Controlled Maturation and Spawning of Captive Black Snook

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Black snook *Centropomus nigrescens* (Fig. 1) is a popular gamefish and an important high-value commercial species along the Eastern Pacific coast (from 33°N to 20°S) (Vega 2004, Cotto *et al*. 2010). This euryhaline, catadromous species is found from southern Baja California and in the mouth of the Gulf of California, Mexico, to northern Colombia (Fig. 2). Adults live in mangrove areas, lagoons, estuaries and freshwater ponds (Robins *et al*. 1991, Escárcega 2010).

TOP. FIGURE 1. *Female black snook broodfish during sampling event at Mote.* LEFT, FIGURE 2. *Geographic range of black snook.* RIGHT, FIGURE 3. *Black snook packaged in shipping bags before sealing.*

Like common snook *C. undecimalis* on the Atlantic coast, black snook is a potential species for aquaculture because of its high demand, size, and culinary value. Currently black snook farming is limited to grow-out farms using wild juveniles; limited reproduction in captivity has been achieved. Previous attempts to reproduce black snook in captivity have met with inconsistent success. Black snook maturation and spawning has only been reported for work done in Ecuador (Carvajal) in 1997, when 8 mature wild females and 5 males were induced to spawn, achieving a 0.7 percent fertilization rate.

The goal of our research was to transport wild-harvested black snook from Costa Rica to Mote Aquaculture and Research Park (Mote) in Sarasota, Florida, and utilize our existing broodstock facility and expertise in centropomid reproduction to identify protocols needed to control maturation and spawning of black snook in captivity on demand.

Black Snook Broodstock Collection

Mature black snook, ranging from ~52 to 78 cm in length and 1.64 to 5.02 kg, were captured with hook and line in Golfo Dulce near Puerto Jimenez, Costa Rica. Fish were transported to

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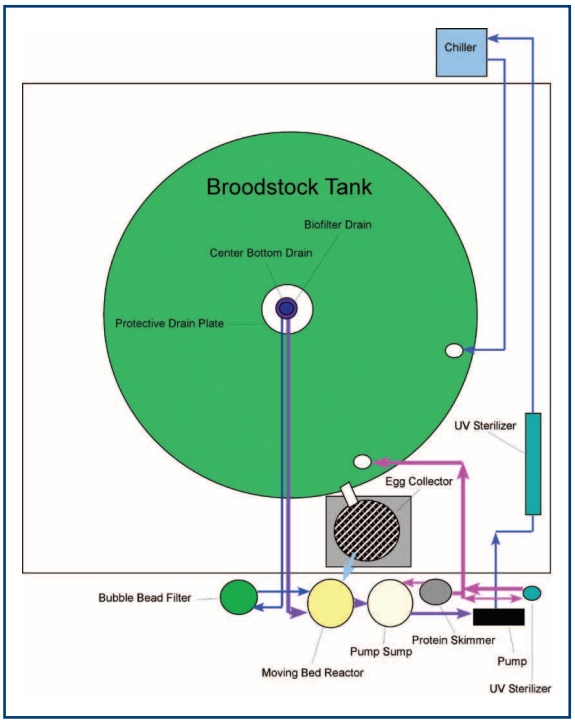
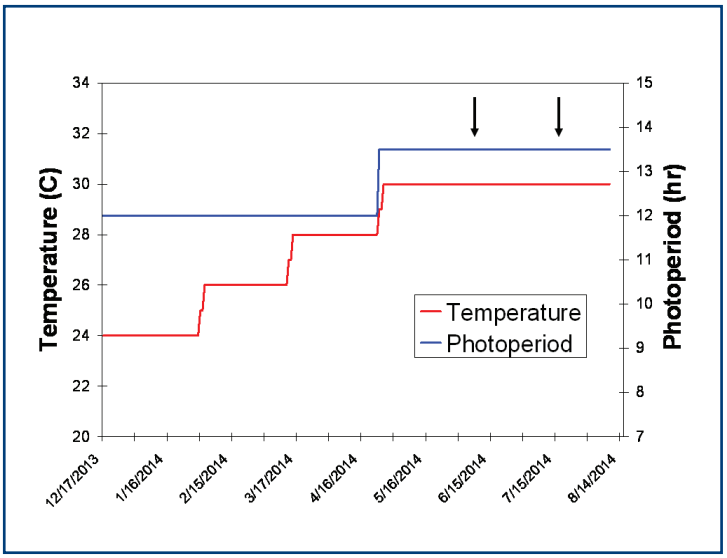
the Tranquility Management, R.L. site, maintained in recirculating systems with 8.9-m³ tanks at a salinity of approximately 32 ppt and allowed to recover from collection for a minimum of two weeks before shipping. While held, fish were fed approximately 100 live shrimp per day until five days before shipping. The current population of 7 females and 6 males were transported in two shipments.

On the shipping date, snook were removed from the acclimation system, placed in a hauling tote and trucked to San Jose, Costa Rica. Upon arrival, each fish was individually packed in a bag with 90 L of oxygenated water, ClorAm-X (3 g) and the head space of the bag filled with pure oxygen before sealing (Fig. 3). The primary (inner) bag containing the fish was then placed in a protective bag liner (boot), which was placed inside an additional outer bag filled with pure oxygen to provide additional integrity in case of punctures. Bags were placed in styrofoam-lined boxes (1.2 m long × 1 m wide × 0.9 m high) with ice packs. Boxes were secured with plastic shrink-wrap, palletized (two boxes per pallet) and shipped as air cargo on a direct flight to Miami International Airport.

Upon arrival in Miami and clearance through customs, bags were opened and dissolved oxygen concentration, pH, salinity and temperature were measured. If the fish in each bag appeared healthy, bags were not punctured. If water quality parameters were within range, the inner and outer bags were refilled with pure oxygen and resealed. Any fish that appeared to be in distress or had lost equilibrium was transferred from the bag into a holding tank to which pure oxygen was supplied through diffusers. Once fish arrived at Mote, water quality was assessed again and water was added to the holding container from the broodstock tank system until water quality was appropriate for fish transfer.

Fish were acclimated and treated with copper sulfate pentahydrate (0.2 ppm) for five weeks to prevent introduction of parasites and pathogens to existing broodstock populations





TOP, FIGURE 4. *Black snook broodstock tank layout at Mote.* BOTTOM, FIGURE 5. *Representation of the photo-thermal cycle used to induce maturation of black snook broodstock. Arrows indicate sampling events when mature females were observed and implanted with GnRH.*

or to the zero-discharge facility at Mote. Following quarantine, weight and length of each fish was measured, sex was determined and fish were implanted with a passive integrated transponder (PIT) tag for identification.

Broodstock System Design and Fish Management

Black snook were added to a broodstock system that had been used previously to mature and spawn captive common snook (Fig. 4). The 48-m³ system is 6.1 m in diameter by 1.83 m deep and water depth is maintained at 1.52 m. To maintain water clarity and chemistry, each tank is equipped with a 0.085-m³ drop filter (Aquaculture Systems Technologies, LLC, New Orleans, LA, USA) for solids removal, a 900-L moving bed reactor containing 0.283-m³ plastic extruded floating media (AMB™ media, EEC, Blue Bell, PA, USA) for biofiltration, a protein skimmer, two 150-W High Output SMART HO UV® units (Emperor Aquatics, Inc®, Pottstown, PA), and 126,000-BTU heater/chiller unit (AquaCal AutoPilot, Inc., St Petersburg, FL, USA). Photoperiod was maintained by four 10,000°K daylight Coralife® bulbs, each on a timer, providing a gradual sunrise and sunset.

Water quality and chemistry were closely monitored to ensure proper conditions for fish. Water quality was measured daily with a YSI Pro Plus meter (YSI Inc., Yellow Springs, OH) and were maintained within the following limits: dissolved oxygen concentration ≥5 and ≤ 9 mg/L, temperature ±1 C of the desired temperature, salinity 35 ± 1 ppt, and pH ≥7.5 and ≤8.4. Water chemistry was assessed once weekly and maintained within the following limits: total ammonia nitrogen <0.5 mg/L, total nitrite-N <1.0 mg/L, and total nitrate-N <50 mg/L. If the concentration of any water chemistry parameter was outside these limits, water was exchanged to alleviate the problem.

Black snook were initially fed fresh frozen shrimp for three days after being added to the tank. Once fish started eating shrimp, Atlantic thread herring *Opisthonema oglinum* was added to the

diet. When all fish were actively feeding, they were fed shrimp and thread herring to satiation daily at a 1:1 ratio until the first sampling. The diet was based on feeding requirements and spawning research conducted at Mote with common snook (Neidig *et al*. 2012). After the first sampling, the daily food ration was adjusted on the basis of tank biomass and feeding behavior. In May 2014, the diet was supplemented with vitamin B1 (40 mg/kg) to prevent potential thiamine deficiency (Amcoff *et al*. 1998, Brown *et al*. 2005).

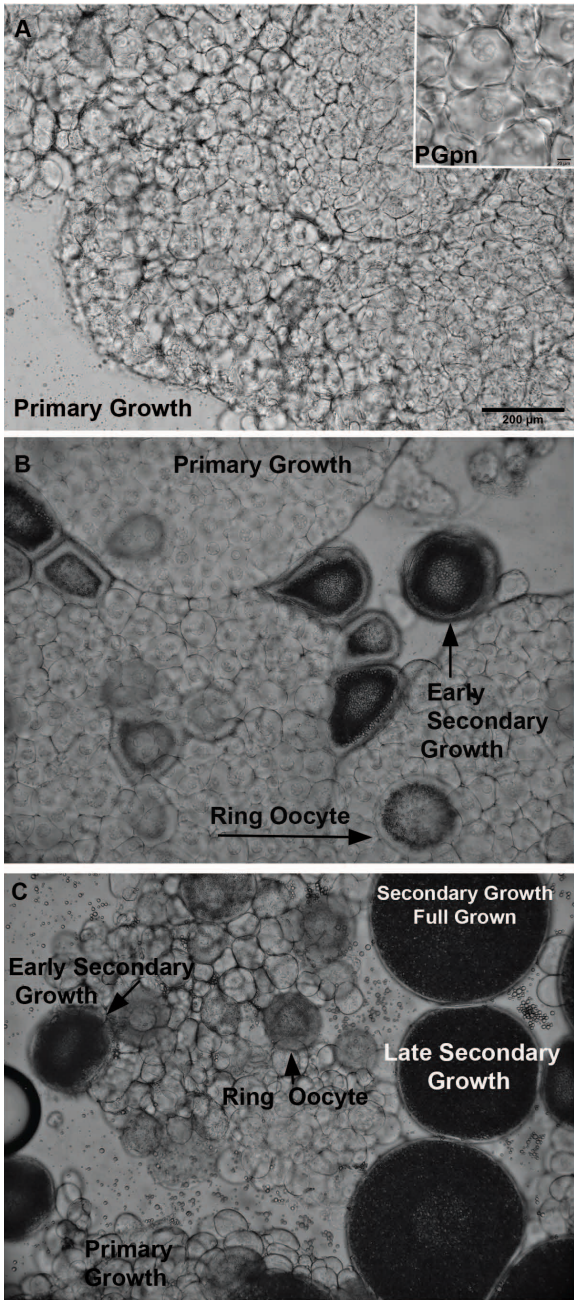
Photo-thermal Manipulation

To influence reproductive cycles, it is necessary to identify the appropriate environmental, hormonal and behavioral cues required by a particular fish species (Zohar and Mylonas 2001, Mylonas *et al*. 2010). There is no published information on the timing of wild spawning for black snook and the only observations of mature fish in Golfo Dulce that we are aware of are from April 2009 and July 2014[1]. Using the average photo-thermal conditions for southwestern Costa Rica, we estimated a target photo-thermal range for maturation at 13 h of light and a water temperature of 29.3 C.

Fish were maintained under a stepwise photo-thermal progression to identify the requirements to induce maturation (Fig. 5). Starting at an initial resting water temperature of 24 C and 12 h of light, temperature was slowly raised by 2 C before the first sampling (24 January 2014). Following each sampling, water temperature was increased by 2 C. Fish were allowed to recover for six weeks between sampling events. During the first three sampling events, no progression to maturation was observed and oocytes in all females were at the primary growth stage (Fig. 6a).

Following sampling on 23 April 2014, temperature was increased to 30 C and day length to 13.5 h (Fig. 5). While sampling on 4 June 2014, two females had oocytes that had advanced to secondary growth, full grown (SGfg, Fig. 6c) stage. Oocytes were sufficiently mature that hormone induction would likely induce spawning. Three of the other five females also had positive progression in oocyte maturation, with oocytes ranging from ringed to late secondary growth (SGl, Figs. 6b and 6c). Photo-thermal conditions were maintained at 30 C and 13.5 h light and on 16 July 2014, six of seven females had oocytes that were staged at secondary growth (SG) with three at SGfg. One more sampling is planned before the fish will be cycled into resting conditions.

Based on observations from earlier sampling periods, 26 C and 12 hr of light will be the resting conditions. At these conditions, fish will not be using energy for maturation and at 26 C black snook continues to feeds vigorously.



ABOVE, FIGURE 6. *Black snook oocytes at different levels of maturation. a) Oocytes of a regressed female with oocytes only at the early stages of primary growth. b) Oocytes from a female that was maturing, with ring oocytes and the early steps of secondary growth. c) Mature oocytes from a female black snook.*

Sampling and Hormone Induced Spawning

To sample broodstock, tanks were drawn down and two dividers made from plastic mesh secured to a polyvinyl chloride (PVC) pipe frame were positioned in the tank to confine all fish into a small area. Fish were captured individually from the restricted area of the tank using a soft-mesh net and placed in a floating, oval polyvinyl tank of seawater where they were anesthetized using 300 mg/L pH-buffered tricaine methanesulfonate (MS-222™) for 1-2 min. Fish were then scanned to obtain the PIT tag number and weight and fork length measured.

The reproductive status of each individual was identified by adapting the classification system for common snook (Neidig *et al*. 2000, Rhody *et al*. 2013). To assess the level of maturation in females, a cannulation biopsy was performed with an 8 fr, premature infant, silastic feeding tube (Fig. 7). An oocyte sample was used to assess the stage of maturation using light microscopy. If females had advanced late secondary growth (SGl) or more mature oocytes, they were implanted with gonadotropin-releasing hormone analog (GnRHa) at 50 µg/kg and returned to the tank. Immature females, those with oocytes at early secondary growth (SGe) or less mature, were given a dose of 25 µg/kg.

GnRHa was delivered using an ethylene and vinyl acetate delivery system (Zohar and Mylonas 2001). Implants were administered with a 12-gauge needle and implanted into the dorsal muscle directly behind the second dorsal spine. All GnRHa implants used in these trials were made by the Institute of Marine and

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FIGURE 7. *Cannulation biopsy performed on a female black snook to assess oocyte maturation.*

FIGURE 8. *Applying gentle pressure to the abdomen of a male black snook to express milt.*

Environmental Technologies (University of Maryland, Baltimore, MD, USA).

To assess male black snook maturation, gentle pressure was applied lengthwise to the abdomen below the testes to assess the presence of flowing milt (Fig. 8). If no milt was obtained, a cannulation biopsy was performed with a 5 fr tube using the procedures described for females to check for milt and determine if the male had undergone sex reversal to a female. No hormone therapy was used for males.

When the evaluation was complete, each fish was revived to the point where it could maintain buoyancy and then returned to the broodstock holding system. Tank water level was increased to full volume and fish were allowed to spawn volitionally.

Egg Collection and Egg Quality Assessment

A skimmer bar on the tank surface was used to direct eggs into an external collection tank containing a 300-µm Nitex® mesh bag. After 24 hours, monitoring of the bag and tank for eggs was initiated, checking both every 2 hours. The 2-hr window between observations was used to reduce potential impacts on spawning or courtship behavior. After a spawn was observed, we continued to monitor the egg collection tank by sieving 9 L of water from the tank every hour to determine when all eggs had been harvested. After all eggs were harvested from the tank, the collector was removed and emptied to assess fertilization rate and spawn volume.

Harvested eggs were added to a graduated *Artemia* hatching cone that was filled to a known volume and gently aerated. When eggs reached at least the blastula stage (approximately 4-5 hr post-fertilization), spawn quality and fertilization rate was assessed. Assessment was carried out by increasing aeration in the hatching cone to a more vigorous level to mix eggs evenly in the cone. Three 10-mL aliquots were collected, aeration returned to a normal, gentle level and every egg in each sample counted to determine the number of eggs/ml in the *Artemia* cone and estimate the spawn size. Fertilized and unfertilized eggs were counted separately to determine fertilization rate. Following the spawn assessment, aeration was removed, allowing non-viable eggs to settle to the bottom of the cone and discarded. The remaining floating fertilized eggs were removed for cleaning by draining through a 500-µm sieve. Eggs were rinsed in three separate buckets containing approximately 6 L of clean full-strength seawater, and moved to a clean hatching cone for distribution to larval tanks.

The broodstock spawned twice (24 and 48 hr) following the June and July sampling events (Table 1). More than 2.5 million eggs (more than 1.2 million fertilized) were collected from the June and July spawns with an overall fertilization rate of 47 percent and mean hatch rate of 96 percent. The second spawn in July was not fertilized, so it was not included in the reported mean hatch rates. At 28 C, black snook eggs (Fig. 9) develop at nearly the same rate as common snook eggs (Yanes-Roca *et al*. 2012)

TABLE 1. Spawning data collected on black snook at Mote.

Sampling Date	*Spawning Date*	*Total Number*	*Fertilization Rate (%)*	*Hatch Rate (%)*
6/4/2014	6/5/2014	1,339,033	50.9	96.6
	6/6/2014	188,700	45.1	94.5
7/16/2014	7/17/2014	834,700	51.9	95.9
	7/18/2014	177,233	0.0	n/a

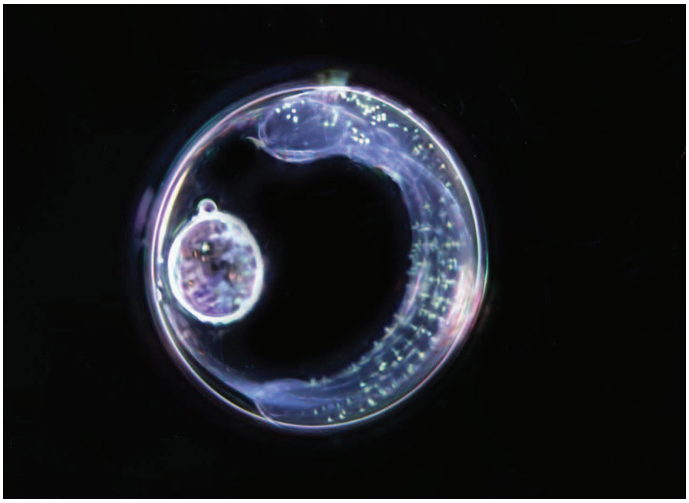

FIGURE 9. *Black snook embryo 10.5 hr after fertilization.*

FIGURE 10. *Newly hatched black snook larvae 15 hr after fertilization.*

and began hatching at 14.25 hr post-fertilization, with all viable eggs hatching after 16 hr. We expected to see increased spawning performance in the July spawn because three females were mature and implanted (versus two mature females in June); however, fish were handled twice during this sampling event because some fish escaped the confined sampling space, which may have resulted in a decrease in spawning success from handling stress.

Egg Hatching and Larval Development

Eggs were stocked directly into hatching cones and newly hatched larvae were transferred to larval-rearing tanks. Hatching tanks were stocked with enough eggs to meet the required larval tank stocking density (see below), assuming a 75 percent hatch rate. To assess hatch rate, larvae and unhatched eggs were homogenized in the hatching tank and three, 10-mL aliquots were collected. When all larvae had hatched, water from hatching cones was decanted into larval rearing tanks and unfertilized eggs and egg casings remained in the bottom of hatching cones.

Because there is no information in the literature on rearing black snook larvae, we adapted the protocols developed at Mote for common snook (Yanes-Roca and Main 2012, Neidig *et al*. 2014). Initial larval stocking densities were 50, 100 and 200 larvae/L in 0.9-m^3 (1.52-m diameter) larval rearing tanks. Fish were sampled at 0, 3, 6, 9 and 15 days post-hatch (DPH) to assess larval growth and development (Figs. 10-12). Black snook larvae appear to grow and develop at similar rates to those of common snook (Yanes-Roca *et al*. 2012). Larvae from the June spawn were reared to 16 DPH and larvae from the July spawn continue to grow from the time this article was written (13 DPH).

Future Goals for Black Snook at Mote

A second black snook broodstock population is being established at Mote to more rapidly develop optimal spawning parameters. We are also working to explore DNA profiling to monitor mating outcomes for the Mote black snook population. This will provide the opportunity to identify parentage of larvae and improve husbandry and spawning strategies for black snook (Rhody *et al*. 2014).

Developing larval rearing and fingerling production protocols to support pilot-scale, experimental stock enhancement releases of black snook in Golfo Dulce is a research goal for this project. Using the aquaculture protocols developed for common snook in Florida, it appears that this goal can be achieved in the next five years. Larval rearing research will focus on identifying optimal husbandry protocols, including live food requirements, environmental parameters, system dynamics and stocking densities.

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Notes

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[1] Nathan Brennan, unpublished data

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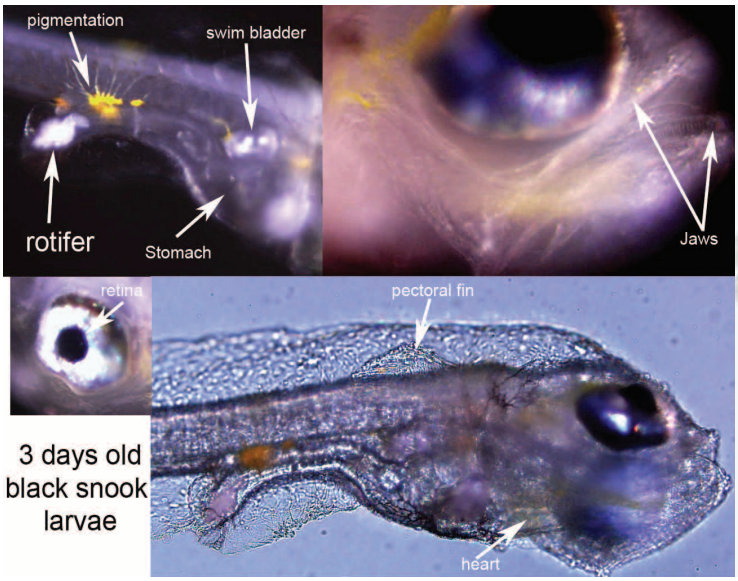

FIGURE 11. *Black snook larvae 3 days after hatching.*

FIGURE 12. *Black snook larvae 9 days after hatching.*

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