Arch Biol Sci. 2017;69(3):491-501 https://doi.org/10.2298/ABS160831124G

Biochemical composition and fatty acid profile of gonads from wild and cultured shortfin corvina (*Cynoscion parvipinnis*) during the early maturation stage

Mayra L. González-Félix[1], Perla Urquidez-Bejarano[1], Martin Perez-Velazquez[1,*], Reina Castro-Longoria[1] and Celia G. Vazquez-Boucard[2]

[1] *Department of Scientific and Technological Research, University of Sonora*, Edificio 7-G, Blvd. Luis Donaldo Colosio s/n, e/Sahuaripa y Reforma, Col. Centro, C.P. 83000, Hermosillo, Sonora, Mexico

[2] *Centro de Investigaciones Biológicas del Noroeste (CIBNOR)*. Av. Instituto Politécnico Nacional 195, Playa Palo de Santa Rita Sur, C.P. 23096, La Paz, B.C.S. Mexico

***Corresponding author**: mperezv@dictus.uson.mx; martin.perez@unison.mx

Received: August 31, 2016; **Revised**: October 13, 2016; **Accepted**: October 14, 2016; **Published online**: November 18; 2016

Abstract: *Cynoscion parvipinnis* is an important fisheries resource of the Gulf of California used locally for human consumption. Its aquaculture potential has recently been demonstrated, and cultured organisms are already available. In this study, the gonadal developmental stage, proximate composition, fatty acid profile and other biochemical and biological indices of wild and cultured fish were characterized. A total of 80 fish, 30 cultured (15 female and 15 male) and 50 wild (31 female and 19 male), captured in November at Santa Rosa, Sonora, Mexico, were analyzed. Histological analyses of the gonads showed that 100% of the wild and cultured fish were at an early maturation stage. Wild fish had greater body weight, total length and gonadosomatic index. Cultured female fish (19.64%) possessed a slightly higher protein content in their gonads than wild females (17.11%), whereas males had similar values (14.00 and 14.83%). Cultured females (10.85%) and males (20.48%) had a significantly greater crude fat content than wild fish (female: 2.05%, male: 11.05%). Palmitic acid was the most abundant fatty acid (FA), ranging from 128.58-164.84 mg of 16:0 g^{-1} of gonad wet weight. After 16:0, highly unsaturated FAs like DHA, ARA and EPA were the major FA in gonadal tissue; n-3 FAs were quantitatively higher than n-6 FAs, while the n-3/n-6 ratios ranged from 2.08-2.81. In general, the biochemical composition of the gonad of wild and cultured organisms at an early maturation stage was quite similar. These data may serve as indicators of dietary requirements for a maturation diet for broodstock culture.

Keywords: *Cynoscion parvipinnis*; gonad developmental stages; fatty acid profile; 17β-estradiol; proximate composition

INTRODUCTION

The shortfin corvina (*Cynoscion parvipinnis*) is a mid-sized sciaenid fish, measuring up to 60 cm in total length, that inhabits coastal sandy bottoms of the Gulf of California, Mexico [1]. It is valued as a food fish, and it is also popular among recreational fishing enthusiasts. Commercial fishery landings of *C. parvipinnis* and other members of the genus *Cynoscion*, such as *C. othonopterus*, all of them known as corvinas, collectively reached 5961 MT in the Mexican Pacific coast in 2013 [2]. Reports indicate that over the past years the shortfin corvina has been overexploited, which along with habitat modification, has led to a decline in their natural populations [3]. Recently, there has been growing interest in the shortfin corvina as a species suitable for aquaculture. Due to its high-quality fillet, aquacultural production of shortfin corvina would enhance food fish supply in a locally well-established market, and at the same time, it could alleviate fishing pressure. Based on juvenile fish obtained from wild broodstock acclimated to and spawned in captivity, promising results have been obtained in comparative growth trials conducted in northwest Mexico, which have elucidated various nutritional aspects of this species [4,5]. However, continuously controlled reproduction over successive generations is a requisite to establish any fish species as a solid candidate for aquaculture. A first step towards the achievement of this goal is gathering information on its reproductive biology. Typically, male and female gonads are examined

© 2017 by the Serbian Biological Society

How to cite this article: González-Félix ML, Urquidez-Bejarano P, Perez-Velazquez M, Castro-Longoria R, Vazquez-Boucard CG. Biochemical composition and fatty acid profile of gonads from wild and cultured Shortfin corvina (*Cynoscion parvipinnis*) during the early maturation stage. Arch Biol Sci. 2017;69(3):491-501.

and classified into developmental stages, either by macroscopic visual cues, such as size and color [6], or by microscopic, histological preparations, which are generally preferred because they are considered more accurate [7]. This information helps determine useful parameters in the management of fisheries resources, e.g., the length of the reproductive season, age/size at first maturity, spawning frequency, etc. [8]. In addition, for aquacultural purposes, it aids in the timing of induced spawning [9].

The composition of fish gonads, in terms of the fatty acid profile, is another important aspect for successful reproduction of fish in captivity. As opposed to saturated and monounsaturated fatty acids, which are a preferred source of catabolic energy, long-chain, polyunsaturated and highly unsaturated fatty acids (PUFA and HUFA, respectively) are usually transferred to fish oocytes [10]. The HUFA docosahexaenoic acid (22:6n-3; DHA), eicosapentaenoic acid (20:5n-3; EPA), and arachidonic acid (20:4n-6; ARA) play roles as constituents of neural tissues, are precursors of hormone-like molecules, and are involved in the development of the embryo's immune system and hatching [11,12]. Therefore, these fatty acids are usually present in relatively high amount in gonads of saltwater fish [13,14]. The relative concentration of some fatty acids, e.g., DHA, could be indicative of gonad maturation, an aspect that deserves to be investigated. On the other hand, in the steroid hormone-driven process of ovarian maturation, 17β-estradiol (E_2) stimulates the synthesis of vitellogenin in the liver of female fish, which is then incorporated into the developing oocytes [15-17]. In some instances, gonad size/weight has been found to correspond with the concentration of E_2 in plasma [18-20], suggesting that E_2 may be used as an indicator of final oocyte maturation in female fish [21,22]. Two additional biochemical components relevant to reproduction are cholesterol and carotenoids. The former serves as a precursor to all steroid hormones, which regulate reproductive physiology [23]. The latter play various roles, as antioxidants, precursors to vitamin A, and are involved in the protection of eggs against light [24], and have been shown to improve egg pigmentation, fertilization rate, and survival of rainbow trout when supplemented in the diet [25].

Because the various aspects of reproductive biology described above are still unknown for the shortfin corvina, the objective of the present investigation was to identify the gonadal developmental stages, proximate composition, fatty acid profile and other biochemical and biological indices of wild and cultured *C. parvipinnis*.

MATERIALS AND METHODS

Wild fish

Wild, adult shortfin corvina with mean body weight (BW) of 1009.69 g for females and 835.93 g for males, were captured in November 2015, using an outboard-powered boat and seine nets at the estuary "Santa Rosa", latitude 28°58' N and longitude 112°10' W, near Kino Bay in the Gulf of California, Sonora, Mexico. Immediately after catching, approximately 1 mL of blood was drawn from the caudal vein of each fish with heparinized 1-cc syringes equipped with 25-gauge needles, and placed into 1.5-mL micro centrifuge tubes (Costar Corning, Incorporated, 1.5 mL Corning, New York, USA), and kept on ice. Subsequently, fish were killed by severing the spinal cord, placed into individually labeled bags, and kept on ice. All fish were transported to the Kino Bay Experiment Station (KBES), University of Sonora at Kino Bay, Sonora, Mexico, for further analysis.

Cultured fish

Juvenile shortfin corvina about 20 g of individual wet weight and 3 months of age after hatching, originating from wild broodstock acclimated to laboratory conditions and spawned in captivity, were obtained from the Center for Reproduction of Marine Species of the State of Sonora (CREMES), Kino Bay, Sonora, Mexico. Fish were transported and held at the KBES in a recirculating culture system consisting of two 10-m^3 fiberglass raceways (each with 8 m, length × 1.5 m, width × 1.0 m, height), in connection with a 3-m^3 sump tank, biofilter, a 1.5-HP pump (Jacuzzi, Model 150MF-T, Little Rock, Arkansas, USA), a sand filter (Jacuzzi, Model L-190-7, Little Rock, Arkansas, USA), a 120-Watt UV light chamber (Rainbow Lifeguard, Model UV97, El Monte, California, USA),

and constant aeration supplied with a 1.0-HP blower (Fuji, Model VFC40, Saddle Brook, New Jersey, USA). Fish were fed a commercial extruded feed with crude protein and lipid contents of 46 and 12%, respectively (Marine MX, Skretting®, Tooele, Utah, USA), for 12 months. After this rearing period and at an approximate age of 15 months, all fish were anesthetized with tricaine methanesulfonate (MS-222, 300 mg L^{-1}; Western Chemical, Inc., Ferndale, WA, USA) prior to blood collection and sacrifice, as previously described, and were then kept on ice for further analysis. The body indices and analyses subsequently described, including histology, proximate composition, fatty acid profile, etc., were performed on all fish unless indicated otherwise.

Fish dissection and tissue preparation

Fish were individually measured (total length (TL); cm) and weighed (BW; g) in order to calculate Fulton's condition factor (K)=(BW × 100)/TL^3 [26]. The fish were then dissected to determine the weights of the viscera, gonads and liver to calculate other body indices, as follows: the viscerosomatic index (VSI) = (viscera weight, g/BW) x 100; the gonadosomatic index (GSI) = (gonad weight, g/BW) x 100, and the hepatosomatic index (HSI) = (liver weight, g/BW) x 100. Gonads from individual fish were partitioned into two similar portions. One portion was stored in Davidson's fixative solution (acetic acid:95% ethanol:formaldehyde:H_2O, at a 1:3:2:3 ratio) for 24 h for histological determination of the gonad developmental stage. The other portion was stored at -84°C and used for the determination of the fatty acid and proximate composition, cholesterol and total carotenoid contents.

Histological analyses of gonads

After fixation in Davidson's solution, the gonads were stored in a 70% ethanol solution for long-term storage, then immersed in ethanol solutions with progressively higher concentration (80, 95, 100%) for dehydration, and then in xylene for clearing. From each gonad, three segments about 1 cm in length, were sliced and embedded in paraffin. Transverse sections were cut, approximately 4 μm thick, placed onto microscope slides, stained with hematoxylin and eosin (H&E), and examined under a light microscope (Leica Microsystems, DM1000, Wetzlar, Germany) equipped with a digital camera (Moticam Pro, 282A), using the Motic Images Plus 2.0 software (Motic, Richmond, BC, Canada) for image acquisition.

Gonad proximate composition and fatty acid analysis

The proximate composition of gonads was determined in terms of crude protein, moisture, ash and crude fat. Using a Dumas Nitrogen Analyzer (Model NDA 702, VELP® Scientifica, Usmate, Italy), crude protein (N factor = 6.25) was analyzed via combustion by the Dumas method (method 968.06) [27]. The contents of moisture and ash were determined by standard methods (methods 930.15 and 942.05, respectively) [27]. Crude fat (CF) was extracted following a previously described method [28], and then quantified gravimetrically after drying a 5-mL aliquot under nitrogen. The remaining lipid extracted was employed for fatty acid analysis. The transesterification reaction of fatty acids was carried out using boron trifluoride. 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 described earlier [29]. Fatty acids were identified by comparison of the retention times to those of known standards, quantified using heptadecanoic acid (17:0) as an internal standard, and expressed as mg g^{-1} of gonad wet weight.

Plasma 17β-estradiol (E2), gonad total carotenoid and cholesterol analyses

Blood samples were centrifuged (Heraeus Fresco 21, Thermo Scientific, Osterode am Harz, Germany) at 850×g for 15 min at 4°C to collect plasma, which was kept in micro centrifuge tubes at -20°C until E_2 analysis. Plasma E_2 concentration was determined by Enzyme-Linked Immune Sorbent Assay (ELISA), following a previously described procedure [30] and employing a commercial kit (MBS044518, My Biosource, San Diego, CA, USA). A fragment of the frozen gonad was used for the determination of total carotenoid and cholesterol content. Gonad

Table 1. Biological parameters of wild and cultured *C. parvipinnis* females and males.

		BW (g)	SE	TL (cm)	SE	K (%)	SE	GSI (%)	SE	VSI (%)	SE	HSI (%)	SE
Wild	♀	1009.69	35.57	47.18	0.70	0.96	0.02	1.16	0.02	9.40	0.29	1.95	0.07
Cultured	♀	448.39	21.57	36.43	0.60	0.92	0.02	0.39	0.04	-	-	-	-
T-test ($P > t$)		-	-	-	-	0.2390		< 0.0001		-	-	-	-
Wild	♂	835.93	49.96	44.07	0.89	0.96[a]	0.03	0.17	0.01	8.10	0.28	2.05	0.10
Cultured	♂	379.28	19.20	35.27	0.76	0.86[b]	0.02	0.05	0.005	-	-	-	-
T-test ($P > t$)		-	-	-	-	0.0091		< 0.0001		-	-	-	-

Values are means and standard errors of the mean (SE) of all fish (Wild female: 31; Cultured female: 15; Wild male: 19; Cultured male: 15). -: Not determined; BW: Body weight; TL: Total length; K: Condition factor; GSI: Gonadosomatic index; VSI: Viscerosomatic index; HSI: Hepatosomatic index.

tissue was immersed and homogenized in isotonic saline solution. Carotenoids were extracted from the crude homogenate using an acetone:methanol solution (2:1) and the concentration was determined by spectrophotometry, by measuring the absorbance at 495 nm [31]. After centrifugation of the crude homogenate at 3000×g for 10 min, the supernatant was used for determination of the total cholesterol content by an ELISA method adapted for small samples [32].

Statistics

Descriptive statistics (means and standard error of the mean, SE) of fish length and weight, body indices, such as K, VSI, GSI, HSI, as well as of biochemical data, i.e. proximate composition and the contents of fatty acids, E_2, total carotenoids and cholesterol, are presented. Excluding fish weight and length data, T-tests were conducted to compare wild and cultured organisms. Statistical analysis was conducted using the Statistical Analysis System (SAS 9.4) software package (SAS Institute Inc., 1999-2000).

RESULTS

Fifty wild shortfin corvinas (31 females and 19 males), in addition to 30 cultured fish (15 females and 15 males), were analyzed. Wild females, with mean BW and TL of 1009.69 g and 47.18 cm, respectively, were larger than their cultured counterparts, with mean BW of 448.39 g and TL of 36.43 cm. Wild males (BW of 835.93 g and TL of 44.07 cm) were also larger than cultured males (BW of 379.28 g and TL of 35.27 cm). Gender differences in size of fish of the same origin indicated that females were larger than males. The mean value of K was 0.96 for both wild females and males, whereas for cultured fish it was 0.92 for females and 0.86 for males. The differences were significant only when comparing wild vs. cultured males. The GSI values of wild fish (1.16 and 0.17% for females and males, respectively) were also statistically greater than those of cultured fish (0.39 and 0.05% for females and males, respectively), and were also greater in females as compared to males of the same origin. The mean values of VSI and HSI were calculated for wild fish only, with a numerically slightly higher VSI value in females (9.40%) vs. males (8.10%), while the means of HSI were relatively similar – 1.95% in females and 2.05% in males (Table 1).

Since stages of gonad development have not yet been histologically classified for the shortfin corvina, histological samples were compared with those described for a related sciaenid species, the spotted seatrout, *Cynoscion nebulosus* [33]. It was determined that gonads were in the early maturation stage for all wild (Fig. 1) and cultured (Fig. 2) male and female fish. For females, this stage is characterized by the presence of cortical alveolar oocytes, while some yolk granule vitellogenic oocytes also may be present. For males, continuous germinal epithelium can be seen throughout the testis, and all stages of spermatogenesis may be present, including spermatozoa in the lumen and ducts.

Although numerically similar, the crude protein content in gonads of wild males (14.83%) was statistically greater than in cultured males (14.00%), while the opposite was observed in cultured females (19.64%), as compared to wild fish (17.11%). The crude fat content also was statistically higher in

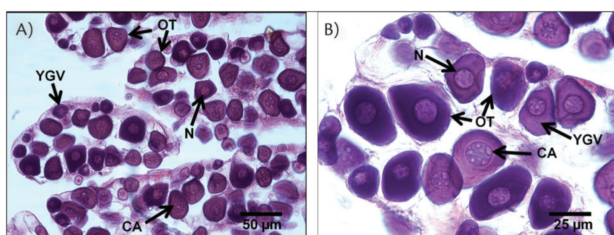

Fig. 1. Ovaries from wild (**A**) and cultured (**B**) *C. parvipinnis* at an early maturation stage, characterized by the presence of yolk granule vitellogenic oocytes (YGV) and cortical alveolar oocytes (CA). OT – oocytes; N – nucleus. **A** – 20x; **B** – 40x.

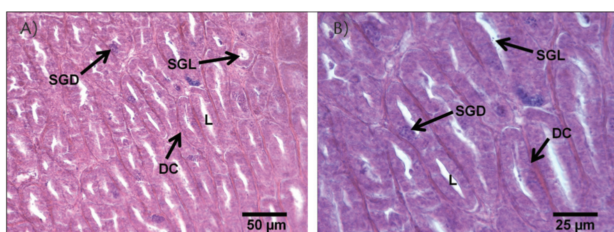

Fig. 2. Testes from wild (**A**) and cultured (**B**) *C. parvipinnis* at an early maturation stage, characterized by well-differentiated ducts (DC) with lumens (L), and the presence of spermatogonia and other stages of spermatogenesis in ducts (SGD) and in lumens (SGL). Magnification: **A** – 20x; **B** – 40x.

the gonads of both cultured males and females (female: 10.85%, male: 20.48%), compared to wild fish (female: 2.05%; male: 11.05%), and it was also higher in males compared to females. Moisture was the major component of the gonads, ranging between 62.52 to 66.94% in cultured corvinas and between 70.08 to 75.56% in wild fish, and it was significantly higher in both male and female fish in comparison to their cultured counterparts. Ash was the smallest component, ranging from 1.09% in wild male gonads, to 2.27% in the gonads of cultured females (Table 2).

Cholesterol and carotenoids were determined only in the gonads of wild fish; in females, cholesterol was higher (10.52 mg g^{-1}) than in males (3.26 mg g^{-1}), but the carotenoids were slightly higher in males than in females (3.13 vs. 2.18 μg g^{-1}). Plasma E_2 concentration in wild females was 0.007 ng mL^{-1} (SE = 0.001), while in cultured females it was 0.009 ng mL^{-1} (SE = 0.001). In wild males, the mean value was 0.006 ng mL^{-1} (SE = 0.002), and for cultured male fish it was 0.007 ng mL^{-1} (SE = 0.001), without significant differences between groups.

As a general trend, it was observed that the fatty acid content was higher in the gonads of cultured fish than in their wild counterparts, with a few exceptions. For instance, in wild female gonads (Table 3), 18:0, 20:4n-6, 22:5n-6, 22:5n-3, 22:6n-3 and 24:4n-6 were more abundant, and 20:4n-6, 22:5n-6, 22:5n-3 and 22:6n-3 also were more abundant in the gonads of wild males (Table 4). In both sexes, PUFA and HUFA were the most abundant type of fatty acids, compared to saturated and monounsaturated fatty acids, although individually, 16:0 proved to be the most abundant fatty acid in the gonads of this species, ranging from 128.58 to 164.84 mg per gram of gonad wet weight. After palmitic acid, highly unsaturated fatty acids like DHA, ARA and EPA were the major fatty acids in gonadal tissue of wild and cultured females and males. Among these, the concentration of EPA was significantly greater in cultured fish, while DHA and ARA were statistically more abundant in wild fish. The concentrations of n-3 fatty acids (wild female: 137.03, cultured female: 140.85, wild male: 130.62 and cultured male: 165.35 mg g^{-1} of gonad wet weight) were quantitatively higher than that of the

Table 2. Proximate composition, cholesterol and carotenoids content in gonads from wild and cultured *C. parvipinnis* females and males.

		CP (%)	SE	CF (%)	SE	CH (%)	SE.	Moisture (%)	SE	Ash (%)	SE	Cholesterol (mg g^{-1})	SE	Carotenoids (μg g^{-1})	SE
Wild	♀	17.11	0.15	2.05	0.08	3.19	0.76	75.56	0.81	2.09^b	0.05	10.52	1.13	2.18	0.29
Cultured	♀	19.64	0.18	10.85	0.70	0.31	0.06	66.94	0.47	2.27^a	0.05	-	-	-	-
T-test ($P > t$)		< 0.0001		< 0.0001		0.0180		< 0.0001		0.0323					
Wild	♂	14.83	0.22	11.05	1.09	2.95	0.59	70.08	0.96	1.09	0.05	3.26	1.54	3.13	0.91
Cultured	♂	14.00	0.63	20.48	2.27	1.25	0.24	62.52	0.25	1.75	0.04	-	-	-	-
T-test ($P > t$)		0.0456		0.0133		0.4064		0.0061		0.0234					

Values are means and standard errors of the mean (SE) of all fish (Wild female: 31; Cultured female: 15; Wild male: 19; Cultured male: 15). -: Not determined; CP: Crude protein; CF: Crude fat; CH: Carbohydrates.

Table 3. Fatty acid profile (mg of fatty acid g^{-1} of gonad wet weight) of gonads from wild and cultured females of *C. parvipinnis*.

	Wild female		Cultured female		
Fatty acid	Mean	SE	Mean	SE	T-test ($P > t$)
16:0	128.58	6.14	143.30	7.17	0.1619
18:0	39.58	2.32	35.23	3.15	0.2902
18:1	88.49	4.93	100.21	5.60	0.1639
18:2n-6	9.42	0.99	48.21	2.67	< 0.0001
18:3n-3	4.19	0.33	10.07	0.60	< 0.0001
20:4n-6	39.33	2.13	7.30	0.35	< 0.0001
20:4n-3	1.72	0.11	5.58	0.49	< 0.0001
20:5n-3	34.50	1.70	57.02	3.22	< 0.0001
22:5n-6	6.52	0.38	2.86	0.19	< 0.0001
22:5n-3	16.44	0.87	11.55	0.70	0.0010
22:6n-3	68.48	3.19	45.32	2.88	< 0.0001
24:4n-6	0.87	0.14	0.35	0.14	0.0277
24:6n-3	0.24	0.05	0.71	0.27	0.0202
Saturates[1]	186.33	8.83	223.42	11.62	0.0196
Monounsaturates[2]	108.91	6.08	112.43	6.13	0.7255
PUFA + HUFA[3]	249.66	11.75	261.21	13.78	0.5633
Total n-3[4]	137.03	5.75	140.85	7.72	0.7052
Total n-6[5]	59.81	2.98	62.23	3.25	0.6275
n-3/n-6	2.34	0.06	2.27	0.07	0.5133

Values are means and standard error of the mean (SE) of all fish (Wild female: 31; Cultured female: 15).
[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, 24:6n-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, 24:6n-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. Fatty acid profile (mg of fatty acid g^{-1} of gonad wet weight) of gonads from wild and cultured males of *C. parvipinnis*.

	Wild male		Cultured male		
Fatty acid	Mean	SE	Mean	SE	T-test (P > t)
16:0	133.64	6.41	164.84	5.26	*(P > t)*
18:0	43.96	1.70	46.14	1.54	0.1314
18:1	94.85	5.07	118.06	4.06	0.0835
18:2n-6	8.65	0.74	57.74	2.00	< 0.0001
18:3n-3	5.75	0.55	12.16	0.46	< 0.0001
20:4n-6	27.67	1.24	8.80	0.87	< 0.0001
20:4n-3	2.19	0.20	5.94	0.38	0.0282
20:5n-3	34.71	2.70	65.67	3.69	0.0005
22:5n-6	5.88	0.29	3.12	0.13	0.0683
22:5n-3	16.89	0.68	14.07	0.51	0.9694
22:6n-3	57.30	3.05	45.83	1.86	0.0036
24:4n-6	0.37	0.12	5.50	3.51	0.1192
24:6n-3	0.46	0.22	4.16	3.02	0.1384
Saturates[1]	201.73	9.75	262.06	8.37	0.0079
Monounsaturates[2]	112.02	5.77	132.15	4.45	0.0312
PUFA + HUFA[3]	227.63	10.20	313.51	16.47	0.0029
Total n-3[4]	130.62	5.48	165.35	10.27	0.0840
Total n-6[5]	46.84	1.79	79.97	4.97	0.0005
n-3/n-6	2.81	0.09	2.08[b]	0.06	< 0.0001

Values are means and standard error of the mean (SE) of all fish (Wild male: 19; Cultured male: 15).
[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

n-6 fatty acids (wild female: 59.81, cultured female: 62.23, wild male: 46.84 and cultured male: 79.97 mg g^{-1} of gonad wet weight). The n-3/n-6 ratios were 2.34 and 2.27 for wild and cultured females, respectively, without significant differences between groups. For males, wild fish had significantly higher n-3/n-6 ratio (2.81 mg g^{-1}) than cultured males (2.08 mg g^{-1}) (Tables 3 and 4).

DISCUSSION

The results presented herein represent, to our knowledge, the first histological evaluation of gonad maturity and gonad biochemical characteristics for *C. parvipinnis*. All wild-caught fish, captured in November 2015 had gonads in the early maturation stage, which can be related to the time of year of fish collection. According to local fishermen's traditional knowledge, *C. parvipinnis* displays a seasonal reproductive migration, with large aggregations of fish starting to occur in the fishing area (estuary "Santa Rosa") in November, when shortfin corvina sport-fishing tournaments are held. Interestingly, *C. othonopterus*, a closely related sciaenid found in the same area of the Gulf of California, performs a well-documented reproductive migration to the Upper Gulf of California and the Colorado River Delta, starting from October through June, with peak spawning events in March and April [34]. If migration of the shortfin corvina is like that of *C. othonopterus*, it is possible that all fish were sampled at the beginning of the migration event, when fish still need to actively feed in preparation for final gonad maturation and spawning. Therefore, they all had gonads in the

early maturation stage. To elucidate not only the biochemical changes taking place during the process of gonad maturation throughout its reproductive migration, but also to obtain a complete classification of the gonad developmental stages, seasonal studies of the reproductive biology of the shortfin corvina are warranted.

Because gonad volume and weight increase as gamete development progresses, the GSI of fish is considered as an indicator of sexual maturity [35]. In wild fish of the present study, GSI means recorded for females and males were 1.16 and 0.17%, respectively, which is consistent with the values reported for females and males (approximately 1.5 and 0.5%, respectively) of the sciaenid *C. nebulosus* captured at the beginning of the reproductive season, presumably also during the early maturation stage [36]. For other members of the family Sciaenidae, GSI values vary with species and degree of gonad maturity. For example, for female and male *Argyrosomus regius* the range is approximately from 0.2 to 5.0% and from 0.05 to 0.70%, respectively, over a whole year [37]. For wild *Sciaenops ocellatus*, values of 0.2-1.0% in wild females and of 0.02-0.40% in wild males have been reported [38]. Over the May-October period, values ranging from approximately 0.8 to 5.0% for females and from 0.1 to 2.5% for males have been reported for *Sciaena umbra* [35]. On the other hand, the concentration of plasma E_2, which has been shown to decrease as gonad maturity progresses in fish, is considered as another indicator of gonad development [39]. In the present study, levels found in wild females and males were 0.007 and 0.006 ng mL^{-1}, respectively. They are somewhat low, as compared to values reported for other sciaenids, e.g., wild *A. regius*, with values ranging from 0.0933 ng mL^{-1} in immature fish to 0.0210 ng mL^{-1} in ripe females, and from 0.0933 to 0.009 ng mL^{-1} in males [37]. For the same species, but in a cultured fish stock, the concentration of plasma E_2 varied from 0.194 to 0.473 ng mL^{-1} from November through July [40]. Once again, seasonal monitoring of the GSI and plasma E_2 in the shortfin corvina would provide a clearer insight into the variation of these parameters.

Histological examination of gonads showed that both cultured female and male fish also possessed gonads in the early maturation stage, as well as similar E_2 plasma concentrations (0.009 and 0.007 ng mL^{-1} for females and males, respectively), as compared to wild fish. However, their size was much smaller (female: 448.39 g cultured vs. 1009.69 g wild; male: 379.28 g cultured vs. 835.93 g wild) and so were their GSI values (female: 0.39% cultured vs. 1.16% wild; male: 0.05% cultured vs. 0.17% wild). One of the reasons the shortfin corvina has attracted interest as a candidate species for aquaculture is its manageable adult size. As compared to large-sized sciaenids, such as totoaba (*Totoaba macdonaldi*), this characteristic has positive implications, such as eliminating the need of high-cost, large facilities for broodstock rearing/spawning, ease of broodstock handling, and presumably a shorter rearing time until reproductive age is attained. It is worthwhile pointing out that cultured fish were exclusively fed the high-quality compound feed described earlier. However, greater growth rates, and possibly shorter time to reach sexual maturity, may be expected by supplementing with fresh-frozen dietary components, such as squid, vitamin/mineral-enriched fish, shrimp, and krill, which are routinely used in broodstock diets of a variety of marine fish species, for example, *Pagrus major* and *Hippoglossus hippoglossus* [24, 41-42].

The indices VSI and HSI, calculated only in wild shortfin corvina, did not show apparent gender differences: VSI values of 9.40 and 8.10% for females and males, respectively, and HSI values of 1.95 and 2.05% for females and males, respectively. For wild red drum (*S. ocellatus*), HSI has been shown to peak in March-April (2.3 and 1.8% for females and males, respectively), when fish actively store lipids, then decline significantly in September-October (0.6% for both sexes) due to nutrient mobilization during reproduction [38]. In recent comparative feeding trials, HSI of the shortfin corvina has varied from 0.78 to 1.45% [4-5]. With respect to the VSI, comparable results (5.49-9.81%) were reported for cultured *A. regius* [43], but lower values, ranging from 2.53 to 2.78%, also were reported for cultured organisms of the same species [44]. While HSI values of the present study are within the range of values previously reported, and VSI data in studies with different species show a large dispersion, the annual variation of the HSI and VSI for *C. parvipinnis* is yet to be determined.

For fish species in which increased energy reserves and ripe gonads account for a significant part of total body weight, especially in females, Fulton's condition factor can serve as an indirect indicator of gonad maturation. This phenomenon has traditionally been shown for various saltwater and freshwater fish species, such as, sardines, anchovies, and sturgeons, in which the highest K values generally coincide with seasonal peaks in reproductive activity [45-47]. In the present study, although differences were detected between wild and cultured males, the variation of K values for both sexes was small, ranging from 0.86 to 0.96. These results are similar to values previously obtained for this species, 0.88-1.1 [4-5] and for other sciaenids, such as *S. umbra* (0.9-1.1), *A. regius* (0.99), and *C. othonopterus* (1.1-1.3) [35,44,48]. Interestingly, significant changes in K were observed over the May-October period for both female and male *S. umbra* [35], an antecedent that prompts further investigation of seasonal changes in K for the shortfin corvina.

In the gonad proximate composition, the content of crude protein in wild females was slightly higher than in cultured fish (19.64 *vs.* 17.11%, respectively), but similar in males (14.83 and 14.00%, respectively). Since both groups of fish had gonads in the early maturation stage, this may explain the similarities. In addition, other studies have described similarities in the biochemical composition of tissues of wild and captive *S. ocellatus* [38]. These values are within the range of results reported for other species, for example, 14.02 to 24.81% crude protein in gonads of female *Epinephelus diacanthus* at different stages of ovarian maturation [49]. There were marked, significant differences in the crude fat content from wild and cultured shortfin corvina in the present study (female: 10.85% cultured vs. 2.05% wild; male: 20.48% cultured vs. 11.05% wild). This result may be attributed to apparent differences in the dietary supply of crude fat, unknown for wild organisms, while captive fish were fed solely a 12% CF compound feed. Large variability of the CF content in gonads at the same or different stages of development also has been observed in several species belonging to various families, including Scombridae, Serranidae and Siganidae, among others, which has been explained in terms of active mobilization of lipid reserves into gonads during vitellogenesis [49-51]. Moisture content, with an overall range from 66.94 to 75.56% for wild and cultured fish in this study, accounted for much of the gonad proximate composition, while ash was the smallest component (1.09-2.27%). These results are consistent with values previously described, e.g. moisture and ash contents of 60.55-71.66% and 0.82-1.20%, respectively, in gonads of wild female *E. diacanthus*, and gonad moisture content of 72.0-79.9% in *Ammodytes hexapterus*, [49,52]. The gonad carbohydrate contents observed in this study for wild and cultured females (3.19 and 0.31%, respectively) or for wild and cultured males (2.95 and 1.25%, respectively) are to some extent similar to the range of values (1.23-1.46%) determined for female *E. diacanthus* [49].

With respect to the gonad fatty acid composition, the overall FA content was numerically higher in cultured fish, which is likely a reflection of their higher lipid content. However, specific fatty acids, such as DHA and ARA, were found at significantly higher concentrations in wild females (DHA: 68.48 mg g^{-1} vs. 45.32 mg g^{-1}; ARA: 39.33 mg g^{-1} vs. 7.30 mg g^{-1}) and males (DHA: 57.30 mg g^{-1} vs. 45.83 mg g^{-1}; ARA: 27.67 mg g^{-1} vs. 8.80 mg g^{-1}). This can be explained in terms of the relatively high abundance of HUFA in natural marine food webs [53], in conjunction with the direct effect dietary FAs have on the fatty acid profile of body tissues, as it has been demonstrated for a number of fishes [54-56], including the shortfin corvina [4]. Furthermore, while saturated and monounsaturated fatty acids are preferentially catabolized as a source of energy, essential fatty acids such as DHA and EPA, can be selectively stored or deposited in tissue, such as gonads [10,57], which also helps explain the observed results. DHA and ARA, along with EPA, were quantitatively the main constituent HUFA in gonads of both wild and cultured *C. parvipinnis*. This pattern agrees well with the observations of many other marine fish species, in which high relative abundance of some or all of these FAs has been detected in the gonads [50,58]. The important physiological role that these FAs play explains their presence in significant amounts in gonadal tissues. For example, EPA and ARA are precursors of thromboxanes, prostaglandins and leukotrienes [11], which not only participate in ovarian and testicular steroidogenesis, but also aid in the regulation of immune and inflammatory processes, hematological and cardiovascular activity, and renal and neural function [59]. In addition,

it has been suggested that ARA is involved in the development of the immune system of the fish embryo, as well as in the process of hatching and early larval performance [12]. In turn, DHA is a major constituent of neural and visual tissues [60].

Cholesterol serves as a precursor to all steroid hormones, which regulate reproductive physiology [23]. For wild *E. diacanthus*, it was reported that cholesterol levels in ovaries decreased from 2.06% in stage I of development, to 1.27% in stage III, suggesting conversion of cholesterol into steroid hormones as gonad maturation progressed [49]. In the present study, the concentration of cholesterol was 10.52 mg g^{-1} for females and 3.26 mg g^{-1} for males. Due to incompatible units, comparison to previous data [49] is unfortunately not possible. However, our results are comparable to the range of values (approximately 4-15 mg g^{-1}) reported for the gonads of female and male *Etroplus suratensis* [61]. Finally, the values of the total carotenoid contents, determined only for wild fish (2.18 μg g^{-1} in females and 3.13 μg g^{-1} in males), were similar to the data determined for *E. diacanthus*, in which values increased with the stage of gonad maturation, from 1.42 μg g^{-1} to 7.98 μg g^{-1} [49]. Carotenoids in fish gonads are thought to play various roles relevant to fish reproduction, such as protection of eggs against light, precursors to vitamin A, and antioxidants [24], and have been shown to improve egg pigmentation, fertilization rate, and survival of rainbow trout when supplemented in the diet [25]. Clearly, more information is needed on the quantitative changes of gonad cholesterol and carotenoids over the reproductive cycle of the shortfin corvina.

CONCLUSIONS

Populations of *C. parvipinnis*, one comprised of wild fish captured in November near Kino Bay, Gulf of California, and one composed of cultured organisms, possessed ovaries and testes in the early maturation stage. The gonadosomatic index, the concentration of gonad total cholesterol and carotenoid, as well as of plasma 17β-estradiol, are reported for the first time for this species. The indices K, VSI and HSI did not show apparent gender differences. Moisture content, with an overall range between 66.94 to 75.56%, was the main component of the gonad proximate composition, while ash was the smallest component (1.09-2.27%). Crude protein and fat ranged from 14.00 to 19.64% and from 2.05 to 20.48%, respectively. In the gonad, the overall FA content was higher in cultured fish. However, specific FAs, such as DHA and ARA, were found at significantly higher concentrations in wild organisms, which along with EPA, were quantitatively the main constituent HUFA in gonads of both wild and cultured fish. In general, the biochemical composition of the gonads of wild and cultured organisms at an early maturation stage was quite similar, and these data may serve as indicators of dietary requirements for a broodstock diet, although they additionally prompt further investigations of seasonal changes in the reproductive biology of the shortfin corvina.

Acknowledgments: Funding for Ms. Urquidez-Bejarano was provided by the Consejo Nacional de Ciencia y Tecnologia (CONACYT-Mexico). The mention of trademarks or proprietary products does not constitute an endorsement of the product by the University of Sonora and does not imply its approval to the exclusion of other products that may also be suitable.

Conflict of interest disclosure: The authors have no conflict of interest related to this work.

REFERENCES

1. Fischer WF, Krupp W, Schneider C, Soomer KE, Carpenter V, Niem VH. Guía FAO para la identificación de especies para los fines de la pesca. Pacífico Centro-Oriental. Vol. 3. Vertebrados. Rome, Italy: Food and Agriculture Organization; 1995. 1813 p.
2. Comisión Nacional de Acuacultura y Pesca (CONAPESCA). Anuario Estadístico de Acuacultura y Pesca. Mazatlán, Sinaloa, Mexico: CONAPESCA; 2013. 297 p.
3. Chao NL. Sciaenidae. In: Fisher W, Krupp F, Schneider W, Sommer C, Carpenter KE, Niem VH, editors. Guía FAO para la identificación de especies para los fines de la pesca. Pacífico Centro-Oriental. Vol. 3.. Vertebrados. Rome, Italy: Food and Agriculture Organization; 1995. p. 1427-518.
4. González-Félix ML, Maldonado-Othón CA, Perez-Velazquez M. Effect of dietary lipid level and replacement of fish oil by soybean oil in compound feeds for the shortfin corvina (*Cynoscion parvipinnis*). Aquaculture. 2016;454:217-28.
5. Minjarez-Osorio C, Castillo-Alvarado S, Gatlin D.M. III, González-Félix M.L, Perez-Velazquez M, Rossi Jr. W. Plant protein sources in the diets of the sciaenids red drum (*Sciaenops ocellatus*) and shortfin corvina (*Cynoscion parvipinnis*): A comparative study. Aquaculture. 2016;453:122-9.

6. Ferreri R, Basilone G, D'Elia M, Traina A, Saborido-Rey F, Mazzola S. Validation of macroscopic maturity stages according to microscopic histological examination for European anchovy. Mar Ecol. 2009;30(Suppl. 1):181-7.
7. Vitale F, Svedäng H, Cardinale M. Histological analysis invalidates macroscopically determined maturity ogives of the Kattegat cod (*Gadus morhua*) and suggests new proxies for estimating maturity status of individual fish. ICES J Mar Sci. 2006;63:485-92.
8. Bagenal TB. Methods for the assessment of fish production in freshwaters. IBP Handbook No. 3. 3rd ed. Oxford, UK: Blackwell Scientific Publications Ltd; 1978. 365 p.
9. Mylonas CC, Fostier A, Zanuy S. Broodstock management and hormonal manipulations of fish reproduction. Gen Comp Endocrinol. 2010;165:516-34.
10. Henderson RJ, Park MT, Sargent JR. The desaturation and elongation of 14C-labelled polyunsaturated fatty acids by pike (*Esox lucius*) in vivo. Fish Physiol Biochem. 1995;14:223-35.
11. Wolfe LS, Horrocks LA. Eicosanoids. In: Siegel GJ, Agranoff BW, Albers RW, Molinoff PB, editors. Basic Neurochemistry. New York, USA: Lippincot-Raven Publishers; 1994. p. 475-90.
12. Sorbera LA, Zanuy S, Carrielo M. A role for polyunsaturated fatty acids and prostaglandins in oocyte maturation in the sea bass (*Dicentrarchus labrax*). In: Vandry H, editor. Trends in comparative endocrinology and neurology: from molecular to integrative biology. New York, USA: Academy of Sciences; 1998. p. 535-7.
13. Henderson RJ, Sargent JR, Hopkins CCE. Changes in the content and fatty acid composition of lipid in an isolated population of the capelin *Mallotus villosus* during sexual maturation and spawning. Mar Biol. 1984;78:255-63.
14. Izquierdo M. Essential fatty acid requirements of cultured marine fish larvae. Aquacult Nutr. 1996;2:183-91.
15. Collier JT, Kaneko T, Hirano T, Grau EG. Seasonal changes in reproductive activity in the Potter's angelfish (*Centropyge potteri*) in Kaneohe Bay, Hawaii. Environ Biol Fishes. 2003;68:49-57.
16. Guerriero G. Seasonal steroids variations and maturity stages in the female chub, *Leuciscus cephalus* L. (Pisces, Cyprinidae). Ital J Zool. 2007;74:317-24.
17. Swanson P, Dickey JT, Campbell B. Biochemistry and physiology of fish gonadotropins. Fish Physiol Biochem. 2003;28:53-9.
18. Aruke A, Goksoyr A. Eggshell and egg yolk proteins in fish: hepatic proteins for the next generation: Oogenic, population, and evolutionary implications of endocrine disruption. Comp Hepatol. 2003;2:4-7.
19. Moncaut N, Nostro FL, Maggese MC. 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. 2003;63:127-37.
20. Berg AH, Westerlund L, Olsson PE. Regulation of Arctic char (*Salvelinus alpinus*) egg shell proteins and vitellogenin during reproduction and in response to l7β-estradiol and cortisol. Gen Comp Endocrinol. 2004;115:276-85.
21. Scott AP, Witthames PR, Turner RJ, Canario AVM. Plasma concentrations of ovarian steroids in relation to oocyte final maturation and ovulation in female plaice sampled at sea. J Fish Biol. 1998;52:128-45.
22. Kokokiris L, Mourot B, Le Menn F, Kentouri M, Fostier A. Endocrine changes during the annual reproductive cycle of the red porgy, *Pagrus pagrus* (Teleostei, Sparidae). Fish Physiol Biochem. 2000;23:1-11.
23. Roberts KD, Bandi L, Calvin HI, Drucker WD, Lieberman S. Evidence that Cholesterol Sulfate is a Precursor of Steroid Hormones. J Am Chem Soc. 1964;86(5):958-9.
24. Izquierdo M, Fernandez-Palacios H, Tacon A. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture. 2001;197:25-42.
25. Harris LE. Effects of a broodfish diet fortified with canthaxanthin on female fecundity and egg color. Aquaculture. 1984;43:179-83.
26. Ricker WE. Computation and interpretation of biological statistics of fish populations. B Fish Res Board Can. 1975;191:1-382.
27. Association of Official Analytical Chemists (AOAC). Official Methods of Analysis. Arlington, VA, USA: Association of Official Analytical Chemists; 2005.
28. Folch J, Lees M, Sloane GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 1957;1:497-509.
29. Lochmann RT, Gatlin DM. III. Essential fatty acid requirement of juvenile red drum (*Sciaenops ocellatus*). Fish Physiol Biochem. 1993;12:221-35.
30. Semenkova T, Barinnikova I, Kime DE, McAllister BG, Buyanova L, Dyubin V, Kolmakov N. Sex steroid profiles in female and male stellate sturgeon (*Acipenser stellatus* Pallas) during final maturation induced by hormonal treatment. J Appl Ichthyol. 2002;18:375-81.
31. Strickland JDH, Parsons TR. A practical handbook of seawater analysis. 2nd ed. Ottawa, Canada: Fisheries Research Board of Canada; 1972. 310 p.
32. Palacios E, Ibarra AM, Ramírez JL, Portillo G, Racotta IS. Biochemical composition of egg and nauplii in White Pacific Shrimp *Penaues vannamei* (Boone), in relation to the physiological condition of spawners in a commercial hatchery. Aquacult Res. 1998;29:183-89.
33. Brown-Peterson NJ. The reproductive biology of spotted seatrout. In: Bortone SA, editor. Biology of the Spotted Seatrout. Boca Raton, FL, USA: CRC Press; 2003. p. 99-134.
34. Rowell K, Flessa KW, Dettman DL, Román M. The importance of Colorado River flow to nursery habitats of the Gulf corvina (*Cynoscion othonopterus*). Can J Fish Aquat Sci. 2005;62:2874-85.
35. Grau A, Linde M, Grau AM. Reproductive biology of the vulnerable species *Sciaena umbra* Linnaeus, 1758 (Pisces: Sciaenidae). Sci Mar. 2009;73(1):67-81.
36. Brown-Peterson NJ, Warren JW. The reproductive biology of spotted seatrout, *Cynoscion nebulosus*, along the Mississippi Gulf Coast. Gulf of Mexico Science. 2001;1:61-73.
37. Abou Shabana NM, Abd El Rahman SH, Al Absawy MA, Assem SS. Reproductive biology of *Argyrosomus regius* (Asso, 1801) inhabiting the south eastern Mediterranean Sea, Egypt. Egypt J Aquat Res. 2012;38:147-56.
38. Craig SR, MacKenzie DS, Jones G, Gatlin III DM. Seasonal changes in the reproductive condition and body composi-

tion of free-ranging red drum, *Sciaenops ocellatus*. Aquaculture. 2000;190:89-102.

39. Hosseinzadeh M, Imanpoor MR, Shabani A, Nekoubin H. Seasonal changes in serum calcium and 17β-estradiol levels in Persian sturgeon, *Acipenser persicus*. J Aquat Res Dev. 2013;4:1-3.
40. Mylonas CC, Mitrizakis N, Papadaki M, Sigelak I. Reproduction of hatchery-produced meagre *Argyrosomus regius* in captivity I. Description of the annual reproductive cycle. Aquaculture. 2013;414-415:309-17.
41. Izquierdo M, Fernandez-Palacios H. Nutritional requirements of marine fish larvae and broodstock. CIHiheam Options Mediterraneennes. 1997;22:243-64.
42. Brown, N. Development of broodstock diets for the Atlantic halibut *Hippoglossus hippoglossus*. Final Report to NRAC. Franklin, Maine, USA: University of Maine, Center for Cooperative Aquaculture Research; 2009. 51 p.
43. Velazco-Vargas JL. Contribución al estudio de las necesidades nutritivas de la corvina (*Argyrosomus regius*, Asso 1801) [dissertation]. Valencia, Spain: Universitat Politecnica de Valencia; 2014. 181 p. Spanish.
44. Chatzifotis S, Panagiotidou M, Papaioannou N, Pavlidis M, Nengas I, Mylonas CC. Effect of dietary lipid levels on growth, feed utilization, body composition and serum metabolites of meagre (*Argyrosomus regius*) juveniles. Aquaculture. 2010;307:65-70.
45. Basilone G, Patti B, Bonanno A, Cuttitta A, Vergara AR, Garcia A. Reproductive aspects of the European anchovy (*Engraulis encrasicolus*): six years of observations in the Strait of Sicily. MedSudMed Technical Documents. 2004:5:67-78.
46. Lenhardt M, Jarić I, Cakić P, Cvijanović G, Gačić Z, Kolarević J. Seasonal changes in condition, hepatosomatic index and parasitism in sterlet (*Acipenser ruthenus* L.). Turk J Vet Anim Sci. 2009;33(3):209-14.
47. Mustać B, Sinovčić G. Reproduction, length-weight relationship and condition of sardine, Sardina pilchardus (Walbaum, 1792), in the eastern Middle Adriatic Sea (Croatia). Period Biol. 2010;112(2):133-8.
48. González-Félix ML, Minjarez-Osorio C, Perez-Velazquez M, Urquidez-Bejarano P. Influence of dietary lipid on growth performance and body composition of the Gulf corvina, *Cynoscion othonopterus*. Aquaculture. 2015;448:401-9.
49. Chandrasekhara A, Krishnan L. Biochemical composition and changes in biological indices associates with maturation of the ovary in the spiny cheek grouper *Epinephelus diacanthus* (Valenciennes, 1828). Indian J Fish. 2011;58:45-52.
50. Suloma A, Ogata HY. Arachidonic acid is a major component in gonadal fatty acids of tropical coral reef fish in the Philippines and Japan. J Aquac Res Dev. 2011;2:2.
51. Sprague M, Dick JR, Medina A, Tocher DR, Bell JG, Muorente G. Lipid and fatty acid composition, and persistent organic pollutant levels in tissues of migrating Atlantic bluefin tuna (*Thunnus thynnus*, L.) broodstock. Environ Pollut. 2012;171:61-71.
52. Robards MD, Anthony JA, Rose GA, Piatt JF. Changes in proximate composition and somatic energy content for Pacific sand lance (*Ammodytes hexapterus*) from Kachemak Bay, Alaska relative to maturity and season. J Exp Mar Biol Ecol. 1999;242:245-58.
53. Dalsgaard J, Michael St. John M, Kattner G, Miiller-Navarra D, Hagen W. Fatty Acid Trophic Markers in the Pelagic Marine Environment. Adv Mar Biol. 2003;46:225-340.
54. Izquierdo MS, Obach A, Arantzamendi L, Montero D, Robaina L, Rosenlund G. Dietary lipid sources for seabream and seabass: growth performance, tissue composition and flesh quality. Aquacult Nutr. 2003;9:397-407.
55. Bell JG, Henderson RJ, Tocher DR, Sargent JR. Replacement of dietary fish oil with increasing levels of linseed oil: modification of flesh fatty acid compositions in Atlantic salmon (*Salmo salar*) using a fish oil finishing diet. Lipids. 2004;39:223-32.
56. Turchini GM, Torstensen BE, Ng WK. Fish oil replacement in finfish nutrition. Rev Aquacult. 2009;1:10-57.
57. Stubhaug I, Lie Ø, Torstensen BE. Fatty acid productive value and b-oxidation capacity in Atlantic salmon tissues (*Salmo salar* L.) fed on different lipid sources along the whole growth period. Aquacult Nutr. 2007;13:145-55.
58. Huynh MD, Kitts DD, Hu C, Trites AW. Comparison of fatty acid profiles of spawning and non-spawning Pacific herring, *Clupea harengus pallasi*. Comp Biochem Physiol B. 2007;146:504-11.
59. Tocher DR, Bell JG, Dick JR, Crampton VO. Effects of dietary vegetable oil in Atlantic salmon hepatocyte fatty acid desaturation and liver fatty acid composition. Lipids. 2003;38:723-32.
60. Sargent JR, Tocher DR, Bell JG. The lipids. In Halver JE, Hardy RW, editors. Fish Nutrition, 3rd ed. San Diego, VA, USA: Academic Press; 2002. p. 181-257.
61. Diwan AD, Krishnan L. Levels of cholesterol in blood serum and gonads in relation to maturation in *Etroplus suratensis* (Block). Indian J Fish. 1986;33(2):241-45.