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Comparative study of lipids and fatty acids in the liver, muscle, and eggs of wild and captive common snook broodstock

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ABSTRACT

In this study, the lipid composition of wild and captive common snook broodstock was investigated to identify potential nutritional deficiencies and formulate suitable diets for captive stocks. Results showed that captive snook incorporated significantly more lipid than their wild counterparts. However, cholesterol and arachidonic acid (ARA) levels were significantly lower compared to wild fish, which may impact steroid and prostaglandin production, reproductive behavior and gametogenesis. In eggs obtained from captive broodstock, high docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) levels, associated with low ARA contents were found. As a result, ARA/EPA ratio in captive eggs was less than half of that in wild eggs with the potential for negative consequences on embryo and larval development. In conclusion, large differences were noticed between wild and captive broodstock that may contribute to the reproductive dysfunctions observed in captive snook broodstock (e.g. incomplete oocyte maturation, low milt production and highly variable egg and larval quality). The wild snook survey also identified the presence of hydrocarbons in the liver, which should be further studied to identify a potential impact on the reproductive performances of a vulnerable population like common snook.

1. Introduction

Dietary lipids and in particular polyunsaturated fatty acids (PUFAs) play a critical role in the successful production of high quality gametes and eggs of marine fish (Izquierdo et al., 2001; Sargent et al., 2002). While a large proportion of dietary lipids are catabolized to fuel reproductive processes, they are also deposited into gametes, especially as yolk reserve in the oocytes (Tocher, 2003). Yolk fatty acid composition directly affects the optimal development of the embryo and yolk-sac larvae by providing docosahexaenoic acid (DHA), essential in neural and visual development, as well as eicosapentaenoic acid (EPA) and arachidonic acid (ARA) which serve as precursors of eicosanoids involved in the modulation of neural, hypothalamic, and immune functions (Bell, 2003; Kamler, 2007; Migaud et al., 2013; Tocher, 2010). ARA is a key PUFA for fish reproduction through the production of prostaglandins that stimulates ovarian and testicular steroidogenesis, final oocyte maturation, ovulation and milt production (Lister and Van Der Kraak, 2008; Norambuena et al., 2013; Sorbera et al., 2001; Wade, 1994). ARA-derived prostaglandins also act as pheromones and influence sexual behavior (Stacey and Sorensen, 2011).

Marine teleosts have lost their ability to synthesize PUFAs, thus, DHA, EPA and ARA are essential fatty acids that must be provided by the diet (Sargent et al., 1997). The low substrate specificity in fatty acid metabolism (several fatty acids are substrates for the same enzyme) explains the greater direct influence of dietary lipids on the final concentrations and cellular functions compared to any other class of nutrients. As a result, the fatty acid profile from fish tissues and eggs reflects the fatty acid profile supplied through the diet (Alasalvar et al., 2002; Sargent et al., 1993, 2002). The comparison of tissues and/or eggs from wild and captive fish allows the identification of potential nutritional deficiencies, which is essential for the development of suitable broodstock diets (Migaud et al., 2013). This strategy has been successful in many species including striped trumpeter *Latris lineata* (Morehead et al., 2001), sea bass *Dicentrarchus labrax* (Alasalvar et al., 2002), white seabream *Diplodus sargus* (Cejas et al., 2003, 2004b), black seabream *Spondyliosoma cantharus* (Rodriguez et al., 2004), Japanese eel *Anguilla japonica* (Oku et al., 2009), black sea bass *Centropristis striata* (Seaborn et al., 2009), highfin amberjack *Seriola rivoliana* (Saito, 2012), greater amberjack *Seriola dumerili* (Rodriguez-Barreto et al., 2012; Saito, 2012) and Senegalese sole *Solea senegalensis* (Norambuena et al., 2012a).

The common snook *Centropomus undecimalis* is an estuarine species found in subtropical and tropical waters, around the Gulf of Mexico and along the western Atlantic coast from Cape Canaveral, Florida, down to Florianopolis, Brazil (Alvarez-Lajonchère and Tsuzuki, 2008). Snook support a valuable recreational fishery in the southeastern United States and are a popular food fish in South America and Mexico. It is a protandric hermaphrodite species with transitional fish observed up

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to 7 years of age (Muller and Taylor, 2006). On the east coast of Florida, the spawning season extends from April to September, with spawning events typically occurring along sandy beaches, inlets and tidal passes of estuaries (Taylor et al., 1998). Habitat loss, increased recreational fishing pressure, and environmental changes (i.e., cold kills) have contributed to a decline in common snook stocks in the Gulf of Mexico (McRae and McCawley, 2011; Muller and Taylor, 2006). Therefore, additional fishery management tools, such as stock enhancement, are being investigated to supplement local fisheries in Florida (Brennan et al., 2008). Intensive aquaculture production is also of interest to increase market availability in South America (Alvarez-Lajonchère and Tsuzuki, 2008).

Despite recent breakthroughs in the spawning of captive common snook broodstock (Ibarra-Castro et al., 2011; Neidig et al., 2000; Rhody et al., 2013, 2014; Yanes-Roca et al., 2009) and advances in larval rearing protocols (Barón-Aguilar et al., 2013; Hauville et al., in press-a, in press-b; Ibarra-Castro et al., 2011; Rhody et al., 2010; Wittenrich et al., 2009), to date, there is still no established large scale production of this species for food or restocking. 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 (Rhody et al., 2013, 2014).

The aim of this study was to compare the lipid composition of muscle, liver and eggs from wild and common snook broodstock maintained in captivity for 3 years, to gain information on broodstock dietary requirements and improve captive spawn quality.

2. Materials and methods

2.1. Captive fish and egg collection

Captive broodstock were collected in Sarasota Bay (27°20′N 82°35′ W), Florida, in Fall 2009, and held indoors in a 4.6 m diameter, 25 m^3, fiberglass tank equipped with a filtration unit. Fish were fed a 50% shrimp, 50% herring diet (Table 1) at 2.5% body weight every other day, and maintained under simulated natural conditions. In May 2012, female broodstock reproductive development was assessed by ovarian biopsy and individuals with oocytes classified in the later stages of the oogenetic cycle (e.g. Secondary Growth Stage, Full-grown Step) (Grier et al., 2009; Neidig et al., 2000; Rhody et al., 2013) were hormonally induced to spawn with gonadotropin-releasing hormone (GnRHa implants, 50 μg/Kg bodyweight, Institute of Marine and Environmental Technologies, University of Maryland, Baltimore, MD, USA). Fish then spawned spontaneously by 32 h post implantation. Eggs were gathered into a collector via skimming of the tank's surface. After collection, eggs were transferred to a conical tank and after 4 h of incubation (past the blastula stage) the non-viable sinking eggs were removed and discarded (fertilization rate 64.1 ± 4.2%). Three viable buoyant egg aliquots were then sampled and rinsed with deionized water before storage at -70 °C. Eggs hatched after 16 h of incubation at 28 °C (hatching rate 82.6 ± 2.8%). In addition, 6 males and 6 females presenting non-mature oocytes, were sacrificed with an overdose of tricaine methanesulfonate (MS 222), weighed, and measured, the otoliths were extracted for age determination, and flesh and liver samples were stored at −70 °C. Hepatosomatic index (HSI) and gonadosomatic index (GSI) were calculated as: (liver or gonad weight (g) / body weight (g)) × 100 (Table 2).

All fish were collected under a Florida Fish and Wildlife Conservation Commission Special Activity License (Contract No. 10087, Permit # SAL 09-522-SR). Animals were sacrificed in accordance with United States legislation concerning the protection of animals used for experimentation. All methods were conducted in accordance with Mote Marine Laboratory's Institutional Animal Care and Use Committee approved protocols (IACUC Approval No. 12-03-KM1).

Table 1
Fatty acid profile (% of total FA) and total fatty acid content (mg/g of dry weight) of the diet fed to the captive broodstock (n = 3).

	Captive broodstock diet		
	Herring	Shrimp	50/50
14:0	4.7 ± 0.1	1.5 ± 0.1	3.9 ± 0.4
15:0	1.2 ± 0.1	1.0 ± 0.0	1.0 ± 0.1
16:0	20.5 ± 0.2	11.6 ± 0.4	18.8 ± 0.9
17:0	1.5 ± 0.0	1.6 ± 0.1	1.5 ± 0.0
18:0	6.0 ± 0.2	6.9 ± 0.3	6.9 ± 0.0
Σ SFA[a]	34.0 ± 0.3	22.8 ± 0.9	32.3 ± 1.4
16:1n−7	5.9 ± 0.1	5.2 ± 0.4	5.5 ± 0.1
18:1n−9	6.4 ± 0.1	7.0 ± 0.2	6.1 ± 0.4
18:1n−7	4.3 ± 0.2	6.0 ± 0.3	5.1 ± 0.4
20:1n−9	0.4 ± 0.1	1.0 ± 0.2	0.8 ± 0.1
Σ MUFA[b]	17.0 ± 0.2	19.4 ± 0.4	17.6 ± 0.1
16:3n−4	0.5 ± 0.0	1.1 ± 0.0	0.5 ± 0.0
18:2n−6	1.4 ± 0.0	1.5 ± 0.1	1.5 ± 0.0
20:4n−6	3.1 ± 0.2	9.2 ± 0.4	4.3 ± 0.2
22:5n−6	1.5 ± 0.1	1.0 ± 0.2	1.6 ± 0.1
20:5n−3	8.6 ± 0.4	15.3 ± 0.3	9.5 ± 0.3
22:5n−3	1.6 ± 0.1	2.1 ± 0.1	1.8 ± 0.1
22:6n−3	22.2 ± 0.6	8.7 ± 0.7	19.9 ± 0.9
Σ n−6[c]	7.5 ± 0.3	13.3 ± 0.4	8.6 ± 0.3
Σ n−3[d]	34.8 ± 1.0	27.1 ± 1.0	33.0 ± 1.1
Σ PUFA[e]	43.7 ± 0.9	42.1 ± 1.0	42.9 ± 1.3
DHA/EPA	2.6 ± 0.1	0.6 ± 0.0	2.1 ± 0.0
ARA/EPA	0.4 ± 0.0	0.6 ± 0.0	0.5 ± 0.0
n−3/n−6	4.7 ± 0.3	2.0 ± 0.1	3.8 ± 0.0
Total FA	117.3 ± 6.9	26.0 ± 1.3	71.8 ± 11.2

SFA: saturated fatty acids, MUFA: mono-unsaturated fatty acid; PUFA: poly-unsaturated fatty acid; DHA: docosahexaenoic acid (22:6n−3); EPA: eicosapentaenoic acid (20:4n−3); ARA: arachidonic acid (20:4n−6).
[a] Includes 12:0.
[b] Includes 15:1.
[c] Includes 18:3n−6, 20:2n−6, 20:3n−6.
[d] Includes 18:3n−3, 18:4n−3, 20:3n−3, 20:4n−3.
[e] Includes 16:2n−4, 18:3n−4.

2.2. Wild fish tissue and egg collection

Wild fish were collected from two close spawning sites (Emerson Point or Rattlesnake Key) in waters around Sarasota, once each in April, June, July and August 2012. Fish were captured with a seine net and held in floating pens until processed. Fish were measured and weighed and their sex and reproductive status assessed. At each time point, 6 sexually mature females (visual observation of mature oocytes after stripping or canulation biopsy) and 6 males (visual observation of milt expression after stripping or canulation) were sacrificed with an overdose of MS 222, placed on ice and quickly brought back to the laboratory where they were processed identically to captive fish. In June and August, no mature males were captured and therefore only female samples could be analyzed. In July, milt was collected from 6 males and stored on ice and eggs were stripped from 6 females. Eggs from 2 females were pooled and the 3 batches of eggs were fertilized in sterile seawater using a drop of milt from each male. After fertilization, eggs were rinsed and stored in sterile seawater in a bag under pure oxygen, secured in a cooler and quickly brought back to the laboratory and transferred to conical tanks to separate viable and non-viable eggs before sampling of 3 aliquots and storage as described previously. The average fertilization rate and hatching rate for the 3 batches were 78.3 ± 6.3% and 83.1 ± 5.1% respectively.

2.3. Proximate, fatty acid and lipid classes analyses

Proximate compositions of flesh and liver samples were determined according to standard procedures (AOAC, 2000). Prior to analysis, samples were minced and blended to ensure homogeneity. Moisture content was determined by drying the samples to constant weight