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# Effect of the enrichment time with the tuna orbital oil emulsion on the fatty acids profile of juveniles of *Artemia franciscana*

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ABSTRACT

*Artemia* is deficient in polyunsaturated fatty acids (PUFAs), particularly in arachidonic (ARA, 20:4*n*-6), eicosapentaenoic (EPA, 20:5*n*-3), and docosahexaenoic acid (DHA, 22:6*n*-3). The aim of this study was to determine the optimal time in which the higher contents of PUFAs in juveniles of *Artemia franciscana* were obtained by the effect of enrichment with the tuna orbital oil emulsion. Six enrichment periods were evaluated: 3, 6, 9, 12, 15 and 18 h, in addition to a control treatment (0 h). The most abundant fatty acids in *A. franciscana* were monounsaturated (43.10% ± 4.35 –52.92% ± 5.82%), followed by saturated (33.83% ± 1.71 –42.33% ± 2.31%) and PUFAs (8.86% ± 2.83% –21.32% ± 2.38%). ARA decreased over the enrichment time; the maximum content was 5.74 ± 0.37% at 3 h, which was not statistically different with respect to the content recorded at 0 h. The highest content of EPA was at 3 h (6.47% ± 1.44%), without significant differences with the content registered at 0 h, while that from 6 h and until 15 h tended to decrease significantly. At 6 h, the content of DHA (8.84% ± 2.72%) was significantly higher compared to the rest of the treatments, which did not differ among themselves, or with the control. After to the 6 h and until 15 h, the content of PUFAs tended to decrease, which could indicate the metabolization of them by *A. franciscana*, coupled with the possible oxidation of these fatty acids in the enrichment solution.

## 1. Introduction

*Artemia* spp. are commonly used to feed larvae and juveniles of farmed fishes, cephalopods, and crustaceans, mainly due to the availability to obtain large biomass in short periods, and easy cultivation. However, *Artemia* has an important deficiency in highly unsaturated fatty acids (HUFAs) as arachidonic (ARA, 20:4*n*-6), eicosapentaenoic (EPA, 20:5*n*-3), and docosahexaenoic acid (DHA, 22:6*n*-3; Sorgeloos, Dhert, & Candreva, 2001; Navarro & Villanueva, 2003), which are essential for the development and maturation of the nervous and sensory systems during larval development of marine fishes (Izquierdo & Koven, 2011), as well as for the proper growth and survival (Watanabe, 1993; Sargent et al., 1999; Okumura, Kurihara, Iwamoto, & Takeuchi, 2005).

In order to increase the concentrations of essential fatty acids (EFA = DHA, EPA and ARA) in live prey, different emulsions rich in polyunsaturated fatty acids (PUFAs) have been developed. The evaluated emulsions in *Artemia* include: Super Selco (McEvoy, Navarro, Amat, & Sargent, 1997), DHA esters microcapsules (Ando & Oomi, 2001), Plus Aquaran (Okumura et al., 2005), Easy DHA Selco, Ori-Green and Ori-Prot (Ait Chattou et al., 2008), as well as emulsions base in oil fish (Hanaee, Ahg, Hanaee, Delazar, & Sarker, 2005; Ait Chattou et al., 2008), oil from ovary of sturgeon (Hafezieh et al., 2010) and tuna orbital oil (TOO, López-Peraza, Hernández-Rodríguez, & Barón-Sevilla, 2018; McEvoy, Navarro, Hontoria, Amat, & Sargent, 1996, 1995). The possibility of supplementing the emulsions or oils extracted from animal tissues with some other source of lipids also has been evaluated, such as the addition of polar lipids of herring to the TOO (McEvoy et al., 1996) and subsequently phosphatidylcholine from soybean (McEvoy et al., 1997). From these studies, the greater bioencapsulation of DHA (14%) and ARA (3%) content in *Artemia* were recorded with the use of TOO adding 12% polar lipids of herring (McEvoy et al., 1996), while for EPA (23.31%) the highest level was obtained with Plus Aquaran (Okumura et al., 2005). Nevertheless, it is important to note that the bioencapsulation of PUFAs was evaluated in *Artemia* metanauplii, with the exception of López-Peraza et al. (2018) who used adults of *A. franciscana*. Metanauplii has a suitable size (0.9–2.6 mm, Soorgelos et al., 1986) for certain crustacean and fish larvae, but not in the case of some organisms such as *Octopus bimaculatus* paralarvae (López-Peraza et al., 2018) and phyllosoma larvae of the rock lobster *Jasus edwardssii* (Smith, Rita, Thompson, Dunstan, & Brown, 2002), which require larger prey.

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On the other hand, standard protocols for *Artemia* enrichment consist in incubations of 18–24 h (at temperature around 28 °C), intense aeration and densities of 100–300 organisms/L. However, it has been shown that under the conditions described above, prolonged enrichment (> 18 h) produced a significant oxidation of PUFAs in the media (McEvoy, Navarro, Bell, & Sargent, 1995). In addition, *Artemia* could metabolize the EFA after ingestion (Guinot et al., 2013; McEvoy et al., 1996, 1995; Navarro, Henderson, McEvoy, Bell, & Amat, 1999). Therefore, the aim of this study was to evaluate the optimal enrichment time where the highest bioencapsulation of PUFAs contained in the TOO emulsion was obtained in juveniles of *A. franciscana*.

## 2. Materials and methods

### 2.1. A. franciscana cultivation

To obtain *A. franciscana* nauplii, the standard hydration-decapsulation-incubation protocol of the cysts (Great Salt Lake Artemia, INVE Aquaculture, lot 7116496181) was followed; using 5% commercial sodium hypochlorite at the rate of 125 mL of chlorine per 10 g of cysts, as a decapsulating solution. The incubation was carried out in a conical container with transparent walls with a useful volume of 15 L of seawater (35 psu) filtered up to 1 μm and maintained at 28 °C. The newly hatched *A. franciscana* (after 24 h of incubation) was transferred to a circular container with a useful volume of 250 L at a density of 2 nauplii/mL for its growth.

The cultivation of *A. franciscana* lasted 9 days, during that period, the individuals were fed with the microalgae *Chaetoceros muelleri*, adjusting the daily ration according to the feeding rate evaluated in preliminaries bioassays. Aeration was constant and the temperature remained at 26 °C using an AMF-500 heater equipped with a thermoregulator. The organic matter deposited on the bottom of the tank was daily removed by a siphon, and 30% seawater exchanges were made. When the *A. franciscana* reached the desired size (aprox. 4 mm of total length), was harvested, and the percentage of final survival was estimated by dividing the final density by the initial density, multiplied by 100.

### 2.2. A. franciscana enrichment

Once the survival was estimated, *A. franciscana* juveniles were transferred to transparent containers with a useful volume of 3 L for their enrichment with the TOO emulsion (Nutrition and Health Omegamex, La Paz, Baja California Sur, Mexico, Table 1). The density of *A. franciscana* in the medium was of 500 juveniles/L. Six enrichment periods were evaluated: 3, 6, 9, 12, 15 and 18 h, and each treatment were prepared in triplicate. The treatments were maintained with constant aeration and at a temperature of 26 °C maintained by a 50 W heater. In addition, before starting the experiment, a sample of *A. franciscana* was taken without enriching with the emulsion (0 h, control treatment) to determine its fatty acid profile.

The enrichment process was carried out following some recommendations of the manufacturer: 1 g of TOO emulsion was added per liter of enrichment media. For this, the emulsion was weighed and dissolved in 1 L of seawater with a domestic blender (Oster), then, the mixture was added in each of the experimental units. At the end of the established period for each treatment, *Artemia* was concentrated with a 500 μm mesh, washed with filtered seawater and stored at −80 °C until analysis. It should be noted that, from 12 h, in the remaining treatments (15 and 18 h), the enrichment media was completely renewed, a procedure commonly used to enrich *Artemia* for long time (McEvoy et al., 1995, 1997).

### 2.3. Analysis of fatty acids

The lipids extraction from *A. franciscana* and the TOO emulsion was carried out following the technique of Folch, Lees, and Stanley (1957) using dichloromethane: methanol as a solvent (Cequier-Sánchez, Rodríguez, Ravelo, & Zarate, 2008) in a ratio of 2:1. For lipids saponification, 100 μL of 90% methanolic KOH (0.3 N) was added, and samples were kept in a water bath at 60 °C for 30 min. Methylation was done by adding esterification reagent 14% boron trifluoride in methanol ($BF_3/CH_3OH$) and kept at 60 °C water bath for 15 min. After esterification, fatty acid methyl esters were dried under a nitrogen gas atmosphere and were stored at −20 °C until analysis (Morrison, 1986). Subsequently, the esters were dissolved in 200 μL of hexane HPLC grade and analyzed using a gas chromatograph (Agilent 6890N) equipped with a mass spectrum detector (Agilent 5973) and an autosampler (Agilent 7683). A capillary column of 30 m length × 250 μm and a film thickness of 0.25 μm (Omegawax® Capillary GC Column, Supelco Inc.) and helium as the carrier gas at a flow rate of 1 mL/min were used. One μL of sample was injected with a Split of 50:1. The initial temperature of the oven was 50 °C, then it was raised to 240 °C at a rate of 5 °C/min and maintained for 10 min.

**Table 1**
Tuna orbital oil emulsion composition according to Nutrition and Health Omegamex, La Paz, Baja California Sur, Mexico.

| Tuna orbital oil (TOO) | |
|---|---|
| | Content (%) |
| DHA | 40 |
| EPA | 8 |
| ARA | 2.3 |
| Saturated | 22 |
| Monounsaturated | 18 |
| n–3 | 53 |
| n–6 | 6.7 |
| **Ingredients per 50 g of emulsion** | |
| TOO | 12.5 g |
| Water | 37.5 g |
| Antifoam | 0.25 g |
| Sodium alginate | 0.5 g |
| Vit. A | 750 UI |
| Vit. E | 100 UI |
| Vit. D2 | 50 UI |
| Astaxantin | 1 mg |

### 2.4. Statistical analysis

The survival data of *A. franciscana* during enrichment, as well as of fatty acids, are presented in percentage values; however, for their statistical analysis, they were transformed with the arccosine function. In order to verify the normality and homogeneity of variance in the data obtained in this work, the Lilliefors and Levene tests were applied, respectively. To evaluate differences in the fatty acid content of the *A. franciscana* through the enrichment time, a One-Way analysis of variance (ANOVA) was applied, and later a Tukey test to identify these differences. Due to the survival data did not meet the assumptions of normality and homogeneity of variance, the nonparametric test of Kruskal Wallis was applied. For survival data, the nonparametric test of Kruskal Wallis was applied. The level of significance for all the tests applied was $P < 0.05$ (Zar, 2010).

## 3. Results

### 3.1. A. franciscana cultivation

Initially, *A. franciscana* nauplii had an average of total length of 0.59 ± 0.33 mm, and 9 days after attained an average of 3.87 ± 0.29 mm, which represents an increase of 6.5- fold compared to their initial size. Their diet started with 100,000 cells/mL and

**Table 2**
Survival (%) of juveniles of *A. franciscana*, pH and dissolved oxygen during the enrichment process with the tuna orbital oil emulsion.

| Time (h) | Survival (%) | DO (mg/L) | pH |
|---|---|---|---|
| **3** | 100.00 ± 0.00 | 7.31 ± 0.25 | 8.04 ± 0.14 |
| **6** | 100.00 ± 0.00 | 7.60 ± 0.17 | 8.12 ± 0.26 |
| **9** | 100.00 ± 0.00 | 7.37 ± 0.56 | 8.07 ± 0.22 |
| **12** | 100.00 ± 0.00 | 7.44 ± 0.41 | 8.32 ± 0.25 |
| **15** | 99.99 ± 0.01 | 7.48 ± 0.43 | 8.10 ± 0.14 |
| **18** | 99.92 ± 0.02 | 7.57 ± 0.11 | 8.15 ± 0.18 |

Results are expressed as the mean ± D.E. ($n$ = 3). A Kruskal Wallis test ($P$ < 0.05) was applied to the survival data.

gradually increased according to the age of the individuals, up to a ratio of 400,000 cells/mL. Final survival in the culture was 65.8%.

### 3.2. Enrichment of the A. franciscana with tuna orbital oil emulsion

There was no mortality (100% survival) of *A. franciscana* at 3, 6, 9 and 12 h of enrichment (Table 2), while in the treatments of 15 and 18 h the survival was 99.99% and 99.92%, respectively. However, there were no statistically significant differences between treatments.

The analysis of the fatty acid composition of the TOO emulsion (Table 3) showed that PUFAs were the most abundant (42.2% ± 1.55%), followed by saturates (29.16% ± 0.86%) and monounsaturates (28.63% ± 0.84%). The most abundant fatty acid in the emulsion was DHA, which registered a value of 30.04% ± 0.51%, followed by 16:0 (19.23% ± 0.07%) and 18:1*n*-9 (17.14% ± 0.12%). ARA content was 2.31% ± 0.00% and that of the EPA was 6.00% ± 0.04%. The DHA/EPA ratio was 5.01, while for EPA/ARA was 2.59. The content of 18:2*n*-6 was 1.11% ± 0.94%, while the values for 18: 3*n*-3 and 18:4*n*-3 were less than 1% (0.76% ± 0.02% and 0.54% ± 0.00%, respectively).

Concerning fatty acid composition of the juveniles of *A. franciscana* (Table 3), it was observed that the monounsaturated fatty acids were the most abundant, with average values between 43.10% ± 4.35% and 52.92% ± 5.82% (6 and 18 h, respectively), followed by saturates (between 33.83% ± 1.71% and 42.33% ± 2.31% at 3 and 12 h respectively) and PUFAs (between 8.86% ± 2.83% and 21.32% ± 2.38% at 15 and 3 h respectively).

The highest total PUFAs content (21.32% ± 2.38%) was recorded at 3 h; however, it only showed significant differences with the contents recorded at 12 and 15 h (9.85% ± 3.04% and 8.86% ± 2.83%, respectively, Table 3). The content of 18:2*n*-6 in enriched *A. franciscana* recorded values ranging from 2.28% ± 0.52% (12 h) to 3.00% ± 0.65% (3 h) without significant differences between treatments, and neither the treatments with the control (4.04% ± 1.15%). The highest content of 18:3*n*-3 was found in unenriched (0 h) *A. franciscana*, which was superior to that registered in the enriched *A. franciscana*, except for that obtained at 3 h (1.18% ± 0.09%). 18:4*n*-3 was not detected at 0 or at 3 h, it was detected after 6 h of enrichment with a maximum value of 0.73% ± 0.39% at 15 h, but without significant differences with the rest of the treatments.

Likewise, the sum of the EFA represented over 65% of the total PUFAs in the treatments, except at 15 h where the sum of these was only 39.41% (Table 4). ARA registered an evident tendency to decrease according to the enrichment time, with a maximum value of 5.74% ± 0.37% at 3 h, which was not statistically different with the value obtained in unenriched *A. franciscana* (5.88% ± 1.62% at 0 h), but it was with respect to the rest of the treatments, where the minimum value (0.92% ± 0.23%) was recorded at 18 h (Table 3, Fig. 1). EPA had an increase at 3 h compared to 0 h, which was where the highest value recorded (6.47% ± 1.44% at 3 h); however, this increase was not significant, since there was no statistical difference. After 6 h, the EPA content tended to decrease, registering the minimum value at 15 h (1.02% ± 0.56%), which was only statistically lower compared of that registered at 0 and 3 h. Afterward, at 18 h (2.82% ± 0.86%) an increase respect to that of 15 h was observed; but, this increase was not significant (Table 3, Fig. 1). The DHA content showed a clear tendency to increase

**Table 3**
Fatty acids composition (% of total fatty acids identified) of the tuna orbit oil (TOO) emulsion and juveniles of *A. franciscana* enriched with TOO at different times (hours).

| Fatty acids | TOO emulsion | *Artemia franciscana* enriched with TOO emulsion (hours) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 3 | 6 | 9 | 12 | 15 | 18 |
| 14:0 | 2.74 ± 0.07 | 4.50 ± 0.89$^{a}$ | 3.01 ± 0.88$^{a}$ | 3.52 ± 0.15$^{a}$ | 3.51 ± 0.01$^{a}$ | 3.41 ± 0.13$^{a}$ | 2.77 ± 0.17$^{b}$ | 2.52 ± 0.23$^{b}$ |
| 15:0 | 1.00 ± 0.02 | 0.20 ± 0.11$^{a}$ | 0.72 ± 0.12$^{a}$ | 0.93 ± 0.05$^{a}$ | 0.96 ± 0.04$^{a}$ | 0.78 ± 0.13$^{a}$ | 0.68 ± 0.01$^{a}$ | 0.69 ± 0.12$^{a}$ |
| 16:0 | 19.23 ± 0.07 | 19.59 ± 0.61$^{a}$ | 20.04 ± 20.15$^{a}$ | 22.40 ± 0.94$^{a}$ | 23.98 ± 0.12$^{b}$ | 24.93 ± 1.90$^{b}$ | 22.53 ± 0.77$^{a}$ | 20.00 ± 1.91$^{a}$ |
| 16:1*n*–7 | 4.67 ± 0.07 | 18.83 ± 1.25$^{c}$ | 16.04 ± 0.94$^{bc}$ | 11.60 ± 1.58$^{a}$ | 11.90 ± 0.63$^{a}$ | 13.29 ± 0.26$^{ab}$ | 13.53 ± 1.43$^{ab}$ | 12.50 ± 1.38$^{a}$ |
| 17:0 | 1.06 ± 0.00 | 0.69 ± 0.08$^{a}$ | 0.81 ± 0.08$^{ab}$ | 1.19 ± 0.08$^{bc}$ | 1.31 ± 0.05$^{bc}$ | 1.15 ± 0.20b$^{c}$ | 1.18 ± 0.13$^{bc}$ | 1.01 ± 0.10$^{abc}$ |
| 17:1*n*–7 | 0.99 ± 0.09 | 0.68 ± 0.00$^{a}$ | 0.72 ± 0.05$^{a}$ | 1.02 ± 0.07$^{a}$ | 1.10 ± 0.02$^{a}$ | 0.92 ± 0.09$^{a}$ | 1.02 ± 0.05$^{a}$ | 1.11 ± 0.55$^{a}$ |
| 18:0 | 5.13 ± 0.70 | 11.24 ± 0.56$^{b}$ | 9.25 ± 0.48$^{ab}$ | 8.44 ± 0.65$^{a}$ | 9.42 ± 0.20$^{ab}$ | 12.07 ± 0.21$^{bc}$ | 11.37 ± 0.73$^{c}$ | 9.94 ± 0.81$^{ab}$ |
| 18:1*n*–9 | 17.14 ± 0.12 | 12.34 ± 1.78$^{a}$ | 15.93 ± 1.48$^{ab}$ | 20.32 ± 1.34$^{bc}$ | 21.82 ± 1.19$^{c}$ | 20.54 ± 2.33$^{bc}$ | 22.20 ± 10.78$^{c}$ | 23.32 ± 1.27$^{c}$ |
| 18:1*n*–7 | 2.17 ± 0.46 | 12.33 ± 1.19$^{b}$ | 12.17 ± 1.12$^{b}$ | 7.67 ± 1.15$^{a}$ | 8.20 ± 0.52$^{ab}$ | 10.92 ± 0.42$^{ab}$ | 13.31 ± 1.13$^{b}$ | 11.05 ± 1.15$^{b}$ |
| 18:2*n*–6 | 1.11 ± 0.94 | 4.04 ± 1.15$^{a}$ | 3.00 ± 0.65$^{a}$ | 2.52 ± 0.07$^{a}$ | 2.47 ± 0.02$^{a}$ | 2.28 ± 0.52$^{a}$ | 2.78 ± 0.48$^{a}$ | 2.73 ± 0.34$^{a}$ |
| 18:3*n*–3 | 0.76 ± 0.02 | 1.48 ± 0.21$^{c}$ | 1.18 ± 0.09$^{bc}$ | 0.64 ± 0.11$^{ab}$ | 0.56 ± 0.05$^{ab}$ | 0.42 ± 0.10$^{a}$ | 0.74 ± 0.38$^{ab}$ | 0.65 ± 0.23$^{ab}$ |
| 18:4*n*–3 | 0.54 ± 0.00 | N.D | N.D | 0.45 ± 0.04$^{a}$ | 0.52 ± 0.01$^{a}$ | 0.24 ± 0.10$^{a}$ | 0.73 ± 0.39$^{a}$ | 0.49 ± 0.07$^{a}$ |
| 20:1*n*–9 | 1.01 ± 0.03 | N.D | N.D | 1.08 ± 0.08$^{a}$ | 1.13 ± 0.01$^{a}$ | 0.83 ± 0.24$^{a}$ | 0.78 ± 0.33$^{a}$ | 0.55 ± 0.01$^{a}$ |
| 20:4*n*–6 | 2.31 ± 0.00 | 5.88 ± 1.62$^{c}$ | 5.74 ± 0.37$^{c}$ | 3.26 ± 0.45$^{b}$ | 2.68 ± 0.28$^{a}$ | 2.23 ± 0.68$^{a}$ | 1.28 ± 0.37$^{a}$ | 0.92 ± 0.23$^{a}$ |
| 20:5*n*–3 | 6.00 ± 0.04 | 5.53 ± 1.04$^{cd}$ | 6.47 ± 1.44$^{d}$ | 4.11 ± 0.93$^{bcd}$ | 3.00 ± 0.63$^{abc}$ | 1.31 ± 0.34$^{ab}$ | 1.02 ± 0.56$^{a}$ | 2.82 ± 0.86$^{abc}$ |
| 22:1*n*–9 | 1.90 ± 0.07 | N.D. | N.D. | 0.76 ± 0.26$^{a}$ | 0.55 ± 0.19$^{a}$ | 0.61 ± 0.03$^{a}$ | 1.34 ± 0.55$^{ab}$ | 3.46 ± 0.72$^{b}$ |
| 22:5*n*–3 | 1.46 ± 0.01 | N.D. | N.D. | 0.60 ± 0.24$^{a}$ | 0.46 ± 0.12$^{a}$ | N.D. | 1.12 ± 0.11$^{b}$ | 0.65 ± 0.08$^{a}$ |
| 22:6*n*–3 | 30.04 ± 0.51 | 2.66 ± 1.03$^{a}$ | 4.93 ± 0.72$^{a}$ | 8.84 ± 2.72$^{b}$ | 5.78 ± 0.99$^{a}$ | 3.36 ± 1.24$^{a}$ | 1.20 ± 0.53$^{a}$ | 4.67 ± 1.93$^{a}$ |
| 24:1*n*–9 | N.D. | N.D. | N.D. | 0.65 ± 0.11$^{a}$ | 0.65 ± 0.13$^{a}$ | 0.71 ± 0.01$^{a}$ | 0.43 ± 0.22$^{a}$ | 0.92 ± 0.14$^{a}$ |
| ΣSAT | 29.16 ± 0.86 | 36.22 ± 2.39$^{ab}$ | 33.83 ± 1.71$^{a}$ | 36.48 ± 1.88$^{ab}$ | 39.19 ± 0.41$^{bc}$ | 42.33 ± 2.31$^{c}$ | 38.52 ± 1.82$^{bc}$ | 34.16 ± 3.17$^{a}$ |
| ΣMONO | 28.63 ± 0.84 | 44.18 ± 4.24$^{a}$ | 44.85 ± 3.53$^{a}$ | 43.10 ± 4.35$^{a}$ | 45.35 ± 2.70$^{a}$ | 47.82 ± 3.39$^{a}$ | 52.62 ± 4.46$^{a}$ | 52.92 ± 5.82$^{a}$ |
| ΣPUFAs | 42.21 ± 1.52 | 19.61 ± 4.8$^{b}$ | 21.32 ± 2.38$^{b}$ | 20.42 ± 4.56$^{b}$ | 15.46 ± 2.09$^{ab}$ | 9.85 ± 3.04$^{a}$ | 8.86 ± 2.83$^{a}$ | 12.93 ± 3.74$^{ab}$ |
| Σ*n*–3 | 38.79 ± 0.58 | 9.68 ± 2.28$^{abc}$ | 12.58 ± 1.60$^{bc}$ | 14.65 ± 2.84$^{c}$ | 10.32 ± 1.77$^{abc}$ | 5.34 ± 1.58$^{ab}$ | 4.81 ± 1.97$^{a}$ | 9.28 ± 2.71$^{abc}$ |
| Σ*n*–6 | 3.42 ± 0.94 | 9.93 ± 0.53$^{b}$ | 8.74 ± 0.94$^{b}$ | 5.77 ± 0.17$^{b}$ | 5.14 ± 0.26$^{b}$ | 4.51 ± 1.26$^{b}$ | 4.06 ± 0.47$^{a}$ | 3.65 ± 0.11$^{a}$ |
| DHA/EPA | 5.01 ± 0.13 | 0.48 ± 0.10$^{a}$ | 0.76 ± 0.16$^{a}$ | 2.15 ± 0.37$^{ab}$ | 1.92 ± 0.08$^{ab}$ | 2.56 ± 0.37$^{b}$ | 1.18 ± 0.74$^{ab}$ | 1.65 ± 0.60$^{ab}$ |
| EPA/ARA | 2.59 ± 0.02 | 0.94 ± 0.10$^{ab}$ | 1.13 ± 0.19$^{ab}$ | 1.26 ± 0.14$^{ab}$ | 1.12 ± 0.12$^{ab}$ | 0.59 ± 0.4$^{a}$ | 0.80 ± 0.24$^{a}$ | 3.06 ± 1.67$^{b}$ |

Results are expressed as the mean ± D.E. ($n$ = 3). Different letters indicate significant differences ($P$ < 0.05, One-Way ANOVA and Tukey test) between treatments. N.D., no detected.

**Table 4**
Proportion of the total essential fatty acids (ΣEFA = ARA, EPA and DHA) in relation to the total of PUFA in the *A. franciscana* enriched with the tuna orbital oil emulsion.

| | Enrichment time (hours) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 9 | 12 | 15 | 18 |
| **ΣEFA** | 14.08 | 17.15 | 16.21 | 11.45 | 6.91 | 3.49 | 8.41 |
| **ΣPUFAs** | 19.61 | 21.32 | 20.42 | 15.46 | 9.85 | 8.86 | 12.93 |
| EFA **(%)** | 71.80 | 80.42 | 79.37 | 74.05 | 70.10 | 39.41 | 65.07 |

during the first hours of enrichment, with a maximum of 8.84% ± 2.72% at 6 h, after, the composition tended to gradually diminish to a lowest value of 1.20% ± 0.53% at 15 h, then increase again to 4.67% ± 1.93% at 18 h. It should be mentioned that the DHA content registered at 6 h, was significantly higher than the rest of the treatments, which did not show significant differences between them, nor with the control treatment (Table 3, Fig. 1).

Regarding to the DHA/EPA ratio, the highest ratio found in juveniles of *A. franciscana* was 2.56 at 12 h, followed by 6 h (2.15), while the lowest was at 0 h. At 15 h (DHA/EPA = 1.18) there was a decrease of 53.9% with respect to the value obtained at 12 h, and then a slight increase to 1.65 at 18 h; however, there were no significant differences between these treatments (12, 15 and 18 h, Table 3).

The EPA/ARA ratio recorded a slight upward trend until 6 h (1.26), then tended to decrease registering a minimum value of 0.59 at 12 h, to subsequently increase reaching a maximum value of 3.06 at 18 h, which was statistically different only with the values recorded in treatments 12 and 15 h (Table 3).

## 4. Discussion

### 4.1. A. franciscana cultivation

Studies where *Artemia* have been grown up to juvenile stages (2.7–5.5 mm of total length according to Sorgeloos, Lavens, Lé, Tackaert, & Versichele, 1986) are scarce, as mentioned above, the organisms are used in the nauplii (newly hatched) and metanauplii phases (0.9–2.6 mm of total length) as food for several marine species in culture. The juveniles of *A. franciscana* recorded slower growth compared to the reported by Lora-Vilchis and Voltolina (2003) and Lora-Vilchis, Cordero-Esquivel, and Voltolina (2004) feeding it with the same microalga, *C. muelleri*. Likewise, survival in this work (65.8%) was lower than that of the authors, who recorded 93% of survival. Similar survival results (> 90%) have been observed when *A. franciscana* feeds with other microalgae such as *Isochrysis galbana* and *Isochrysis* sp. (Evjemo & Olsen, 1999; Lora-Vilchis et al., 2004), which is probably related to the differences in the biochemical composition of the algae.

### 4.2. A. franciscana enrichment

The TOO emulsion used in the present work contained 30.04% of DHA with respect to the total of fatty acids identified, however, despite the high content of DHA in the emulsion, the maximum value found in juveniles of *A. franciscana* enriched was 8.84% ± 2.72% at 6 h, lower than previously reported by McEvoy et al. (1996, 1997) with TOO (12.3%–14.0%) and Guinot et al. (2013) with marine lecithin oil (13%). Nonetheless, it is important to note that these authors analyzed the fatty acid profiles in *Artemia* metanauplii, and in the present study, juveniles of 3.87 ± 0.29 mm were used, so the age or size of *Artemia* could have influenced in the bioencapsulation efficiency of fatty acids. In this sense, it has been observed that, in general, the content of PUFAs decreases according to the age of *Artemia* (Naz, 2008; Ritar, Smith, Dunstan, Brown, & Hart, 2003), probably due to the fact that the younger *Artemia* (metanauplii) still has vitelline reserves, which are consumed during ontogenetic development. The species is another factor that can influence the bioencapsulation of fatty acids by *Artemia*. However, it is difficult to make comparisons between individuals belonging to the same species, since in the literature it is very common that the specific name is not indicated, it is only referred as *Artemia* or *Artemia* sp.

The EPA and ARA content of juveniles of *A. franciscana* recorded a similar trend; the highest values were found at 0 and 3 h, and subsequently the contents tended to decrease according to the enrichment

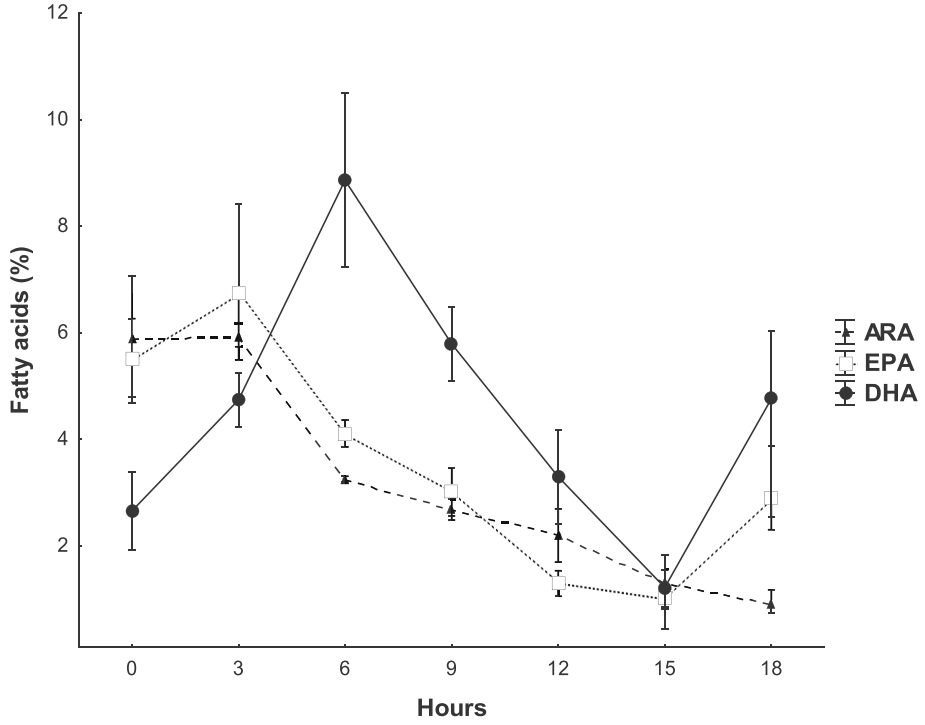


**Fig. 1.** ARA, EPA and DHA proportion (% of total fatty acids identified) of *A. franciscana* enriched with tuna orbital oil emulsion. See statistical analysis in Table 2.

time. The above suggests that the initial content (0 and 3 h) of EPA and ARA could be due mainly to the contribution of the microalga *C. muelleri* and not precisely by the TOO emulsion, since it has been reported that this microalgae can have contents of EPA from 7% to 16% and ARA from 3.5% to 5.9% (Lemus et al., 2006; Cazares-Salazar, 2014), hence the contents of these EFA are greater in these treatments. In addition to the EPA and ARA, the DHA also tended to decrease according to the enrichment time, particularly after 6 h. This is probably due to the fact that *A. franciscana* metabolized the ingested EFA (Navarro et al., 1999). Moreover, despite the fact that the TOO emulsion contained astaxantin as antioxidant (Table 1), cannot be ruled out the possible oxidation of these during the treatments. Since as mentioned previously, the enrichment media was renewed after 12 h, which could indicate that before the renewal, the EFA could be in oxidation process, so were no longer available in the media in sufficient quantities (McEvoy et al., 1997, 1995; Navarro et al., 1999). After the renewal of the enrichment media, the EFA were again available; therefore, *A. franciscana* began its incorporation, which was reflected until 18 h, since the DHA and EPA had a slight increase (without significant differences) with respect to that found at 15 h. All of the above indicates that renewal or adding new enrichment media after 12 h was unnecessary because, as noted, this did not have an immediate effect on the bioencapsution on PUFAs.

It is important to note that an adequate DHA/EPA ratio in the diet of the organisms is elementary to optimize the use of both fatty acids, because it has been observed that when the concentration of EPA is high, the incorporation of DHA into phospholipids is reduced, and an increase in DHA in the diet, decreases markedly the incorporation of EPA into phospholipids (*i.e.,* phosphatidylethanolamine, Izquierdo, Socorro, Arantzamendi, & Hernández-Cruz, 2000).

The recommended DHA/EPA ratio to satisfy the nutritional requirements of marine fishes is 2 (Sargent, McEvoy, & Bell, 1997), but this applies as long as the basic requirements of DHA are met. This is due to its importance for the adequate development of the visual capacity of the larvae, which leads to an increase in the efficiency for the capture of prey, thus promoting better growth and survival (Bell, Batty, & Dick, 1995; Furuita, Takeuchi, & Uematsu, 1998; Izquierdo & Koven, 2011; Masuda et al., 1999; Sargent et al., 1999; Watanabe, Izquierdo, Takeuchi, Satoh, & Kitajima, 1989). The DHA/EPA ratio of the TOO emulsion used in this study was high (5.01) which is attributable to the high content of DHA in the emulsion. In *A. franciscana* enriched at 6, 9 and 12 h, the DHA/EPA ratios were around 2; however, in treatments 9 and 12 h, both DHA and EPA content were significantly lower compared to that recorded at 6 h; therefore, the proportions between them were remained, so that the DHA/EPA relationships in the treatments were similar to each other, and close to the requirements described for various species of marine fishes.

Also, an adequate EPA/ARA ratio in the diet of the organisms is of great importance, since prostaglandins and leukotrienes produced from ARA have an stimulating effect, while those of the EPA a inhibitory effect in several physiological processes (*i.e.,* immune response, maturation, spawning, etc.) in organisms (Sargent et al., 1999; Tocher, 2003). The EPA/ARA ratio recommended in the diet of marine fishes is species-specific; however, commonly are in the range of 3.5–5 (Izquierdo & Koven, 2011). In the present work, the highest EPA/ARA ratio (3) was recorded at 18 h, being this ratio lower than that recommended for marine fishes. But, in the same way as in the DHA/EPA ratio, this relationship applies when the basic requirements of EPA and ARA in the organisms in culture are met (Sargent et al., 1997), so it is important to know the specific nutritional requirements of the different species.

On the other hand, the EFA contents obtained at 6 h of enrichment were superior to those registered by López-Peraza et al. (2018), who also used the species of *A. franciscana* in an advanced stage of development (8 mm of total length), and the TOO emulsion Omegamex in an enrichment period of 12 h. These results suggest that, in addition to age, the enrichment time and the protocol used in the present work had an effect on the effectiveness of the bioencapsulation of the EFA contained in the TOO emulsion by *A. franciscana.*

## 5. Conclusion

In general, the proportion of PUFAs in the enriched juveniles of *A. franciscana* tended to decrease according to the time of enrichment, probably due to the metabolization of them by *A. franciscana*, as well as the possible oxidation of these nutrients in the enrichment media. These results agree with previous reports, where short enrichment periods (< 6 h) optimized the bioencapsulation of the PUFAs in *Artemia.* According to the results obtained, we recommend enriching juveniles of A. franciscana with tuna orbital oil for a period of 6 h to obtain the highest DHA content, and only 3 h for EPA and ARA content. Those juveniles can further be used as a living food to assess the nutritional physiology of organisms under culture.

## CRediT authorship contribution statement

**Mario Nieves-Soto:** Resources, Formal analysis, Conceptualization. **Ricardo Lozano-Huerta:** Investigation. **Diana J. López-Peraza:** Conceptualization, Investigation, Visualization, Writing - original draft, Supervision, Project administration, Funding acquisition, Project administration. **María A. Medina-Jasso:** Conceptualization, Writing - review & editing. **Miguel A. Hurtado-Oliva:** Writing - review & editing. **José F. Bermudes-Lizárraga:** Writing - review & editing.

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## References

Ait Chattou, M., Roo, J., Socorro, J., Estefanell, J., Fernández-Palacios, H., & Izquierdo, M. S. (2008). Use of different commercial *Artemia* sp. enrichments in *Octopus vulgaris*, parlarval rearing at initial planktonic phase: Effects on growth, survival and fatty acid composition of the paralarvae. *XIII international symposium on fish nutrition and feeding*.

Ando, J., & Oomi, Y. (2001). Positional distribution of highly unsaturated fatty acids in triacyl-*sn*-glycerols of *Artemia* nauplii enriched with docosahexaenoic acid ethyl ester. *Lipids, 36*(7), 733–740. https://doi.org/10.1007/s11745-001-0779-4.

Bell, M., Batty, R. S., & Dick, J. R. (1995). Dietary deficiency of docosahexaenoic acid impairs vision at low light intensities in juvenile herring (*Clupea harengus* L.). *Lipids, 30*, 443–449. https://doi.org/10.1007/bf02536303.

Cazares-Salazar, A. (2014). *Efecto de dietas mixtas de microalgas marinas en el cultivo y composición de ácidos grasos esenciales del copépodo Pseudodiaptomus euryahalinus*Master dissertation. Baja California: Center for Scientific Research and Higher Education at Ensenada. http://cicese.repositorioinstitucional.mx/jspui/handle/1007/71.

Cequier-Sánchez, E. C., Rodríguez, C., Ravelo, A. G., & Zarate, R. (2008). Dichloromethane as solvent for lipid extraction, lipid classes and fatty acid assessment from samples of different nature. *Journal of Agricultural and Food Chemistry, 56*, 4297–4303. https://doi.org/10.1021/jf073471e.

Evjemo, J. O., & Olsen, Y. (1999). Effect of food concentration on the growth and production rate of *Artemia franciscana* feeding on algae (*T. iso*). *Journal of Experimental Marine Biology and Ecology, 242*, 273–296.

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry, 226*, 497–509.

Furuita, H., Takeuchi, T., & Uematsu, K. (1998). Effect of eicosapentaenoic and docosahexaenoci acids on growth, survival and brain development of larva Japanese flounder *Paralichthys olivaceus*. *Aquaculture, 161*, 269–279.

Guinot, D., Monroig, Ó., Navarro, J. C., Varó, I., Amat, F., & Hontoria, F. (2013). Enrichment of *Artemia* metanauplii in phospholipids and essential fatty acids as a diet for common octopus (*Octopus vulgaris*) paralarvae. *Aquaculture Nutrition, 19*, 837–844. https://doi.org/10.1111/anu.12048.

Hafezieh, M., Kamarudin, M. S. S., Saad, C. R. B., Sattar, M. K. A., Agh, N., Valinassab, T., et al. (2010). Effects of enriched *Artemia urmiana* with HUFA on growth, survival, and fatty acids composition of the Persian sturgeon larvae (*Acipenser persicus*). *Iranian Journal of Fisheries Sciences, 9*(1), 61–72.

Hanaee, J., Ahg, N., Hanaee, M., Delazar, A., & Sarker, S. D. (2005). Studies on the enrichment of *Artemia urmiana* cysts for improving fish food value. *Animal Feed Science and Technology, 120*, 107–112. https://doi.org/10.1016/j.anifeedsci.2005.01.010.

Izquierdo, M., & Koven, W. (2011). Lipids. In G. J. Holt (Ed.). *Larval fish nutrition* (pp. 47–81). Chichester, U. K: Wiley-BlackWell/John Wiley and Sons.

Izquierdo, M. S., Socorro, J., Arantzamendi, L., & Hernández-Cruz, C. M. (2000). Recent advances in lipid nutrition in fish larvae. *Fish Physiology and Biochemistry, 2*, 7–107.

Lemus, N., Urbano, T., Arredondo-Vega, B., Guevara, M., Vásquez, A., Carreón-Palau, L., et al. (2006). Crecimiento y perfil bioquímico de *Chaetoceros muelleri* cultivada en sistemas discontinuos y semicontinuos. *Ciencias Marinas, 32*(3), 597–603.

López-Peraza, D. J., Hernández-Rodríguez, M., & Barón-Sevilla, B. (2018). Fatty acid profile, proteolytic activity and survival of *Octopus bimaculatus* paralarvae fed with enriched *Artemia franciscana*. *Molluscan Research, 38*(1), 1–11. https://doi.org/10.1080/13235818.2018.142848.

Lora-Vilchis, M. C., Cordero-Esquivel, B., & Voltolina, D. (2004). Growth of *Artemia franciscana* fed *Isochrysis* sp. and *Chaetoceros muelleri* during its early life stages. *Aquaculture Research, 35*, 1086–1091. https://doi.org/10.1111/j.1365-2109.2004.01123.x.

Lora-Vilchis, M. C., & Voltolina, D. (2003). Growth and survival of *Artemia franciscana* (kellogg) fed with *Chaetoceros muelleri* lemmerman and *Chorella capsulata* guillard. *Revista de Investigaciones Marinas, 24*(3), 241–246.

Masuda, R., Takeuchi, T., Tsukamoto, K., Sato, H., Shimizu, K., & Imaizumi, K. (1999). Incorporation of dietary docosahexaenoic acid into the central nervous system of the yellowtail *Seriola quinqueradiata*. *Brain, Behavior and Evolution, 53*, 173–179.

McEvoy, L. A., Navarro, J. C., Amat, F., & Sargent, J. R. (1997). Application of soya phosphatidylcholine in tuna orbital oil enrichment emulsions for *Artemia*. *Aquaculture International, 5*, 517–526.

McEvoy, L. A., Navarro, J. C., Bell, J. G., & Sargent, J. R. (1995). Autoxidation of oil emulsions during the *Artemia* enrichment process. *Aquaculture, 134*, 101–112.

McEvoy, L. A., Navarro, J. C., Hontoria, F., Amat, F., & Sargent, J. R. (1996). Two novel *Artemia* enrichment diets containing polar lipid. *Aquaculture, 144*, 339–352.

Morrison, K. (1986). *Techniques of lipidology: Isolation, analysis and identification of lipids.* Holland: American Elsevier.

Navarro, J. C., Henderson, R. J., McEvoy, L. A., Bell, M. V., & Amat, F. (1999). Lipid conversions during enrichment of *Artemia*. *Aquaculture, 174*, 155–166.

Navarro, J. C., & Villanueva, R. (2003). The fatty acid composition of *Octopus vulgaris* paralarvae reared with live and inert food: Deviation from their natural fatty acid profile. *Aquaculture, 219*, 613–631.

Naz, M. (2008). The changes in the biochemical compositions and enzymatic activities of rotifer (*Brachionus plicatilis*, Müller) and *Artemia* during the enrichment and starvation periods. *Fish Physiology and Biochemistry, 34*, 391–404. https://doi.org/10.1007/s10695-007-9199-5.

Okumura, S., Kurihara, A., Iwamoto, A., & Takeuchi, T. (2005). Improved survival and growth in *Octopus vulgaris* paralarvae by feeding large type *Artemia* and Pacific sandeel, *Ammodytes personatus*. *Aquaculture, 244*, 144–157.

Ritar, A. J., Smith, G. G., Dunstan, G. A., Brown, M. R., & Hart, P. R. (2003). *Artemia* prey size and mode of presentation: Effects on the survival and growth of phyllosoma larvae of southern rock lobster (*Jasus edwardsii*). *Aquaculture International, 11*, 163–182. https://doi.org/10.1016/j.aquaculture.2004.11.044.

Sargent, J. R., McEvoy, L. A., & Bell, J. G. (1997). Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. *Aquaculture, 155*, 117–127.

Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J., et al. (1999). Lipid nutrition of marine fish during early development: Current status and future directions. *Aquaculture, 179*, 217–229.

Smith, G. G., Rita, A. J., Thompson, P. A., Dunstan, G. A., & Brown, M. R. (2002). The effect of embryo incubation temperature on indicators of larval viability in Stage I phyllosoma of the spiny lobster. *Jasus edwardsii. Aquaculture, 209*, 157–167. https://doi.org/10.1016/S0044-8486(01)00758-X.

Sorgeloos, P., Dhert, P., & Candreva, P. (2001). Use of brine shrimp, *Artemia* sp. in marine fish larviculture. *Aquaculture, 200*, 147–159.

Sorgeloos, P., Lavens, P., Lé, P., Tackaert, W., & Versichele, D. (1986). *Manual para el cultivo y uso de artemia en acuicultura.* Universidad del Estado en Gent, Bélgica-Facultad de Agronomíawww.fao.org/docrep/field/003/AB474S/AB474S00.htm.

Tocher, D. R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science, 11*, 107–184. https://doi.org/10.1080/713610925.

Watanabe, T. (1993). Importance of docosahexaenoic acid in marine larval fish. *Journal of the World Aquaculture Society, 24*, 152–161.

Watanabe, T., Izquierdo, M. S., Takeuchi, T., Satoh, S., & Kitajima, C. (1989). Comparision between eicosapentaenoic and docosahexaenoic acids in terms of essential fatty efficacy in larval red sea bream. *Nippon Suisan Gakkaishi, 55*, 1635–1640.

Zar, J. H. (2010). *Biostatistical analysis.* Englewood Cliffs, N: J. Pearson Prentice Hall.