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Fatty acid and proximate composition of wild male and female king angelfish (*Holacanthus passer*) gonads during the ripe and spent developmental stages

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Abstract

Overfishing of king angelfish *Holacanthus passer* reduced populations in the Gulf of California. This study aimed to determine the fatty acid and proximate composition of male and female gonads, and the E2 concentration in plasma of wild organisms with different stages of gonadal development captured during early and mid-summer at El Datil, near Kino Bay, Sonora, Mexico, to help establish basic reproductive aspects and possibly to help develop the aquaculture of this ornamental species. Out of the 45 adult specimens analyzed, 66.7% were females and 33.3% were males; 16.7% of the females had ripe gonads while 83.3% had spent gonads, and 20% of males had ripe gonads while 80% had spent gonads, indicating they were reproducing earlier, likely in the spring. Females had smaller size but numerically greater gonadosomatic index (GSI) and condition factor (K) than males. Ripe and spent females showed no statistical differences in weight, length or K; however, ripe females showed significantly higher GSI (P = 0.0005) than spent females, 3.43 *vs*. 0.87%. Lipid content was higher in ripe gonads, with higher content of fatty acids. In both sexes, DHA was quantitatively the major fatty acid, (10.83-16.28 mg of DHA g^{-1} of gonad wet weight). The n-3/n-6 ratios varied from 1.99 to 3.54, lower for ripe organisms due to a higher content of ARA and n-6 derivatives. Gonad DHA content changed in relation to its developmental stage and it might serve as an additional maturation index. Information on the chemical composition and fatty acid profile of the gonads can be used as indicators of dietary lipid and essential fatty acid requirements of broodfish, which may contribute to the formulation of a balanced diet for the culture of this ornamental species.

Keywords: fatty acid profile, gonad developmental stages, gonads, *Holacanthus passer*, king angelfish.

Introduction

The growing demand of coral reef species for exhibition in aquariums around the world has made the marine ornamental aquaculture a very profitable economic activity (Wabnitz *et al.*, 2003; Bruckner, 2005; Livengood and Chapman, 2007). The supply of organisms that populate the exhibits however, is questionable, since it relies on extraction from their natural habitats, quite often captured illegally (Lango-Reynoso *et al.*, 2012). The king angelfish, *Holacanthus passer* (Valenciennes, 1846), also known as Passer angelfish, is among the highly valued species in the international ornamental market. A member of the Pomacanthidae family, this non-migratory tropical species is distributed in the Pacific Ocean, from the north coast of Baja California, Mexico, to the north coast of Peru, and the Galapagos Islands in Ecuador (Arellano-Martínez *et al.*, 1999; Aburto-Oropeza *et al.*, 2000; Pyle *et al.*, 2010). Adults have an attractive dark blue color with a lateral vertical white stripe behind the pectoral fin, while juveniles are characterized by their yellow or orange coloration, shimmering-blue vertical stripes and blue rimming of the dorsal and anal fins, although they undergo changes in coloration as they mature. Females and males are isomorphic, except for their pelvic fins, which are white in males and yellow in females (Thomson *et al.*, 2000; Aquarium of the Pacific, 2016). This fascinating and contrasting coloration of *H. passer* is what drives the commercial interest and the reason why it is an economically important species for aquaria exhibition, particularly for Mexico and Costa Rica, where juveniles are captured to be exported to the United States of America and other countries around the world, fetching prices of over US$200.00 dollars per individual (LiveAquaria, 2016).

Overfishing has caused a decline in king angelfish populations (Sánchez-Alcántara *et al.*, 2006), thus, in the Gulf of California, the species was included in the list of protected species by the Mexican norm NOM-059-SEMARNAT-2001 (Mexico, 2002) as the first conservation effort of the government. Additionally, it is well known that the reproduction of marine finfish under controlled conditions also reduces the impact of heavy fishing pressure on wild populations (Mehdi and Ehsan, 2011; Arantes *et al.*, 2012), but successful reproduction in captivity requires the knowledge of several aspects of the adult life stage of an organism (Izquierdo *et al.*, 2001; Olivotto *et al.*, 2011). For *H. passer*, Arellano-Martínez *et al.* (1999) established that it is a partial spawner with asynchronous development of the gonads; its reproductive cycle is dictated by five phases of gonadal development, with sexual maturity and reproductive activity attained during the period of April to November.

Seasonal variations in the reproductive cycle of teleost fish are influenced by exposure to environmental stimuli acting on the hypothalamus-pituitary-gonad axis (Mylonas and Zohar, 2001; Zohar *et al.*, 2010); when sexual maturity has been attained and under the adequate environmental conditions, two types of

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gonadotropins (GtH) are produced by the pituitary gland and released into the bloodstream, namely, the follicle stimulating hormone (FSH or GtH I), which controls both, vitellogenesis and spermatogenesis, and the luteinizing hormone (LH or GtH II), which controls the final oocyte maturation and spermiation. In the female gonad during vitellogenesis, the FSH and LH promote the synthesis of sexual steroids such as testosterone, which subsequently, will be aromatized into 17β-estradiol (E2; Nagahama, 1994; Mylonas *et al.*, 2000; Mylonas and Zohar, 2001). This steroid hormone induces the biosynthesis of vitellogenin in the liver of mature female fish, which is subsequently incorporated into the developing oocyte (Collier *et al.*, 2003; Swanson *et al.*, 2003; Guerriero, 2007). A correspondence between plasma concentration of E2 and the size or weight of the gonads has been established (Aruke and Goksoyr, 2003; Moncaut *et al.*, 2003; Berg *et al.*, 2004), therefore, it is considered as an indicator of female gonadal maturation or final oocyte maturation in female fish (Scott *et al.*, 1998; Kokokiris *et al.*, 2000).

Reproductive success in fish requires the physiological allocation of lipid resources for storage, usually in liver, muscle and intraperitoneal fat, for later use in gonadal maturation, namely ovogenesis and spermiogenesis (Abdel-Aziz and El-Nady, 1993; Sargent, 1995; Luzzana *et al.*, 1996). Polyunsaturated and highly unsaturated fatty acids (PUFA and HUFA, respectively) play an important role in gonadal maturation, egg quality, and larval growth (Izquierdo *et al.*, 2001). Eicosapentaenoic acid (20:5n-3; EPA) and arachidonic acid (20:4n-6; ARA) are precursors of thromboxanes, prostaglandins, and leukotrienes (Wolfe and Horrocks, 1994), hormone-like molecules that play a role in immune and inflammatory responses, haematological and cardiovascular activity, renal and neural function, as well as in reproduction, specifically in ovarian and testicular steroidogenesis (Tocher, 2003). ARA is also thought to be involved in the embryonic development of the immune system, as well as in hatching and early larval performance (Sorbera *et al.*, 1998). Docosahexaenoic acid (22:6n-3, DHA) on the other hand, plays a major role as a constituent of neural and visual tissues (Sargent *el al.*, 2002), in consequence, mostly saturated and monounsaturated fatty acids are preferentially catabolized to meet energy demands, while HUFA are transferred to the eggs (Henderson *et al.*, 1995), therefore, the concentration of specific fatty acids such as DHA in gonads perhaps could be used as an additional indicator of gonadal maturation, although this requires further investigation. The aim of this study was to determine the fatty acid and proximate composition of male and female king angelfish (*H. passer*) gonads, and the concentration of E2 in plasma of wild organisms with different stages of gonadal development captured during the summer time in the area of El Datil, a location near Kino Bay, Sonora, Mexico.

Materials and Methods

Animals

A scientific collection permit (SGPA/DGVS/ 00809/14, issued on January 30th, 2014) was obtained through the Mexican Agency for the Environment and Natural Resources (SEMARNAT) to retrieve wild adult female and male king angelfish, which were acquired by SCUBA diving at El Datil, latitude 28°42′ N and longitude 112°17′ W, near Kino Bay, Sonora, Mexico. A total of 45 organisms were captured during July and August 2014 using fish landing nets. They were kept in a submersible cage and transported live in a circular tank of 200 L, provided with aeration and clean seawater, to the Kino Bay Experiment Station of the Department of Scientific and Technological Research of the University of Sonora, in Kino Bay, Sonora, Mexico. Upon arrival blood was collected from the caudal vein of all fish using 3 ml syringes, plasma was then separated by centrifuging (Heraeus Fresco 21, Thermo Scientific, Osterode am Harz, Germany) the blood at 850 *g* for 15 min at 4°C, and then stored in microcentrifuge tubes at -20°C until E2 was determined. Fish were then euthanized with an overdose of tricaine methanesulfonate (MS-222) prior to collection of biological indexes: individual weight (IW, g) and length (IL, cm) were measured to determine the condition factor (K) = (wet body weight × 100) / total length^3, cm; Ricker, 1975). Gonads were then dissected from all fish and weighed to calculate the gonadosomatic index (GSI, %) = (gonad wet weight, g /total wet weight, g) × 100; after this, each gonad was divided into two similar sections, one of them was used for the determination of the fatty acid and proximate composition and stored in an ultrafreezer (ThermoFisher Scientific, Isotemp Basic U86-13A41, Waltham, MA, USA) at -84.0ºC, and the other one for the histological determination of the developmental stage of the gonad and stored in Davidson's fixative solution (acetic acid:95% ethanol:formaldehyde:H_2O at a 1:3:2:3 ratio).

Proximate composition and fatty acid analysis

Crude protein (N factor = 6.25) was analyzed (method 968.06; Association of Official Analytical Chemists - AOAC, 2005) via combustion by the Dumas method with a Dumas Nitrogen Analyzer (Model NDA 702, VELP® Scientifica, Usmate, Italy). Moisture (method 930.15; AOAC, 2005) and ash (method 942.05; AOAC, 2005) were determined by standard official methods. Crude fat (CF) was extracted by the method of Folch *et al.* (1957) and quantified gravimetrically after drying a 5 ml aliquot under nitrogen. The remaining lipid extracted after the CF determination was used for fatty acid analysis. Fatty acids were transesterified with boron trifluoride and fatty acid methyl esters (FAME) were analyzed with a Varian 3800 gas chromatograph equipped with a 30 m × 0.25 mm fused silica capillary column and a flame ionization detector as previously described (Lochmann and Gatlin, 1993). Fatty acids

were identified by comparison of retention times to those of known standards, and quantified by using an internal standard (heptadecanoic acid, 17:0); they were expressed as mg g^{-1} of gonad wet weight.

Histological and plasma 17β-estradiol (E2) analyses

Gonads from each fish stored in Davidson's fixative solution were fixed during 24 h, after which they were transferred to a 70% ethanol solution for long-term storage. Three segments of each gonad (~1 cm length) were sliced, embedded in paraffin, and transverse sections were processed to 4 µm slides and stained with hematoxylin and eosin. The slides were examined under a light microscope (Leica Microsystems, DM1000, Wetzlar, Germany) equipped with a digital camera (Moticam Pro, 282A) and the Motic Images Plus 2.0 software (Motic, Richmond, BC, Canada) was used for image acquisition. Gonads were further analyzed for assessing their developmental stage according to the description provided by Arellano-Martínez *et al.* (1999). Additionally, plasma E2 was measured by Enzyme-Linked Immune Sorbent Assay (ELISA) according to the procedure described by Semenkova *et al.* (2002) using a commercial kit (MBS044518, My Biosource, San Diego, CA, USA).

Statistical analyses

Data presented here are means and standard error of the mean (S.E.) of biological parameters such as individual weight and length, GSI, K, E2, in addition to proximate compositions and fatty acid concentrations of female and male gonads of the identified developmental stages detected in *H. passer* captured during this season, which were ripe and spent. The non-parametric Kruskal-Wallis analysis was used to test differences among ripe and spent female and male gonads for these variables ($P < 0.05$) using the Statistical Analysis System (SAS Institute Inc., 2013, Software Release 9.4, Cary, NC, USA) software package.

Results

Gonad histology

A total of 45 adult specimens of *H. passer* were captured during July and August 2014. Of the captured king angelfish, a total of 30 organisms were females and 15 were males. Through the histological analyses of the gonads and the description provided by Arellano-Martinez *et al.* (1999), it was established that 16.7% of the females had ripe gonads, while 83.3% had spent gonads; in ripe ovaries, mature oocytes were evident and predominant, some oocytes were in a hydrated or pre-hydrated state and the lumen was not visible, whereas in the spent ovaries early oocytes were abundant and atresia was evident in some of them (Fig. 1). In the case of males, 20% had ripe gonads while 80% had spent gonads. The ripe testis showed tubules filled with spermatozoa, while in spent testis the tubules were practically empty, with residual spermatozoa in lumen and ducts (Fig. 2).

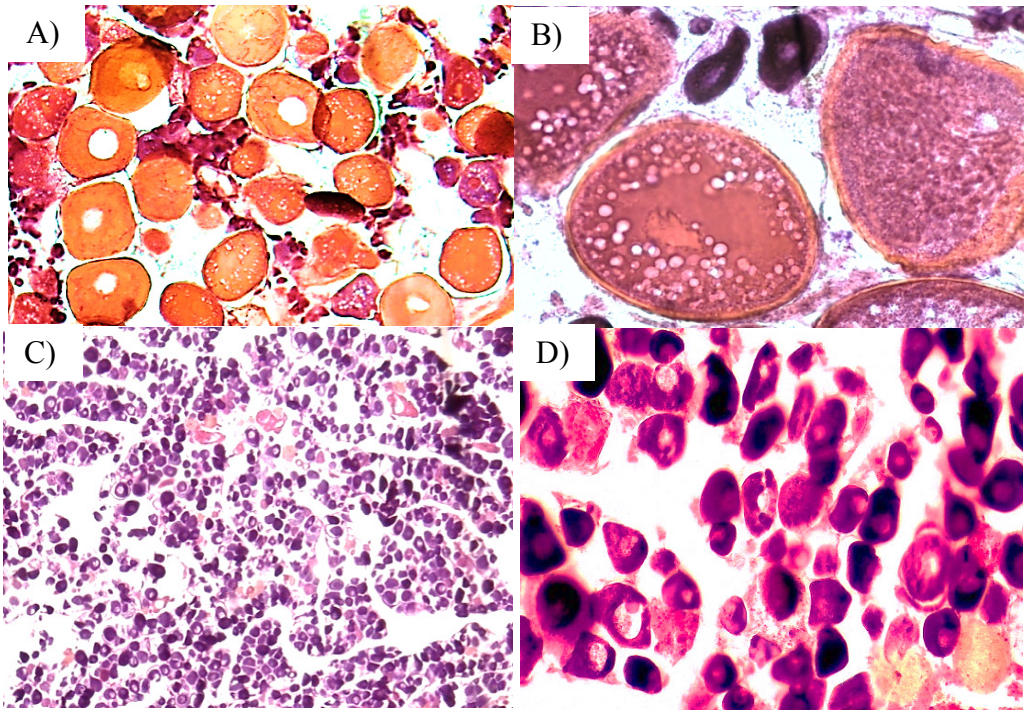

Figure 1. Developmental stages of wild *H. passer* female gonads. A) Ripe ovaries 4X, B) Ripe ovaries 20X, C) Spent ovaries 4X, D) Spent ovaries 20X.

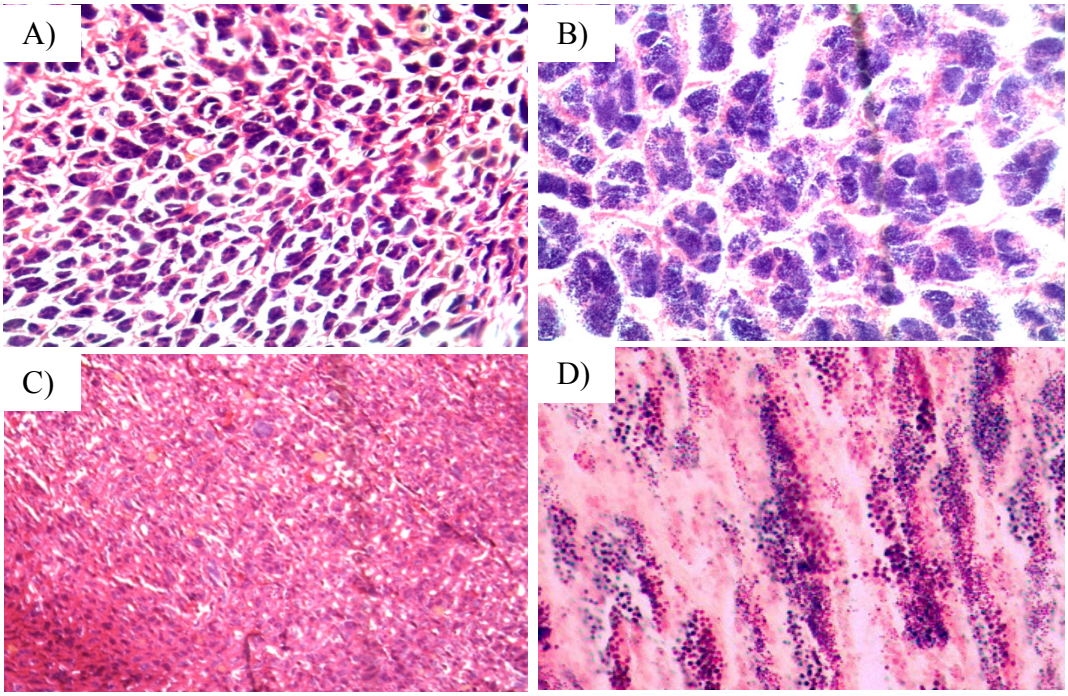

Figure 2. Developmental stages of wild *H. passer* male gonads. A) Ripe testis 4X, B) Ripe testis 20X, C) Spent testis 4X, D) Spent testis 20X.

Biological parameters

Females had notably smaller size, but numerically greater GSI and K values than males. Ripe and spent females showed no statistical differences in weight, length or K; however, ripe females showed a significantly higher GSI (P = 0.0005) than spent females (3.43 *vs*. 0.87%, respectively). On the other side, ripe males were significantly heavier (P = 0.0141), larger (P = 0.0208), and they also had a significantly higher GSI (P = 0.0440) than spent males, but there were no significant differences (P = 0.0604) in their K (Table 1).

Table 1. Means and standard error of the mean (S.E.) of biological parameters, gonadosomatic index (GSI), and condition factor (K) of wild female and male *Holacanthus passer* captured at El Datil during June and July 2014.

		Relative abundance (% of total)	Weight (g)	S.E.	Total length (cm)	S.E.	GSI (%)	S.E.	K (%)	S.E.
Ripe	♀	16.7	492.62	73.41	24.12	1.22	3.43[a]	0.19	3.42	0.25
Spent	♀	83.3	448.53	32.11	24.22	0.56	0.87[b]	0.08	3.05	0.08
P < 0.05 Chi-square		-	0.4197		0.9778		0.0005		0.1726	
Ripe	♂	20.0	1069.79[a]	90.47	33.03[a]	0.78	0.15[a]	0.03	2.95	0.07
Spent	♂	80.0	766.70[b]	26.34	30.27[b]	0.42	0.09[b]	0.01	2.76	0.04
P < 0.05 Chi-square		-	0.0141		0.0208		0.0440		0.0604	

[a,b]Mean values of females or males of different developmental stages (Ripe ♀: 5; Spent ♀: 25; Ripe ♂: 3; Spent ♂: 12) with different superscripts within the same column, are significantly different (P < 0.05).

Proximate composition and E2 concentration

The proximate composition of the gonads of both sexes showed that moisture was the major component, ripe and spent females having a higher content than males, and ash followed the same trend but it was the minor component (Table 2). On the other hand, crude protein and crude fat content of ripe gonads, as expected, were higher in mature females and males than in the spent gonads; however, none of the components of the proximate composition were significantly different in ripe or spent fish, except for the ash content of male gonads, which was significantly higher (P = 0.0126) in spent males than in ripe ones. E2 plasma concentration was analyzed only in ripe and spent females. In ripe females values ranged from 6.47 to 6.93 ng ml^{-1}, with a mean of 6.68 ng ml^{-1} (S.E. = 0.13). In spent females, values ranged from 3.52 to 4.10 ng ml^{-1}, with a mean of 3.84 ng ml^{-1} (S.E. = 0.15), which was significantly lower (P = 0.0323) than that of ripe females.

Table 2. Means and standard error of the mean (S.E.) of the proximate composition of gonads from wild female and male *Holacanthus passer* captured at El Datil during June and July 2014.

		Protein (%)	S.E.	Crude fat (%)	S.E.	Moisture (%)	S.E.	Ash (%)	S.E.
Ripe	♀	18.66	1.04	6.08	0.21	75.90	0.49	2.11	0.10
Spent	♀	17.92	0.48	4.97	0.38	76.72	0.23	2.14	0.04
P < 0.05 Chi-square		0.4107		0.1261		0.0997		0.7838	
Ripe	♂	14.34	1.53	11.80	1.47	71.98	0.23	1.56[b]	0.02
Spent	♂	13.81	0.40	11.26	0.75	72.10	0.22	1.81[a]	0.04
P < 0.05 Chi-square		0.4132		1.0000		0.6439		0.0126	

[a,b]Mean values of females or males of different developmental stages (Ripe♀: 5; Spent ♀: 25; Ripe ♂: 3; Spent ♂: 12) with different superscripts within the same column, are significantly different (P < 0.05).

Fatty acid profile

As a general trend, the content of fatty acids was higher in ripe gonads than in the spent counterpart, and in ripe female gonads the content of some fatty acids was significantly higher than in spent gonads (Table 3), for instance, 16:0, 18:0, 18:1, 20:4n-6, 20:5n-3, 22:5n-6 and 24:4n-6. In male gonads similar trends were observed but statistical differences were not evident (Table 4). In both sexes PUFA and HUFA were the most abundant type of fatty acids; moreover, DHA proved to be, quantitatively, the major fatty acid in gonadal tissue in this species, with values ranging from 10.83 mg DHA g^{-1} of gonad wet weight in spent males, to 16.28 mg DHA g^{-1} of gonad wet weight in ripe females. The concentration of n-3 fatty acids was quantitatively higher than that of the n-6 fatty acids, and the n-3/n-6 ratios were 3.20 and 3.54 for spent females and males, respectively, and 1.99 and 2.85 for ripe females and males. In addition, in ripe fish higher contents of ARA and derivatives of the n-6 family of fatty acids were evident.

Table 3. Means and standard error of the mean (S.E.) of fatty acids (mg of fatty acid g^{-1} of gonad wet weight) from ripe and spent gonads of wild *H. passer* females captured at El Datil during June and July 2014.

Fatty acid	Ripe ♀		Spent ♀		P < 0.05
	Mean	S.E.	Mean	S.E.	Chi-square
16:0	2.79[a]	0.67	1.02[b]	0.20	0.0115
18:0	1.30[a]	0.29	0.54[b]	0.08	0.0095
18:1	1.98[a]	0.45	0.89[b]	0.15	0.0315
18:2n-6	0.14	0.04	0.10	0.02	0.1715
18:3n-3	0.21	0.04	0.16	0.04	0.1818
20:4n-6	0.95[a]	0.36	0.35[b]	0.06	0.0404
20:4n-3	1.02	0.17	0.68	0.11	0.0560
20:5n-3	0.53[a]	0.12	0.25[b]	0.04	0.0202
22:5n-6	9.12[a]	1.43	4.73[b]	0.52	0.0140
22:5n-3	2.12	0.68	1.48	0.42	0.1330
22:6n-3	16.28	0.89	14.02	0.77	0.0880
24:4n-6	3.52[a]	0.39	2.33[b]	0.24	0.0406
24:5n-3	5.89	1.55	3.98	0.47	0.2748
24:6n-3	0.64	0.19	0.52	0.09	0.3934
Saturates[1]	4.48[a]	1.02	1.82[b]	0.32	0.0169
Monounsaturates[2]	3.62[a]	0.67	1.77[b]	0.25	0.0406
PUFA + HUFA[3]	41.42[a]	4.59	29.30[b]	2.37	0.0344
Total n-3[4]	27.13	2.61	21.48	1.59	0.1165
Total n-6[5]	13.91[a]	1.94	7.61[b]	0.80	0.0169
n-3/n-6	1.99[b]	0.11	3.20[a]	0.22	0.0078

[a,b]Mean values of females of each of the developmental stages (Ripe♀: 5; Spent ♀: 25) with different superscripts within the same row, are significantly different (P < 0.05). [1]Saturates: 14:0, 16:0, 18:0, 20:0. [2]Monounsaturates: 14:1, 16:1, 18:1, 20:1, 22:1, 24:1. [3]PUFA+HUFA: 16:2, 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:3n-6, 20:4n-6, 20:4n-3, 20:5n-3, 21:5n-3, 22:5n-6, 22:5n-3, 22:6n-3, 24:4n-6, 24:5n-3, 24:6n-3. [4]Total n-3: 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 21:5n-3, 22:5n-3, 22:6n-3, 24:5n-3, 24:6n-3. [5]Total n-6: 18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6, 22:5n-6, 24:4n-6.

Table 4. Means and standard error of the mean (S.E.) of fatty acids (mg of fatty acid g^{-1} of gonad wet weight) from ripe and spent gonads of wild *H. passer* males captured at El Datil during June and July 2014.

Fatty acid	Ripe ♂		Spent ♂		P < 0.05
	Mean	S.E.	Chi-square	S.E.	Chi-square
16:0	1.24	0.74	0.98	0.64	0.1489
18:0	0.65	0.30	0.53	0.23	0.3473
18:1	1.12	0.69	0.92	0.61	0.1124
18:2n-6	0.10	0.05	0.08	0.04	0.3460
18:3n-3	0.19	0.09	0.09	0.03	0.1467
20:4n-6	0.22	0.11	0.19	0.11	0.1927
20:4n-3	0.73	0.23	0.55	0.14	0.3865
20:5n-3	0.33	0.16	0.33	0.17	0.5156
22:5n-6	6.10	1.99	3.23	0.80	0.2482
22:5n-3	1.15	0.28	0.87	0.31	0.3123
22:6n-3	14.71	1.12	10.83	0.96	0.0833
24:4n-6	1.79	0.45	1.32	0.36	0.4701
24:5n-3	4.26	1.44	2.21	0.53	0.1489
24:6n-3	0.17	0.01	0.34	0.10	1.0000
Saturates[1]	2.12	1.18	1.75	1.04	0.1117
Monounsaturates[2]	2.00	0.93	1.48	0.79	0.1489
PUFA + HUFA[3]	30.33	5.92	20.63	3.39	0.1489
Total n-3[4]	21.78	3.36	15.50	2.05	0.1489
Total n-6[5]	8.26	2.39	4.90	1.26	0.3123
n-3/n-6	2.85	0.41	3.54	0.62	0.9263

[a,b]Mean values of males of each of the developmental stages (Ripe ♂: 3; Spent ♂: 12) with different superscripts within the same row, are significantly different (P < 0.05). Saturates: 14:0, 16:0, 18:0, 20:0. [2]Monounsaturates: 14:1, 16:1, 18:1, 20:1, 22:1, 24:1. [3]PUFA+HUFA: 16:2, 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:3n-6, 20:4n-6, 20:4n-3, 20:5n-3, 21:5n-3, 22:5n-6, 22:5n-3, 22:6n-3, 24:4n-6, 24:5n-3, 24:6n-3. [4]Total n-3: 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 21:5n-3, 22:5n-3, 22:6n-3, 24:5n-3, 24:6n-3. [5]Total n-6: 18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6, 22:5n-6, 24:4n-6.

Discussion

The aquaculture of ornamental fish represents an opportunity to reduce the impact that the extraction of organisms for commercialization has on the natural populations and, at the same time, it also represents the opportunity to provide jobs and a source of income for people. In order to achieve a closed cycle in aquaculture, that is, to become independent of the wild organisms that the environment provides, which are mostly broodstock, a method to keep the broodfish in an ideal setting needs to be developed. This method needs to include the provision of an adequate culture environment that would promote the development of the gonads, as well as a diet that would meet the nutritional requirements for the optimal allocation of nutrients to the maturation of the gonad.

The determination of dietary requirements may follow a traditional approach, such as feeding fish graded levels of a particular nutrient for a period of time and establishing the level that promotes the best growth response as the dietary requirement, although nowadays this approach may include other parameters into consideration, such as the maximum tissue protein retention, a nitrogen balance, or other more suitable parameters (Ogino, 1980a, b; Wilson and Poe, 1985). Another traditional approach is to determine the concentration of essential nutrients in tissues, which helps drawing conclusions about the nutrient needs (Kaushik, 1979; Tacon and Cowey, 1985). For instance, when the established essential amino acids requirements of *Ictalurus punctatus* were regressed against the essential amino acids present in its body, a regression coefficient of 0.96 was obtained (Wilson and Poe, 1985), indicating that the composition of the tissues in the body may very well be used as a pattern of the requirement for essential nutrients. Thus, this last approach was used in this study for the male and female king angelfish gonads in an effort to determine the requirement for essential fatty acids drawn from their fatty acid profile.

Out of the 45 adult specimens of *H. passer* analyzed, 66.7% were females and 33.3% were males; 16.7% of the females had ripe gonads while 83.3% had spent gonads, and 20% of the males had ripe gonads while 80% had spent gonads. These wild organisms were captured during July and August at the location of El Datil, near Kino Bay, Sonora, Mexico. During this time of the year Arellano-Martinez *et al.* (1999) reported that in king angelfish collected from Cueva de Leon (24°02′ N, 110°24′ W) in South Baja California, most of the female gonads were ripe and a small portion were still developing; their reproductive activity took place from June to November, and was well correlated with their GSI. For males they observed that some gonads were developing or ripe in July and August, and some males also had spawning and spent gonads, but no correlation was established between the GSI and the reproductive activity of males because of the low weight the male gonads reach during maturation. Cueva de Leon and El Datil are different geographical areas with different temperatures and conditions; local temperatures

at El Datil and the Kino Bay area are higher than those observed at Cueva de Leon during the same period of time, and seawater generally reaches temperatures above 25ºC in late April to early May at El Datil. It is well known that, among the environmental parameters, temperature plays one of the leading roles in gonad maturation and spawning of fish (Zohar, 1989; Bye, 1990), which partly explains why a large percentage of female and male gonads in this study were already spent and a small percentage was still ripe, indicating that fish were reproducing earlier than populations in southern areas, most likely in the spring, but a yearly study is required to confirm this.

One could expect that, as the gonad develops into a fully ripe gonad and its weight increases, the GSI percentages also increase. In this study, the GSI in *H. passer* females with ripe gonads was 3.42%, significantly higher than that of females with spent gonads with 0.87%. For the same gender Arellano-Martínez and Ceballos-Vázquez (2001) reported the largest GSI value of 2.45% in June, decreasing to 2.0% in July at Cueva de Leon, which are lower values than the ones reported in this study. The GSI values they reported are means of all females captured in that period, whereas in this study, GSI are means for females with a similar state of gonadal development; in addition, females in their study averaged 16.19 cm whereas in this study, both, ripe and spent females were larger, averaging 24.12 and 24.22 cm, respectively, which explains the larger gonad size and thus, the larger GSI. The GSI values observed in this study are also consistent with values reported for other species, for example, *Dentex dentex* females with a GSI of 4.1 and 0.7% in mature and resting females, respectively (Chatzifotis *et al.*, 2004). On the other hand, ripe males were significantly heavier and larger than spent males in this study, and the GSI for ripe gonads (0.15%) also was significantly higher than in spent gonads (0.08%). Both ripe (33.03 cm) and spent (30.27 cm) males, also were larger than males reported by Arellano-Martínez and Ceballos-Vázquez (2001), with a mean length of 20.12 cm and a GSI of 0.10% in June and 0.12% in July, very similar GSI values to the ones recorded in this study, which confirms their observation of a very low weight of the male gonads, even during the mature stage. Conversely, for *D. dentex*, GSI values of 3.4 and 0.2% were reported in mature and spent males, respectively (Chatzifotis *et al.*, 2004), which makes evident the wide variety of morphological features characterizing the gonads of teleost fish. The morphology of this species is characterized by a small visceral cavity, which may partly explain the small GSI values observed in males.

The Fulton's condition factor or K is an indirect estimate of the robustness of fish, common values reported for marine fish are usually above 1.0, lower values indicate less robust fish and higher values more robust fish (González-Félix *et al.*, 2015, 2016; Perez-Velazquez *et al.*, 2016). In this study, K values for *H. passer* ranged from 2.76 to 3.42, an indication of the overall robustness of all organisms. Although not statistically significant, females and males with ripe gonads showed slightly higher K values than their spent counterparts, which is explained by the fact that this index is an estimate of the body weight-to-length ratio, and decreases sensibly as body weight decreases or length increases. Hence, smaller K values in this study corresponded to spent females that had smaller gonad/body weight, and to spent males that also had smaller gonad/body weight and smaller body length. For this species, Arellano-Martínez and Ceballos-Vázquez (2001) reported K values close to 4.3 for females, and close to 4.0 and 4.3 for males during June and July. These authors suggested that for this species, the reproductive activity is inversely related to the nutritional status, thus higher K values were observed during the inactive reproductive period that coincided with smaller GSI values. In this study, wild organisms were sampled only for two months in the summer, and K values of mature females and males, 3.42 and 2.95, respectively, as well as of spent females and males, 3.05 and 2.76, respectively, were smaller than K values reported by Arellano-Martínez and Ceballos-Vázquez (2001). These lower values are explained by the smaller lengths and weights of the organisms sampled in their study, compared to the ones in our study; but, since a limited period of time was monitored here, the verification of the inverse relationship between GSI and K could not be performed for this work. The E2 analysis could be performed only in plasma samples of ripe and spent females, and their mean values of 6.68 and 3.84 ng ml^{-1}, respectively, are comparable, though slightly higher, to values reported for three different stages of ovarian development of wild female Persian sturgeon, *Acipenser persicus*, they were the vitellogenic stage III (E2: 5.33 ng ml^{-1}), the ripe stage IV (E2: 1.98 ng ml^{-1}) and the ovulation stage V (E2: 2.31 ng ml^{-1}; Hosseinzadeh *et al.*, 2013), while Semenkova *et al.* (2002) reported that E2 concentration in plasma of stellate sturgeon (*Acipenser stellatus*) ranged from 1.22 to 2.05 ng ml^{-1} and decreased to 0.16 ng ml^{-1} after ovulation, a similar trend for the E2 values observed in this study.

The proximate composition of *H. passer* male gonads showed that, in spite of their low weight, their lipid content was proportionally higher than in the female gonads. Numerically, but not statistically significant, the lipid content was higher in ripe gonads, which coincided with the higher content of fatty acids. In both sexes of this species, DHA was quantitatively the major fatty acid, ranging from 10.83 up to 16.28 mg of DHA g^{-1} of gonad wet weight. The n-3 fatty acids were quantitatively higher than n-6 fatty acids, and the n-3/n-6 ratios ranged from 1.99 up to 3.54. The lower ratios observed in ripe organisms (Tables 3 and 4) can be attributed not to the lower content of n-3 fatty acids, but to the higher content of ARA and derivatives of this fatty acid from the n-6 family, which would reduce the ratio, and, as previously suggested, ARA is the second major component of gonads in many fish species (Suloma and Ogata, 2011). Similar DHA contents have been reported in mature male (35.97% of total FAME) and female (21.65% of total FAME) gonads of spawning herring, *Clupea harengus pallasi* (Huynh *et al.*, 2007), and in the male (22.0% of total FAME) and

female (25.0% of total FAME) gonads of Atlantic bluefin tuna (*Thunnus thynnus*) broodstock during reproductive migration, where n-3/n-6 ratios of 5.4 and 6.4, respectively, were observed (Sprague *et al.*, 2012). In other species, consistently high DHA contents have been observed, but not necessarily the highest, such as in *Salmo trutta labrax*, where DHA content in female gonads was reported to be 15.55% of total FAME with a 5.23 n-3/n-6 ratio (Aras *et al.*, 2003). *D. dentex* also had comparable n-3/n-6 ratios, ranging from 2.56 up to 7.50 in females caught in different seasons of the year, with DHA contents ranging from 7.4 to 25.9 mol%, while males had ratios ranging from 3.70 to 4.30 and DHA contents ranging from 10.5 to 13.2 mol% (Chatzifotis *et al.*, 2004). Knowing the essential fatty acid content of the broodfish gonads can help establish their nutritional status, which is of utmost importance since it can markedly affect ovarian maturation, reproductive performance, and offspring quality (Izquierdo *et al.*, 2001). Furthermore, information on the chemical composition and fatty acid profile of the gonads can be used as indicators of dietary lipid and essential fatty acid requirements of broodfish, which may contribute to the formulation of a balanced diet for the culture of this ornamental species. On the other hand, knowledge obtained on the stages of gonadal development of local king angelfish during the period of this study could help establish basic reproductive aspects, like the size or age at which the organisms reach sexual maturity in this area, and the length of their reproduction season, which is relevant for the development of a protection program.

We concluded that, during the months of July and August, close to 80% of wild *Holacanthus passer* captured from El Datil in the Kino Bay area, had spent gonads, and in about 20% of them the gonads were ripe, which indicates that fish were reproducing earlier than populations in southern areas, most likely in the spring. The quantitative composition of fatty acids of the gonad confirmed that they are a very rich source of HUFA of both, the n-3 and n-6 family. DHA was quantitatively the most important fatty acid of all, and its concentration in the gonad changed in relation to its developmental stage, therefore, it might serve as an additional maturation index. A seasonal study is warranted to confirm these observations, and to describe the remaining stages of gonad development throughout the year in this location for this species.

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References

Abdel-Aziz SH, El-Nady FS. 1993. Lipid dynamics in the common torpedo, *Torpedo torpedo*, from the south eastern Mediterranean. *J Fish Biol*, 43:155-162.

Aburto-Oropeza O, Sala E, Sánchez-Ortiz C. 2000. Feeding behavior, habitat use, and abundance of the angelfish *Holacanthus passer* (Pomacanthidae) in the southern Sea of Cortés. *Environ Biol Fishes*, 57:435-442.

Aquarium of the Pacific. Available on: http://www.aquariumofpacific.org/onlinelearningcenter/species/king _angelfish. Accessed on: January 14th 2016.

Arantes FP, Borçato FL, Sato Y, Rizzo E, Bazzoli N. 2012. Reproduction and embryogenesis of the mandi-amarelo catfish, *Pimelodus maculatus* (Pisces, Pimelodidae), in captivity. *Anat Histol Embryol*, 42:30-39.

Aras NM, Haliloğlu HI, Ayik Ö. 2003. Comparison of fatty acid profiles of different tissues of mature trout (*Salmo trutta labrax*, Pallas, 1811) caught from Kazandere Creek in the Çoruh Region, Erzurum, Turkey. *Turk J Vet Anim Sci*, 27:311-316.

Arellano-Martínez M, Ceballos-Vázquez BP, García-Domínguez F, Galván-Magaña F. 1999. Reproductive biology of the king angelfish *Holacanthus passer* Valenciennes 1846 in the Gulf of California, Mexico. *Bull Mar Sci* 65:677-685.

Arellano-Martínez M, Ceballos-Vázquez BP. 2001. Reproductive activity and condition index of Holacanthus passer (Teleostei: Pomacanthidae) in the Gulf of California, Mexico. *Rev Biol Trop*, 49:939-943.

Aruke A, Goksoyr A. 2003. Eggshell and egg yolk proteins in fish: hepatic proteins for the next generation: oogenic, population, and evolutionary implications of endocrine disruption. *Comp Hepatol*, 2:4-7.

Association of Official Analytical Chemists. 2005. Official Methods of Analysis of the Association of Official Analytical Chemists. Arlington, VA: AOAC.

Berg AH, Westerlund L, Olsson PE. 2004. Regulation of Arctic char (*Salvelinus alpinus*) egg shell proteins and vitellogenin during reproduction and in response to l7β-estradiol and cortisol. *Gen Comp Endocrinol*, 115:276-285.

Bruckner AW. 2005. The importance of marine ornamental reef fish trade in the wider Caribbean. *Rev Biol Trop*, 53:127-138.

Bye VJ. 1990. Temperate marine teleosts. *In*: Munro AD, Scott AP, Lam TJ (Ed.). *Reproductive Seasonality in Teleosts: Environmental Influences*. Boca Raton, FL: CRC Press. pp. 125-143.

Chatzifotis S, Muje P, Pavlidis M, Agren J, Paalavuo M, Molsa H. 2004. Evolution of tissue composition and serum metabolites during gonadal development in the common Dentex (*Dentex dentex*). *Aquaculture*, 236:557-573.

Collier JT, Kaneko T, Hirano T, Grau EG. 2003. Seasonal changes in reproductive activity in the Potter's angelfish (*Centropyge potteri*) in Kaneohe Bay, Hawaii. *Environ Biol Fishes*, 68:49-57.

Folch J, Lees M, Sloane GH. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem*, 1:497-509.

González-Félix ML, Minjarez-Osorio C, Perez-Velazquez M, Urquidez-Bejarano P. 2015. Influence

of dietary lipid on growth performance and body composition of the Gulf corvina, *Cynoscion othonopterus*. *Aquaculture*, 448:401-409.

González-Félix ML, Maldonado-Othón CA, Perez-Velazquez M. 2016. Effect of dietary lipid level and replacement of fish oil by soybean oil in compound feeds for the shortfin corvina (*Cynoscion parvipinnis*). *Aquaculture*, 454:217-228.

Guerriero G. 2007. Seasonal steroids variations and rnaturity stages in the fernale chub, *Leuciscus cephalus* L. (Pisces, Cyprinidae). *Ital J Zool*, 74:317-324.

Henderson RJ, Park MT, Sargent JR. 1995. The desaturation and enlongation of ^{14}C-labelled polyunsaturated fatty acids by pike (*Esox lucius*) in vivo. *Fish Physiol Biochem*, 14:223-235.

Hosseinzadeh M, Imanpoor MR, Shabani A, Nekoubin H. 2013. Seasonal changes in serum calcium and 17β-estradiol levels in Persian sturgeon, *Acipenser persicus*. *J Aquac Res Dev*, 4:1-3.

Huynh MD, Kitts DD, Hu C, Trites AW. 2007. Comparison of fatty acid profiles of spawning and non-spawning Pacific herring, *Clupea harengus pallasi*. *Comp Biochem Physiol B*, 146:504-511.

Izquierdo MS, Fernández-Palacios H, Tacon AGJ. 2001. Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture*, 197:25-42.

Kaushik S. 1979. Application of a biochemical method for the estimation of amino acid needs in fish: quantitative arginine requirements in different salinities. *In*: Halver JE, Tiews K, Heenemann IH (Ed.). *Finfish Nutrition and Fishfeed Technology*. Berlin: GmbH and Co. pp. 197-207.

Kokokiris L, Mourot B, Le Menn F, Kentouri M, Fostier A. 2000. Endocrine changes during the annual reproductive cycle of the red porgy, *Pagrus pagrus* (Teleostei, Sparidae). *Fish Physiol Biochem*, 23:1-11.

Lango-Reynoso F, Castañeda-Chávez M, Zamora-Castro JE, Hernández-Zárate G, Ramírez-Barragán, Solís-Morán E. 2012. La acuariofilia de especies ornamentales marinas: un mercado de retos y oportunidades. *Lat Am J Aquat Res*, 40:12_21.

LiveAquaria. Available on: http://www.liveaquaria.com/product/prod_display.cfm?c=15+18+416&pcatid=416. Accessed on: May 25th 2016.

Livengood EJ, Chapman FA. 2007. The ornamental fish trade: an introduction with perspectives for responsible aquarium fish ownership. Gainesville, FL: University of Florida, IFAS, Florida Cooperative Extension Service. pp. 1-7.

Lochmann RT, Gatlin DM III. 1993. Essential fatty acid requirement of juvenile red drum (*Sciaenops ocellatus*). *Fish Physiol Biochem*, 12:221-235.

Luzzana U, Serrini G, Moretti VM, Grimaldi P, Paleari MA, Valfre F. 1996. Seasonal variations in fat content and fatty acid composition of male and female coregonid 'bondella' from Lake Maggiore and landlocked shad from Lake Como (Northern Italy). *J Fish Biol*, 48:352-366.

Mehdi Y, Ehsan MS. 2011. A review of the control of reproduction and hormonal manipulations in finfish species. *Afr J Agric Res*, 6:1643-1650.

Mexico. Secretaría de Medio Ambiente y Recursos Naturales. 2002. Norma Oficial Mexicana NOM-059-ECOL-2001. Protección ambiental; Especies nativas de México de flora y fauna silvestres; Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio; Lista de especies en riesgo. *Diario Oficial de la Federación, Mexico*, 06 de marzo de 2002. 2ª sec, pp. 95-190.

Moncaut N, Nostro FL, Maggese MC. 2003. Vitellogenin detection in surface mucus of the South American cichlid fish *Cichlasoma dimerus* (Heckel, 1840) induced by estradiol-17beta. Effects on liver and gonads. *Aquat Toxicol*, 63:127-137.

Mylonas C, Blaise O, Gothilf Y, Steven C, Alok D, Zohar Y. 2000. Gonadotropin-releasing activities in striped bass (*Morone saxatilis*) of the three native forms of gonadotropin-releasing hormone (GnRH) and of novel agonists. *In*: Norberg B, Kjesbu OS, Taranger GL, Andersson E, Stefansson SO (Ed.). *Fish Reproductive Physiology*. Bergen, Norway: University of Bergen. pp. 451.

Mylonas C, Zohar Y. 2001. Use of GnRHa-delivery systems for the control of reproduction in fish. *Rev Fish Biol Fisher*, 10:463-491.

Nagahama Y. 1994. Endocrine control of gametogenesis. *Int J Dev Biol*, 38:217-229.

Ogino C. 1980a. Protein requirements of carp and rainbow trout [in Japanese]. *Bull Jap Soc Sci Fish*, 46:385-388.

Ogino C. 1980b. Requirements of carp and rainbow trout for essential amino acids [in Japanese]. *Bull Jap Soc Sci Fish*, 46:171-174.

Olivotto I, Planas M, Simões N, Holt JG, Alessandro-Avella M, Calado R. 2011. Advances in breeding and rearing marine ornamentals. *J World Aquacult Soc*, 42:135-166.

Perez-Velazquez M, Minjarez-Osorio C, González-Félix ML. 2016. Effect of dietary lipid level on growth performance, feed utilization, and body composition of totoaba, *Totoaba macdonaldi* (Gilbert, 1890). *Aquac Res*:1-11. doi:10.1111/are.13002.

Pyle R, Allen G, Myers R, Zapata F, Robertson R, Rocha LA, Craig MT. 2010. *Holacanthus passer*. *In*: The IUCN Red List of Threatened Species 2010: e.T165880A6155998. Available on: http://dx.doi.org/10.2305/IUCN.UK.2010-4.RLTS.T165880A6155998.en. Accessed on: March 21st 2016.

Ricker WE. 1975. Computation and interpretation of biological statistics of fish populations. *Bull Fish Res Board Can*, 191:1-382.

Sánchez-Alcántara I, Aburto-Oropeza O, Balart E, Cupul-Magaña A, Reyes-Bonilla H, Sánchez-Ortiz C. 2006. Threatened fishes of the world: Holacanthus passer Valenciennes, 1846 (Pomacanthidae). *Environ Biol Fishes*, 77:97-99.

Sargent JR. 1995. Origin and functions of egg lipids: nutritional implications. *In*: Bromage NR, Roberts RJ (Ed.). *Broodstock Management and Egg and Larval Quality*. Oxford, UK: Blackwell Science. pp. 353-372.

Sargent JR, Tocher DR, Bell JG. 2002. The lipids. *In*: Halver JE, Hardy RW (Ed.). *Fish Nutrition*. 3rd ed. San

Diego, CA: Academic Press. pp. 181-257.

Scott AP, Witthames PR, Turner RJ, Canario AVM. 1998. Plasma concentrations of ovarian steroids in relation to oocyte final maturation and ovulation in female plaice sampled at sea. *J Fish Biol*, 52:128-145.

Semenkova T, Barinnikova I, Kime DE, McAllister BG, Buyanova L, Dyubin V, Kolmakov N. 2002. Sex steroid profiles in female and male stellate sturgeon (*Acipenser stellatus* Pallas) during final maturation induced by hormonal treatment. *J Appl Ichthyol*, 18:375-381.

Sorbera LA, Zanuy S, Carrielo M. 1998. A role for polyunsaturated fatty acids and prostaglandins in occyte maturation in the sea bass (*Dicentrarchus labrax*). *In*: Vandry H (Ed.). *Trends in Comparative Endocrinology and Neurology: from Molecular to Integrative Biology*. New York, NY: Academy of Sciences. pp. 535-537.

Sprague M, Dick JR, Medina A, Tocher DR, Bell JG, Muorente G. 2012. Lipid and fatty acid composition, and persistent organic pollutant levels in tissues of migrating Atlantic bluefin tuna (*Thunnus thynnus*, L.) broodstock. *Environ Pollut*, 171:61-71.

Swanson P, Dickey JT, Campbell B. 2003. Biochemistry and physiology of fish gonadotropins. *Fish Physiol Biochem*, 28:53-59.

Tacon AGJ, Cowey CB. 1985. Protein and amino acid requirements. *In*: Tytler P, Calow P (Ed.). *Fish Energetics: New Perspectives*. London: Croom Helm. pp. 155-183.

Thomson DA, Findley LT, Kerstitch AN. 2000. *Reef Fishes of the Sea of Cortez: the Rocky-shore Fishes of the Gulf of California*. Austin, TX: University of Texas Press. 407 pp.

Tocher DR, Bell JG, Dick JR, Crampton VO. 2003. Effects of dietary vegetable oil in Atalntic salmon hepatocyte fatty acid desaturation and liver fatty acid composition. *Lipids*, 38:723-732.

Wabnitz C, Taylor M, Green E, Razak T. 2003. From ocean to aquarium: the global trade in marine ornamental species. UNEP-WCMC Biodiversity Series, 17:1-64.

Wilson RP, Poe WE. 1985. Relationship of whole body and egg essential amino acid patterns to amino acid requirement patterns in channel catfish, *Ictalurus punctatus*. *Comp Biochem Physiol B*, 80:385-388.

Wolfe LS, Horrocks LA. 1994. Eicosanoids. *In*: Siegel GJ, Agranoff BW, Albers RW, Molinoff PB (Ed.). Basic Neurochemistry. New York, NY: Raven Press. pp. 475-490.

Zohar Y. 1989. Fish reproduction: its physiology and artificial manipulation. *In*: Shilo M, Sarig S (Ed.). *Fish culture in Warm Water Systems: Problems and Trends*. Boca Raton, FL: CRC Press. pp. 65-119.

Zohar Y, Muñoz-Cueto JA, Elizur A, Kah O. 2010. Neuroendocrinology of reproduction in teleost fish. *Gen Comp Endocrinol*, 165:438-455.