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Parental contribution and spawning performance in captive common snook *Centropomus undecimalis* broodstock

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ABSTRACT

Common snook are a species relatively new to aquaculture and to date, virtually no information is available on captive broodstock spawning characteristics. Understanding basic and fundamental data such as broodstock contribution of captive mass spawning snook is important, not only for the development of a successful selective breeding program for the species, but also for restocking wild fisheries and maintenance of local genetic variation. A scoping study was undertaken to explore the potential of DNA profiling for monitoring mating outcomes in captive snook. Spawning success was monitored among wild harvested broodstock that were undergoing hormonal treatment to induce spawning. The broodstock were maintained in three separate tanks (Tank A: 18 males and 15 females; Tank B: 22 males and 11 females; Tank C: 40 males and 16 females) and were subject to different handling stresses. Sixteen mass spawning events were studied across the three tanks over a 15 month period. DNA profiling of eight microsatellite markers was employed to detect and quantify individual parental contributions for 2,154 larvae obtained from the three tanks. The panel of loci was generally robust and allowed unambiguous assignment of 89% of larvae to a single family. All spawns occurred within approximately 24 to 72 hours post-implantation and only females implanted with gonadotropin releasing hormone analogue (GnRHa) were found to contribute. Overall, spawning performance among the three tanks was highly variable in terms of the total number of eggs produced (from 86,300 to 2,378,000 per spawn), fertilization success (from 17.0 to 87.3%) and hatch rate (from 47.8 to 98.1%). Three-day larval survival ranged from approximately 25.9 to 90.1% in tank A and 19.9 to 74.2% in tank C. During this study, new information regarding requirements for broodstock husbandry, mating patterns and spawning periodicity of captive common snook broodstock were obtained. Spawn contribution data 1) provided a confirmation of GnRHa treatment efficacy in female snook with a minimum stage of oogenesis (late secondary growth-SGl) required for successful spawning; 2) identified a potential impact of handling on maturation and spawning in male and female broodstock; 3) confirmed that, through photothermal conditioning, captive common snook broodstock can spawn over consecutive days and several times per year, including outside of their natural spawning season.

1. Introduction

Saltwater recreational fishing is a multibillion (US) dollar industry in the United States, with the economic output in Florida alone valued at $4.9 billion in 2011 (American Sport Fishing Association, 2013). The common snook *Centropomus undecimalis* is one of the three most popular gamefish in Florida, making them a vital part of an economically important sport fishery (Muller and Taylor, 2012). However, red tide events, cold kills, habitat loss and increased sport fishing pressure have left wild stocks vulnerable; prompting the Florida Fish and Wildlife Conservation Commission (FWC) to regularly assess the condition of wild stocks (Muller and Taylor, 2012). Bag limits and limitations on size and seasons for snook harvest have been implemented and a sizeable investment has been apportioned for fisheries managers to develop an effective marine fish stocking technology for rapid restoration of depleted stocks (Tringali and Leber, 1998; Tringali et al., 2008a). In Florida, aquaculture technologies are being developed at marine fish hatcheries to increase the production of fingerlings for release (Alvarez-Lajonchère and Taylor, 2003) and investigate stock enhancement as a fisheries management tool (Brennan et al., 2008). In Central and South America, common snook, along with several other *Centropomus* species, are also popular food fish and as such considered as emerging species for intensive aquaculture in North and South America. Collectively, they support high value commercial fisheries in those regions (Alvarez-Lajonchère and Tsuzuki, 2008). Despite recent breakthroughs

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in the spawning of captive common snook broodstock (Ibarro-Castro et al., 2011; Rhody et al., 2013; Yanes-Roca et al., 2009) and advances in larval rearing protocols (Barón-Aguilar et al., 2013; Ibarra-Castro et al., 2011; Rhody et al., 2010; Wittenrich et al., 2009; Yanes-Roca et al., 2012), to date, there is still no established large scale production of this species for food or restocking for recreational fisheries. To date, major reproductive bottlenecks of captive snook broodstock include the failure of females to ovulate without hormonal manipulation, reduced milt production in males and inconsistent supply of high quality eggs and larvae.

Common snook mating behavior in the wild is believed to be complex and is still not fully understood (Taylor et al., 2000; Trotter et al., 2012; Yanes-Roca et al., 2009). Initial successes in developing spawning procedures with wild common snook involved the induction of ovulation with hormones and strip spawning to obtain eggs (Neidig et al., 2000). In more recent years, studies focused on photothermal conditioning and hormonal manipulation to induce oocyte maturation, ovulation and volitional spawning of captive broodstock. Snook broodstock were successfully induced to spawn in captivity by implanting females with gonadotropin-releasing hormone agonists (GnRHa) (Ibarro-Castro et al., 2011; Rhody et al., 2010, 2013). To improve aquaculture technologies and increase the production of common snook fingerlings for food consumption or fisheries enhancement studies, a better understanding of the environmental, behavioral and social conditions that promote spontaneous spawning is required.

Genetic management in aquaculture and supplemental stocking programs also requires careful consideration, since maintenance of genetic diversity is a key biological requisite for population resilience and productivity (Brown et al., 2005; Duchesne and Bernatchez, 2002; Tringali et al., 2008a). With a restricted number of breeding individuals available, conservation of single-locus and quantitative genetic variation in managed populations requires maintenance of sufficiently large (genetic) effective population sizes (Lorenzen et al., 2010; Tave, 1993). Common snook are mass-spawners with spawning naturally taking place in relatively large breeding groups. Although this reproductive strategy can allow for the production of a large quantity of offspring, individual mating patterns and reproductive success cannot be easily monitored or assessed under standard captive culture conditions (Gruenthal and Drawbridge, 2012). Further, this mating tactic can potentially lead to highly skewed levels of individual parental contributions and large variability in family size. In such conditions, census population size is extremely unlikely to be a reliable indicator of the underlying effective population size.

Maintaining genetic variation and pedigree information has proven to be a critical component of the successful commercial-scale culture of many freshwater and marine fish species. The development of DNA-based genetic markers has had a revolutionary impact on terrestrial and aquatic animal breeding and selection (Georges, 2001; Liu and Cordes, 2004; Migaud et al., 2013). In the field of fisheries and aquaculture, microsatellite markers have been used successfully in a number of ways including characterization of genetic stocks (Cushman et al., 2012; Heard, 2012), broodstock selection (Hayes et al., 2007), mapping economically important quantitative traits and identifying genes responsible for these traits (Chistiakov et al., 2006). DNA profiling of parents and offspring have allowed for individuals to be assigned to family groups in many mass spawning species including barramundi, *Lates calcarifer* (Domingos et al., 2014; Frost et al., 2006; Loughnan et al., 2014), European sea bass, *Dicentrarchus labrax* (Massault et al., 2010) and Atlantic cod, *Gadus morhua* (Herlin et al., 2008). Genetic markers have also been used extensively as a management tool to identify and monitor hatchery fish reared for stock replenishment including species such as brown trout, *Salmo trutta* (Taggart and Ferguson, 1986), red drum, *Sciaenops ocellatus* (Gold et al., 2010; Saillant et al., 2009; Tringali et al., 2008b) and white seabass, *Atractoscion nobilis* (Gruenthal and Drawbridge, 2012). A number of DNA microsatellites suitable for genotyping have been developed for use in population assignment studies of common snook in Florida (Seyoum et al., 2005; Wilson et al., 1997). The isolation and characterization of 27 polymorphic loci for common snook were initially developed to study potential genetic differences among wild populations originating from Florida's Atlantic and Gulf of Mexico waters (Seyoum et al., 2005; Tringali et al., 2008a).

To date, no data on spawning dynamics, including individual spawning performance, have been reported for common snook in captivity. As a first step in this direction, we initiated a scoping study employing microsatellite-based DNA profiling to assign parentage within a subset of captive broodstock maintained at Mote Marine Laboratory, Sarasota, Florida, USA. Screening was carried out on parents and offspring from three brood tanks in which hormonal treatments for volitional spawning were being trialed. In addition to an assessment of the DNA profiling technique, the study provided useful insights into the efficacy of hormonal treatments and fish husbandry on the spawning performance of individuals and its implications for monitoring and maintaining genetic variation within the captive stock.

2. Materials and methods

2.1. Broodstock collection and maintenance

Wild adult common snook were collected from three locations in Sarasota County (Florida, USA) and transported to Mote Aquaculture Research Park (Sarasota, Florida, USA) in 2009. Following a 40-day quarantine period, broodstock were PIT-tagged and a fin tissue sample was taken from each individual for DNA analysis. Tissue samples were stored in 95% ethanol until the time of processing.

Collected broodstock were divided among three separate, indoor, photoperiod (10-15H light) and temperature (20–30 °C) controlled recirculating tank systems (A, B, C) (Fig. 1). Tank A contained 18 males and 15 females; Tank B had 22 males and 11 females; whereas Tank C had 40 males and 16 females (Table 1). Systems A and B each consisted of a 4.6 m diameter, green, fiberglass tank with a total system volume of 28 m^3 whereas tank C was 6.1 m in diameter and had a total system volume of 48 m^3. Salinities were maintained at 35‰. Temperature was controlled in each tank system by cycling water through an individual heater/chiller unit (AquaCal, St. Petersburgh, FL, USA). Filtration included a drop filter (Aquaculture Systems Technologies, New Orleans, LA, USA) for the collection of fine solids, 900-l moving bed for bio-filtration, protein skimmer and an ultraviolet light (UV) sterilization unit.

2.2. Broodstock sampling and hormonal induction

Over the study period (from April 2011 to June 2012, 15 months), snook in tanks A and C were handled a total of seven times. In contrast, snook in tank B were handled much more frequently (monthly intervals), to obtain blood samples from each individual for seasonal sex steroid profiling. Snook in tanks A and C were not blood sampled during the study. The procedures described below were conducted at all handling events.

To sample the broodstock the tank water level was lowered. Two dividers, made from plastic mesh stretched across a polyvinyl chloride (PVC) pipe frame, were used to corral the fish into a section of the tank. From this restricted section, individual fish were netted into a 500-l tank containing 200-l of water and anesthetized with tricaine methanesulfonate (MS-222) at a concentration of 300 ppm for approximately 1-2 minutes. All male and female common snook were weighed (to the nearest gram) and measured (total length, TL, mm). Female broodstock were biopsied and the reproductive status of each individual was assessed using a classification proposed by Grier et al. (2009) and adapted to common snook according to Rhody et al. (2013). Not all handling events corresponded with the hormonal induction of females. In total, two sampling events in tank A, one in tank B and five in tank C utilized hormonal implantation. Only females determined to have oocytes

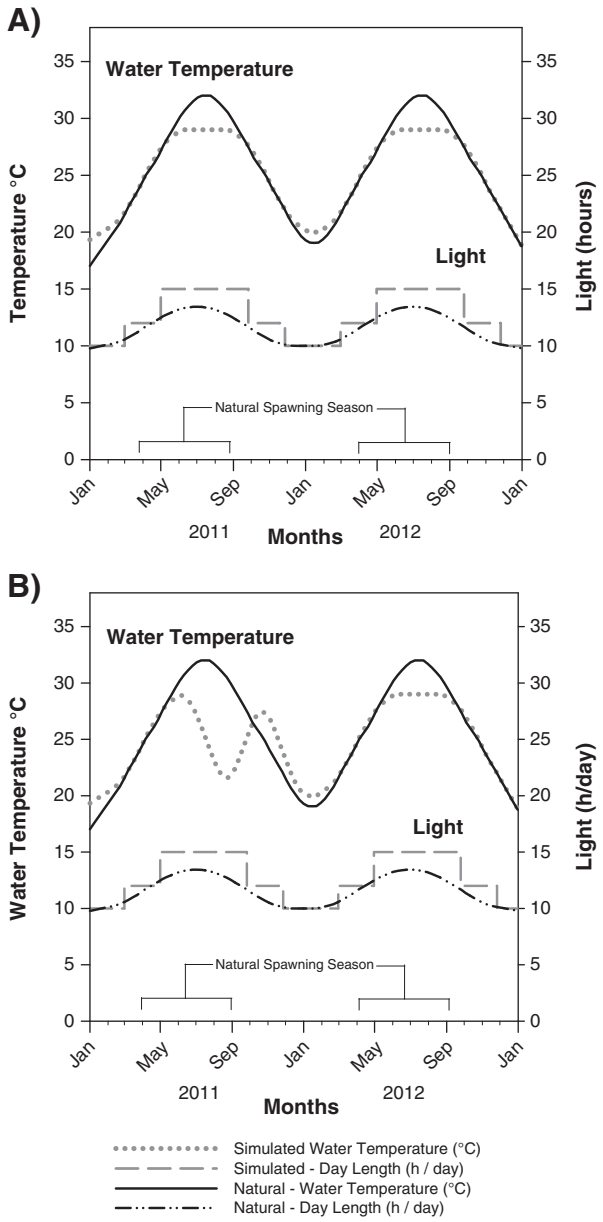

Fig. 1. Schematic representation of ambient natural and artificial (simulated) environmental conditions associated with the annual reproductive cycle of wild common snook located on the Gulf coast of Florida and captive common snook broodstock held at Mote Aquaculture Research Park, Sarasota, FL from the years 2011 to 2012. Simulated photo-thermal cycle used to mature and spawn captive broodstock in (A) Tanks A and B. Simulated photo-thermal cycle used to mature and spawn captive broodstock in (B) Tank C showing out-of-season spawning (→). Natural ambient cycle of day length (light h/day) (— —) and water temperature (°C) (—) in Tampa Bay, FL. Imposed photo-thermal cycle used to mature and spawn captive broodstock including day length (light h/day) (— ·· —) and water temperature (°C) (■ ■ ■).

in early secondary growth (SGe) or the later stages of the oogenetic cycle were implanted with GnRHa (Institute of Marine and Environmental Technologies, University of Maryland, Baltimore, MD, USA) at a dosage of 50 μg/kg bodyweight. Males were not implanted during this study.

2.3. Larval sampling

A total of 16 mass spawning events were documented during the study. Broodstock typically spawned during early to late evening where a spawning event or 'spawn' was defined as the sum of eggs or offspring produced during a single evening. Following each nightly spawning event, eggs were transferred from the broodstock tank to 100-l hatching tanks. At 4–6 h post fertilization (blastula stage), aeration was removed and non-viable (sinking) eggs were discarded. At 17 h post fertilization, approximately 250 newly hatched larvae (volumetrically measured) were stocked into individual microcosms. Accuracy of initial stocking ranged from 231 to 268 larvae per microcosm. The microcosms (25 for each spawn), made of PVC (2.6 cm diameter), were held in a shallow rectangular recirculating raceway tank system equipped with UV sterilization. The base of each microcosm was covered with a 200 μm nylon mesh to prevent the escape of larvae, while allowing circulation of the water. Temperature in the raceway tank was maintained at 28 ± 1 °C.

To assess daily larval survival for each spawn, the total number of live larvae from each of five microcosms were counted on days 1, 2 and 3 post-hatch (total of 15). An additional 150, three-day-old snook larvae were randomly sampled from the remaining ten microcosms (20 larvae per microcosm) and individually stored in 95% ethanol until they could be genotyped.

2.4. DNA extraction

Genomic DNA was extracted from all 122 snook broodstock (fin clip biopsy) within the three spawning tanks and approximately 2,200 three-day-old snook larvae (whole animal) using the PureGene DNA Extraction kit (Qiagen, USA) according to the manufacturer's instructions. Each sample was digested with 300 μl of cell lysis buffer, 2 μl of proteinase K (20 mg/μl), and incubated overnight at 55 °C. To increase DNA yield, 5 μl of glycogen (5 mg/ml) was added to the precipitation step and the DNA re-suspended with 30 μl of hydration solution. DNA was quantified using a Nanodrop 1000 Spectrophotometer (Thermo Scientific, USA). Adults and larvae yielded an average of 500 ng/μl and 10 ng/μl of pure DNA respectively. All samples were stored at 4 °C prior to PCR amplification.

2.5. DNA microsatellites and PCR amplification

Eight polymorphic microsatellite markers, Cun01, Cun08, Cun19, Cun10A, Cun18, Cun11, Cun14 and Cun16 (Seyoum et al., 2005) were assayed in three robust PCR multiplexes (detailed in Table 2). Each 12.5 μl PCR reaction consisted of 0.3 U of GoTaq (Promega, Madison, WI, USA), 2.5 μl 5 x GoTaq Buffer, 0.2 mM each dNTP, 3 mM $MgCl_2$, 1.25 mg/ml BSA, 0.8 μM each primer, and 5 to 20 ng DNA template (150 ng/μl for adults). Thermal cycling parameters for all amplifications were: 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C 30 s, and a final extension of 7 min at 72 °C. PCR reaction products were stored at 4 °C until genotyped.

Table 1
Description of captive common snook broodstock populations held at Mote Aquaculture Research Park. Weight and fork length (FL) ± SEM.

Broodstock tank	Total no. fish/tank	Total no. males/females	Male : female sex ratio	Female mean weight (kg)	Female mean length (cm)	Male mean weight (kg)	Male mean length (cm)	Tank biomass (kg/m^3)
A	33	18/15	1.2 : 1	3.4 ± 0.4[a]	68.4 ± 2.4[a]	2.1 ± 0.1[a]	60.1 ± 1.1[a]	3.2
B	33	22/11	2 : 1	2.5 ± 0.3[a]	60.9 ± 2.3[a]	2.1 ± 0.2[a]	59.1 ± 1.5[a]	2.6
C	56	40/16	2.5 : 1	3.4 ± 0.3[a]	68.9 ± 2.0[a]	2.7 ± 0.2[b]	63.5 ± 1.6[a]	3.4

Means with different letters in the same column are significantly different ($P < 0.05$).

Table 2
Details of the eight polymorphic microsatellite markers used in the present study.

Locus	Primer sequence (5'–3')	Annealing temperature (°C)	Repeats	No. of alleles	Allele size range	Multiplex	Primer label	H_E (unbiased)	H_o	Fis *
Cun01	F: AAGTTCGCTCCCTCTCACTT R: GATACACATTGCCCTCAAG	55	$(AC)_{26}$	12	117–149	1	NED	0.767	0.779	−0.015
Cun08	F: TTCTTTTCACTGCTTCTGTCTG R: GTAAACCCGGTCGATTCTTC	55	$(TG)_9(TA)_{10}$	13	168–202	1	HEX	0.799	0.803	−0.006
Cun19	F: AGCCAGCGAAGGCCAATGT R: AGCCAGCGAACACACACTCA	55	$(TG)_{16}/(TG)_9$	6	152–164	1	FAM	0.516	0.533	−0.033
Cun10A	F: CCCCAAGGATCGTCTATCTC R: TAACATTTCACACGGTGCTG	58	$(CA)_{18}$	23	158–216	2	FAM	0.922	0.959	−0.040
Cun18	F: CTGACAGCTCAGTGCGTCT R: TGTAAACAGAAGGCGGTCAT	58	$(TG)_{12}$	9	111–135	2	HEX	0.757	0.836	−0.104
Cun11	F: CACATGCAAAGAGACTGCAC R: GGAGGGAAAAACGACTTGAT	55	$(AC)_6(ATGT)$ $(GC)_3(AC)_{13}$	17	150–204	3	NED	0.795	0.754	0.052
Cun14	F: GCCTGTCATCTCACTGGGTA R: GTCTTTGATCTGCCCGTTTA	55	$(TG)_{36}$	13	173–215	3	FAM	0.738	0.746	−0.011
Cun16	F: TATTGCTGTGAGCAGATGGA R: ATAGCCTGCAGTTCTTGGAA	55	$(TG)_6(CG)$ (TG)3/(TG)4	7	119−141	3	HEX	0.752	0.721	0.041

* Fis: Computed as in Weir and Cockerham (1984).

2.6. Genotyping and parentage analyses

Each PCR multiplex was screened with 1 μl of each PCR product being added to 12 μl Hi-Di formamide containing 0.86 μl Home Made ROX DNA size standard (De Woody et al., 2004). Microsatellite alleles were detected and sized on an ABI 3130xl genetic analyzer (Applied Biosystems, Carlsbad, CA, USA) and scored with GeneMapper v4.0 (Applied Biosystems, Carlsbad, CA, USA). The potential presence of null alleles and genotype errors in the broodstock were tested with Micro-Checker version 2.2.3 (Van Oosterhout et al., 2004). All adult samples were screened twice to maximize genotyping accuracy. The genotyping data were initially interpreted using the exclusion based program Probmax (Danzmann, 1997) and further confirmed with the Family Assignment Program, FAP (Herlin et al., 2007; Taggart, 2007). Both programs provided the user with an assignment mode that allows for the identification of all possible parental combinations for each offspring; however, a predictive mode to calculate the resolving power of specific genotypic data sets was only available in FAP.

Table 3
Computation of the resolving power of microsatellite panels within the three experimental tanks. The proportion of offspring per family that should be unambiguously assignable to a single family are given. Eight and seven loci options are considered. The calculations, performed using FAP (Taggart, 2007), were based on the known parental genotypes within each spawning tank and assume that all sire x dam combinations were equally likely to occur. Figures in parentheses refer to the number of potential different mating combinations possible, given the numbers of sires and dams present in each tank.

		Tank A (270)	Tank B (242)	Tank C (640)
All 8 loci	Mean:	0.99	0.99	0.95
	SD:	0.02	0.02	0.06
	Min:	0.87	0.88	0.67
	Max:	1.00	1.00	1.00
7 loci (exc. Cun14)	Mean:	0.97	0.96	0.91
	SD:	0.04	0.04	0.10
	Min:	0.77	0.82	0.41
	Max:	1.00	1.00	1.00

3. Results

3.1. Genotyping

All broodstock were genotyped across the eight loci on two separate occasions. No genotyping inconsistencies were found between these two screenings. FAP predictive mode was used to compute the power of the eight locus microsatellite panel to unambiguously assign parentage in each of the three experimental tanks (Table 3). Mean assignment rates among families within tanks ranged from 95% to 99%. Similar statistics were also calculated for seven loci (dropping the problematic Cun14 locus; see below). Mean predicted resolving power was reduced to 91–97% among tanks (Table 3).

Of 2,200 offspring that were initially screened for eight loci, complete genetic profiles were obtained for 2,154 individuals. Of these 74% assigned to at least one family under the stringent exclusion model (i.e. assuming no genotyping error at all) – with 26% not assigning to any expected family. Using a more realistic model for practical genotyping, i.e. allowing up to one allele mismatch across the eight loci composite genotype per progeny, 98% of individuals were assigned to families; 89% unambiguously to a single family. Thus, actual assignment rates were in line with predicted expectations (see above). Inspection of the data for these single match assignments showed that 54% of the identified genotyping errors occurred for locus Cun14, and involved miscalling of alleles from adjacent bins. The remainder of genotyping miscalls were spread evenly across all other loci and again involved either alleles resolving on bin boundaries or weak samples where allelic dropout resulted in heterozygous individuals being mis-scored as homozygous for the smaller allele. There was no evidence of null alleles segregating. A further 9% of progeny assigned to multiple families. In all these multiple-match cases, at least one of the families implicated was a confirmed spawning pair (from unambiguous single family matches). Finally, approximately 2% of progeny ($n = 44$) required mismatches at 2–4 alleles to be permitted in order to assign to potential parental crosses. Multiple families were identified for each offspring and, in all cases, implicated at least one confirmed

Table 4
Relationship between the timing of hormonal implantation, oocyte stage of development and total female spawn contribution for sixteen mass spawning events observed in three captive common snook broodstock populations.

Oocyte development (Stage)	Oocyte development (Step)	Abbreviation	No. females implanted (***n***)	No. females spawned (***n***)	Females implanted (%)	Females spawned (%)	Total spawn contribution (%)	No. Fish spawned 1 day post-implantation (***n***)	No. Fish spawned 2 days post-implantation (***n***)	No. Fish spawned 3 days post-implantation (*n*)
Secondary growth (SG)	Early	SGe	5	0	10.4	0	0	0	0	0
	Late	SGl	9	7	18.8	77.8	20.0	6	8	5
	Full-grown	SGfg	32	26	66.7	81.3	74.2	23	13	3
Oocyte maturation (OM)	Eccentric germinal vesicle	O Megv	1	1	2.1	100.0	2.9	1	0	0
	Germinal vesicle migration	OMgvm	1	1	2.1	100.0	2.9	1	1	1
	Preovulatory	OMpov	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

spawning pair. Invariably, inspection of trace files revealed poorly resolved/ambiguous profiles at one or more loci. These individuals were not considered further. Assignments were made using both PROBMAX and FAP software, and results concurred.

3.2. Hormonal induction

During the study period, all spawns occurred within approximately 24–72 hours post implantation and only females implanted with GnRHa were found to contribute (Table 4). Progeny testing revealed

Table 5
Common snook broodstock spawning performance in three mass spawning tanks. Data presented represents number of eggs collected, fertilization rate, hatch rate and three day larval survival ± SEM (n = 3).

Broodstock tank	Spawning event (mm/dd/yyyy)	Number of males / females spawned	Total male/female spawn contribution (%)	Number of eggs collected	Fertilization rate (%)	Hatch rate (%)	Survival 1 DPH (%)	Survival 2 DPH (%)	Survival 3 DPH (%)
A	Jun 15, 2011	9/4	50/26	190,000 ± 11,000	69.3 ± 2.1	47.8 ± 5.5	91.8 ± 2.1	87.6 ± 4.3	79.3 ± 2.6
	Jun 16, 2011	10/6	55/40	204,700 ± 31,700	17.0 ± 3.0	78.2 ± 3.7	97.3 ± 0.5	95.8 ± 1.9	90.1 ± 1.9
	May 22, 2012	3/2	17/13	272,000 ± 15,159	60.7 ± 0.5	56.3 ± 2.9	92.2 ± 1.2	61.4 ± 4.1	25.9 ± 2.3
	May 23, 2012	2/3	11/20	239,100 ± 5,253	65.1 ± 2.3	50.6 ± 3.9	67.5 ± 3.6	60.9 ± 2.0	60.7 ± 2.3
	May 24, 2012	2/2	11/13	79,500 ± 4,642	70.1 ± 4.1	91.0 ± 0.6	97.0 ± 0.8	77.3 ± 3.2	76.3 ± 3.1
B	May 24, 2012	2/1	9/9	86,300 ± 4,439	34.4 ± 4.8	64.3 ± 6.2	80.6 ± 3.9	74.6 ± 4.8	68.5 ± 3.8
C	May 12, 2011	19/10	48/63	2,075,300 ± 60,103	62.5 ± 2.1	78.8 ± 0.2	76.4 ± 7.7	74.2 ± 4.1	59.9 ± 2.5
	Jul 6, 2011	6/4	15/25	964,600 ± 39,443	68.8 ± 1.9	84.0 ± 2.3	81.1 ± 2.6	78.9 ± 2.0	53.0 ± 3.1
	Jul 7, 2011	12/5	30/31	1,310,000 ± 47,849	55.7 ± 1.0	86.0 ± 2.0	90.2 ± 1.8	59.7 ± 1.9	27.5 ± 2.7
	Jul 8, 2011	11/3	28/19	656,730 ± 8,806	40.4 ± 2.7	98.1 ± 0.4	72.3 ± 4.9	64.4 ± 3.2	49.4 ± 5.2
	Nov 10, 2011	13/2	33/13	583,400 ± 22,385	63.9 ± 0.4	93.0 ± 1.9	65.3 ± 5.1	45.9 ± 3.5	28.5 ± 1.6
	Apr 6, 2012	15/4	38/25	906,100 ± 8,662	22.8 ± 1.4	90.9 ± 2.2	93.4 ± 1.9	88.1 ± 3.1	19.9 ± 2.1
	Apr 7, 2012	10/4	25/25	145,500 ± 6,936	55.4 ± 2.7	53.2 ± 1.8	76.7 ± 0.5	43.9 ± 7.2	27.7 ± 5.9
	Jun 6, 2012	10/4	25/25	2,378,000 ± 53,655	78.7 ± 1.4	69.8 ± 2.4	79.9 ± 3.3	68.7 ± 1.1	52.0 ± 5.4
	Jun 7, 2012	10/4	25/25	1,043,200 ± 15,247	63.6 ± 1.0	97.1 ± 0.5	85.5 ± 4.3	79.0 ± 1.4	74.2 ± 2.3
	Jun 8, 2012	5/2	13/13	375,000 ± 27,221	87.3 ± 1.7	83.6 ± 2.2	79.7 ± 3.0	77.8 ± 5.7	22.2 ± 1.9

Table 6
Female (A) and male (B) spawn contribution observed from 2011 to 2012 in a single captive common snook broodstock population (Tank A). Results were determined by exclusion based parentage using 8 DNA microsatellites for genotyping individual larvae (n = 5 spawns).

Date	Jun 15, 2011	Jun 16, 2011	May 22, 2012	May 23, 2012	May 24, 2012	Total No. Offspring	Percentage (%)
A) No. of larvae sired by females Female ID – Tank A							
F4		**91**				**91**	**10.9**
F5			**25**	**2**		**27**	**3.2**
F6	**106**	**5**		**8**	**1**	**120**	**14.4**
F9	**11**	**14**				**25**	**3.0**
F11	**14**	**28**				**42**	**5.0**
F12	**39**	**10**				**49**	**5.9**
F14			**150**	**147**	**175**	**472**	**56.7**
F15		**6**				**6**	**0.7**
B) No. of larvae sired by males Male ID – Tank A							
M1	**17**	**1**				**18**	**2.2**
M3	**1**	**8**				**9**	**1.1**
M4	**3**	**2**				**5**	**0.6**
M6	**1**	**3**	**10**			**14**	**1.7**
M8		**4**	**19**			**23**	**2.8**
M9	**17**	**57**				**74**	**8.9**
M10	**102**	**15**	**146**	**149**	**151**	**563**	**67.7**
M13	**9**	**14**				**23**	**2.8**
M15	**17**	**10**				**27**	**3.2**
M17		**40**		**8**		**48**	**5.8**
M18	**3**				**25**	**28**	**3.4**

Table 7
Female (A) and male (B) spawn contribution observed from 2011 to 2012 in a single captive common snook broodstock population (Tank C). Results were determined by exclusion based parentage using 8 DNA microsatellites for genotyping individual larvae (n = 10 spawns).

Date	May 12, 2011	Jul 6, 2011	Jul 7, 2011	Jul 8, 2011	Nov 10, 2011	Apr 6, 2012	Apr 7, 2012	Jun 6, 2012	Jun 7, 2012	Jun 8, 2012	Total no. offspring	Percentage (%)
A) No. oflarvae sired by females Female ID – Tank C												
F29			1								1	0.1
F30	3	1				1	9				14	1.3
F31			9			9	150	1	6		175	16.1
F32	9										9	0.8
F34	13	37	8	80	42		1	65	47	41	334	30.7
F36	13	20	21	3		5		6	5		73	6.7
F37	12										12	1.1
F38	2										2	0.2
F39	1										1	0.1
F40	3										3	0.3
F41	4										4	0.4
F42	22	36	24	37	112	9	2	61	72	85	460	42.3
B) No. oflarvae sired by males Male ID – Tank C												
M39	6	20		3							29	2.5
M40	3								3	4	10	0.9
M42				1							1	0.1
M45	3	32		61	20		1				117	10.0
M46	3	19	2	5	34	8					71	6.1
M48						2					2	0.2
M49						1			6	7	14	1.2
M50					1						1	0.1
M51			2			1	2	79			84	7.2
M52	17		4		4	14	2				41	3.5
M54					1			6	72		79	6.8
M55			8	33		48	2				100	8.6
M56	2			1	48			3	26	3	83	7.1
M58	4				8	2					14	1.2
M59	5		21		5						31	2.7
M60	16		2			2	7	1	6		34	2.9
M62						1	6				7	0.6
M64	1		6			1					8	0.7
M65	2			2				29	3		36	3.1
M66	3		1	3							7	0.6
M67	1		6								7	0.6
M68					1						1	0.1
M69					12			1			13	1.1
M70				7		2	24				33	2.8
M71	2		2				3	12	2		21	1.8
M72	1										1	0.1
M73	6										6	0.5
M74	7		10	3		5					38	3.3
M76	1					5	116		8	43	173	14.8
M77				1	3	1			5	39	50	4.3
M78								2			2	0.2
M80					17	24					41	3.5

that offspring sampled at three days post-hatch were only spawned from females that, at the time of hormonal implantation, had a minimum oocyte developmental stage of late Secondary Growth (SGl). Total parental contribution from females with oocytes in SGl was 20.0% and 74.2% for females with oocytes staged as Secondary Growth full grown (SGfg). Progeny were identified from 35 of 48 (73%) hormonally treated females (Table 4). No progeny were detected from the five implanted females identified as having the majority of their oocytes in early Secondary Growth (SGe). Total spawn contribution from females implanted at different stages of oocyte development is presented in Table 4.

3.3. Spawn performance

From April 2011 to June 2012, five spawning events occurred in tank A, one in tank B and 10 in tank C (Table 5). In tank A, successive spawning over a maximum of three consecutive days was observed in six female and eight male broodstock with one female and four males contributing in both years 2011 and 2012 (Table 6A, B). In tank C, successive spawning over consecutive days was observed in five females and eighteen males with > 50% spawning at multiple times in the same year (Table 7A, B). Results from the November 2011 spawning event in tank C showed photo-thermal conditioning of snook outside their natural spawning season (April to September in Florida) is possible (Table 7A, B; Fig. 1).

Overall, spawning performances between the three tank groups were highly variable in terms of the total number of eggs produced (from 86,300 to 2,378,000 eggs/spawn), fertilization (from 17.0 to 87.3%) and hatch rate (from 47.8 to 98.1%) (Table 5). The mean number of eggs produced per spawn in tank A was 197,060 ± 32,643 (SEM) whereas in tank C production averaged almost five times that at 1,043,783 ± 224,999 (SEM) eggs per spawn. Three day larval survival ranged from approximately 25.9% to 90.1% in tank A and 19.9% to 74.2% in tank C (Table 5). Mean larval survival in tanks A and C was recorded as 66.5% and 41.4% respectively. Only a single spawn was obtained from tank B therefore, no comparisons could be made with tanks A and C.

3.4. Parental contribution

Tank A: Approximately 50% of males had a detectable contribution in 2011 but only 11-17% contributed in 2012 (Table 5). A similar pattern

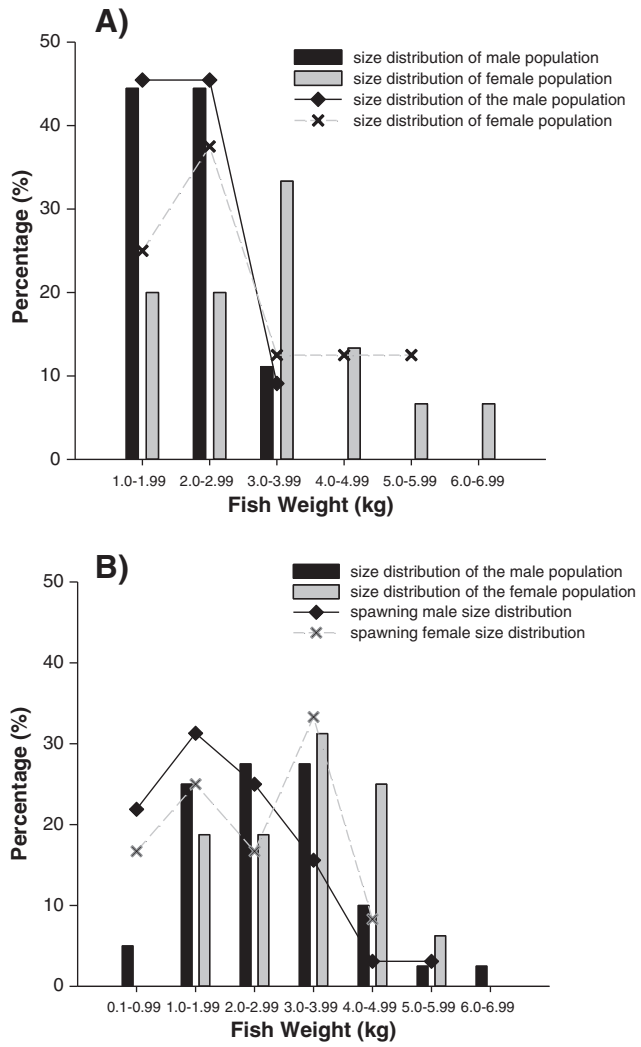

Fig. 2. Relationship between the total numbers of offspring produced from 2011 to 2012 plotted against parental size distribution of female/male population (sire weight — dam weight in kg) for common snook broodstock in (A) Tank A and (B) Tank C.

was observed for females with many more spawning in 2011 than in 2012. Overall, parental contributions were highly skewed, particularly in 2012. Two dams were responsible for 47.6% of the offspring (202 out of 424 sampled) accounted for in 2011 from tank A (Table 6A). Similarly, in 2012, a total of 93.0% (472 out of 508 sampled) were assigned to a single dam. Neither of the two females that were predominant contributors in year one contributed in year two. The opposite was found among the male broodstock in tank A. A single male sired up to 36% of assigned fry in year one (117 out of 324) and the same male sired 88% (446 out of 508) in year two (Table 6B). Five males made substantial contributions (siring 18–74 offspring) in 2011 while low levels of contribution (1–8 offspring) were detected for another five males (Table 6B). Offspring were identified from two to six (13–40%) of the total fifteen female broodstock and from two to ten (11–50%) of the total eighteen male broodstock in tank A (Table 6A, B).

In contrast to tank A, only one spawn occurred in tank B with 100% of the offspring assigned to a single female and two males (data not shown). Tank C: Male and female total spawn contribution was highly variable between spawning events in tank C. Throughout 2011 and 2012 spawning seasons, offspring were assigned to ≤63% of female and ≤48% of male broodstock in tank C (Table 5). Four of twelve females were repeatedly the top contributors, siring 1,042 larvae or 95% of the total larvae assigned in tank C (Table 7A). Low levels of contribution were identified from the additional eight spawning female broodstock (1-14 offspring). Based on results obtained from preliminary studies evaluating the effect of hormonal therapy on milt production in male common snook broodstock (Rhody, unpublished), the number of males spawning in tank C was much higher than expected despite low fertilization rates observed across some spawning events (Table 5). Thirty-two of forty males spawned (80%) overall; although the average number of males spawning per event was 28% (Table 7B).

3.5. Impact of broodstock size on reproductive success

A single factor one way ANOVA (SPSS 19, IBM, USA) showed the average weight of captive female snook broodstock in tanks A, B and C were not significantly different (Table 1, $P = 0.11$). In contrast, a statistical difference in weight was detected among males. Variations in these metrics were determined using Duncan's post hoc test where males in tank C were significantly heavier than males in tanks A and B ($P = 0.04$, $F = 3.2$, $df = 2$). Lengths were not significantly different for females ($P = 0.06$) and males ($P = 0.10$) in all tanks (Table 1). Size distributions of contributing males and females were varied and no size-assortative mating was observed in tank A or tank C (Fig. 2A, B). In tank A, there was no significant correlation between male or female size and contribution to offspring (male regression, $P = 0.92$, $R^2 = 0.001$, $n = 11$; female regression, $P = 0.09$, $R^2 = 0.39$, $n = 8$) (Fig. 3A, B). In tank C, there was no correlation between male size and number of offspring sired (regression, $P = 0.30$, $R^2 = 0.03$, $n = 32$; Fig. 3C) however; a correlation between female size and contribution to offspring (regression, $P = 0.01$, $R^2 = 0.45$, $n = 12$) was observed (Fig. 3D).

4. Discussion

While most genetic studies on snook populations have been directed toward conservation of wild stocks (Seyoum et al., 2005; Tringali et al., 2008a), the present study focused on using genetic markers for the improved management of captive common snook broodstock. In this molecular based assessment, new information on requirements for broodstock husbandry, mating patterns and spawning periodicity of captive common snook broodstock were obtained. More specifically, spawn contribution data 1) provided a confirmation of GnRHa treatment efficacy in female snook with a minimum stage of oogenesis (late secondary growth-SGl) required for successful spawning; 2) identified a potential impact of handling on maturation and spawning in male and female broodstock; 3) confirmed that, through photothermal conditioning, captive common snook broodstock can spawn over consecutive days and several times per year including outside of their natural spawning season.

Overall, the eight loci microsatellite panel performed well, giving robust data for all the parents and vast majority of offspring. The rate of genotyping errors within the offspring was 1.7%, mostly attributable to one locus (Cun14) where there was ambiguity between some adjacent allele bins. In future work, it may be prudent to replace Cun14 with an alternative marker. The exclusion based assignment worked well when one allelic mismatch was tolerated, an approach that is routinely taken (Pompanon et al., 2005), given the inevitable low level of error expected.

Since fish are held under enclosed conditions, reproductive bottlenecks observed in common snook broodstock, such as lack of spontaneous spawning, poor fertilization and low fecundity could be linked to one or more missing environmental cues. Bottlenecks include few individuals initiating and completing the gametogenic cycle even with the use of hormonal therapies and low spermatogenic activity in males, with reduced milt production evidenced by inability or difficulty in stripping male snook (Rhody, unpublished). The relationship between the developmental stage of the gonads and required timing of hormonal stimulation in teleosts is well described (Mylonas et al., 2010; Zohar and

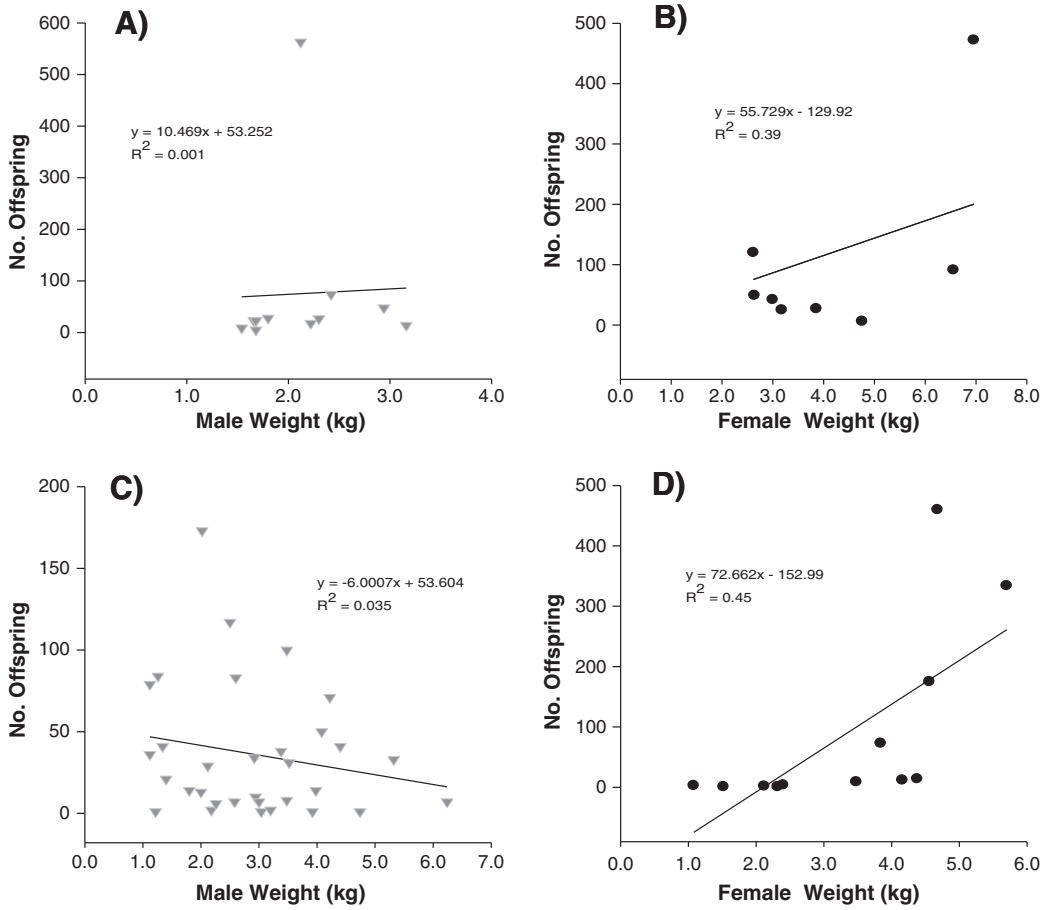

Fig. 3. Scatterplot of male (▼) and female (●) body size (kg) versus contribution to offspring. Male (A) and female (B) body size (kg) versus contribution to offspring in Tank A (n = 11, n = 8). Male (C) and female (D) body size (kg) versus contribution to offspring in Tank C (n = 32, n = 12).

Mylonas, 2001); however, few studies in aquaculture species have linked hormonal treatment to the effective spawn contribution from individual broodstock during mass spawning events. Hormonal treatments given to immature individuals or to adults too early in the reproductive cycle are usually ineffective (Mylonas et al., 2010). In the present study, parentage assignment of the progeny showed GnRHa hormonal stimulation was effective when performed with females whose oocytes were classified in the later stages of vitellogenesis including Late Secondary Growth (SGl) or beyond. These results confirmed that the timing of implantation must be correlated with the stage of oocyte development in order to optimize broodstock spawning potential in common snook. Captive common snook males were not implanted during the spawning trials. Average fertilization rate from all spawns combined was 56.9% indicating hormonal stimulation is not required for male spermiation to occur; however, future work may aim to investigate the effect of hormonal manipulation on male spawn performance and quality.

Parental contribution has already been studied in a range of teleost species with many reports showing skewed contribution (Coleman and Jones, 2011), especially in captive male broodstock, potentially leading to a reduction in genetic diversity (Borrell et al., 2011; Liu et al., 2012). This was confirmed in the present study, although in some of the spawning events and tanks, up to 50% of the female and/or male snook broodstock significantly contributed to the progeny. This is a very interesting finding. First, results confirmed the suitability of the environmental conditioning of captive snook broodstock and the efficacy and requirement of hormonal therapies in female broodstock. Second, results showed multiple males fertilized egg batches in the absence of hormonal treatments. These results are important for future aquaculture and restocking programs where genetic variability must be maximized. Selection programs in all livestock require the generation of as many families as possible to select from and improve commercially important traits, such as growth, disease resistance and yield. During the present study, progeny were identified from 35 of 48 (73%) hormonally treated females (Table 4). A total of 40 families were produced in tank A and 87 families in tank C, all from a relatively small broodstock population of males and females (Table 1). The most represented family was from tank A with 337 progeny, 40.5% of the total assigned. This type of dominance has been seen in other mass spawning fish species, such as Atlantic cod (Herlin et al., 2008) and Japanese flounder *Paralichthys olivaceus* (Sekino et al., 2003).

It must be acknowledged that parental contribution to individual spawns may have been much higher given the variable fertilization rates (17–83%), hatch rates (50–97%) and survival to 3 DPH (20–90%). This variability is similar to most non-domesticated marine fish studied. In well domesticated aquaculture fish species, such as European sea bass (Vandeputte et al., 2009) and Atlantic salmon, *Salmo salar* (Vasemagi et al., 2012) domestication and selection has reduced such variability along with better husbandry and captive spawning technologies.

Parental contribution was highly variable between spawning seasons, events and tanks with fewer individuals contributing towards the end of the season (Tables 6 and 7). Previous field studies conducted with wild spawning snook showed that as the spawning season advances, egg quality decreases, thus having a direct impact on spawn quality including fertilization and hatch rate as well as larval survival (Yanes-Roca et al., 2009). Further investigation is needed to document patterns of egg quality across spawning seasons in captive common snook broodstock to better understand this relationship. Data presented

here shows a lack of size-assortative mating, due to sexual dimorphism in snook, as they are protandric hermaphrodites (Taylor et al., 2000). Interestingly, during the course of the study no sex change in males was observed in any of the captive broodstock populations. Overall, the spawning population matched the size distribution of the entire captive broodstock population, suggesting reproductive success in captive common snook is not linked to this factor. A positive correlation between female size and contribution to offspring was observed. These findings are similar to other studies documenting that larger females have higher fecundity; a possible mechanism that could allow enhanced contributions by some individuals to population replenishment (Beldade et al., 2012).

Broodstock contributions varied among the three snook populations. Only one spawn was documented from broodstock in tank B compared to multiple spawns observed from the other two broodstock groups (A and C). Over the study period, broodstock in tank B were blood sampled monthly as part of another study to monitor the seasonal reproductive cycle through sex steroid profiling, leading them to be handled almost two times more frequently than broodstock in tanks A and C. In tank B, a lack of spermiation in males and oocyte maturation in females was observed throughout most of the trial. The single spawn obtained from broodstock in tank B was recorded near the end of the study following a four month period where no handling occurred. The absence of reproductive activity in tank B could have been caused by the stress of monthly handling which involved cannulation biopsies and repeated blood sampling. Research has shown tolerance and physiological response to stress varies among fish species (Schreck et al., 2001). Stress factors, such as handling and hormonal induction, are known to have a negative effect on maturation and spawning in fish (Barton, 2002; Wendelaar Bonga, 1997). Such effects may have altered spawning behavior (McConnachie et al., 2012; Morgan et al., 1999), advanced or delayed oocyte maturation and ovulation (Watanabe et al., 2005), induced follicular atresia (Micale et al., 1999), altered gamete and offspring viability (Bobe and Labbé, 2010) and reduced egg production (Bogevik et al., 2012; Milla et al., 2009). In captive common snook, extensive handling appears to have a negative impact on maturation and spawning and further investigations are needed to define the limits so optimal broodstock management strategies can be implemented.

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