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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

Table 2
Proximate composition (% of wet weight) of flesh and liver, weight (kg), fork length (cm), hepatosomatic index (HSI), gonadosomatic index (GSI) and age (years) from wild and captive common snook broodstock (n = 6). Superscript letters indicate significant differences within a row.

		Female					Male		
		Wild April	Wild June	Wild July	Wild August	Captive	Wild April	Wild July	Captive
Lipid	Flesh	1.0 ± 0.1^{b}	0.7 ± 0.1^{ab}	0.5 ± 0.0^{a}	0.7 ± 0.1^{ab}	2.2 ± 0.5^{c}	0.7 ± 0.1^{ab}	0.5 ± 0.0^{a}	1.0 ± 0.1^{b}
	Liver	6.0 ± 1.0^{b}	9.4 ± 3.2^{bc}	6.3 ± 1.8^{b}	15.8 ± 4.6^{c}	9.8 ± 2.0^{bc}	3.4 ± 0.3^{a}	4.5 ± 0.9^{a}	6.6 ± 1.0^{b}
Protein	Flesh	20.1 ± 0.3^{ab}	19.4 ± 0.2^{a}	19.2 ± 0.5^{a}	21.1 ± 0.5^{b}	22.0 ± 0.2^{b}	20.1 ± 0.4^{ab}	18.9 ± 0.2^{a}	21.9 ± 0.2^{b}
	Liver	14.8 ± 0.8^{ab}	17.3 ± 1.3^{b}	18.0 ± 1.6^{b}	13.0 ± 0.9^{a}	13.3 ± 0.9^{a}	13.2 ± 0.4^{a}	18.4 ± 2.1^{b}	12.9 ± 0.7^{a}
Moisture	Flesh	78.2 ± 0.4^{b}	79.3 ± 0.2^{b}	79.5 ± 0.3^{b}	76.2 ± 0.3^{a}	75.0 ± 0.4^{a}	78.6 ± 0.3^{b}	79.8 ± 0.2^{b}	76.4 ± 0.4^{a}
	Liver	73.6 ± 0.9^{b}	69.8 ± 3.1^{ab}	75.0 ± 1.8^{b}	65.9 ± 4.1^{a}	66.6 ± 1.5^{a}	74.2 ± 0.6^{b}	74.4 ± 0.7^{b}	69.6 ± 1.1^{ab}
Ash	Flesh	1.2 ± 0.1	1.2 ± 0.0	1.2 ± 0.1	1.2 ± 0.0	1.4 ± 0.1	1.3 ± 0.0	1.2 ± 0.1	1.3 ± 0.0
	Liver	1.4 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.0	1.3 ± 0.1	1.1 ± 0.1
Weight		3.04 ± 0.55	4.32 ± 0.84	3.32 ± 0.77	3.79 ± 0.48	2.05 ± 0.17	2.22 ± 0.24	3.22 ± 0.91	2.19 ± 0.19
Fork length		668 ± 39	722 ± 64	604 ± 64	726 ± 27	593 ± 18	617 ± 20	556 ± 44	583 ± 25
HSI		0.9 ± 0.1^{bc}	0.9 ± 0.1^{bc}	0.7 ± 0.1^{ab}	1.2 ± 0.1^{c}	0.7 ± 0.1^{ab}	0.8 ± 0.1^{b}	0.3 ± 0.0^{a}	0.6 ± 0.1^{ab}
GSI		1.6 ± 0.6^{ab}	4.1 ± 0.7^{bc}	5.2 ± 1.5^{c}	5.0 ± 0.8^{c}	0.7 ± 0.2^{a}	0.9 ± 0.4^{ab}	1.2 ± 0.3^{ab}	0.7 ± 0.2^{a}
Age (mean)		6.3 ± 0.9	5.7 ± 0.8	6.0 ± 1.0	6.0 ± 0.7	6.7 ± 0.5	6.3 ± 0.4	7.5 ± 1.2	8.8 ± 1.3
Age (range)		4–10	3–8	3–9	5–9	5–8	5–8	4–12	6–14

(105 °C for 24 h). Ash content was determined after 24 h in crucibles at 600 °C. Crude protein content (Nx6.25) was determined using the automated Kjeldahl method (Tecator Kjeltec Auto 1030 analyzer, Foss, Warrington, U.K.). Crude lipid content was determined after extraction according to Folch et al. (1957).

Separation of lipid classes was performed by double development high-performance thin-layer chromatography using methyl acetate/isopropanol/chloroform/methanol/0.25% aqueous KCl (25:25:25:10:9, by volume) as the first development to separate polar lipids and isohexane/diethyl ether/acetic acid (85:15:1, by volume) as the secondary development to separate neutral lipids (Henderson and Tocher, 1992). Lipid classes were visualized by charring at 160 °C for 20 min after spraying with 3% (w/v) aqueous cupric acetate in 8% (v/v) phosphoric acid and quantified by densitometry with a tungsten lamp at 370 nm using a CAMAG-3 TLC scanner (CAMAG, Muttenz, Switzerland) with winCATS Planar Chromatography Manager software. Identification of individual classes was confirmed by comparison and reference to retention factors of authentic standards run alongside samples.

Fatty acid composition was determined by gas–liquid chromatography after preparation of fatty acid methyl esters (FAMEs) according to Morrison and Smith (1964). FAMEs were separated and quantified on a gas chromatograph (Shimadzu GC-2014, Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with a Phenomenex ZB-WAX plus capillary column (30 m long, 0.53 mm internal diameter, 1.0 μm thickness; Phenomenex, Torrance, CA, USA) with on-column injection and flame ionization detection, using helium as carrier gas (4 mL min^{-1}) and injector and detector temperatures of 250 and 260 °C respectively. Temperature was held at 160 °C for 5 min then increased up to 220 °C at 3 °C per minute and maintained at this temperature for 30 min. FAME peaks were identified by comparison with known standards (Supelco, Inc., Bellefonte, Pennsylvania, USA).

2.4. Statistics

Statistical analysis was performed using MINITAB® version 16.0. Data were analyzed by two-way ANOVA (sex and origin for Tables 2 through 6, and origin and tissue in Table 7) followed by a Tukey's post hoc test with 95% confidence. Non-homogenous data were arcsine square root transformed before analysis. No statistical analysis was performed on hydrocarbon data as this lipid class was not observed in all samples and more data would be required. All results are presented as means ± SEM. Only fatty acids contributing to at least 1% in one group are represented.

3. Results

3.1. Broodstock morphometrics and proximate composition

Morphometric and lipid class composition data are presented in Table 2. There was no statistical difference in age, weight and length among the groups used in this study. No difference in HSI or GSI was found between captive males and captive females. HSI from July males was the lowest (0.3 ± 0.0) of all fish groups, though not statistically

Table 3
Lipid class composition (%) of flesh from wild and captive common snook broodstock (n = 6). Superscript letters indicate significant differences within a row.

	Female					Male		
	Wild April	Wild June	Wild July	Wild August	Captive	Wild April	Wild July	Captive
PC	25.3 ± 1.6^{bc}	27.3 ± 1.8^{bc}	29.7 ± 0.5^{c}	19.9 ± 1.2^{b}	15.4 ± 1.1^{a}	29.3 ± 1.6^{c}	31.5 ± 0.2^{c}	20.7 ± 1.3^{b}
PS	3.2 ± 0.3^{ab}	3.6 ± 0.2^{b}	5.0 ± 0.2^{c}	4.5 ± 0.3^{c}	2.3 ± 0.6^{a}	3.8 ± 0.2^{b}	5.3 ± 0.2^{c}	3.1 ± 0.3^{ab}
PI	4.8 ± 0.4^{ab}	5.9 ± 0.4^{b}	6.1 ± 0.2^{b}	5.8 ± 0.3^{b}	3.5 ± 0.5^{a}	6.4 ± 0.5^{b}	8.2 ± 0.3^{c}	5.1 ± 0.3^{ab}
PE	11.7 ± 0.7^{bc}	12.7 ± 0.8^{c}	13.4 ± 0.5^{c}	9.5 ± 0.7^{b}	7.3 ± 0.3^{a}	14.1 ± 1.0^{c}	14.3 ± 0.3^{c}	9.3 ± 0.6^{b}
Total polar*	51.1 ± 3.4^{b}	55.8 ± 2.7^{bc}	64.3 ± 1.3^{cd}	51.9 ± 2.5^{b}	33.9 ± 3.2^{a}	59.7 ± 3.1^{c}	69.1 ± 0.2^{d}	45.3 ± 2.4^{ab}
DAG	2.2 ± 0.3^{b}	1.6 ± 0.4^{ab}	0.4 ± 0.2^{a}	2.0 ± 0.1^{b}	2.7 ± 0.2^{b}	2.0 ± 0.2^{b}	0.8 ± 0.3^{a}	2.4 ± 0.3^{b}
CHOL	12.1 ± 0.7^{b}	14.4 ± 1.2^{bc}	17.7 ± 0.7^{c}	12.3 ± 0.4^{b}	8.6 ± 0.5^{a}	13.8 ± 0.6^{b}	16.6 ± 0.5^{c}	10.1 ± 0.8^{a}
FFA	4.9 ± 0.4^{a}	4.5 ± 0.4^{a}	5.3 ± 0.5^{a}	14.9 ± 0.7^{b}	4.8 ± 0.5^{a}	5.0 ± 0.6^{a}	5.6 ± 0.1^{a}	4.7 ± 0.7^{a}
TAG	24.1 ± 4.1^{bc}	19.8 ± 4.3^{b}	8.5 ± 1.3^{a}	15.7 ± 3.0^{b}	43.9 ± 3.7^{d}	14.6 ± 3.4^{b}	4.9 ± 0.6^{a}	28.8 ± 2.1^{c}
W + SE	5.6 ± 0.6^{b}	3.9 ± 0.7^{a}	3.9 ± 0.6^{a}	3.2 ± 0.2^{a}	6.3 ± 1.9^{b}	5.0 ± 0.6^{ab}	3.0 ± 0.2^{a}	8.8 ± 1.8^{bc}
HC	nd	nd	nd	nd	nd	nd	nd	nd
Total neutral	48.9 ± 3.4^{c}	44.2 ± 2.7^{bc}	35.8 ± 1.3^{ab}	48.1 ± 2.5^{c}	66.1 ± 3.2^{e}	40.4 ± 3.1^{b}	30.9 ± 0.2^{a}	54.7 ± 2.4^{cd}

PC: phosphatidylcholine; PS: phosphatidylserine; PI: phosphatidylinositol; PE: phosphatidylethanolamine; DAG: diacylglycerol; CHOL: cholesterol; FFA: free fatty acid; TAG: triacylglycerol; W + SE: wax and sterol ester; HC: hydrocarbon; nd: not detected.
* Includes lysophosphatidylcholine, sphingomyelin, phosphatidylglycerol and pigmented material.

Table 4
Lipid class composition (%) of liver from wild and captive common snook broodstock (n = 6). Superscript letters indicate significant differences within a row.

	Female					Male		
	Wild April	Wild June	Wild July	Wild August	Captive	Wild April	Wild July	Captive
PC	16.0 ± 1.4^{bc}	13.3 ± 2.2^{b}	21.7 ± 2.6^{c}	5.8 ± 0.9^{a}	9.1 ± 1.5^{ab}	17.6 ± 0.7^{bc}	14.3 ± 1.5^{b}	10.0 ± 1.7^{ab}
PS	1.9 ± 0.3^{ab}	1.3 ± 0.5^{a}	3.0 ± 0.7^{b}	1.1 ± 0.2^{a}	1.2 ± 0.5^{a}	2.8 ± 0.3^{b}	3.9 ± 0.6^{b}	1.6 ± 0.5^{a}
PI	2.8 ± 0.5^{b}	2.5 ± 0.6^{b}	5.2 ± 0.9^{c}	0.7 ± 0.1^{a}	1.7 ± 0.6^{ab}	3.2 ± 0.3^{b}	3.6 ± 0.6^{bc}	1.6 ± 0.5^{ab}
PE	9.4 ± 0.9^{b}	8.8 ± 1.4^{ab}	11.9 ± 1.4^{b}	3.6 ± 0.9^{a}	5.7 ± 1.0^{a}	10.3 ± 0.7^{b}	10.0 ± 1.2^{b}	6.3 ± 1.2^{ab}
Total polar*	43.0 ± 3.2^{bc}	37.6 ± 6.6^{bc}	51.0 ± 6.2^{c}	22.2 ± 2.6^{a}	28.8 ± 5.0^{ab}	48.7 ± 1.9^{c}	45.6 ± 4.8^{c}	31.7 ± 5.0^{ab}
DAG	2.7 ± 1.0^{ab}	3.7 ± 1.0^{bc}	1.7 ± 0.8^{a}	4.9 ± 0.6^{c}	5.3 ± 0.8^{c}	2.1 ± 0.2^{ab}	0.9 ± 0.9^{a}	4.6 ± 1.0^{c}
CHOL	12.4 ± 1.7^{b}	8.7 ± 1.6^{ab}	13.4 ± 1.9^{b}	5.5 ± 0.5^{a}	8.1 ± 1.1^{ab}	13.3 ± 0.7^{b}	13.8 ± 1.7^{b}	8.9 ± 1.3^{ab}
FFA	20.6 ± 1.8^{b}	20.6 ± 0.7^{b}	15.7 ± 1.4^{ab}	28.4 ± 0.8^{c}	20.1 ± 1.5^{b}	18.1 ± 0.8^{ab}	18.4 ± 1.5^{ab}	20.6 ± 1.2^{b}
TAG	9.8 ± 2.8^{b}	18.9 ± 6.9^{c}	10.2 ± 5.1^{b}	29.3 ± 4.1^{d}	27.1 ± 5.9^{cd}	7.0 ± 4.1^{ab}	4.9 ± 4.9^{a}	23.0 ± 4.4^{c}
W + SE	11.0 ± 2.1^{b}	7.4 ± 0.4^{a}	6.8 ± 1.1^{a}	7.6 ± 1.8^{ab}	10.7 ± 1.7^{b}	10.3 ± 1.7^{b}	10.9 ± 1.8^{b}	11.2 ± 1.5^{b}
HC	0.5 ± 0.9	3.1 ± 0.5	1.3 ± 0.3	2.1 ± 0.7	nd	0.3 ± 0.1	3.2 ± 0.4	nd
Total neutral	57.0 ± 5.5^{ab}	62.4 ± 6.6^{b}	49.0 ± 6.2^{a}	77.8 ± 2.6^{c}	71.2 ± 5.0^{bc}	51.2 ± 4.1^{a}	52.1 ± 5.4^{a}	68.3 ± 5.0^{bc}

Abbreviations as in Table 3.
* Includes lysophosphatidylcholine, sphingomyelin, phosphatidylglycerol and pigmented material.

different from captive males, captive females, and July females. HSI from August females was the greatest (1.2 ± 0.1) of all fish groups, yet not statistically different from April and June females. GSI was the lowest for captive fish (0.7 ± 0.2), though not statistically different from wild males and females caught in April. The highest GSI values were observed for July and August females (5.2 ± 1.5 and 5.0 ± 0.8 respectively), though not statistically different from June females.

In flesh samples, lipid content was significantly higher for captive females (2.2 ± 0.5%) compared to all the other groups (average of 0.7 ± 0.1%). Liver lipid content was statistically higher for captive males compared to wild males, however captive female liver lipid content was not statistically different from that of wild females. Liver protein content was significantly higher in wild June females and wild July males and females. In contrast, in these same three groups, flesh protein content was lower, though not statistically different from April males and females.

3.2. Lipid classes

Lipid class composition of flesh and liver is represented respectively in Tables 3 and 4. In flesh, captive females presented significantly lower total polar lipid content, and higher total neutral lipid content compared to wild females. Likewise, captive males presented lower total polar lipid content and higher total neutral lipid content compared to wild males. In addition, levels of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and cholesterol (CHOL) were significantly lower, and levels of triacylglycerols (TAG) significantly higher in captive female flesh samples compared to wild females and captive males compared to wild males. Among wild males, significantly lower total polar lipid content and higher total neutral lipid content were observed in April males compared to July males. Among wild females, the highest total polar lipid content and the lowest total neutral lipid content were observed in July females, though not significantly different than levels in June females.

Table 5
Fatty acid profile (% of total FA) and fatty acid content (mg/g of dry weight) of flesh from wild and captive common snook broodstock (n = 6). Superscript letters indicate significant differences within a row.

	Female					Male		
	Wild April	Wild June	Wild July	Wild August	Captive	Wild April	Wild July	Captive
14:0	1.8 ± 0.3^{ab}	1.6 ± 0.2^{a}	1.4 ± 0.1^{a}	2.0 ± 0.2^{ab}	2.6 ± 0.2^{b}	1.4 ± 0.2^{a}	1.3 ± 0.3^{a}	2.8 ± 0.2^{b}
15:0	1.3 ± 0.3	1.0 ± 0.1	0.6 ± 0.1	1.1 ± 0.1	0.6 ± 0.0	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.0
16:0	21.6 ± 0.9^{ab}	20.9 ± 0.4^{a}	20.5 ± 0.5^{a}	21.9 ± 0.9^{ab}	23.8 ± 0.2^{b}	21.9 ± 0.4^{ab}	20.9 ± 0.8^{a}	23.1 ± 0.8^{b}
18:0	5.2 ± 0.2^{a}	5.7 ± 0.2^{ab}	5.6 ± 0.3^{ab}	5.3 ± 0.3^{a}	5.4 ± 0.2^{a}	5.9 ± 0.4^{b}	6.2 ± 0.3^{b}	5.5^{a} ± 0.2
Σ SFA[1]	30.9 ± 1.3^{a}	30.0 ± 0.7^{a}	28.8 ± 0.5^{a}	31.2 ± 1.2^{ab}	33.1 ± 0.2^{b}	30.7 ± 0.8^{a}	29.8 ± 1.2^{a}	32.7 ± 0.9^{b}
16:1n−7	5.8 ± 0.7^{b}	4.8 ± 0.5^{ab}	4.2 ± 0.4^{a}	6.2 ± 0.8^{b}	5.5 ± 0.5^{b}	3.9 ± 0.5^{a}	3.3 ± 0.6^{a}	5.4 ± 0.5^{b}
18:1n−9	10.9 ± 1.0^{a}	10.8 ± 0.9^{a}	10.1 ± 0.3^{a}	12.0 ± 0.8^{ab}	13.5 ± 0.6^{b}	11.0 ± 1.3^{a}	10.6 ± 1.2^{a}	12.9 ± 0.8^{b}
18:1n−7	3.3 ± 0.3^{b}	2.8 ± 0.3^{ab}	2.5 ± 0.1^{a}	3.4 ± 0.4^{b}	2.8 ± 0.2^{ab}	2.5 ± 0.3^{a}	2.3 ± 0.2^{a}	2.7 ± 0.2^{ab}
Σ MUFA[2]	20.7 ± 1.8^{ab}	18.9 ± 1.6^{a}	17.2 ± 0.5^{a}	22.3 ± 2.1^{b}	22.7 ± 1.2^{b}	18.0 ± 1.9^{a}	16.8 ± 2.1^{a}	22.1 ± 1.6^{b}
16:3n−4	1.6 ± 0.3^{b}	1.4 ± 0.1^{ab}	1.6 ± 0.1^{b}	1.6 ± 0.1^{b}	1.1 ± 0.1^{a}	1.2 ± 0.1^{a}	1.4 ± 0.2^{ab}	1.0 ± 0.1^{a}
18:2n−6	1.4 ± 0.1^{b}	1.1 ± 0.1^{a}	1.5 ± 0.2^{b}	1.2 ± 0.1^{a}	1.2 ± 0.0^{a}	1.3 ± 0.1^{ab}	1.2 ± 0.1^{a}	1.3 ± 0.1^{ab}
20:4n−6	7.4 ± 0.7^{b}	8.9 ± 1.0^{bc}	11.4 ± 0.7^{c}	7.7 ± 1.1^{b}	5.6 ± 0.6^{a}	8.8 ± 1.0^{bc}	10.8 ± 1.0^{c}	5.6 ± 0.6^{a}
22:5n−6	2.6 ± 0.4^{a}	3.0 ± 0.3^{ab}	3.9 ± 0.3^{b}	2.5 ± 0.4^{a}	2.5 ± 0.2^{a}	3.5 ± 0.4^{b}	4.2 ± 0.4^{b}	2.6 ± 0.3^{a}
20:5n−3	3.7 ± 0.3^{ab}	4.4 ± 0.1^{b}	3.8 ± 0.3^{ab}	4.9 ± 0.4^{b}	3.6 ± 0.1^{ab}	3.5 ± 0.2^{ab}	3.1 ± 0.2^{a}	3.5 ± 0.1^{ab}
22:5n−3	3.7 ± 0.4^{b}	3.7 ± 0.3^{b}	3.0 ± 0.2^{a}	3.5 ± 0.4^{b}	2.8 ± 0.2^{a}	3.6 ± 0.2^{b}	2.9 ± 0.2^{a}	3.2 ± 0.2^{ab}
22:6n−3	18.5 ± 2.7^{ab}	18.7 ± 1.6^{ab}	19.5 ± 1.4^{b}	15.5 ± 2.0^{a}	20.0 ± 0.8^{b}	21.2 ± 2.0^{b}	20.3 ± 1.7^{b}	20.5 ± 1.3^{b}
Σ n−6[3]	12.3 ± 1.0^{bc}	14.1 ± 1.2^{c}	17.8 ± 1.0^{d}	12.5 ± 1.3^{bc}	10.2 ± 0.8^{a}	14.6 ± 1.3^{cd}	17.0 ± 1.4^{d}	10.4 ± 0.9^{a}
Σ n−3[4]	27.3 ± 2.4^{ab}	27.7 ± 1.4^{ab}	27.2 ± 1.2^{ab}	25.1 ± 2.0^{a}	27.4 ± 0.7^{ab}	29.5 ± 1.8^{b}	27.1 ± 1.7^{ab}	28.2 ± 1.4^{b}
Σ PUFA[5]	42.2 ± 3.1^{ab}	44.1 ± 2.2^{ab}	47.4 ± 0.7^{b}	40.4 ± 3.1^{a}	39.7 ± 1.4^{a}	46.0 ± 2.9^{b}	46.3 ± 2.8^{b}	40.6 ± 2.2^{a}
DHA/EPA	5.2 ± 0.9^{bc}	4.3 ± 0.3^{ab}	5.4 ± 0.8^{bc}	3.2 ± 0.3^{a}	5.6 ± 0.2^{bc}	6.1 ± 0.8^{c}	6.9 ± 1.0^{c}	5.9 ± 0.3^{c}
ARA/EPA	2.0 ± 0.3^{ab}	2.1 ± 0.3^{b}	3.1 ± 0.4^{c}	1.6 ± 0.2^{a}	1.6 ± 0.1^{a}	2.6 ± 0.4^{bc}	3.6 ± 0.4^{c}	1.6 ± 0.2^{a}
n−3/n−6	2.2 ± 0.1^{ab}	2.0 ± 0.2^{ab}	1.6 ± 0.2^{a}	2.1 ± 0.2^{ab}	2.7 ± 0.1^{b}	2.1 ± 0.1^{ab}	1.6 ± 0.1^{a}	2.8 ± 0.2^{b}
Total FA	37.4 ± 4.1^{bc}	27.2 ± 2.1^{ab}	23.3 ± 1.5^{a}	40.1 ± 9.2^{bc}	56.7 ± 13.7^{cd}	31.0 ± 3.6^{b}	22.3 ± 3.8^{a}	70.7 ± 35.7^{cd}

Abbreviations as in Table 1.
[1] Includes 12:0.
[2] Includes 15:1, 20:1n−9.
[3] Includes 18:3n−6, 20:2n−6, 20:3n−6.
[4] Includes 18:3n−3, 18:4n−3, 20:3n−3, 20:4n−3.
[5] Includes 16:2n−4, 18:3n−4.

Table 6
Fatty acid profile (% of total FA) and fatty acid content (mg/g of dry weight) of liver from wild and captive common snook broodstock (n = 6). Superscript letters indicate significant differences within a row.

	Female					Male		
	Wild April	Wild June	Wild July	Wild August	Captive	Wild April	Wild July	Captive
14:0	1.7 ± 0.3^{ab}	1.8 ± 0.3^{ab}	1.4 ± 0.3^{a}	2.4 ± 0.3^{ab}	3.8 ± 0.6^{b}	1.3 ± 0.2^{a}	1.2 ± 0.2^{a}	3.3 ± 0.4^{b}
15:0	1.3 ± 0.5^{ab}	2.1 ± 0.6^{b}	0.8 ± 0.2^{a}	1.7 ± 0.1^{b}	0.9 ± 0.1^{a}	0.7 ± 0.1^{a}	1.0 ± 0.2^{a}	0.8 ± 0.0^{a}
16:0	23.7 ± 1.5^{bc}	24.6 ± 1.7^{bc}	22.5 ± 2.0^{b}	29.6 ± 0.4^{c}	23.1 ± 1.1^{b}	22.0 ± 1.1^{ab}	22.8 ± 1.1^{b}	20.8 ± 0.7^{a}
17:0	1.0 ± 0.2^{a}	1.8 ± 0.2^{b}	1.4 ± 0.2^{ab}	1.7 ± 0.1^{b}	1.0 ± 0.0^{a}	0.9 ± 0.1^{a}	1.2 ± 0.1^{ab}	1.0 ± 0.0^{a}
18:0	5.8 ± 0.5^{a}	8.3 ± 1.6^{b}	10.6 ± 1.2^{bc}	6.2 ± 0.6^{ab}	5.5 ± 0.2^{a}	6.1 ± 0.4^{ab}	7.7 ± 0.6^{b}	6.0 ± 0.1^{ab}
Σ SFA[1]	33.6 ± 1.7^{ab}	38.5 ± 1.4^{bc}	36.9 ± 1.3^{b}	41.5 ± 0.6^{c}	34.4 ± 1.4^{ab}	31.0 ± 1.5^{a}	33.9 ± 1.3^{ab}	31.8 ± 1.0^{a}
16:1n − 7	7.5 ± 1.2^{bc}	6.5 ± 1.3^{b}	4.4 ± 1.1^{a}	9.9 ± 0.6^{c}	7.3 ± 0.7^{b}	4.3 ± 0.6^{a}	3.4 ± 0.6^{a}	6.1 ± 0.6^{b}
18:1n − 9	12.7 ± 1.4^{b}	12.5 ± 1.5^{b}	13.1 ± 0.9^{b}	16.0 ± 0.5^{c}	13.5 ± 1.4^{b}	9.7 ± 1.4^{a}	8.2 ± 1.2^{a}	13.4 ± 1.9^{b}
18:1n − 7	5.0 ± 1.1^{bc}	3.9 ± 0.5^{b}	3.7 ± 0.6^{ab}	5.5 ± 0.3^{c}	4.2 ± 0.2^{b}	3.2 ± 0.3^{a}	2.9 ± 0.3^{a}	3.9 ± 0.4^{b}
Σ MUFA[2]	26.0 ± 1.9^{c}	23.5 ± 2.6^{bc}	21.7 ± 2.6^{b}	32.1 ± 0.9^{cd}	26.2 ± 2.1^{c}	17.9 ± 2.3^{a}	15.0 ± 2.0^{a}	24.7 ± 3.0^{bc}
16:3n − 4	1.4 ± 0.4^{b}	1.9 ± 0.4^{b}	0.9 ± 0.2^{a}	1.7 ± 0.1^{b}	0.9 ± 0.1^{a}	0.8 ± 0.1^{a}	0.9 ± 0.2^{a}	0.8 ± 0.1a
18:2n − 6	1.2 ± 0.2^{a}	1.0 ± 0.1^{a}	1.3 ± 0.2^{ab}	1.1 ± 0.1^{a}	1.5 ± 0.1^{b}	1.0 ± 0.1^{a}	1.1 ± 0.1^{a}	1.4 ± 0.1^{b}
20:4n − 6	5.4 ± 0.8^{b}	5.6 ± 0.8^{b}	7.4 ± 1.1^{bc}	2.9 ± 0.2^{a}	3.3 ± 0.8^{a}	8.0 ± 0.9^{c}	9.1 ± 0.9^{c}	4.3 ± 1.0^{ab}
22:5n − 6	1.4 ± 0.2^{bc}	1.1 ± 0.2^{b}	2.1 ± 0.5^{cd}	0.5 ± 0.0^{a}	1.4 ± 0.1^{bc}	2.4 ± 0.3^{d}	2.6 ± 0.2^{d}	1.6 ± 0.2^{c}
20:5n − 3	2.0 ± 0.2^{ab}	2.7 ± 0.3^{bc}	2.3 ± 0.3^{b}	2.6 ± 0.4^{bc}	1.8 ± 0.3^{a}	2.3 ± 0.2^{b}	1.7 ± 0.3^{a}	2.1 ± 0.2^{ab}
22:5n − 3	3.0 ± 0.4^{bc}	1.8 ± 0.1^{ab}	1.8 ± 0.2^{ab}	1.3 ± 0.1^{a}	3.0 ± 0.2^{bc}	3.5 ± 0.3^{c}	2.2 ± 0.3^{b}	3.1 ± 0.2^{c}
22:6n − 3	15.3 ± 2.5^{b}	13.4 ± 3.3^{b}	15.1 ± 2.7^{b}	5.9 ± 0.9^{a}	16.6 ± 2.0^{bc}	23.4 ± 2.6^{d}	23.2 ± 2.3^{d}	20.2 ± 3.1^{cd}
Σ n − 6[3]	9.0 ± 0.8^{b}	8.7 ± 0.8^{b}	11.9 ± 1.6^{bc}	5.5 ± 0.3^{a}	7.2 ± 0.9^{ab}	12.3 ± 1.1^{c}	13.7 ± 1.0^{c}	8.2 ± 1.0^{b}
Σ n − 3[4]	21.7 ± 2.7^{b}	19.1 ± 3.0^{b}	20.1 ± 2.6^{b}	10.8 ± 0.7^{a}	23.4 ± 2.0^{bc}	30.2 ± 2.0^{c}	28.0 ± 1.9^{c}	27.2 ± 2.8^{c}
Σ PUFA[5]	33.1 ± 3.2^{b}	30.7 ± 3.3^{b}	33.7 ± 3.7^{b}	19.3 ± 0.9^{a}	32.9 ± 2.7^{b}	44.1 ± 2.8^{c}	43.3 ± 2.5^{c}	37.4 ± 3.7^{bc}
DHA/EPA	7.3 ± 0.8^{b}	5.8 ± 1.8^{ab}	8.3 ± 2.6^{bc}	2.7 ± 0.9^{a}	11.0 ± 2.1^{cd}	11.2 ± 1.9^{cd}	16.0 ± 2.5^{d}	10.2 ± 1.8^{c}
ARA/EPA	2.6 ± 0.2^{ab}	2.3 ± 0.5^{ab}	4.1 ± 1.4^{bc}	1.3 ± 0.3^{a}	2.1 ± 0.5^{a}	3.8 ± 0.7^{b}	6.4 ± 1.2^{c}	2.1 ± 0.5^{a}
n − 3/n − 6	2.4 ± 0.1^{b}	2.2 ± 0.2^{ab}	1.7 ± 0.1^{a}	2.0 ± 0.1^{ab}	3.3 ± 0.2^{c}	2.5 ± 0.1^{b}	2.1 ± 0.1^{ab}	3.4 ± 0.1^{c}
Total FA	165.5 ± 36.0^{b}	240.6 ± 63.7^{bc}	169.9 ± 39.8^{b}	352.7 ± 68.7^{c}	277.7 ± 67.4^{bc}	94.5 ± 16.2^{a}	106.2 ± 23.4^{a}	187.7 ± 34.6^{b}

Abbreviations as in Table 1.
[1] Includes 12:0.
[2] Includes 15:1, 20:1n − 9.
[3] Includes 18:3n − 6, 20:2n − 6, 20:3n − 6.
[4] Includes 18:3n − 3, 18:4n − 3, 20:3n − 3, 20:4n − 3.
[5] Includes 16:2n − 4, 18:3n − 4.

Table 7
Fatty acid profile (% of total FA) and fatty acid content (mg/g of dry weight) of flesh, liver and eggs from wild and captive common snook female broodstock (n = 6 for flesh and liver, n = 3 for eggs). Superscript letters indicate significant differences within a row.

	Wild July			Captive		
	Flesh	Liver	Eggs	Flesh	Liver	Eggs
14:0	1.4 ± 0.1^{a}	1.4 ± 0.3^{a}	1.9 ± 0.1^{ab}	2.6 ± 0.2^{b}	3.8 ± 0.6^{c}	2.4 ± 0.1^{b}
16:0	20.5 ± 0.5^{ab}	22.5 ± 2.0^{b}	21.7 ± 0.7^{b}	23.8 ± 0.2^{c}	23.1 ± 1.1^{bc}	17.6 ± 0.3^{a}
17:0	0.7 ± 0.1^{a}	1.4 ± 0.2^{c}	1.0 ± 0.0^{b}	0.7 ± 0.0^{a}	1.0 ± 0.0^{b}	0.8 ± 0.0^{a}
18:0	5.6 ± 0.3^{b}	10.6 ± 1.2^{c}	5.2 ± 0.1^{b}	5.4 ± 0.2^{b}	5.5 ± 0.2^{b}	4.0 ± 0.1^{a}
Σ SFA[1]	28.8 ± 0.5^{ab}	36.9 ± 1.3^{c}	30.6 ± 0.6^{b}	33.1 ± 0.2^{bc}	34.4 ± 1.4^{bc}	25.4 ± 0.3^{a}
16:1n − 7	4.2 ± 0.4^{a}	4.4 ± 1.1^{a}	7.6 ± 0.3^{c}	5.5 ± 0.5^{ab}	7.3 ± 0.7^{bc}	6.2 ± 0.1^{b}
18:1n − 9	10.1 ± 0.3^{a}	13.1 ± 0.9^{b}	16.9 ± 0.3^{c}	13.5 ± 0.6^{b}	13.5 ± 1.4^{b}	12.9 ± 0.2^{b}
18:1n − 7	2.5 ± 0.1^{a}	3.7 ± 0.6^{ab}	4.6 ± 0.2^{bc}	2.8 ± 0.2^{a}	4.2 ± 0.2^{b}	3.8 ± 0.1^{b}
Σ MUFA[2]	17.2 ± 0.5^{a}	21.7 ± 2.6^{b}	29.5 ± 0.2^{c}	22.7 ± 1.2^{b}	26.2 ± 2.1^{bc}	23.3 ± 0.3^{b}
16:2n − 4	0.6 ± 0.0^{a}	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}	0.8 ± 0.0^{ab}	1.1 ± 0.1^{b}	0.9 ± 0.0^{b}
16:3n − 4	1.6 ± 0.1^{b}	0.9 ± 0.2^{a}	1.4 ± 0.0^{b}	1.1 ± 0.1^{ab}	0.9 ± 0.1^{a}	0.9 ± 0.0^{a}
18:2n − 6	1.5 ± 0.2^{ab}	1.3 ± 0.2^{a}	2.2 ± 0.5^{b}	1.2 ± 0.0^{a}	1.5 ± 0.1^{ab}	1.9 ± 0.0^{b}
20:4n − 6	11.4 ± 0.7^{c}	7.4 ± 1.1^{bc}	5.4 ± 0.3^{b}	5.6 ± 0.6^{b}	3.3 ± 0.8^{a}	3.8 ± 0.2^{a}
22:5n − 6	3.9 ± 0.3^{c}	2.1 ± 0.5^{ab}	2.1 ± 0.2^{ab}	2.5 ± 0.2^{b}	1.4 ± 0.1^{a}	1.9 ± 0.0^{a}
20:5n − 3	3.8 ± 0.3^{c}	2.3 ± 0.3^{ab}	2.4 ± 0.4^{ab}	3.6 ± 0.1^{c}	1.8 ± 0.3^{a}	4.2 ± 0.2^{cd}
22:5n − 3	3.0 ± 0.2^{b}	1.8 ± 0.2^{a}	2.7 ± 0.1^{b}	2.8 ± 0.2^{b}	3.0 ± 0.2^{b}	3.2 ± 0.1^{b}
22:6n − 3	19.5 ± 1.4^{b}	15.1 ± 2.7^{a}	14.5 ± 0.2^{a}	20.0 ± 0.8^{b}	16.6 ± 2.0^{ab}	27.3 ± 0.4^{c}
Σ n − 6[3]	17.8 ± 1.0^{c}	11.9 ± 1.6^{b}	10.6 ± 1.0b	10.2 ± 0.8^{b}	7.2 ± 0.9^{a}	8.7 ± 0.1^{ab}
Σ n − 3[4]	27.2 ± 1.2^{b}	20.1 ± 2.6^{a}	20.8 ± 0.6^{a}	27.4 ± 0.7^{b}	23.4 ± 2.0^{ab}	36.3 ± 0.3^{c}
Σ PUFA[5]	47.4 ± 0.7^{c}	33.7 ± 3.7^{a}	33.6 ± 0.5^{a}	39.7 ± 1.4^{b}	32.9 ± 2.7^{a}	47.0 ± 0.3^{c}
DHA/EPA	5.4 ± 0.8^{a}	8.3 ± 2.6^{b}	6.5 ± .7^{b}	5.6 ± 0.2^{a}	11.0 ± 2.1^{bc}	6.6 ± 0.3^{b}
ARA/EPA	3.1 ± 0.4^{c}	4.1 ± 1.4^{cd}	2.3 ± 0.6^{bc}	1.6 ± 0.1^{b}	2.1 ± 0.5^{bc}	0.9 ± 0.1^{a}
n − 3/n − 6	1.6 ± 0.2^{a}	1.7 ± 0.1^{a}	2.1 ± 0.3^{ab}	2.7 ± 0.1^{b}	3.3 ± 0.2^{bc}	4.2 ± 0.1^{c}
Total FA	23.3 ± 1.5^{a}	169.9 ± 39.8^{cd}	193.4 ± 17.2^{d}	56.7 ± 13.7^{b}	277.7 ± 67.4^{de}	169.0 ± 13.9^{c}

Abbreviations as in Table 1.
[1] Includes 12:0, 15:0.
[2] Includes 15:1, 20:1n − 9.
[3] Includes 18:3n − 6, 20:2n − 6, 20:3n − 6.
[4] Includes 18:3n − 3, 18:4n − 3, 20:3n − 3, 20:4n − 3.
[5] Includes 18:3n − 4.

In liver, captive males presented significantly lower total polar lipid content, and higher total neutral lipid content compared to wild males. However, levels were more variable among wild females with the highest total polar lipid content observed in July females, though not significantly different from that of June and April females. July females also presented the lowest total neutral lipid content, though not significantly different from that of April females. In addition, the presence of hydrocarbons was detected in wild liver samples, while no observation was made in captive liver samples and flesh samples from both wild and captive fish.

3.3. Fatty acid profiles

Fatty acid profiles and total fatty acid content of flesh and liver samples are presented in Tables 5 and 6 respectively. In flesh samples, total of saturated fatty acids (SFAs) and mono-unsaturated fatty acid (MUFA) was significantly higher in captive males compared to wild males and in captive females compared to April, June and July females. No significant difference in total SFA was observed among wild fish. Levels of ARA in captive females and males were similar (5.6 ± 0.6% of total fatty acids (TAF)) and significantly lower than that of wild fish (average of 9.2 ± 0.9% of TFA). DHA levels were not different in males of both origins, while among females, a significantly lower level of DHA was observed in August females compared to July and captive females, with April and June presenting intermediate levels. ARA/EPA ratio was significantly lower in captive males (1.6 ± 0.2), compared to wild males (average of 3.1 ± 0.4). It was significantly also lower for captive females (1.6 ± 0.1), compared to June and July females (average of 2.6 ± 0.4) and not statistically different from April and August females.

In liver, no difference in total SFA was observed among males, while captive females showed levels similar to that of all other groups, except August females that showed a significantly higher total SFA. Total MUFA was significantly greater in captive males compared to wild males, while captive females presented a total MUFA level not statistically different from that of April, June and August females, but significantly higher than that of July females. As in flesh, liver ARA levels in captive males (4.3 ± 1.0% of TFA) were significantly lower than that of wild males (average of 8.6 ± 0.9% of TFA). ARA levels in captive females (3.3 ± 0.8% of TFA) were not different from those of August females, but significantly lower than those of other female groups (average of 6.1 ± 0.9% of TFA). DHA levels in captive females were not statistically different to those of captive males, however, DHA levels in wild females (average of 12.4 ± 2.7% of TFA) were significantly lower than those of wild males (average of 23.3 ± 2.5% of TFA). DHA/EPA ratio was not statistically different among males from all groups, while it was significantly higher in captive females compared to wild females. As in flesh, ARA/EPA ratio of captive males (2.1 ± 0.5) was significantly lower than that of wild males (3.8 ± 0.7 and 6.4 ± 1.2 respectively). ARA/EPA ratio in captive females was significantly lower than that of July females, but not different from that of other wild female groups. Wild males incorporated a significantly higher total level of PUFA (average of 43.7 ± 2.7% of TFA), compared to that of females from all groups (average of 29.9 ± 3.5% of TFA).

Overall, in both flesh and liver, between captive females and captive males, there was no significant difference in total SFA, total MUFA, total PUFA, and ARA, EPA or DHA content and resulting ratios. In flesh, among wild females and males, from the same time group (April females–April males, July females–July males), there was no significant difference in total SFA, total MUFA, total PUFA, and ARA, EPA or DHA contents and resulting ratios. However, in liver tissue, total MUFA, total PUFA and DHA contents were significantly higher in April males compared to April females and July males compared to July females, resulting in higher DHA/EPA ratios in April males compared to April females and July males compared to July females, while ARA/EPA ratios were not significantly different among April fish or among July fish.

Fatty acid profile and total fatty acid content of flesh, liver and egg samples from July females and captive females are compared in Table 7. No significant differences were observed in flesh and liver total SFA between wild and captive females, however captive eggs contained significantly lower SFA levels compared to wild eggs (25.4 ± 0.3 and 30.6 ± 0.6% of TFA respectively). Total MUFA were significantly lower in captive eggs compared to wild eggs (23.3 ± 0.3 and 29.5 ± 0.2% of TFA respectively), although not different between wild and captive liver tissue and significantly higher in captive flesh tissue compared to wild flesh tissue (22.7 ± 1.2 and 17.2 ± 0.5% of TFA respectively). ARA contents were significantly higher in wild fish tissues and eggs compared to captive fish tissues and eggs (11.4 ± 0.7 and 5.6 ± 0.6% of TFA respectively in flesh, 7.4 ± 1.1 and 3.3 ± 0.8% of TFA respectively in liver, and 5.4 ± 0.3 and 3.8 ± 0.2% of TFA respectively in eggs). No significant differences were observed between wild and captive fish flesh and liver EPA contents; however, EPA contents were significantly lower in wild eggs than in captive eggs (2.4 ± 0.4 and 4.2 ± 0.2% of TFA respectively). A similar pattern was observed in DHA incorporation with no significant difference in flesh and liver DHA content between wild and captive fish, however, DHA contents were significantly lower in wild eggs compared to captive eggs (14.5 ± 0.2 and 27.3 ± 0.4% of TFA respectively). Consequently, ARA/EPA ratio in wild eggs was significantly higher than that in captive eggs (2.3 ± 0.6 and 0.9 ± 0.1 respectively), while there was no significant difference between DHA/EPA ratios. Total PUFA was not significantly different in wild and captive liver tissue, however, total PUFA in wild flesh tissue was significantly higher than that in captive flesh tissue (47.4 ± 0.7 and 39.7 ± 1.4% of TFA respectively) and total PUFA in wild eggs was significantly lower than that in captive eggs (33.6 ± 0.5 and 47.0 ± 0.3% of TFA respectively).

4. Discussion

Results from this study highlighted numerous differences in lipids between wild and captive snook broodstock with potential consequences on reproductive success and egg quality.

Captive fish presented a significantly higher flesh lipid content compared to their wild counterparts. This is the consequence of feeding a high lipid diet combined with reduced physical activity as already reported in several other marine fish species, including white seabream (Cejas et al., 2003, 2004b), black seabream (Rodriguez et al., 2004), greater amberjack (Rodriguez-Barreto et al., 2012; Saito, 2012) and Senegalese sole (Norambuena et al., 2012a). In fish, excess energy is mainly stored as neutral lipid and more particularly as TAG (Sargent et al., 2002), explaining the high TAG content (>40%) in the flesh of captive females. In this study, despite the significantly higher lipid and TAG content of captive females, captive eggs contained a significantly lower total FA content compared to wild eggs (13% reduction). Rodriguez-Barreto et al. (2012) made a similar observation between wild and cultured greater amberjack with cultured fish presenting higher total lipid content in flesh and liver, but lower content in gonads. The accumulation of lipids in teleost eggs is a complex process that is not yet fully understood (Hiramatsu et al., 2015). Several species-specific phospholipoglycoproteins (vitellogenins) are involved and their synthesis is controlled by a series of regulating hormones. Estrogen is believed to be the most potent steroid, stimulating the synthesis of vitellogenins by the liver. Vitellogenins are then released into the bloodstream and actively incorporated into maturing oocytes through receptor-mediated endocytosis (Hiramatsu et al., 2015; Lubzens et al., 2010; Tocher, 2003). In captive broodstock, a disruption of the endocrine reproductive axis is commonly observed, requiring the use of hormonal therapies (injection/implant) to induce final gonad maturation and spawning (Mylonas et al., 2010; Zohar and Mylonas, 2001). This disruption has been confirmed in common snook where lower estrogen and androgen levels were measured in captive broodstock compared to wild fish (Rhody et al., in review). Therefore, while high lipid content is

observed in the flesh and liver of captive fish, low estrogen levels likely impact vitellogenesis, affecting egg final total lipid content.

Among wild fish, no clear trend of TAG utilization during the reproduction period was detected, even though the lowest TAG levels in the flesh were observed in July, which is considered the peak of the snook spawning season. Wild snook keep feeding throughout the spawning season and though Almansa et al. (2001) demonstrated the use of lipid reserve during ovarian maturation of captive seabream fed during the spawning period, the wild snook diet seems to cover the nutritional needs of brooders. This would explain the lack of depletion of TAG reserves, the low flesh lipid content and low levels of perivisceral fat. Additional data would be necessary to investigate the mobilization of reserves during the spawning season. Another difference in lipid classes among wild and captive fish was noticed with regard to CHOL levels. CHOL is a simple lipid that does not contain any fatty acid and teleost fish have the ability to synthesize it (Leaver et al., 2008; Tocher, 2003). In humans, the role of ARA in CHOL regulation has been recently studied, demonstrating the regulation of reverse cholesterol transport by ARA metabolites (lipoxins) (Demetz et al., 2014; Spite, 2014). In addition, ARA lipoxygenated or epoxygenated products are involved in the expression of the steroidogenic acute regulatory (StAR) gene (Stocco et al., 2001). StAR proteins are involved in CHOL transfer from the outer to the inner mitochondrial membrane, where the first step of steroid production occurs and a strong correlation between StAR gene tissue-specific expression and tissue capacity to produce steroids has been reported (Castillo et al., 2015). In Senegalese sole, ARA and CHOL levels in blood were correlated with dietary ARA levels (Norambuena et al., 2013). CHOL has been identified as the main precursor of sex steroid hormones in fish which play major roles in the final oocyte maturation, meiosis resumption and sexual behavior (Diotel et al., 2011; Tokarz et al., 2013). Therefore, the lower levels of CHOL observed in captive fish may be a consequence of the lower ARA levels and may contribute to the reproductive dysfunction reported in captive snook (e.g. incomplete ovarian maturation, reduced milt volume as compared to wild males, and low quality eggs). In wild fish, the presence of hydrocarbons in the liver is of concern. Hydrocarbon contaminants have been found to have a detrimental effect on vitellogenesis with repercussion on circulating hormones and plasma vitellogenin, estrogenic and antiestrogenic effects as well as delay in oocyte maturation (Nicolas, 1999). In vulnerable populations such as common snook, any reduction in reproductive success can seriously impact wild stock recruitment and further investigation is therefore critical. Previous research has demonstrated the existence of hydrocarbon detoxification mechanisms in fish (Lee et al., 1972) and the lack of hydrocarbons in captive fish samples suggest a successful detoxification after three years in captivity unless the contamination of wild fish occurred after the acquisition of the captive broodstock (e.g. BP Deepwater Horizon oil spill in April 2010) (Weisberg et al., in press).

Dietary fatty acids and their cyclooxygenase and lipoxygenase metabolites are known to impact on oocyte maturation and spermatogenesis as well (Cerda et al., 1997; Sorbera et al., 2001). Lower ARA contents in captive broodstock, as observed in the present study, have also been reported in captive broodstock of white sea bream (Cejas et al., 2004b), black sea bream (Rodriguez et al., 2004), Senegalese sole (Norambuena et al., 2012a) and greater amberjack (Rodriguez-Barreto et al., 2012; Saito, 2012). As mentioned previously, ARA is a precursor of prostaglandins that is thought to stimulate the later stages of gametogenesis (e.g. ovulation) as well as influencing mating behavior (e.g. pheromones). ARA and EPA compete for the same enzymes involved in the production of prostaglandins (Sargent et al., 1999a). ARA forms 2-series prostaglandins, while EPA forms the less biologically active and antagonistic 3-series prostaglandins (Bell et al., 1994; Tocher et al., 1996). Therefore, in addition to absolute content, the relative proportion of each fatty acid should be taken into consideration (Izquierdo et al., 2000, 2001; Sargent et al., 1999a). Indeed, in turbot, *Scophthalmus maximus*, changes in the dietary ARA/EPA ratio modified the proportion of prostaglandins produced (J.G. Bell et al., 1995; Bell et al., 1994). In addition, significantly higher levels of 2-series prostaglandins and lower levels of 3-series prostaglandins were measured in wild Senegalese sole compared to captive broodstock that had lower ARA content, had lower ARA/EPA ratio and presented reproductive dysfunctions (Norambuena et al., 2012b). Therefore, the lower ARA content and ARA/EPA ratios in captive snook broodstock may impact on prostaglandin synthesis with potential negative consequences on captive snook reproduction. In addition, in Senegalese sole, increased ARA levels and ARA/EPA ratios were correlated with increased plasma steroid levels in males (11-ketotestosterone and testosterone), but no effect was observed in females (estradiol) (Norambuena et al., 2013). Moreover, in sea bass, it was demonstrated that a diet high in n − 3 fatty acids promoted female reproductive performance, while a diet with a higher level of ARA and lower n-3 content improved fertilization rate (Asturiano et al., 2001). Therefore, dietary ARA levels and ARA/EPA ratios seem to be of particular importance in male gonad maturation and quality, and the lower values observed in the captive males in this study most likely contributed to the poor milt production (quantity and quality) reported in captivity. Among wild fish, no clear seasonal variation in flesh and liver fatty acid profiles was observed during the spawning season even though ARA content was significantly higher in July during the peak of the natural spawning season. Fuiman and Faulk (2013) studied the transfer of dietary ARA to the eggs in red drum *Sciaenops ocellatus* and demonstrated a rapid diet–egg connection, supporting the hypothesis that batch-spawners migrate to their spawning ground to take advantage of a diet promoting gonad maturation and quality. Therefore, it seems as though snook spawning ground diets are able to sustain gamete production throughout the spawning season with potentially a higher supply of ARA during the peak of the spawning season.

In addition to their impact on gonad maturation, spawning behavior and sperm quality, dietary fatty acids also influence egg quality and larval survival. Indeed, many studies demonstrated the importance of egg and yolk-sac lipid reserves for both energy and structural development of embryos and larvae from warm and temperate waters, including red drum (Vetter et al., 1983), red sea bream *Pagrus major* (Koven et al., 1989), gilthead sea bream *Sparus aurata* (Koven et al., 1989; Rønnestad et al., 1994), common dentex *Dentex dentex* (Mourente et al., 1999), white seabream (Cejas et al., 2004a) and Atlantic Bluefin tuna *Thunnus thynnus* (Morais et al., 2011). After hatching, MUFAs are preferentially used for energy while SFAs and PUFAs are incorporated into structural phospholipids (Kamler, 2007; Sargent et al., 2002). DHA is the main fatty acid in neural and visual membranes and a deficiency has been shown to strongly impair larval development (M.V. Bell et al., 1995; Benítez-Santana et al., 2007; Neuringer et al., 1988). DHA and EPA compete in the formation of phospholipid structures with a higher biological value for DHA than EPA (Rodriguez et al., 1998; Sargent et al., 1999b). Therefore, as for ARA and EPA, the DHA:EPA ratio needs to be considered in addition to absolute content. In this study, DHA and EPA levels were significantly higher in captive eggs, however DHA/EPA ratios were similar in the eggs. It is interesting to note that flesh and liver DHA and EPA levels were not different between wild and captive females. The selective transfer and accumulation of DHA and to a lesser extent EPA into fish eggs have been demonstrated and the DHA and EPA rich captive diet probably leads to this large deposition in captive eggs (Johnson, 2009; Sargent et al., 2002; Wiegand, 1996). The higher level of EPA incorporated into the eggs, combined with the lower ARA content, leads to an ARA/EPA ratio less than half that of wild eggs, leading to possible modification in eicosanoid production and subsequent pathways (Bell, 2003).

Overall, the present results highlight lipid imbalances in captive broodstock, especially in ARA levels. Therefore, an ARA dietary supplementation may be of interest, with potential benefits to reproductive success and egg quality. Additional studies are required to determine the optimal level of supplementation and to achieve an adequate ARA/EPA ratio taking into account a probable rapid diet–egg transfer. In

addition, lowering the dietary EPA content would most likely benefit egg quality as well. The presence of hydrocarbon in the liver of wild fish should be further investigated to identify the source and potential impact on fish reproduction. The study of spawning grounds diet would also be of interest, allowing for the monitoring of the resource as a shift in prey availability due to changing environmental conditions could impact snook reproductive success.

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