following hormone induction (semi-natural) were attempted. The dosages per injection (mL kg⁻¹) and maximum number of injections per trial are presented with the time interval (h) between injections in parentheses.

Chorulon[®] was used to stimulate reproduction for these trials, as it appeared to have the greatest efficacy in previous artificial spawning trials. Dosages administered were 1.0 mL kg⁻¹, with a regimen of two to four injections given once per day (24 h interval). Treated fish were placed in a slightly saline (2 mg L⁻¹ NaCl) recovery tank for 5–10 min post injection before being placed in blue 1665 L spawning tanks with constant aeration and the spawning structures described previously.

Fish were monitored for at least 1 week following injections for evidence of spawning, and structures were checked every 24 h for egg deposition. When eggs were detected on the spawning structures, they were removed if possible and hatched in hatching jars or 76 L aquaria. Occasionally, eggs were not detected

before hatch or could not be removed. These were allowed to hatch in the spawning tanks and the larvae were removed using Wisconsin plankton nets.

Broodstock were fed regularly with tilapia. Water temperatures and dissolved oxygen concentrations were monitored throughout the study.

Results

Spawning trials

Results from all spawning trials are presented in Table 1. There was no evidence of natural pond spawning during this study in 2005 and 2006. Average morning water temperatures in breeding ponds ranged from 28.1 to 32.1 °C during the presumed

spawning season, which Bacheler (2002) defined as late-spring to summer in Carite Reservoir, Puerto Rico. Average morning dissolved oxygen concentrations ranged from 4.8 to 7.5 mg L⁻¹. Weekly checks of spawning structures showed no evidence of bigmouth sleeper egg deposition. Monthly seining from May through August produced no evidence of bigmouth sleeper juveniles, and no juveniles were collected when the ponds were drained in October of each year. Broodstock appeared healthy and well-fed at pond draining. Natural pond spawning was discontinued in 2007.

Initial attempts at artificial spawning using Ova-prim[®] in 2006 were not successful during seven trials using a total of 41 females and 33 males. No free-flowing eggs were obtained of sufficient development or number to attempt fertilization. No males exhibited free-flowing milt before hormone injection, but 40% of males had milt flow after injection. Broodstock survival was over 90% initially following injection, and swelling at injection sites was minimal. However, the stress and damage associated with hand stripping resulted in high delayed mortality within 2 weeks following each trial.

Successful reproduction was achieved in 2007 using artificial spawning techniques. During five trials, 46 females and 43 males were induced using Chorulon[®] or Ovaprim[®]. Twelve (26%) females released eggs during these trials. Both Ovaprim[®] and Chorulon[®] induced ovulation, but total fecundity and hatch rates appeared to be higher for Chorulon[®]-induced fish. A regimen of 1.0 mL kg⁻¹ Chorulon[®] with up to four separate injections spaced 24 h apart was most effective. An estimated 10 200 larvae were hatched during the 2007 artificial spawning trials. Again, the stress of injection and handling resulted in high mortality of brood fish following artificial spawning trials.

The first artificial spawning trial of 2008 produced about 4000 larvae. More importantly perhaps, a female injected with Chorulon[®] spawned volitionally on the vertical wall of a glass aquarium during the injection period. Because of this finding, the semi-natural trials were initiated, yielding greater fecundities and hatching success than the artificial spawning trials. In 2008, 54 females and 46 males were injected with a regimen of 1.0 mL kg⁻¹ Chorulon[®] spaced 24 h apart with up to four injections. Spawning fish generally released eggs following the third injection. An estimated 62 000 larvae were produced using semi-natural spawning in 2008.

Only semi-natural spawning trials were conducted in 2009. Five trials using 50 females and 39 males and a regimen of 1.0 mL kg⁻¹ spaced 24 h apart with up to four injections were conducted. An estimated 217 000 larvae were produced in 2008 using the semi-natural approach. Two to three injections were typically best. During the fifth trial, a second spawn which produced 10 000 larvae occurred 8 days after the end of the injection period. It was not clear if this was from a female that had spawned during the primary trial. Broodstock mortality following all semi-natural spawning trials was minimal.

Another breakthrough in 2009 was the first spawning of captive brood stock. Adult bigmouth sleepers that were collected in 2008, held in 1665 L hold tanks, and fed tilapia for 1 year were successfully spawned in 2009. These fish were cycled into the semi-natural spawning trials with success. Because these fish were generally larger than wild-caught broodstock, it appeared that fecundities were greater as well.

Adult spawning coloration and larval characteristics

Clear differences in male and female coloration were observed in hormone-injected fish before spawning, and later confirmed in river fish during June field collections. Males displayed darkened skin along their lateral surfaces with contrasting yellow spots that began slightly anterior to the pectoral fins and continued into the caudal fin. Yellow spots were also present on dorsal and anal fins. Red coloration appeared on the tips of several spines on the anterior dorsal fin and to a lesser extent, on the dorsal side of the caudal fin. Additionally, numerous red spots were visible beginning at the head and eyes, which gradually disappeared close to the dorsal fin. Females also exhibited some or all of these colorations, but generally lacked dark coloration on the sides, thereby making the yellow spots less noticeable. Coloration on the dorsal fin, if present, appeared light red or orange. These patterns appeared to be more common on smaller and younger female fish, and duller or lacking on larger fish.

In addition, a redness and swelling of the genital papillae, particularly in females, seemed to be predictive of reproductive readiness. When gently squeezed to check for flowing gametes, both males and females demonstrated an erectile function in genital papillae as a necessary precursor to the release of eggs or milt during artificial spawning trials.



Figure 2 Photo of 1-day-old bigmouth sleeper larva.

Based on eggs fertilized at 1400 h and larvae observed at 1000 h the following morning, it appears that larvae hatch and are free swimming in <20 h post fertilization at a temperature of 23–24 °C. Larvae maintain their position within the water column through short swimming bursts towards the surface, alternating with head-first descents. Larvae at hatch are 1.0–1.5 mm TL with a large yolk sac, unpigmented eyes and no apparent mouth (Fig. 2).

Discussion

Bigmouth sleepers can be successfully induced to spawn using Chorulon[®] injections followed by either active hand stripping or passive spawning in tanks. A Chorulon[®] injection regimen of up to three 1 mL kg⁻¹ injections, each separated by 24 h, appeared to be most effective, as the majority of eggs obtained and successfully fertilized followed the third injection. Semi-natural spawning was the most effective spawning technique, as breeding pairs typically spawned voluntarily using a series of hormone injections. The eggs were placed on vertical or overhead surfaces. Fecundity and hatching success both appeared to be highest for semi-natural spawns, and post-spawning survival of broodstock was greatly improved using this technique.

The main factor determining whether induction was successful in wild broodstock appears to be spawning readiness of individual fish at the time of collection, which may vary by several months depending on environmental conditions in different habitats. Natural reproductive cycles of bigmouth sleepers are correlated to water temperature (Harris 2007), and spawning success in the present study

corresponded to peak spawning periods in the source reservoir (Bacheler 2002). Seasonal trends in GSI and vitellogenin, oestradiol 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 (Harris 2007). If broodstock were collected too far before or after peak spawning, they would have been less likely to spawn.

In addition, broodstock collected in 2006 were not injected immediately and likely had some stress-related egg resorption (Rottman, Shireman & Chapman 1991), as well as unnatural environmental stimuli in holding tanks (temperature changes, reduced flow, etc.). Consequently, wild broodstock should be collected at the height of a population's reproductive season and injected with hormones immediately after collection, as was performed in subsequent years. However, the successful spawning of captive broodstock, combined with the greatly reduced broodstock mortality associated with semi-natural spawning, presents an opportunity to streamline production by eliminating the step of broodstock collection from the wild. This will greatly reduce time and labour requirements, as well as the impact on wild populations. Although nothing is known about bigmouth sleeper longevity, it is assumed that individual broodstock can be held and spawned for at least 2–3 years before a replacement is needed.

Diel timing of injections may also be important, as several eleotrid species commonly spawn in the early morning hours to midday (Auty 1978; Herbert & Graham 2004). In these trials, evening injections appeared to be more successful, possibly because hormone injections were timed to better correlate with diel hormone fluctuations (Zohar & Billard 1984). Early morning spawning, together with hatching of larvae in <20 h, has been documented in other eleotrid species (Auty 1978) and amphidromous gobies (Iguchi & Mizuno 1990, 1991; Moriyama, Yanagisawa, Mizuno & Omori 1998; Delventhal 2000). Early morning spawning followed by late evening hatching would synchronize larval emergence and journey downstream with darkness hours of 22:00–04:00 hours, thereby reducing predation by sight-feeders on larvae (Auty 1978; McKaye *et al.* 1979). Adelsberger (2009) supported this, reporting evidence that bigmouth sleeper larvae drift at night in Puerto Rico streams.

Larvae emerge quickly in a hatchery setting, with free-swimming larvae observed in <20 h following

fertilization. The small size of larvae is similar to other eleotrids: including *Dormitator latifrons* (Todd 1975), *Hypseleotris compressus* (Auty 1978) and *Dormitator maculatus* (Nordlie 2000). Auty (1978) described small size, rapid development, unpigmented eyes, large yolk sac and an undeveloped mouth as characteristic of marine pelagic development. In the case of bigmouth sleepers and other peripherally freshwater species, the adaptation to spawning in freshwater requires that larvae are carried downriver to estuarine or marine areas to feed. Furthermore, the upward swimming behaviour of bigmouth sleeper larvae is similar to that described for two amphidromous gobies, *Sicyopterus stimpsoni* and *Sicyopterus lagocephalus* (Keith 2003), and the eleotrid, *D. latifrons* (Todd 1975). Balon and Bruton (1994) asserted that this behaviour is beneficial for movement downstream to the ocean.

Sexually dimorphic coloration in bigmouth sleepers has not been described previously, but has been noted in other eleotrids and gobies (Auty 1978; Teixeira 1994; Makeeva 2002). Reddish coloration in genital papillae of gravid females has also been documented in marble goby (Cheah *et al.* 1994). The erectile function in genital papillae appeared to be an indicator of spawning readiness, and this may have utility in reducing handling and associated stress before ovulation.

Spawning structures used in this study were too large to be used in aquaria, and hence most spawning occurred in the larger holding tanks. Smaller structures such as those used for desert goby *Chlamydogobius eremius* (Ralph & Ralph 1988) or the eleotrid *Micropercops cinctus* (Makeeva 2002) could be used in aquaria with smaller bigmouth sleepers. Aquarium spawning would facilitate the observation of broodstock pairs and easier removal and hatching of egg masses.

Although passive pond spawning was not realized during this study, it may still prove useful for hatchery production of bigmouth sleepers. The shallow depth of the ponds combined with lack of shade, low elevation and frequent water shortages resulted in high March water temperatures (28.1–32.1 °C). In addition, pond bottoms were covered in a thick layer of sediment due to many years of use without adequate drying or reshaping. It is also possible that eggs or larvae were missed due to their rapid hatch times or small size at hatch. Additional research may find that passive pond spawning is possible under different environmental conditions.

This research was the first step in the creation of hatchery protocols for bigmouth sleepers. There is still much to learn about the hatchery production of this species, but it is clear that bigmouth sleepers can be spawned artificially and semi-naturally using the spawning agent Chorulon[®]. If larval rearing and fingerling production protocols can be determined, hatchery propagation of bigmouth sleepers will be a useful tool in fisheries management and conservation efforts in the Caribbean.

Acknowledgments

The authors thank the Puerto Rico Department of Natural and Environmental Resources (DNER) and Federal Aid in Sport Fish Restoration (SFR) for the funding of this research (SFR Project F-53R). Many employees at the University of Arkansas at Pine Bluff provided assistance with field and lab work, especially S. Garcia, C. Adelsberger and K. Olivieri. Drs S. Lochmann, M. Eggleton and T. Sink provided helpful guidance while the manuscript was in draft.

References

- Adelsberger C.M. (2009) *Natural life history characteristics of bigmouth sleeper *Gobiomorus dormitor* in Puerto Rico rivers*. Master's thesis, University of Arkansas at Pine Bluff, Pine Bluff, AR, USA.
- Auty E.H. (1978) Reproductive behavior and early development of the empire fish *Hypseleotris compressus* (Eleotridae). *Australian Journal of Marine Freshwater Research* **29**, 585–597.
- Bachelor N.M. (2002) *Ecology of bigmouth sleepers *Gobiomorus dormitor* (Eleotridae) in a Puerto Rico reservoir*. Master's thesis, North Carolina State University, Raleigh, NC, USA.
- Bachelor N.M., Neal J.W. & Noble R.L. (2004) Reproduction of a landlocked diadromous fish population: bigmouth sleepers *Gobiomorus dormitor* in a reservoir in Puerto Rico. *Caribbean Journal of Science* **40**, 223–231.
- Balon E.K. & Bruton M.N. (1994) Fishes of the Tatinga River, Comoros, with comments on freshwater amphidromy in the goby *Sicyopterus lagocephalus*. *Ichthyological Exploration of Freshwaters* **5**, 25–40.
- Bedarf A.T., McKaye K.R., Van Den Berghe E.P., Perez L.J.L. & Secor D.H. (2001) Initial six-year expansion of an introduced piscivorous fish in a tropical Central American lake. *Biological Invasions* **3**, 391–404.
- Cheah S.H., Senoo S., Lam S.Y. & Ang K.J. (1994) Aquaculture of a high-value freshwater fish in [Malaysia]: the marble or sand goby (*Oxyeleotris marmoratus*, Bleeker). *Naga* **17**, 22–25.
- Churchill T.N., Noble R.L., Gran J.E. & Alicea A.R. (1995) *Largemouth bass recruitment in Lucchetti Reservoir*. Final

- report, Federal Aid in Sportfish Restoration Project F-16, Study 2, Puerto Rico Department of Natural and Environmental Resources, Puerto Rico.
- Darnell R.M. (1962) *Fishes of the RioTamesi and Related Coastal Lagoons in East-Central Mexico*, Vol. 8. University of Texas: Mexico Publications of the Institute of Marine Science, pp. 299–365.
- Delventhal N. (2000) Breeding *Awaous (Euctenogobius) flavus*. Gobiidae.com database portal. Available at <http://www.gobiidae.com/breeding.awaous.htm> (accessed 15 November 2007).
- Harris N.J. (2007) *Evaluation of hatchery spawning techniques, natural reproductive cycles, and growth rates of the bigmouth sleeper *Gobiomorus dormitor* in Puerto Rico*. Master's thesis, University of Arkansas at Pine Bluff, Pine Bluff, AR, USA.
- Herbert B.W. & Graham P. (2004) Breeding and fecundity of the endemic Australian gudgeon, sleepy cod *Oxyeleotris lineolatus* (Steindachner 1867) (Eleotridae). *Aquaculture* **236**, 241–252.
- Holmquist J.G., Schmidt-Gengenbach J.M. & Yoshioka B.B. (1998) High dams and marine-freshwater linkages: effects on native and introduced fauna in the Caribbean. *Conservation Biology* **12**, 621–630.
- Iguchi K. & Mizuno N. (1990) Diel changes of larval drift among amphidromous gobies in Japan, especially *Rhinogobius brunneus*. *Journal of Fish Biology* **37**, 255–264.
- Iguchi K. & Mizuno N. (1991) Mechanisms of embryonic drift in the amphidromous goby *Rhinogobius brunneus*. *Environmental Biology of Fishes*. **31**, 295–300.
- Keith P. (2003) Biology and ecology of amphidromous Gobiidae of the Indo-Pacific and Caribbean regions. *Journal of Fish Biology* **63**, 831–847.
- Lindquist D.G. (1980) Bigmouth Sleeper, *Gobiomorus dormitor*. In: *Atlas of North American Freshwater Fishes* (ed. by D.S. Lee, C.R. Gilbert, C.H. Holcutt, R.E. Jenkins, D.E. McAllister & J.R. Jr Stauffer), NC State Museum of Natural History, Raleigh, NC, USA, p. 784.
- Luong V.C., Yi Y. & Lin C.K. (2005) Cove culture of marble goby (*Oxyeleotris marmorata* Bleeker) and carps in Tri An Reservoir in Vietnam. *Aquaculture* **244**, 97–107.
- Makeeva A.P. (2002) Embryonic development of *Micropercops cinctus* (Eleotridae). *Journal of Ichthyology* **42**, 627–638.
- Masson I. & Stone N. (2005) *Developing methods for harvesting rosy red fathead minnow eggs*. Master's thesis, University of Arkansas at Pine Bluff, Pine Bluff, AR, USA.
- McKaye K.R., Weiland D.J. & Lim T.M. (1979) Comments on the breeding biology of *Gobiomorus dormitor* (Os-teichthyes: Eleotridae) and the advantage of schooling behavior to its fry. *Copeia* **1979**, 542–544.
- Moriyama A., Yanagisawa Y., Mizuno N. & Omori K. (1998) Starvation of drifting goby larvae due to retention of free embryos in upstream reaches. *Environmental Biology of Fishes* **52**, 321–329.
- Neal J.W., Noble R.L., Lilyestrom C.G., Churchill T.N., Alicea A.R., Ashe D.E., Holliman F.M. & Waters D.S. (1999) *Freshwater sportfish community investigations and management*. Final report. Federal Aid in Sportfish Restoration Project F-41-2, Puerto Rico Department of Natural and Environmental Resources, Puerto Rico.
- Neal J.W., Noble R.L., Bachelier N.M., McGee M. & Lilyestrom C.G. (2001) *Freshwater sportfish community investigations and management*. Final report. Federal Aid in Sportfish Restoration Project F-41-2, Puerto Rico Department of Natural and Environmental Resources, Puerto Rico.
- Neal J.W., Noble R.L., Olmeda M.L. & Lilyestrom C.G. (2004) Management of tropical freshwater fisheries with stocking: the past, present, and future of propagated fishes in Puerto Rico. In: *Propagated Fish in Resource Management. American Fisheries Society Symposium 44* (ed. by M.J. Nickum, P.M. Mazik, J.G. Nickum & D.D. MacKinlay), pp. 197–206. American Fisheries Society, Bethesda, MD, USA.
- Nordlie F.G. (2000) Patterns of reproduction and development of selected resident teleosts of Florida salt marshes. *Hydrobiologia* **434**, 165–182.
- Ralph D. & Ralph R. (1988) Breeding Australia's desert goby. *Tropical Fish Hobbyist* **36**, 38–40.
- Rottman R.W., Shireman J.V. & Chapman F.A. (1991) *Capturing, handling, transporting, injecting and holding brood fish for induced spawning*. Southern Regional Aquaculture Center Publication No. 422. Available at <https://srac.tamu.edu/> (accessed 9 November 2010).
- Tavarutmaneegul P. & Lin C.K. (1988) Breeding and rearing of sand goby (*Oxyeleotris marmoratus* Blk.) fry. *Aquaculture* **69**, 299–305.
- Teixeira R.L. (1994) Abundance, reproductive period, and feeding habits of eleotrid fishes in estuarine habitats of north-east Brazil. *Journal of Fish Biology* **45**, 749–761.
- Todd E.S. (1975) Vertical movements and development of the prolarvae of the eleotrid fish, *Dormitator latifrons*. *Copeia* **3**, 564–568.
- Zohar Y. & Billard R. (1984) Annual and daily changes in plasma gonadotropin and sex steroids in relation to teleost gonad cycles. *Transactions of the American Fisheries Society* **113**, 444–451.