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Research Article

Effects of different dietary lipids concentrations on the egg production and egg quality produced by *Macrobrachium acanthurus* females

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ABSTRACT. The sexual maturation and reproduction of crustaceans are very demanding processes for energy and nutrients. Females require the nutrients to deposit them into the eggs and allow the healthy development of embryos and early larvae survival. The lipids are essential nutrients related to the sexual maturation and the egg production, as they are sources of metabolic energy for the gonad development, as well structural molecules for the embryonic tissue formation. This work presents the effects of different dietary lipid levels (10, 12.5, 15, 17.5 and 20% of a mixture of krill and cod liver oil as lipid sources), on the growth, egg production, and egg quality, of freshwater prawn *Macrobrachium acanthurus* females fed during 60 days. The results showed that an inclusion higher than 15% of lipids, improved the egg production and the content of protein and lipids significantly. A lipid inclusion between 15 and 17.5% might be optimal for maturation and egg production of the females of *M. acanthurus*.

Keywords: *Macrobrachium acanthurus*, nutrient requirements, egg production, egg quality, growth, reproduction.

INTRODUCTION

The sexual maturation and reproduction of crustaceans are very demanding processes for energy and nutrients (NCR, 2011), and thus, dietary requirements are generally higher in mature adults than in non-reproductive adults or juveniles (Wouters *et al.*, 2001). Notably, the females require mobilizing the nutrients from the hepatopancreas to the ovaries, deposit them into the eggs (Rodríguez-González *et al.*, 2009) and allow the healthy development of embryos and early larvae survival (Wouters *et al.*, 2001). Unbalance or incomplete diets might cause poor reproductive performances or even stop animals from reproducing (Bray & Lawrence, 1992).

The lipids are essential nutrients related to the sexual maturation and the egg production, as they are sources of metabolic energy for the gonad development and structural molecules for the early tissue formation. The lipid requirements for crustaceans during the sexual maturation stage have been mainly reported for penaeid shrimps (Wouters *et al.*, 2001) and the fresh-water prawn *Macrobrachium rosenbergii* (D'Abramo & New, 2010), but the knowledge for other species remain mostly unknown.

In the other hand, the freshwater prawn *M. acanthurus* represents a valuable economic resource for the communities of fishermen at the states of Veracruz and Tabasco, Mexico. The overfishing and water contamination are affecting the distribution of the natural populations and decreasing the abundance of this species. The culture of *M. acanthurus* has been suggested as an alternative to fisheries (Yamasaki-Granados *et al.*, 2012). However, there is scarce information on the reproduction conditions (García-Guerrero *et al.*, 2013) that might allow the development of an efficient and economically feasible production of this species. The nutritional requirements for the reproductive stage have not yet reported and just Villafuerte *et al.* (2016) found that females of *M. acanthurus* fed on diets with inclusion of krill meal and oil (protein and lipid sources, respectively), matured faster and reduce their growth performances than females fed on fish meal and oil. As well, the females

fed on the diet with krill products produced eggs with higher content of lipid and protein. So, with the objective to contribute to the knowledge of nutrient requirements during the reproductive stage of *M. acanthurus* females, this work presents the effects of different dietary levels of lipid on the growth, egg production and egg quality of females fed during 60 days.

MATERIALS AND METHODS

Individuals collection

Individuals of *M. acathurus* were collected at the Jamapa River, Municipality of Boca del Río, State of Veracruz, Mexico (19°02'33"N, 96°14'10"W), during May of 2015. After collected, the organisms were sorted into females and males, then packed in a plastic bag for transportation. Once at the Laboratorio de Producción Acuícola (UNAM-FES Iztacala), the individuals were stocked in 200 L glass tanks equipped with gravel filters, continuous aeration and thermostats to maintain the temperature around 30°C. The females were fed on a diet with fish and krill meals (40% crude protein) and fish oil (16% crude lipids) until the feeding trial started.

Diet formulation

Five diets were formulated with increasing lipid levels of 10, 12.5, 15, 17.5 and 20%. These levels were selected according to previous results reported by Villafuerte *et al.* (2016). The diets were isonitrogenous (40% crude protein) and isoenergetic (400 kcal 100^{-1} g). The energy was kept in the diets by modifying the levels of a carbohydrates mixture as the levels of lipids increased (Teshima *et al.*, 2006). Krill oil (AkerBioMarine AS, Oslo, Norway) and cod liver oil (Drotasa, S.A. de C.V., México) were used as lipid sources to provide the different levels of inclusion, in a 1:1 mixture. Soybean lecithin (Abastecedora de Productos Naturales, S.A. de C.V., Mérida, México) and the cholesterol (94%, Sigma-Aldrich Co., St. Louis, MO, USA) was added at the same level to all diets. As protein sources, defatted fish (Vimifos S.A. de C.V., Sonora, México) and krill (AkerBioMarine AS, Oslo, Norway) meals and casein (Hegard de México, México) were used. As a source of carbohydrates, the diets included a mixture (2:3:7:13) of dextrin, α-starch, glucose, and sucrose. The diet formulations are shown in Table 1. Diets were prepared by mixing all the powdered ingredients for 20 min, then the oil mixture, cholesterol and soybean lecithin were added and remixed for other 20 min. Finally, water (40%, v/w) was added to produce a wet dough, which was passed through a meat mincer to produce pellets of 5 mm diameter. The pellets were dried at 60°C for 6 h and then, were kept at -24°C until used.

Feeding trial

For the feeding trial, glass tanks of 20 L were used, and each equipped with a gravel filter, continuous aeration and a temperature of 30 ± 1°C. Each diet was fed to six females and each of them represented a replication. One female (mean initial weight of 0.4 ± 0.1 g) was randomly stocked in each tank. Then, the females were fed 8% of their body weight in only one fed at night. Every 15 days, the females were weighted, and the size of the ration was modified accordingly. Water parameters (mean ± SD) through the feeding trial were of dissolved oxygen, 5.9 ± 0.1 mg L^{-1}; temperature, 28 ± 1°C and total hardness, 194 ± 15 mg L^{-1}. The feeding trial lasted for 60 days, and at the end, the females were weighted to obtain the growth performance and then, allocated in 80 L glass tanks with a male, to allow them to pair. Females were checked on daily basis. Once the eggs were detected, the organism was sacrificed with an overdose of the anesthetic MS-222 (ethyl 3-aminobenzoate, methanesulfonic acid, Sigma-Aldrich Co., St. Louis, MO, USA). Then, the eggs were collected, counted and measured (length and width) with a stereoscopic microscope (model SMZ1500, Nikon Instruments, Japan) equipped with a micrometer. The volume of eggs was calculated with the formula $V = 3.1416 \times \text{length} \times \text{width}^2$. The eggs were kept for proximate composition analysis. Females were dissected, and samples of hepatopancreas, muscle, and ovaries were taken for the proximate composition. All samples were kept at -24°C until analysis.

Chemical analysis

Diets were evaluated by the techniques reported by the AOAC (1990) for moisture, ash and protein content, while lipids by the technique reported by Blight & Dyer (1959). Samples of tissues from females and eggs were analyzed for protein (Micro Lowry, Peterson's Modification Total Protein Kit (Sigma Aldrich Co., St. Louis, MO, USA) and lipid (by the technique of Blight & Dyer, 1959).

Statistical analysis

The obtained data were analyzed for normality (W test of Shapiro & Wilk) and homoscedasticity (Barlett´s test) according to Zar (1999). As data showed to be normal and homoscedastic, one-way ANOVA test was used (Prism 6 for Mac OS X, GraphPad Software Inc., USA). Significant differences among the treatments were determined by a Fisher LSD test (Zar, 1999), with a significance level of 5% ($P < 0.05$) for each set of comparisons.

Table 1. Formulation and proximate composition of the experimental diets with different levels of lipids for *Macrobrachium acanthurus* females.

Ingredients (g 100^{-1} g)	Lipid inclusion (%)				
	10	12.5	15	17.5	20
Fish meal[1]	15	15	15	15	15
Krill meal[1]	15	15	15	15	15
Casein	20	20	20	20	20
Soybean lecithin	0.5	0.5	0.5	0.5	0.5
Cholesterol	1	1	1	1	1
Oil mixture[2]	10	12.5	15	17.5	20
Carbohydrate[3]	22.6	16.9	11.1	5.3	0
Vitamin mixture[4]	1.9	1.9	1.9	1.9	1.9
Mineral mixture[5]	5	5	5	5	5
Glucosamine HCl	0.8	0.8	0.8	0.8	0.8
Na succinate	0.3	0.3	0.3	0.3	0.3
Na citrate	0.3	0.3	0.3	0.3	0.3
Dibutyl hydroxyl toluene	0.1	0.1	0.1	0.1	0.1
Wheat gluten	5	5	5	5	5
α-Cellulose	2.5	5.7	9	12.3	15.1
Proximate composition (%)					
Protein[6]	46	43.3	41.6	42.5	43
Lipid[6]	10.08	12.6	15.24	17.65	20.38
Moisture	16.6	14.5	14.2	15.5	16.6
Ash[6]	5.2	4.7	5.1	6.3	5.9

[1]Defatted meals, [2]Krill oil: cod liver oil (1:1), [3]Dextrine: α-starch: glucose: sucrose (1:3:7:13), [4]In mg 100^{-1} g: ρ-aminobenzoic acid, 15.08; biotin, 0.63; inositol, 632.0; niacin, 63.2; Ca pantothenate, 94.8; pyridoxine HCl, 18.96; riboflavin, 12.64; thiamine HCl, 6.32; menadione, 6.34; b-carotene, 15.17; calciferol, 1.90; cyanocobalamin, 0.13; L-ascorbyl-phosphate Mg, 55.11; folic acid, 1.26; choline chloride, 948.0, [5]In mg 100 g^{-1}: K_2HPO_4, 1169; $Ca_3(PO4)_2$, 1591; $MgSO_4$ .7H^2O, 1778.5; NaH_2PO_4. $2H_2O$, 461.5, [6]On dry weight basis.

RESULTS

The growth performance of the females fed different levels of lipids are shown in Table 2. Weight gain (WG), specific growth rate (SGR), feed intake (FI) and fed efficiency rate (FER) did not show significant differences among the groups. Values increased up to a level of inclusion of 15% of lipid, after which, decreased.

Regarding the contents of protein in the tissues of the females, there was a tendency of decreasing values in the hepatopancreas (Fig. 1a) as the levels of lipid increased in the diet. Values were significantly lower in the females fed the diets with 17.5 and 20% lipid inclusion. Protein content in the ovaries showed a similar trend, as significantly lower values were obtained in the ovaries of females fed the diets with 15, 17.5 and 20% of lipids in the diets (Fig. 1b). In the muscle, contents of protein showed significantly lower values in the treatments with 15 and 20% of lipid inclusion (Fig. 1c).

In the other hand, lipid contents on the hepatopancreas are shown in Fig. 2a, a significantly higher value was observed at the females fed the diet with 20% lipid inclusion. In the ovaries (Fig. 2b), a significantly higher value was observed at the females fed with 15% lipid inclusion, after which the contents decreased significantly in the females fed the diets with 17.5 and 20%. Finally, lipid content in the ovaries was significantly higher in females fed the diet with 15% of lipid inclusion, than the content observed in the treatment with 10 and 12.5% (Fig. 2c). Finally, contents of lipids increased as the level of inclusion in the diet increased, and a significantly higher value was observed in the females fed the diet with 20% lipid inclusion.

The number of eggs produced per female (Fig. 3a) increased as the level of lipid increased, and a significantly higher value was observed on the females fed the diet with 20% when compared with the treatments 10, 12.5% and an initial sample taking from a wild-captured female. Regarding the size of the eggs (show as a volume, Fig. 3b), no significant differences

Table 2. Growth performance of *Macrobrachium acanthurus* females fed different levels of lipid inclusion in the diet. Data are the means of seven replicates ± SD. No significant differences were observed at this level ($P < 0.05$). [1]Weight gain = [(final weight - initial weight)/initial weight] × 100, [2]Specific growth rate = [(ln final weight - ln initial weight)/60] × 100, [3]Feed efficiency ratio = weight gain (g)/ total feed ingested (dry weight basis).

	Lipid inclusion (%)				
	10	12.5	15	17.5	20
Weight gain (%)	100 ± 33	112 ± 38	157 ± 51	115 ± 31	133 ± 41
Specific growth rate (%/day)	1.1 ± 0.3	1.2 ± 0.3	1.5 ± 0.4	1.3 ± 0.2	1.4 ± 0.3
Feed intake (g/female/day)	0.04 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
Feed efficiency ratio	0.19 ± 0.04	0.17 ± 0.02	0.22 ± 0.06	0.17 ± 0.05	0.17 ± 0.03

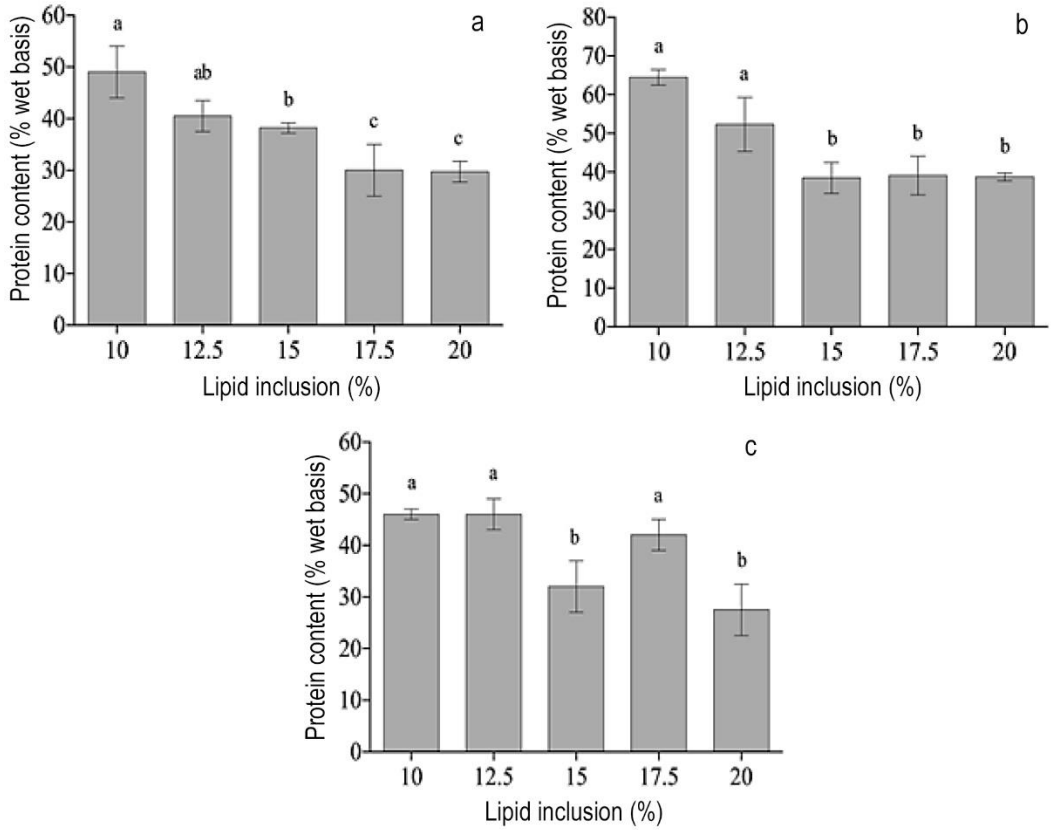

Figure 1. Protein contents of a) hepatopancreas, b) ovaries, and c) muscle of females of *Macrobrachium acanthurus*, fed diets with different inclusions of lipids for 60 days. Each bar represents the mean of three replicates ± SD. Bars with different letter differ significantly ($P < 0.05$).

were observed among the treatments, but slightly lower volume was observed in the eggs produced by females fed the diet with 20% lipids. The protein content of the eggs (Fig. 3c) did not show significant differences among the treatments, although females fed the diets with 15 and 17.5% produced eggs with higher protein. Lipid content of the eggs (Fig. 3d) showed a significantly increased in those produced by females fed on 15, 17.5 and 20% lipid content.

DISCUSSION

The freshwater prawn *Macrobrachium acanthurus* has been suggested as potential species to be cultured (Kutty & Valenti, 2010) and feeding plays a vital role during the production process. However, the information regarding the nutrient requirements for this species is almost unknown to develop a diet. The protein requirements are, by far, the most studied nutrient for species of *Macrobrachium* distributed in Mexico (Casas-Sánchez *et al*., 1995; Benítez-Mandujano &

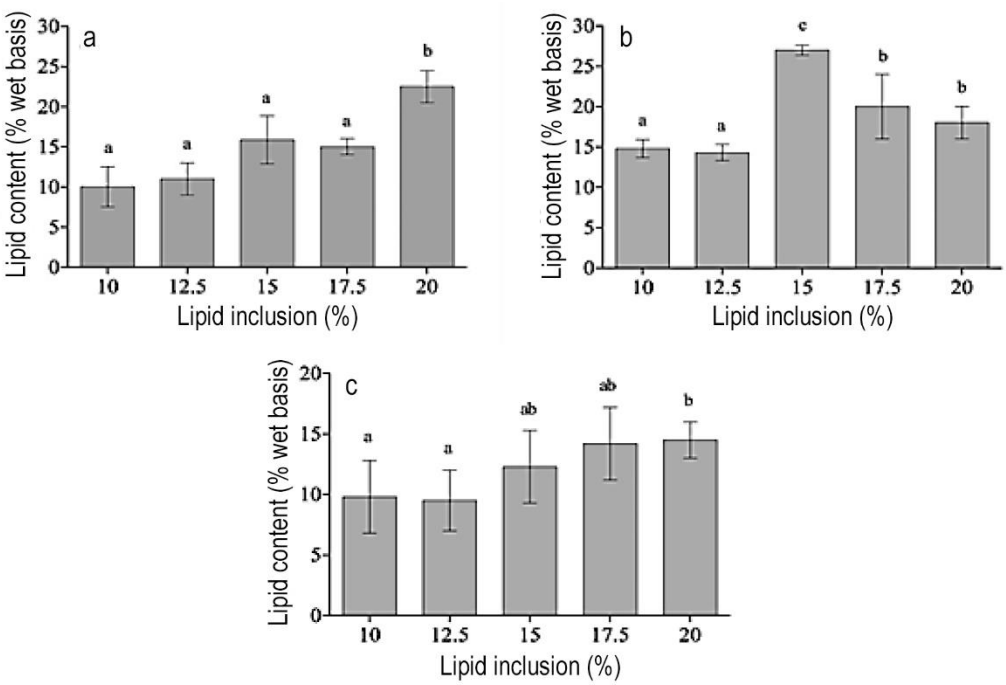

Figure 2. Lipid contents of a) hepatopancreas, b) ovaries, and c) muscle of females of *Macrobrachium acanthurus*, fed diets with different inclusions of lipids for 60 days. Each bar represents the mean of three replicates ± SD. Bars with different letter differ significantly ($P < 0.05$).

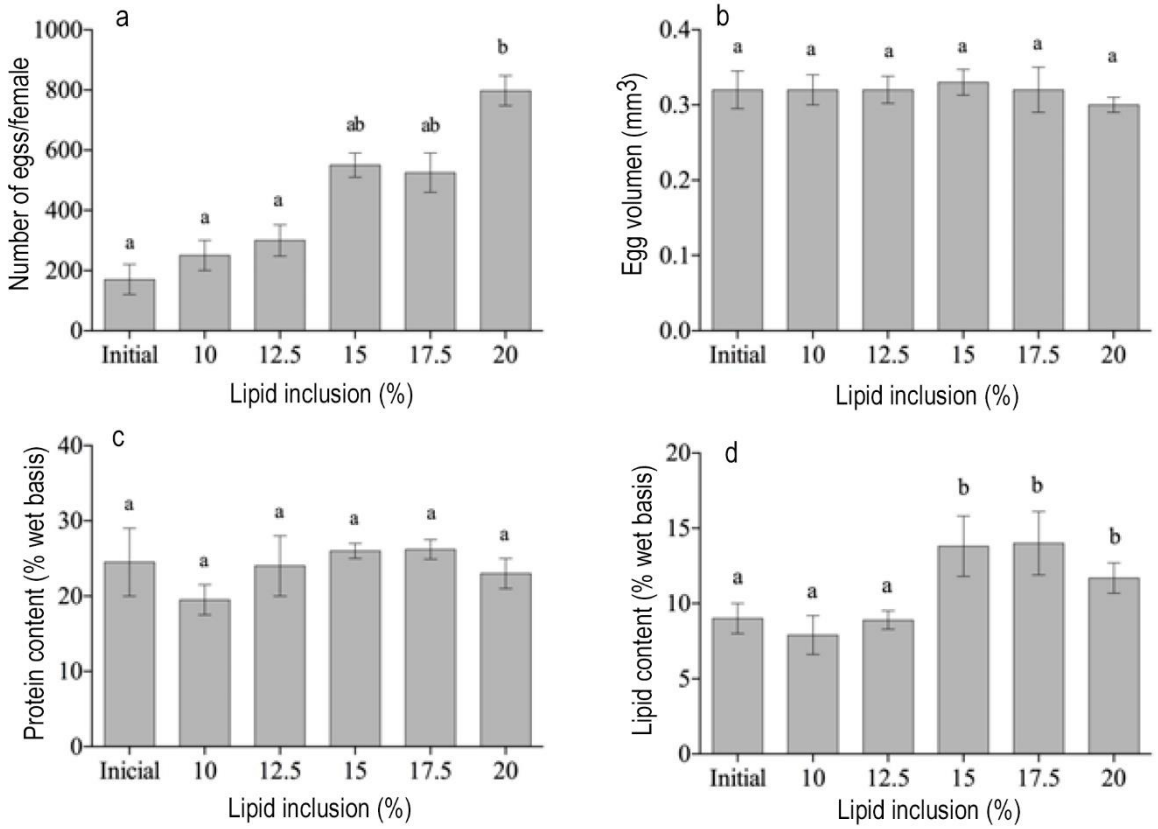

Figure 3. a) Number, b) volume, c) protein, and d) lipid contents of the eggs produced for females of *Macrobrachium acanthurus*, fed diets with different inclusions of lipids for 60 days. Each bar represents the mean of three replicates ± SD. Bars with different letter differ significantly ($P < 0.05$).

Ponce-Palafox, 2014; Villafuerte *et al.*, 2016; Méndez-Martínez *et al.*, 2017). The dietary lipids, on the contrary, have not received so much attention, despite to be an essential nutrient and energy source, particularly during the maturation and reproductive stages of females (Harrison, 1990; Wouters *et al.*, 2001). The results obtained from the feeding trial with females of *M. acanthurus* suggested that levels above 15% of inclusion of a mixture of krill and fish oils are necessary to improve the production and quality of eggs.

The maturation and the reproduction are highly demanding processes that require energy and nutrient from the female to produce the eggs (NCR, 2011). According to Harrison (1990), the members of the genus *Macrobrachium* usually show a decline in growth rate when females enter in sexual maturity. The growth performance (as weight gain and specific growth rate) was higher than the reported by Villafuerte *et al.* (2016) on females of *M. acanthurus* fed on diets with 10% lipid content from different sources. However, values are lower than those reported for other crustacean species of the same initial weight, such as *M. rosenbergii* (Teshima *et al.*, 2006) and *Cherax quadricarinatus* (Hernández-Vergara *et al.*, 2003). An indication that females were in the process of sexual maturation at the end of the 60 days feeding period with their respective diets.

Lipids are essential molecules in the metabolism, reproduction of crustaceans and usually, the hepato-pancreas is the primary site for their processing and storage. During maturation, ovaries are additional sites for lipid metabolism (Harrison, 1990); but according to Teshima *et al.* (1988), lipids usually are transferred from the hepatopancreas to the ovaries to cover the energy expenses of oogenesis and vitellogenesis. We observe that lipid content in the hepatopancreas increased as the lipid does in the diets, with a significantly higher value in the females fed the diet with 20% inclusion. An indication that this level is higher than necessary for the reproductive process, as the lipid has been storage in both, hepatopancreas and muscle, instead of mobilizing them to the ovaries. Interestingly, the protein content in the hepatopancreas and ovaries, decreased as the lipid increased in the diet, showing the mobilization from hepatopancreas to the ovaries, and then deposited in the eggs. Subramoniam (2011), reported that the egg yolk of crustaceans is composed of large quantities of the protein vitellogenin linked to lipids, forming a complex referred to as lipovitellin. Dietary protein was the same in all the diets, so with the increment of egg production in the females with higher lipid inclusions, protein must be mobilized at a higher rate than the lipid.

The inclusion of the higher levels of lipids (15-20%) improved eggs produced per female. For the first time, a direct relationship between dietary lipid levels and the number of eggs is reported. Still, the number of eggs produced is lower than those reported by Tamburus *et al.* (2012) for wild females collected in Brazil. It seems that regional differences between the populations led to these differences, as wild-caught females from the collection area of this experiment (showed in Figure 3 as Initial) produced lower numbers of eggs. Mejía-Ortiz *et al.* (2001) reported a similar number of eggs produced in wild-caught females from Huitzilapan River, a location near where we collected our organisms.

In the other hand, several parameters have been used to determine the egg quality of the crustaceans (Rodríguez-González *et al.*, 2009) and we decided to use the volume and contents of protein and lipid, as seems to be the most commonly used parameters. Despite the dietary lipid inclusion, egg volume did not show differences among the treatments and values were similar to those reported for wild females of *M. acanthurus* (Mejía-Ortiz *et al*., 2001; Tamburus *et al.*, 2012) and females fed on different sources of lipids (Villafuerte *et al*., 2016).

Regarding the protein content of the eggs, was not affected by the dietary lipid fed to the females and the contents were similar to those reported previously by Villafuerte *et al.* (2016). Lipid content of the eggs was influenced by the dietary lipid inclusion, as levels higher than 15% of dietary lipids improved the condition of the eggs. However, it might be necessary to see if such levels of lipids influence the hatching rates and the early larval survival.

For the first time, the effect of different levels of dietary lipids in the growth and the egg production and quality from *M. acanthurus* females is reported. We concluded that a lipid inclusion between 15 and 17.5% might be optimal for maturation and egg production of the females, as well an increment in the protein and lipid contents of the eggs. Even the 20% lipid inclusion allowed females to produce more eggs, the lipid deposition in the hepatopancreas and muscle might have adverse effects in the long term for the organisms.

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