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Comparison of fatty acids profile of the gonads and eggs of *Lutjanus guttatus* (Perciformes: Lutjanidae) obtained from wild and captive broodstock.

Comparación del perfil de ácidos grasos de las gónadas y huevos de Lutjanus guttatus (Perciformes: Lutjanidae) obtenidas de reproductores silvestres y de cautiverio

Comparação do perfil de ácidos graxos das gônadas e ovos de Lutjanus guttatus (Perciformes: Lutjanidae) obtidos de animais reprodutores silvestres e em cativeiro

Jonathan Chacón-Guzmán
jonathan.chacon.guzman@una.cr
Pacific Marine Park Program
Universidad Nacional
Heredia, Costa Rica
Orcid: https://orcid.org/0000-0002-8449-2334

Milagro Carvajal-Oses
Milagro.carvajal.oses@una.cr
Pacific Marine Park Program
Universidad Nacional
Heredia, Costa Rica
Orcid: https://orcid.org/0000-0002-8294-9863

Pedro Toledo-Agüero
ptoledo@ucn.cl
Departament of Aquaculture
Universidad Católica del Norte
Coquimbo, Chile
Orcid: https://orcid.org/0000-0003-3844-1824

Héctor Flores-Gatica
hflores@ucn.cl
Departament of Aquaculture
Universidad Católica del Norte
Coquimbo, Chile
Orcid: https://orcid.org/0000-0002-8020-8076

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Abstract

The proportions of fatty acids present in eggs and gonads of wild and captive *Lutjanus guttatus* (Steindachner, 1869) were evaluated to assist in obtaining nutritional information necessary to improve the diets provided in aquaculture production centers of this species. Samples of eggs and gonads were obtained from broodstock (1.0±0.1 kg) kept in the Pacific Marine Park (PMP), Costa Rica, and from individuals caught by fishermen in their natural environment. The samples were taken in triplicate during the period spanning from August to September 2015. Captive snappers were fed a fresh diet. Spawns and gonads were obtained from two groups of 16 specimens each of captive and wild fish, both groups having a sex ratio of 1:1 male to female. Gonads from females were selected according to their maturity stage, and male gonads were sampled based on the quantity, viscosity and color of semen. There were no statistically significant differences ($p \geq 0.05$) between egg diameters and oil droplets size; viability rates, expressed as percentages, were considered to be high and appropriate for comparison (91.2% captive, 86.1% wild).

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There were also no statistically significant differences in the fatty acid composition of eggs obtained from wild and captive females (p=0.2188), or in gonads from males (p=0.6179) and wild and captive females (p=0.1153). The presence of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was observed in all of the samples analyzed, while arachidonic acid (ARA) was found in amounts ranging from 0.38 to 5.07% and was not present in the eggs of wild females or in the gonads of captive females.

Keywords: Fatty acids; Eggs; Gonads; Wild; Captivity.

Resumen

Se evaluaron las proporciones de ácidos grasos presentes tanto en huevos como en gónadas de *Lutjanus guttatus* (Steindachner, 1869) silvestres y en cautiverio, con el fin de brindar la información nutricional necesaria para mejorar las dietas aplicadas en los centros de producción acuícola de esta especie. Las muestras de huevos y gónadas fueron obtenidas tanto de reproductores (1.0±0.1 Kg) mantenidos en el Parque Marino del Pacífico, Costa Rica; como de los capturados, en el medio natural, por los pescadores. Las muestras fueron tomadas durante el periodo comprendido entre agosto y septiembre del 2015 y por triplicado. Los pargos en cautiverio fueron alimentados con base en dieta fresca. Los desoves y gónadas fueron obtenidos de lotes de 16 ejemplares en cautiverio y silvestres, ambos con una proporción de 1:1 macho y hembra. Las gónadas muestreadas de las hembras se seleccionaron de acuerdo con el estado de madurez; y la de los machos según la cantidad, viscosidad y color del semen. No existieron diferencias, estadísticamente significativas, ($p \geq 0.05$) entre los diámetros de los huevos y las gotas de aceite; los porcentajes de viabilidad se consideraron altos y aptos (91.2 % cautiverio, 86.1 silvestres) para la comparación. No se dieron diferencias, estadísticamente significativas, en la composición de ácidos grasos en los huevos obtenidos de hembras silvestres y de cautiverio (p=0.2188), ni en gónada de machos (p=0.6179) y hembras silvestres y de cautiverio (p=0.1153). Se observó la presencia del ácido eicosapentanoico (EPA) y del ácido docosahexanoico (DHA) en todas las muestras analizadas y el ácido araquidónico (ARA) se encontró en valores que variaron entre 0.38 y 5.07 %, y no estuvo presente en huevos silvestres ni en la gónada de hembras cautivas.

Palabras claves: Ácido graso; huevos; gónadas; silvestres; cautiverio.

Resumo

Foram avaliadas as proporções dos ácidos graxos presentes tanto em ovos quanto em gônadas Lutjanus Guttatus (Steindachneri, 1869) silvestres e em cativeiro, de modo a fornecer informações nutricionais para melhorar a alimentação aplicada nos centros de produção de aquicultura da espécie. As amostras de ovos e gônadas foram obtidas de reprodutores (1,0 ± 0,1 kg), mantidos no Parque Marinho do Pacífico, na Costa Rica, e dos capturados, no ambiente natural, por pescadores. As amostras foram coletadas no período de agosto a setembro de 2015 e em triplicado. Os pargos em cativeiro foram alimentados com dieta fresca. A desova e as gônadas foram obtidas de lotes de 16 exemplares cativos e silvestres, ambos com uma proporção de 1: 1 macho e fêmea. As gônadas amostradas das fêmeas foram selecionadas de acordo com o estado de maturidade; e a dos machos de acordo com a quantidade, viscosidade e cor do sêmen. Não houve diferenças estatisticamente significantes ($p \geq 0,05$) entre os diâmetros dos ovos e as gotas de óleo; as porcentagens de viabilidade foram consideradas altas e adequadas (91,2% em cativeiro, 86,1 silvestres) para a comparação. Não houve diferenças estatisticamente significativas na composição de ácidos graxos nos ovos obtidos a partir de fêmeas silvestres e em cativeiro ($p = 0,2188$), ou em gônadas

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de machos (p = 0,6179) e de fêmeas silvestres e em cativo (p = 0,1153). Observou-se a presença de ácido eicosapentaenoico (EPA) e ácido docosahexaenoico (DHA) em todas as amostras analisadas e ácido araquidônico (AA) encontrado em valores que variaram entre 0,38 e 5,07% e não estava presente em ovos silvestres e nem em gônada de fêmeas cativas.

Palavras-chave: Ácido graxo; ovos; gônadas; silvestre; cativeiro.

For several decades, species of the genus *Lutjanus* have been successfully cultivated, especially in Asia and Australia; while in America, mass production of artificially reared finglings and large-scale commercial cultivation began in the second half of the 2000s thanks to advances made with *Lutjanus guttatus* (Doi, Kohno, Taki, Ohno and Singhagraiwa, 1994; Emata, Eullaran and Bagrinao, 1994; Avilés et al., 1996, Field, 1997; Watanabe et al., 1998; Herrera-Ulloa, Chacón-Guzmán, Zúñiga-Calero, Fajardo-Espinoza and Jiménez-Montealegre, 2009; Abdo De la Parra, Rodríguez-Ibarra, Rodríguez-Montes de Oca and Velasco-Blanco, 2015).

L. guttatus known as rose spotted snapper (pargo manchado, flamingo or lunarejo in spanish), has been one of the species of greatest interest for marine pisciculture in Latin America, its distribution in the Pacific Ocean ranges from Mexico to Peru; it possesses biological characteristics that potentiate its natural reproduction in captivity and farming in floating cages; it holds a high demand in international markets, especially in the United States, where crop specimens are sold whole (approx. 400 g) or in butterfly fillet at 5 to 8 USD/pound (Allen, 1995; Fischer et al., 1995; Herrera -Ulloa et al., 2009; Chacón-Guzmán, 2010).

In several countries, this potential crop species has encouraged research aimed at comprehending and controlling the different aspects of its life cycle in captivity (Abdo-De la Parra, Rodríguez-Ibarra, Rodríguez-Montes de Oca, Velasco-Blanco and Ibarra- Castro, 2015). In Costa Rica, the first large-scale productions were recorded since 2005, farming in floating cages in 2006, national Market fresh produce sales in 2007 and export in 2009 (Herrera-Ulloa et al., 2009; Herrera-Ulloa, Chacón-Guzmán, Zúñiga-Calero and Jiménez-Montealegre, 2010).

However, despite significant progress, the larval stage remains one of the critical phases in the artificially reared finglings production process as this stage presents the highest mortality rate (Abdo-De la Parra et al., 2010). To increase survival at this stage, the nutrition of the broodstock plays a preponderant role due to its effect on the fertility rate and the quality of eggs (Watanabe, Arakawa, Kitajuma and Fajita, 1984).

In its natural environment *L. guttatus* is described as a carnivorous species that feeds mainly on fish and crustaceans (Rojas, 1997; Rojas-Herrera and Chiappa-Carrara, 2002; Rojas, Maravilla y Chicas, 2004). In captivity, the natural diet has been imitated by using marine species such as sardines, shrimp, squid, tuna and other fish, which has resulted in floating eggs of acceptable quality, viability and hatching rates above 90% (Herrera-Ulloa et al., 2009).

However, one must consider that egg quality; regarding its biochemical content to meet the nutritional demand of the embryo; is related to the nature and content of pigments, vitamin C, inorganic substances

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(phosphorus, iron and calcium), organic compounds (polyunsaturated fatty acids or PUFAs), among others; and the age of the female. Therefore, under these premises, fresh diets do not always offer the same proportions of nutrients found in the natural environment diet, as is the case with PUFAs, required for proper growth and development; the most important being docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3). (Kjørsvik, Mangor-Jensen and Holmefjord, 1990; Sargent, McEvoy and Bell, 1997).

Thus, when *L. guttatus* are farmed in captivity it is necessary to include in their diet the required n-3 and n-6 polyunsaturated acids as these are necessary for sound biological function and adequate cellular structure (Sargent et al., 1995); they are precursors to prostaglandins (Murdoch, Hansen and McPherson, 1993) and contribute in the reproductive phases in females, influence gonadal development, fecundity and egg quality (Navas et al., 1997; Bruce et al., 1999).

The objective of the present study is to determine and compare the proportions of fatty acids present in the eggs and gonads of wild and captive rose spotted snappers, with the purpose of acquiring data to improve the diets supplied to *L. guttatus* broodstock in captivity.

Methodology

Samples of eggs and gonads (1.0 ± 0.1kg) from *L. guttatus* broodstock were collected between August and September of 2015, both from specimens held in captivity in the Pacific Marine Park (PMP), Puntarenas, Costa Rica, and wild specimens caught by artisanal fishermen using the longline technique in the Gulf of Nicoya, Costa Rica.

Captive broodstock (n=16) were captured 22 months prior to initiating the investigation at 9°46'05"N and 84°52'16"E coordinates in the Gulf of Nicoya. They were marked with nano transponders [Trovan®, ID-100A (1.25), purchased from Inversiones Ekida Inc., 420-2050, San Pedro de Montes de Oca, Costa Rica] and then acclimatized in indoor rooms exposed to dim artificial light and natural light (approximate natural photoperiod of 12 hours light per 12 hours of darkness). Natural spawning (n=3) took place in 20 m^3 circular fiberglass tanks equipped with a Pentair Aquatic Eco-System® recirculation system (double UV-65 watt sterilizers lamps, RK2 foam fractionator, biological filter and mechanical filter) with used water replacement of 40 L/min and 5% daily exchange with previously filtered (5µm) and UV sterilized (UV-128 watts, TT PE 4250 HO Astral®) water. The broodstock were fed squid (*Illex argentinus*) (38% m/m), Pacific thread herring (*Opisthonema libertate*) (58% m/m) and crystal shrimp (*Farfatepenaeus brevirostris*) (4% m/m) at a ration (BW/day) equivalent to 4% of their body weight (BW), once a day, 5 times a week except Sundays and Thursdays.

Wild broodstocks (n=16) were captured at 9°49'05"N and 84°50'26"E coordinates in the Gulf of Nicoya, 5 km from the area where the fish held in captivity were caught. On the same day of capture, ten specimens were induced to spawn using human chorionic gonadotropin (hCG) according to the methodology described by Herrera-Ulloa et al. (2009), and placed in 10 m^3 circular fiberglass tanks, provided with the same type of recirculation system as employed with the captivity broodstock.

In both groups (captive and wild), spawns occurred between 03:00 and 05:00 a.m. The eggs were diverted through a superficial lateral tube to a 150 L harvesting

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tank having a 500 µm central sieve drain, nine hours later eggs were collected and individually counted in a 1 L graduated glass cylinder. Eggs were counted by the volumetric displacement method: after introducing the eggs, five minutes were given for floating eggs to separate from non-floating ones. To determine the number of floating eggs per mL, three one-milliliter samples were counted. The total number of eggs, floating and non-floating, was determined by multiplying the quantity of eggs per mL by the total milliliters equivalent to the egg column measured in the graduated glass cylinder.

From the column of floating eggs, 3 samples (n=30) were taken and examined under a microscope to determine the diameter of the egg and oil droplet size as well as the survival rate as defined based on the criteria established by Silva and Castelló (2005).

To obtain the gonad samples, six fish from each group were sacrificed (3 males and 3 females). 5 mL of clove oil (*Syzygium aromaticum*) was used to surgically sedate the fish prior to dissection.

Gonads sampled from females were selected according to their maturity stage, with predominance of oocytes with average diameters of 425±25 µm and that of males according to the quantity, viscosity and color of the semen.

For its preservation, both eggs and gonads were washed with distilled water and preserved under vacuum at -40º C. Each gonad sample was taken from three broodstock specimens, according to their origin and gender.

Fatty acids analysis: for total lipids extraction, the sample mass was directly measured in a 250 mL glass centrifuge tube, 50 mL of petroleum ether was added and vortexed for 1 minute, then centrifuged for 10 minutes at 4000 rpm. The product was decanted in a 250 mL glass flask and the organic extract evaporated to dryness in a rotary evaporator at 40°C and the residue weighed.

Subsequently, fatty acid profiling was performed by gas chromatography using a Shimadzu GC-2014-FID gas chromatograph equipped with AOC-20i auto injector and an open tubular column (Varian CP-SIL 88 fused silica WCOT, 100 m x 0.25 mm df=0.2 µm).

Egg diameter and oil droplet size data were subjected to one-way analysis of variance (ANOVA) to detect differences between spawns ($p<0.05$). To statistically compare the fatty acids content in eggs and gonads (both sexes), the Kruskal-Wallis non-parametric test was used (Zar, 1999).

Results

Because the manipulation of fish while in captivity, prior to natural spawning, can induce excessive stresses that prevent the final detonation of eggs, it was decided to perform ovarian biopsies only to wild females and abdominal massages to wild males for obtaining semen (see Table 1). Semen quality was determined by macroscopic inspection.

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Table 1.
Characteristics of the ovarian samples of wild broodstock used for spawning and extraction of gonads.

Fish ID	Weight (kg)	Sex	Oocytes size (µm)	Semen quality	Destined Use*
1	0.96	Male	----------	Normal **	Spawn
2	1.01	Male	----------	Normal	Spawn
3	0.98	Male	----------	Normal	Spawn
4	0.99	Male	----------	Normal	Spawn
5	1.05	Male	----------	Normal	Spawn
6	1.04	Female	400	----------	Spawn
7	0.9	Female	425	----------	Spawn
8	0.91	Female	425	----------	Spawn
9	1.05	Female	450	----------	Spawn
10	1.04	Female	450	----------	Spawn
11	1.09	Male	----------	Normal	Gonad
12	1.00	Male	----------	Normal	Gonad
13	1.03	Male	----------	Normal	Gonad
14	0.92	Female	425	----------	Gonad
15	0.91	Female	400	----------	Gonad
16	1.09	Female	425	----------	Gonad

Notes: * Destined use: indicates the use that was given to the fish, either for reproduction (spawn) or gonad extraction; ** Normal: white, creamy and abundant semen. Only males having semen with these characteristics were selected.
Source: Own elaboration from present study data

The compared spawns did not show significant statistical differences ($p \geq 0.05$) with respect to egg diameter and oil droplet size. Viability rates (percentages) were similar in captive fish spawns but lower in the induced spawns of wild fish, although wild fish produced a greater number of total eggs (see Table 2).

Table 2.
Spawn quantity and quality from fish held in captivity and wild fish induced with hCG hormone.

	Captive 1		Captive 2		Captive 3		Captive x̄		Wild	
		VR[1] (%)		VR (%)		VR (%)		VR (%)		VR (%)
Spawn quantity	234,000		198,000		103,000		178,333		360,000	
Viable eggs	206,000	88.0	189,000	95.5	93,000	90.3	162,667	91.2	310,000	86.1
Defective eggs	28,000	12.0	11,000	4.6	15,000	9.7	18,000	8.8	50,000	13.9
Egg diameter (µm)	750.0±25.4		751.7±22.7		750.0±25.4		750.0±24.5		750.0±19.7	
Oil droplet size (µm)	0.117±0.02		0.118±0.02		0.117±0.02		0.117±0.02		0.116±0.02	

Notes: [1]VR= viability rate expressed as a percentage.
Source: Own elaboration from present study data

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The number of fatty acids detected was lower in the gonad of wild *L. guttatus* females than in the rest of the sampled specimens; palmitic acid is present in the highest proportion followed by oleic acid which was only found in wild and male captive specimens (see Table 3). On the other hand, 18:2n-6 acid was present in all samples of captive fish and in the eggs of wild fish. No trend was observed in relation to the abundance and presence of this fatty acid, there were no statistically significant differences in its abundance in the eggs of wild and captive females (p=0.2188), nor in male gonads (p=0.6179) and wild and captive female gonads (p=0.1153).

With respect to n-3 fatty acids (see Table 3), 18:3n-3 was present in the samples of reproductive tissues and eggs of captive fish with low levels in the male and female gonad, and higher level in the eggs. The presence of EPA was observed in all analyzed samples. Samples obtained from wild fish showed lower EPA values than those from captive fish tissue (see Table 3). The gonads of wild males contained 63.3% of the value of EPA found in captive males.

DHA was the HUFA (highly unsaturated fatty acid) found in greater proportion in all analyzed samples, with values ranging from 10.01 to 15.89%. The lowest values are found in the gonads of wild and captive males (see Table 3), however, eggs also showed high concentrations of this HUFA.

Arachidonic acid (ARA) was found in values that ranged from 0.38 to 5.07% and was absent in wild eggs and in captive *L. guttatus* female gonads (see Table 3).

Saturated fatty acids predominated in all analyzed samples, and their sum totaled values close to 50%. Regarding n-3 and n-6, a greater presence of n-3 series fatty acids was observed in all the samples analyzed, with the highest levels found in captive fish. On the other hand, the proportion of PUFAs was found to range from 17 to 27%, being lower in the gonads of both captive and wild *L. guttatus* males. The gonads of females and eggs of wild females had the highest proportions of PUFAs. In relation to HUFAs, males had the lowest values of total HUFAs while the values of females and eggs varied between 20.26 and 24.38%. The gonads of wild females had the highest value of these fatty acids (see Table 1).

The EPA: ARA ratio varied in all samples, with a high value in eggs obtained from captive females, compared to the DHA: EPA ratio where similar values were observed throughout all analyzed samples (see Table 3).

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Table 3.

Fatty acids profile (% of total fatty acids) of eggs and gonads of wild and captive L. guttatus from the analysis of unified samples, taken from three specimen categories: H-S= wild females; H-C= captive females; M-S= wild males; M-C= captive males, --= Undetected.

Fatty acid	Eggs H-S	Eggs H-C	Gonad M-S	Gonad M-C	Gonad H-S	Gonad H-C
13:00	--	--	0.39	0.01	1.91	--
12:00	--	0.04	0.18	--	--	--
14:00	2.83	2.72	3.06	7.74	2.96	4.11
15:00	--	0.25	1.21	0.52	--	--
16:00	26.51	24.49	27.39	28.05	26.59	28.39
16:1n-7	6.39	6.72	4.75	7.21	6.84	5.74
17:00	1.5	0.55	2.04	0.73	--	0.77
17:1n-7	1.19	--	0.9	--	--	--
18:00	11.25	11.6	15.8	8.72	12.31	11.12
18:1 cis (Mixture of oleic acid, vaccenic acid, petroselinic acid)	--	3.01	--	--	--	23.78
18:1n-9	22.23	--	24.75	21.17	21.9	--
18:2n-6	2.21	0.17	--	3.17	--	0.14
18:4n-6	--	--	--	0.55	2.96	--
18:3n-3	--	0.52	--	0.24	--	0.24
18:2n-7 conjugated	--	0.43	--	--	--	1.12
20:00	--	0.16	--	--	--	0.09
20:3n-9	3.81	0.17	--	--	--	0.11
20:2n-6	--	--	0.57	0.37	--	--
20:4n-6	--	0.38	2.64	1.35	5.07	--
20:1n-9,cis-5	--	--	0.5	0.38	--	--
20:1n-9,cis-11	--	--	1.31	2.06	--	--
20:5n-3	6.07	7.32	3.9	6.16	5.49	6.62
22:00	--	2.37	--	--	--	2.03
22:6n-3	13.37	15.89	10.01	11.36	13.82	13.53
Saturated	42.45	42.43	50.07	45.77	43.77	46.51
Monoinsaturated	29.81	31.73	30.4	30.82	28.74	29.79
n-3	19.44	23.73	13.91	17.76	19.31	20.39
n-6	2.21	0.55	3.21	5.44	8.03	0.14
n-3 HUFA	19.44	23.21	13.91	17.52	19.31	20.15
EPA:ARA	--	2.18	1.48	4.56	1.08	--
DHA:EPA	2.20	2.17	2.56	1.84	2.51	2.04
PUFAs	27.70	25.31	17.12	23.20	27.34	24.11
HUFAs	23.25	23.76	16.55	18.87	24.38	20.26

Source: Own elaboration from present study data

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Discussion

In nature or under culture conditions, the quality fish gametes can be variable as a consequence of the influence of a significant number of external factors (Bobe and Labbé, 2010). In this context, the quality of eggs and larvae of *L. guttatus* would be related to the nutritional quality of the diet with which the broodstock are fed and conditioned. In captivity the effective management of the methods employed in obtaining sexual products is another factor that can condition the results.

Hormone-induced spawning, although capable of providing direct control over the final stages of the fish's reproductive cycle, as well as being an adequate source of eggs of good quality, is more variable and unpredictable when compared to natural spawning (Marte 1989; Papanikos et al. 2003). Natural spawning guarantees a constant supply of fertile eggs with which constant production of artificially reared finglings can be developed for commercial purposes (Dumas et al., 2004). However, according to Tamaru, Carlstrom, Fitzgerald y Ako (1996) the percentage of fertilization in hormone-induced spawning can vary considerably from 32.6 to 99.9%. In the present study, the viability rate, despite being lower in wild fish (86.1%) compared to captive fish (91.2%), is considered suitable in terms of quality. The results given in Table 1 and 2 show that the quality characteristics of spawns and gonads based on the stages of maturity of the fish were similar and did not show significant differences ($p \geq 0.05$) in aspects that generally infer differences in the quality of the eggs such as diameter and oil droplet size. The values obtained for these variables were close to those previously reported for the same specie (Lagos 2000; Mejía-Narváez et al., 2009).

Terán et al. (2004) indicate that oocytes of 300 to 400 microns in diameter are in the process of final vitellogenesis and require exogenous gonadotropin for ovulation in *Lutjanidae*. Hence, the stage of gonadal maturity (oocyte diameter) of wild females in this study were considered suitable to induce spawning to obtain the samples and compare them to captive broodstock that spawned good quality fertile eggs which reached maturation stages earlier and consequently their development advanced faster than other seeds. Similarly, the macroscopic quality of male semen was considered normal. It can be inferred that the stage of maturity and the quality of sexual products was similar.

Studied *L. guttatus* broodstock were fed a fresh diet obtained from the Pacific coast of Costa Rica. Thus, it was necessary to study and determine its nutritional quality prior use as feed in order to obtain optimal production of viable larvae and juveniles, as has been done for other marine fish species (Benetti et al., 2008).

It has already been mentioned that in marine fish, adequate supplementation of essential fatty acids in the diet of broodstock is important for good egg quality (Navas et al., 1997; Bruce et al., 1999). This supplementation should be carried during vitellogenesis due to the direct relationship that exists between the quality of the lipids present in the oocyte and the composition of fatty acids in the diet the broodstock (Fernández-Palacios et al., 1995; Wilson, 2009; Tocher, 2010). In this study, no differences are observed in the levels of PUFAs in gonad and eggs of *L. guttatus*.

Lipid sources in fish eggs are the chorion (lipoproteinic layer), the vitelli that also

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contains glycogen and free amino acids and used mainly as an energy source (Fyhn and Govoni, 1995), the lipid globule that contains triglycerides and used as a source of energy and fatty acids (Parra, Rønnestad and Yúfera, 1999). These nutrients are the main sources of energy from the formation of the gastrula to the hatching stage of the embryo (Vetter, Houdson and Arnold, 1983).

Under these considerations it is necessary to approach the natural feeding condition of *L. guttatus* broodstock to achieve a quality of gametes suitable for the successful intensive production of larvae and juveniles. One way to realize this is by utilizing analytical information obtained from samples of the natural environment of the species intended for reproduction management under controlled conditions and to compare with data obtained under captivity conditions as has been practiced for *Spondyliosoma cantharus* (Rodríguez et al., 2004), *Seriola dumerili* (Rodríguez-Barreto et al., 2012) or *Cynoscion parvipinnis* (González-Félix, Urquidez-Bejarano, Perez-Velazquez, Castro-Longoria and Vazquez-Boucard, 2017).

The focus in fish reproduction management has centered around lipid quality due to the importance of these nutrients in tissue formation and as a source of energy in the larval stages of fish development. Of all PUFAs the most important are 18:3n-3, ARA, EPA, and DHA (Civera-Cerecedo, Álvarez-González and Moyano-López, 2004). Due to the above, the lip content of broodstock feed must be suitable to guarantee the transfer of these nutrients to the offspring via the gametic route, as was considered in this study by selecting fresh feeds with adequate levels of these fatty acids as defined in the literature. In cultivation experiences carried out with *Diplodus sargo* (Sparidae), when comparing ovarian fatty acids content in wild and captive fish, it was recognized that the diet supplied to the broodstock was inappropriate (Cejas et al., 2003). Likewise, Mourente and Odriozola (1990) showed that the composition of fatty acids in eggs of *Sparus aurata* (Sparidae) had significant differences due to the influence of fatty acid levels in broodstock diet.

According to the metabolic interactions of PUFAs, the nutritional requirements of DHA, EPA and ARA cannot be considered independently, on the contrary, it is important to consider the proportion of DHA:EPA and DHA:ARA (Sargent, Bell, McVoy , Tocher and Estévez, 1999). In marine fish the content of ARA and the DHA: EPA ratio correspond to important factors that influence egg viability and possibly also its symmetry (Pickova, Dutta, Larsson and Kiessling, 1997). The results obtained show such proportions to be similar in eggs procured from the natural environment and under captivity conditions, which could indicate the potential viability of *L. guttatus* larvae and juveniles.

The optimal dietary ratio of DHA: EPA for sea bass larvae is 2:1 and that of EPA: ARA is 1:1; in turbot and halibut larvae the DHA: EPA ratio is 2:1, but EPA: ARA in these species is 10:1 or greater (Sargent et al., 1999).

In *Paralichthys adspersus* (Paralichthyidae) eggs the amount of n-3 HUFA increases with the addition of a greater amount of fatty acids in the diet of broodstock, while the proportions of DHA:EPA and EPA:ARA are 2:1 and 4:1, respectively. These criteria were associated with egg quality (Wilson, 2009).

The results of this study on *L. guttatus* show that the DHA: EPA ratio in eggs from wild broodstock was 2.2:1 and 2.17:1 from captive broodstock; these values resemble

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those reported for marine fish. The EPA: ARA ratio was significantly lower in wild eggs (1.6:1) and very high in captive eggs (19.3:1). Wilson (2009) recommends the incorporation of n-3 HUFA in the diet of broodstock at levels of 4.1% of total lipids, and a total fatty acid content of 20 to 25% as this allows an adequate quality of spawns and improves the fertilization rate.

It is necessary to investigate the levels of vitamins, such as E and C, and amino acids required by larvae and juveniles of *L. guttatus*. On the other hand, to improve egg quality and larvae viability, efforts need to be directed to improving the proportion of DHA: EPA and ARA: EPA in feed, which allow good neural and visual function, eicosanoids promote achieving efficient physiological functions (Bell, Farndale, Bruce, Navas and Carrillo, 1997).

When analyzing the sexual products obtained in this study, it was determined that both broodstock groups generated sexual products suitable for the comparison of fatty acids in eggs and gonads of wild *L. guttatus*. It can be concluded that it is necessary to know the proportions of DHA: EPA and ARA: EPA required for adequately feeding specimens in captivity. In addition, it's suggested to study and determine their nutritional quality prior to use as feed to achieve optimal larval and juvenile production. Finally, it is mandatory to investigate and define the nutritional needs of marine fish broodstock to ensure highly viable offspring, and on the other hand, to optimize the use of nutrients of high cost and reduced access such as fishmeal and fish oil, which have suitable fatty acids profiles for marine fish.

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