Ongrowing and enhancement of n-3 HUFA profile in adult *Artemia*: Short vs long-time enrichment

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Abstract

The use of adult *Artemia* is uncommon in aquaculture and limited to feeding adults of species not accepting inert diets. The aim of the present study was the development of a standardized technique for adult *Artemia* production in small volumes (25-100 L) and the assessment of ongrowing and enrichment diets for enhancement of n-3 HUFA content. We firstly assessed the effect of initial nauplii density (1, 2, and 3 nauplii mL⁻¹) and food dose (x1, x2, and x3 quantities of mixed *Tisochrysis lutea*, *Spirulina* and Prolon) for production of *Artemia*. Intermediate nauplii densities and food doses yielded the best overall results (44-46% survival; 5.6-5.9 mm length; 365-456 µg *Artemia*⁻¹). Secondly, four diets (mixtures of *Tisochrysis lutea*, *Phaeodactylum tricornutum*, *Spirulina* sp., Prolon and Red Pepper) were tested for ongrowing. Also, ten short-time enrichments (STE; 30 h) and a long-time enrichment (LTE; 9 days) were also assayed for n-3 HUFA enhancement. The improvement in n-3 HUFA was rather limited in STE. However, LTE on a mixture of *P. tricornutum*, Prolon and Red Pepper (regardless of further STE) in ongrowing *Artemia* yielded the highest contents in total fatty acids and in 20:4n-6, 20:5n-3, 22:5n-3 and 22:6n-3. The quality of LTE *Artemia* was based on an equilibrated dietary source of protein (*Spirulina*), 20:5n-3 (*P. tricornutum*) and 22:6n-3 (Red Pepper). STE *Artemia* had a quality similar or even higher than that of captured zooplankton and its use in our laboratory for the feeding of seahorses significantly enhanced breeding and newborn quality.

Abbreviations: AA – arachidonic acid; DHA – docosahexaenoic acid; EFA – essential fatty acid; EPA – eicosapentaenoic acid; FA – fatty acid; HUFA – highly unsaturated fatty acids; LTE – long-time enrichment; PCA – principal component analysis; STE – short-time enrichment

Keywords: *Artemia*, ongrowing, microalgae, enrichment, fatty acids, HUFA, seahorse.

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Introduction

Early developmental stages of *Artemia* are commonly used for feeding of various larval organisms, including fish and crustaceans. Most developed juveniles and adults in production systems successfully accept inert diets or formulated feed and hence ongrown adult *Artemia* is rarely used. However, for some organisms such as seahorses, require a supply of live or frozen zooplankton throughout the whole rearing system (Olivotto et al., 2011).

The nutritional quality of *Artemia* is limited (Léger et al., 1986), particularly in regards to n-3 HUFA. This is a concern as n-3 HUFA are essential to most marine vertebrates (Sargent et al., 1995, 2002). The natural diet of marine fish and crustaceans is rich in n-3 HUFA, commonly reflected in the fatty acids profile of eggs (Tocher and Sargent, 1984; Bruce et al., 1999; Tocher, 2003). Providing enhanced quality prey can enhance breeding and newborn quality in various species (Sargent et al., 1999; Mazorra et al., 2003; Tlusty et al., 2005; Lim et al., 2008; Ahn et al., 2011).

Production of high quality adult *Artemia* should ideally be based on both an effective ongrowing procedure and an efficient enrichment technique, based on mixtures of different ingredients (microalgae, commercial products). Generally, *Artemia* is ongrown in large tanks, suitable for supplying high quantities of biomass (Dhont et al., 1993; Lim et al., 2008). Some circumstances require much lower quantities and consequently, small culture volumes are more suitable for ongrowing. Factors such as cyst strain, temperature and food conversion efficiency strongly influence biomass production in proportion to initial nauplii density (Sorgeloos et al., 1980).

Young *Artemia* is n-3 HUFA deficient (Thinh et al., 1999; Vismara et al., 2003) and an issue with its enrichment is the retroconversion of DHA into EPA, occurring particularly in younger individuals (Navarro et al., 1999). Therefore, the nutritional value of adult *Artemia* is superior to that of nauplii or young stages (Léger et al., 1986). Hence, the enhancement of *Artemia* quality would be more efficient in adults, depending on the duration and type of enrichment applied.

This study describes a simple technique for the production of adult *Artemia* in small volume units and assesses the effect of several ongrown and enrichment diets (STE and LTE). A further purpose of the present study was the stable production of high quality *Artemia* to feed seahorse breeders maintained at our facilities (Planas et al., 2008, 2010). Results from the study were used to establish standard production and enrichment procedures in our facilities for the rearing and breeding of seahorses in captivity, which resulted notoriously enhanced.

Material and methods

Cultures of microalgae

The strains for the cultures of *Tisochrysis lutea* (former *Isochrysis galbana* T-Iso) and *Phaeodactylum tricornutum* were obtained from the culture collection of the Instituto de Ciencias Marinas de Andalucía (Cádiz, Spain) (CCMM *Isochrysis* aff. *galbana* T-ISO, strain 05/0401)

(Lubián and Yúfera, 1989) and the Cambridge Culture Collection of Algae and Protozoa (CCAP, strain 1052/6), respectively. Cultures were grown on Walne medium (Walne, 1966) at 28 °C, under a continuous light regime of about 60 $\mu\text{E m}^{-1} \text{s}^{-1}$ provided by daylight fluorescent tubes. Microalgae were firstly cultured in 5 L flasks followed by 70 L plastic bags and harvested during the middle stationary phase.

Production of adult Artemia

Initially, two experiments were run in duplicate to establish an experimental procedure for *Artemia* ongrowing. EG *Artemia* cysts (Inve, Belgium) were hatched (0.8 g L⁻¹) in 20 L seawater at 28 °C. Freshly hatched nauplii were filtered, washed, and transferred to 30 L methacrylate cylinders (20 cm diameter; 1 m height). The *Artemia* was cultivated for 15 days in 25 L of highly aerated seawater at 26 °C and 38 ppt salinity. Mixtures of the microalgae *Tisochrysis lutea*, Prolon (Inve, Belgium; 17% protein, 10% ash; 6% moisture) and freeze dried *Spirulina* (= *Arthrospira*) (Iberfrost, Spain; 60% protein, 8% ash, 7% moisture), were fed to the *Artemia*. The initial culture volume (10 L) was daily increased (5 L) from days 2-4 to reach a final and constant volume of 25 L. From day 5 onwards, the culture media was renewed daily (about 200% water exchange), early in the morning before feeding. This cultivation procedure was established according to previous experimental trials.

- Experiment I: Initial nauplii density

Three densities of *Artemia* nauplii were assayed: 25,000 (x1), 50,000 (x2), and 75,000 (x3) nauplii cylinder⁻¹ (1, 2, and 3 nauplii mL⁻¹, respectively). The general procedure and the food dose applied to treatment x1 are summarized in Table 1. From day 5 of culture, treatments x2 and x3 received double and triple the food dose used in treatment x1, respectively.

- Experiment II: Food daily ratio

According to the results achieved in Experiment I, the initial density of nauplii in this assay was established at 2 nauplii mL⁻¹ (50,000 nauplii cylinder⁻¹). The ongrowing procedure was the same as for Experiment I except for feeding ratios.

Three daily ratios of food (x1, x2 and x3) were evaluated. Since day 5 of culture, the dose of food supplied to treatment x2 was the same as for treatment x2 in Experiment I (Table 1), whereas treatments x1 and x3 received half and triple the amount of food offered in treatment x2, respectively.

In both experiments, NO₂, NO₃ and NH₃-NH₄ levels were checked using Sera Kits (Germany), both before and after each daily water exchange (200%). Total length (n= 30) and dry weight of *Artemia* (n= 100) were analysed at different time intervals. In Experiment II, fatty acids content in 15 d old *Artemia* was also analysed.

Artemia ongrowing and enrichment

Results from Experiments I and II were used to establish final culture conditions. Four ongrowing diets (A, B, C and D) were assayed in 100 L methacrylate cylinders (115 x 35 cm) for

18 days at 26 °C. The initial culture volume (50 L) was increased by 10 L per day (including microalgae volume addition) from days 2-5 to reach a final constant volume of 95 L. From day 5 onwards, the culture media was renewed daily (about 200% water exchange) through different mesh size (days 5-9: 250 µm; days 10-18: 500 µm), early in the morning before feeding.

The ongrowing diets consisted of different combinations of live microalgae (*T. lutea* or *P. tricornutum*), freeze dried *Spirulina*, Prolon (culture and enrichment diet from Inve, Belgium) and Red Pepper (enrichment diet from Bernaqua, Belgium; 6% protein, 3% ash, 68% moisture) as shown in Table 2. Diets A and B were based on diets x2 and x3 in Experiment II, respectively. The composition of diet D was the same as for diet C but included LTE; days 10 – 18). This consisted of an extra daily dose of Red Pepper (100 mg L⁻¹) supplied at 6 pm alongside the ongrowing diet.

Ten STE (A1-D10; Table 3) were carried out in 18 days-old *Artemia* cultivated on ongrowing diets A, B, C and D. For this, different combinations and doses of *T. lutea*, *P. tricornutum*, Prolon, Easy DHA Selco (enrichment diet from Inve, Belgium; 0% protein, 1% ash, 30% moisture), Red Pepper and 5% v/v *Artemia* Condition Enhancer (Inve, Belgium) were assayed. ACE (500 mg L⁻¹) was supplied 6 hours after the beginning of enrichment. For the enrichment, *Artemia* adults were collected early in the morning from the ongrowing units, then filtered, washed and transferred to 25 L units (200 *Artemia* L⁻¹) and enriched at 26 °C for 30 h. Enrichment diets were administered in three daily doses (0, 6 and 24 h).

Duplicate samples of *Artemia* were taken for dry weight, lipid content and fatty acids analysis. The samples were filtered, rinsed with tap water, transferred to cryotubes, lyophilised, weighed and maintained at -80 °C within a nitrogen atmosphere.

For the purpose of comparison with adult enriched *Artemia*, mixtures of wild mysidaceans *Leptomysis* spp. (70%) and *Siriella* spp. (30%) were captured twice in Toralla Island (Vigo, Spain) in late spring. These were either submitted to starvation for 5 days or enriched for 20 hours. The enrichment consisted of a first dose of Prolon (50 mg L⁻¹) and Red Pepper (125 mg L⁻¹) for 16 hours, and a second dose of Prolon (100 mg L⁻¹) and Red Pepper (250 mg L⁻¹) for 4 hours. Samples were taken in duplicate for lipid content and fatty acids analysis.

Lipids were extracted from samples of microalgae, adult *Artemia* and mysidaceans according to Bligh and Dyer (1959) as described in Valladares and Planas (2012). Aliquots of each algae culture of known concentration were centrifuged, resuspended with 0.5 M ammonium formate solution, freeze-dried and stored at -80 °C until further analyses. Total lipid content was quantified gravimetrically (Herbes and Allen, 1983). Fatty acid (FA) composition of lipids was analysed by gas-chromatography (GC) according to Christie (1982). Lipids were transmethylated (Lepage and Roy, 1986) and fatty acids analysed by GC (Perkin Elmer, Clarus 500 gas chromatograph) as described in Planas et al. (2010). Samples were analysed in duplicate.

Data analysis

Statistical analyses were performed using the software package Statistica 8.0 (StatSoft). The results are provided as mean $\pm$ SD. One-way analysis of variance (ANOVA) and post-hoc SNK test were used to check differences among treatments. Repeated measures ANOVA (RM-ANOVA) was used to check differences of LTE effect with time. A significance level of 0.05 was used in statistical tests. A principal component analysis was applied to FA data, which were mean centred and normalized to unit column standard deviation (SD) before calculation (Støttrup et al., 2013).

Results

Artemia cultivation procedure

The final ongrowing efficiency in Experiment I was not significantly affected by the initial density of *Artemia* nauplii (ANOVA, $p > 0.005$) except for total biomass yield (ANOVA, $p = 0.007$) (Table 4), which increased with increasing initial nauplii densities. Although not statistically significant, most parameters analysed were higher in treatment x2. For that reason, treatment x2 was selected to run Experiment II, in which the highest overall success was achieved in treatment x3, especially for *Artemia* dry weight, total biomass and ongrowing efficiency (ANOVA, $p < 0.05$) (Table 4). Treatment x1 yielded the worst results overall in both experiments.

Most mortality occurred on days 6-10 in Experiment I and 3-10 in Experiment II. In treatment x3 of Experiment II, a huge increase in the levels of NO_2 , NO_3 and $\text{NH}_4\text{-NH}_3$ (16.5, 40 and 0.27 mg L^{-1} , respectively) occurred during the last days of ongrowing, resulting in a further noticeable mortality. For these reasons, treatment x2 was selected for the studies on the effect of ongrowing diets and enrichments.

Total lipid content and FA profiles in adult *Artemia* from all treatments in both experiments did not differ significantly (ANOVA, $p > 0.05$). Total lipids ranged from 18.15 to 21.03% dry weight. The highest FA content corresponded to 16:0, 18:1n-7, 18:2n-6 and 20:5n-3, in decreasing order. DHA (22:6n-3) and n-3 HUFA content ranged between 4.95-10.97 and 19.8-30.1 mg g^{-1} , respectively.

Fatty acids in diets and adult Artemia

The FA composition of microalgae and commercial products used in the ongrowing and enrichment of *Artemia* is provided in Table 5. Dried *Spirulina* had the lowest lipid content and the most unbalanced FA profile, characterized by high levels in 16:0, 18:3n-6 and 18:2n-6, and by the absence of highly unsaturated fatty acids. Microalgae *P. tricornutum* and *T. lutea* showed high EPA and DHA levels, respectively. The former was also rich in 14:0, 18:1n-7 and 18:4n-3, and the latter was rich in saturates 16:0 and 16:1n-7.

The enrichers Prolon, Red Pepper and DHA Selco showed the highest n-3 HUFA content. Prolon was rich in DHA, 16:0, 18:1n-9 and to a lesser extent in EPA. Red Pepper was particularly rich in 16:0 and DHA. Finally, DHA Selco showed the most equilibrated FA profile with high content in 18:1n-9, DHA and 16:0.

Adult *Artemia* ongrown on tested diets (A–D) showed clear differences in the FA content, especially in n-3 HUFA (Table 6). *Artemia* fed on diets A and B did not differ significantly in lipid content and FA content. In both treatments, adult *Artemia* was rich in 16:0, 18:1n-9, 18:2n-6 and 20:5n-3. *Artemia* from diet C showed significantly increased content of 16:1n-7, 18:0 and 20:5n-3 compared to treatments A and B but showed lower levels of 18:2n-6, 18:3n-3, 18:4n-3, 20:4n-6 and 22:6n-3. Finally, the highest content of total lipids, total FA and n-3 HUFA were achieved in *Artemia* from diet D, due to a huge increase in 20:4n-3, EPA and especially in 22:5n-3 and DHA. Other FA content in *Artemia* fed on diets C and D was not statistically different. For these reasons, diet D was selected for the study on the evolution of FA content in *Artemia* submitted to LTE.

Regarding STE, treatments A1 and B2 (based on *T. lutea* and Prolon) contained the lowest total lipid and n-3 HUFA content (Table 7). A 2-fold increase of Prolon dose in both on-growing diets A and B and corresponding enrichments did not significantly increase the absolute content of fatty acids.

Enrichments C3 to C7 were conducted on *Artemia* previously cultured on diet C (Spirulina, *P. tricornutum* and Prolon) using different enrichment combinations of *T. lutea*, Prolon, Red Pepper and Easy DHA Selco. The FA content in enrichments A1, B2 and C3 were very similar, with small but significant increases mainly in 16:0, 16:1n-7, 18:2n-6, 18:3n-3 and EPA in the latter. In C4 – C7 *Artemia*, the FA contents were almost identical, with significantly higher n-3 HUFA (DHA, 22:5n-3 and EPA), 16:0 and 18:0 levels than in enrichments A1, B2 and C3. The most interesting difference among enrichments C4 – C7 was a slight increase in DHA in C4 *Artemia* (Prolon + Easy DHA Selco). Increasing levels of Red Pepper in enrichments C5 – C7 (Prolon + Red Pepper) did not result in substantially different fatty acid content.

Finally, D8 – D10 *Artemia* provided the highest levels in total lipids, saturates (16:0, 18:0), n-6 fatty acids (18:2n-6 and 20:4n-6), n-3 HUFA (EPA, 22:5n-3 and DHA), and DHA/EPA ratio. Increases in essential fatty acids (EFA) such as ARA, EPA and DHA were highly significant when compared to all other enrichments. Although differences among D8, D9 and D10 were scarce, the highest levels in EFA were achieved when Red Pepper was included in the enrichment (D10 and particularly D9).

Artemia ongrown for 18 days on diet D which included LTE between days 10 – 18, was analysed for changes in FA content over time (Figure 1). Total lipids increased from 20.7 at day 9 to 24.8, 26.9 and 26.8 mg g⁻¹ at days 12, 15 and 18, respectively. Fatty acids 16:1n-7, 18:0, 18:1n-7 and EPA remained constant during the whole LTE period but the content of all other fatty acids increased progressively until day 15 (6 days of LTE) and then decreased slightly until

day 18 (9 days of LTE). For most FA, significant statistical differences (RM-ANOVA) were found between days 9 and 12 but FA content at days 12, 15 and 18 were similar. However, total FA content was always highest at day 15. Subsequent STE using different combinations of Prolon and Red Pepper did not significantly increase FA content reached at day 15 (Fig. 1).

The score plots from a principal component analysis (PCA) on the FA content in adult *Artemia* revealed huge differences among diets and enrichments (Fig. 2). In the score plots (Fig. 2a, 2c), samples from diets A, B and C and their corresponding STE samples (right in the diagram), were clearly separated from the samples of *Artemia* from diet D submitted to LTE (left in the diagram). FA located to the left in the loadings plot (Fig. 2b, 2d) were those found in higher concentrations in both STE and LTE D-type *Artemia*, while those located to the right in the diagram corresponded to FA found in higher concentrations in ongrownn and STE *Artemia* from A, B and to a lesser extent, C treatments. The scores plot from the PCA on A- and B-type enriched *Artemia* (A1 and B2) were very similar, but the scores from the PCA on A- and B-ongrownn *Artemia* (Day 18 from diets A and B) resembled those from the PCA on C-type *Artemia*, both enriched (C4 - C7) and non-enriched (Diet C, day 18). The interpretation of the plots was supported by raw data (Tables 6 and 7), confirming a strong positive effect of LTE on n-3 HUFA content, particularly in EPA, DHA and ARA, together with increasing DHA/EPA and DHA/ARA ratios.

Fatty acids in wild mysidaceans

Total lipids in the mixture of wild mysidaceans (11.95%) were relatively stable both after the enrichment (13.49 – 14.15% dry weight) and during the 5-day starvation period (10.96 - 14.35% dry weight). Initially, EPA (14.47 mg g⁻¹) and DHA (13.13 mg g⁻¹) content was high compared to adult enriched *Artemia* previously ongrownn on diets A, B and C, but much lower than in D-type *Artemia*. Wild mysidaceans were also rich in 16:0 (13.65 mg g⁻¹) but poor in 18:1n-9 (6.20 mg g⁻¹) and 18:2n-6 (1.44 mg g⁻¹). DHA/EPA and DHA/ARA ratios were 0.91 and 5.54, respectively. As for total lipids, the FA profile and DHA/EPA and DHA/ARA ratios did not differ substantially after undergoing enrichment or starvation.

Discussion

In the present study, experiments were carried out to set up a standardized procedure for the small-volume production of high nutritional quality adult *Artemia* and to assess how diet and enrichment influenced the fatty acids profile in adult *Artemia*.

In the preliminary experiments for adult *Artemia* production, it was used a mixture of *T. lutea*, Prolon (Inve) and freeze dried *Spirulina*, considering different initial nauplii densities and food doses. *T. lutea* was selected as a basic ingredient of the diet, whereas *Spirulina* and Prolon were included in the diet due to their high protein content (Ciferri, 1983) and n-3 HUFA, respectively. In addition, *Spirulina* is very rich in the antioxidant astaxanthin (Tlustý et al., 2005).

The best conditions for the production of adult *Artemia* at 26°C in 25 L seawater were achieved with initial densities of 2 - 3 nauplii mL⁻¹ (Treatments x2 and x3 in Experiment 1, respectively) and a daily food dose equivalent to treatment x2 in Experiment I. Increasing initial nauplii densities and especially food ratios (Treatment x3 in Experiment II) would be feasible for a period of time but accompanied by rising levels of metabolic waste products (NO₂, NO₃ and NH₄-NH₃) and further crash of the culture. For these reasons, treatment x2 in both experiments was selected as the best option for culture. The overall performance of that culture conditions was as follows: 5.6 – 5.9 mm in length, 365 - 456 µg dry weight *Artemia*⁻¹, with 44-46% final survival. The final yield was very similar to that described by Dhont and Lavens (1996) for closed systems and low initial nauplii density. However, it was higher than those previously reported for smaller volumes and higher initial nauplii densities (Espinosa-Fuentes et al., 1997; Maldonado-Montiel and Rodríguez-Canché, 2005).

The nutritional quality of *Artemia* biomass produced in semi-intensive or super-intensive systems is analogous to natural produced biomass except for the lipid content and fatty acids profile (Léger et al., 1986; Dhont and Lavens, 1996). The final FA profile in adult *Artemia* is partially dependent on the composition of the diet (Léger et al., 1986; Vismara et al., 2003). In this study, increased feed ratios (Diet A vs diet B) did not result in differences on FA content. *Artemia* fed on diets A and B, based on *T. lutea*, Proton and *Spirulina*, reflected the low content in 16:1n7 of *T. lutea*, and the high content of 18:1n9 and 18:2n-6 in Proton and 18:3n6 (GLA; γ-linolenic acid) in *Spirulina*. GLA is a major fatty acid in many *Spirulina* strains, comprising more than 25% of total FA (Cohen et al., 1983). In addition, the content of EPA and DHA was low in *Artemia* fed on diets A and B, resulting in n-3/n-6 values lower than in *Artemia* fed on diets C and D.

Substituting the microalgae *T. lutea* (Diets A, B and C) for *P. tricornutum* (Diet D) improved the levels of 16:1n7 and EPA but reduced those of DHA. These changes were due to *P. tricornutum* being rich in 16:1n7 and deficient in DHA. Nevertheless, long-time incorporation of a compound highly rich in DHA (Diet D) drastically enhanced the DHA content in *Artemia* (from 4.37 ± 2.00 mg g⁻¹ on diet C to 18.84 ± 0.32 mg g⁻¹ on diet D). An important advantage of diet D compared to other diets and enriching mixtures tested was the enhancement of ARA (from 1.71 ± 0.31 up to 5.16 ± 0.19 mg g⁻¹ in *Artemia* fed on diets C and D, respectively), an essential FA that plays an important role in gonad maturation and newborn quality of some fish species (Sargent et al., 1995, 2002; Mazorra et al., 2003; Furuita et al., 2007). The EPA/ARA ratio also seems to be important during ovarian development (Mazorra et al., 2003).

The importance of DHA for marine fishes is reflected in its very high content in eggs (Anderson et al., 1990). Although some fish and crustaceans are capable of synthesizing DHA and EPA from 18:3n-3 (Kanazawa et al., 1979), this is not common in marine environments. In contrast to freshwater species, physiologically essential n-3 HUFAs (EPA and DHA) in marine fish or crustaceans need to be obtained from dietary sources as they cannot be synthesized *de novo* from 18:3n-3 (Sargent et al., 1995; Tocher, 2003). Accordingly, n-3 HUFA requirements in

marine fish (Sargent et al., 1989) would only be fulfilled by administering diets with adequate n-3 HUFA levels.

One of the main goals of recent research in optimizing live food has been focused on the enhancement of n-3 HUFA content, especially in *Artemia* nauplii and metanauplii, common prey in the feeding schedule of larval rearing of many marine species (Léger et al., 1986; Lavens and Sorgeloos, 2000). Pre-adult and adult *Artemia* are suitable for juveniles and adults of some marine species such as lobsters, shrimps or prawns, whose rearing is mainly based on live prey (Léger et al., 1986; Bengtson et al., 1991). Although the use of adult enriched *Artemia* would ensure the administration of controlled and relatively stable FA content, its use in marine rearing systems is uncommon due partially to economic reasons.

The use of the *P. tricornutum* (EPA source), Spirulina (Protein source) and Red Pepper (main DHA source) yielded *Artemia* rich in protein with an equilibrated proportion of EPA and DHA. The quality (n-3 HUFA content) of LTE *Artemia* was based on the equilibrium between the microalgae. Those ingredients provided *Artemia* quality similar Red Pepper was used in LTE experiments due to its high DHA/EPA ratio, though other similar commercially available products rich in n-3 HUFA would likely provide good results. LTE were carried out on *Artemia* juveniles based on daily increasing doses of Dry Selco (Inve, Belgium). At 7 days old, the *Artemia* contained n-3 HUFA levels (64.3 mg g⁻¹ dry weight) up to five times higher than those from STE, and EPA and DHA contents of 44.2 and 16.5 mg g⁻¹ dry weight, respectively (Léger et al., 1986; Dhont et al., 1993). These contents are higher than those achieved in this study for n-3 HUFA (50.05-58.03 mg g⁻¹ dry weight) and EPA (27.59-29.77 mg g⁻¹ dry weight) on STE and LTE D-type *Artemia*, but are lower than those for DHA (17.04 - 20.59 mg g⁻¹ dry weight). Such differences among both studies are very likely due to dissimilarities between FA profiles of enrichers, strain origin, and enrichment conditions (salinity, dissolved oxygen or temperature) that might also affect *Artemia* physiology and FA metabolism (Léger et al., 1987; Navarro et al., 1999).

In LTE *Artemia*, the inclusion of Red Pepper considerably enhanced the content of ARA, EPA and DHA, and DHA/EPA and DHA/ARA ratios. DHA/EPA ratio is considered a good indicator of prey quality, and according to Tocher and Sargent (1984), the optimal ratio is about 2:1. The highest DHA/EPA values were obtained with diet D submitted to enrichments D9 and D10 (0.69 – 0.71). However, Sargent et al. (1999) suggested an optimal level of 6.5 – 12.5% DHA. The use of LTE with diet D would ensure such levels and consequently this is the recommended treatment. According to the results of this study, the duration of LTE should be at least 3 days to ensure the best outcome.

Most adult fish and crustaceans are ongrownn on pellets or artificial diets but others like seahorses only accept live food. For the latter, adult *Artemia* can contribute significantly to partially or totally cover the daily food ratio. Mysidaceans are one of the most-used frozen organisms to feed several cultivated species. These organisms have an excellent nutritional value, partially due to their FA profile. Our analyses on *Leptomysis* and *Siriella* species showed

that they can remain alive for a few days without food, and still provide high levels of n-3 HUFA and excellent DHA/EPA ratios. Interestingly, LTE *Artemia* showed similar or even better FA profiles (higher DHA content) and ratios compared to mysidaceans, confirming their high nutritional value.

Compared to other prey, adult *Artemia* produced on optimized culture and enrichment procedures, whether used alone or as a supplement, have two main advantages. They have a relatively stable, known, biochemical composition, and are continuously available. Those facts are particularly important for our purposes since the final objective of this study was the stable production of high quality *Artemia* for the feeding of seahorse breeders maintained at our facilities (Planas et al., 2008, 2010). Adult seahorses are typically reared on live or frozen zooplankton (Olivotto et al., 2011). In our laboratory (IIM, CSIC), *Artemia* is the main prey for the feeding of seahorse breeders, being occasionally supplemented with wild caught mysidaceans. Breeders and ongrowing seahorses (*Hippocampus guttulatus*) were originally fed on STE *Artemia* (treatments A1 and B2). With that *Artemia* as unique food, seahorse growth was efficiently sustained but newborn quality was lower than expected. This low quality was reflected in the high rate of mouth malformations in newborn seahorses (Planas et al., 2009) and the deficient energetic status in early developing juveniles, resulting in very low initial survivals (Olivotto et al., 2011). Substituting STE with LTE *Artemia* drastically increased more than 3-folds the levels of total n-3 HUFA, EPA and DHA in eggs and newborn (unpub. data), and enhanced newborn quality whereas malformations were avoided, improving largely survival (e.g. up to 85% survival at day 30 in *H. guttulatus*) and growth rate in juveniles (Planas et al., 2012). Currently, LTE *Artemia* (enrichments D9 or D10) is routinely used in our facilities as the main enrichment procedure in *Artemia* for the ongrowing and breeding of three seahorse species (*H. guttulatus*, *H. abdominalis* and *H. reidi*). Furthermore, the application of the ongrownn technique to a 25 L cultivation unit ensures the production of adult *Artemia* biomass required to feed 30-60 seahorse breeders for one week (Quintas et al., 2007). We have not tested LTE *Artemia* in species other than seahorses. However, the scope of the present study and their applicability are not restricted to seahorses but also to other species demanding prey rich in n-3 HUFA. Also, the different enrichment procedures assayed would be also useful for experimental purposes requiring different and reliable fatty acids profiles in *Artemia*.

In summary, this study describes a simple and reliable technique for the production of adult *Artemia* in small volumes. It also shows the high quality of LTE *Artemia* (minimum 3 days; 6-9 days recommended), regardless of further STE. The end quality of the *Artemia* produced containing 5.16-5.94 mg ARA g⁻¹, 28.46-29.77 mg EPA g⁻¹ and 18.84-20.59 mg DHA g⁻¹). was similar to, or even higher than, that of wild mysidaceans.

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Table 1.

Culture conditions for the ongrowth of *Artemia* in treatment x1 of Experiment I. Treatments x2 and x3 received 2 and 3-folds the food dose supplied in treatment x1, including *T. lutea* addition from day 8 onwards). *T. lutea* culture was approx 8×10^6 cells mL^{-1} .

Days	<i>T. lutea</i> L	Proton mg L^{-1}	<i>Spirulina</i> sp. mg L^{-1}	Water Exchange / Mesh size	Volume L	Aeration
1	9 am: 3	-	-	-	10	Weak
2 - 4	9 am: 5				15 - 20 - 25	
5 - 7	9 am: 2.5	9 am: 5 6 pm: 10	9 am: 12.5 6 pm: 25	Flow rate: 6 L min^{-1} for 20 min Mesh: $250 \mu\text{m}$	25	Moderate
8 - 15		9 am: 10 6 pm: 20	9 am: 25 6 pm: 50	Flow rate: 10 L min^{-1} for 20 min Mesh: $500 \mu\text{m}$		High

Table 2.

Composition and daily doses of diets A, B, C and D for the on-growing of *Artemia* in 95 L seawater. * From day 10.

Days (Vol)	Time	Ongrowing diets			
		A	B	C	D
1 – 4 (60-90 L)	9 am	<i>Tisochrysis lutea</i> (5 L)		<i>Phaeodactylum tricornutum</i> (4-6 L)	
5 – 7 (95 L)	9 am	<i>Tisochrysis lutea</i> (3-4 L) <i>Spirulina</i> sp. (25 mg L ⁻¹) Prolon (10 mg L ⁻¹)		<i>Phaeodactylum tricornutum</i> (8 L)	
	6 pm	<i>Spirulina</i> sp. (50 mg L ⁻¹) Prolon (20 mg L ⁻¹)		<i>Phaeodactylum tricornutum</i> (4 L) <i>Spirulina</i> sp. (15 mg L ⁻¹) Prolon (6 mg L ⁻¹)	
		<i>Tisochrysis lutea</i> (3-4 L)			
8 – 18 (95 L)	9 am	<i>Spirulina</i> sp. (25 mg L ⁻¹) Prolon (10 mg L ⁻¹)	<i>Spirulina</i> sp. (50 mg L ⁻¹) Prolon (20 mg L ⁻¹)	<i>Phaeodactylum tricornutum</i> (8 L)	
	6 pm	<i>Spirulina</i> sp. (50 mg L ⁻¹) Prolon (20 mg L ⁻¹)	<i>Spirulina</i> sp. (100 mg L ⁻¹) Prolon (40 mg L ⁻¹)	<i>Phaeodactylum tricornutum</i> (4 L) <i>Spirulina</i> sp. (30 mg L ⁻¹) Prolon (12 mg L ⁻¹) Red Pepper (100 mg L ⁻¹)*	

Table 3.

Ingredients and doses used in STE *Artemia* (18 days) previously ongrownn on diets A, B, C and D (STE: A1 - D10). Tiso – *T. lutea*; P – Prolon; DHA Selco – Easy DHA Selco; RP – Red Pepper.

Diet of ongrowing	Short-time enrichment (STE)				
	Ingredients	Type	0 – 6 Hours	6 - 24 Hours	24 - 30 Hours
A	Microalgae + Prolon	A1	Tiso (0.2 L L ⁻¹) P (50 mg L ⁻¹)	Tiso (0.2 L L ⁻¹)	Tiso (0.6 L L ⁻¹)
B		B2	Tiso (0.2 L L ⁻¹) P (100 mg L ⁻¹)		
C			C3	Tiso (0.5 L L ⁻¹)	P (100 mg L ⁻¹)
	Prolon + DHA Selco	C4	P (100 mg L ⁻¹)	P (200 mg L ⁻¹)	DHA Selco (250 mg L ⁻¹)
	Prolon + Red Pepper	C5			RP (150 mg L ⁻¹)
		C6			RP (300 mg L ⁻¹)
		C7			RP (600 mg L ⁻¹)
	D	Prolon	D8	RP (250 mg L ⁻¹)	RP (500 mg L ⁻¹)
Red Pepper		D9			
		Prolon + Red Pepper	D10	P (50 mg L ⁻¹) RP (125 mg L ⁻¹)	P (100 mg L ⁻¹) RP (250 mg L ⁻¹)

Table 4.

The effect of initial nauplii density (Experiment 1) and food dose (Experiment II) on the final performance (survival, length, dry weight, total biomass and culture efficiency) in *Artemia* on-growing. Different letters (a – c) between treatments indicate significant differences (Post-hoc SNK Test, $p < 0.05$).

	EXPERIMENT I - Initial density (nauplii ml ⁻¹)				EXPERIMENT II - Food Dose			
	x1	x2	x3	p (ANOVA)	x1	x2	x3	p (ANOVA)
Survival (%)	64 ± 8 ^a	46 ± 8 ^a	47 ± 4 ^a	0.136	33 ± 4 ^a	44 ± 2 ^a	48 ± 8 ^a	0.117
Length (mm)	5.1 ± 0.6 ^a	5.6 ± 0.1 ^a	5.5 ± 0.5 ^a	0.591	4.6 ± 0.8 ^a	5.9 ± 0.1 ^{ab}	6.9 ± 0.3 ^b	0.045
Dry Weight (µg <i>Artemia</i> ⁻¹)	330 ± 87 ^a	456 ± 97 ^a	399 ± 6 ^a	0.369	210 ± 49 ^a	365 ± 66 ^a	610 ± 28 ^b	0.009
Total Biomass (g DW)	6.3 ± 1.0 ^a	12.6 ± 0.3 ^b	16.9 ± 1.8 ^c	0.007	4.1 ± 0.4 ^a	9.5 ± 1.3 ^b	17.5 ± 2.3 ^c	0.007
Efficiency (µg produced per initial nauplii)	202 ± 67 ^a	216 ± 39 ^a	199 ± 44 ^a	0.945	64 ± 4 ^a	139 ± 27 ^a	284 ± 3 ^b	0.002

Table 5.

Main fatty acids (mg g⁻¹) and lipid content (Dry weight percentage) in on-growing and enrichment diets (Two replicates). Main fatty acids are given in bold.

Fatty Acids	<i>Tisochysis lutea</i>	<i>Phaeodactylum tricornutum</i>	<i>Spirulina</i> sp.	Prolon	Red Pepper	DHA Selco
14:0	13.68	16.32	1.08	15.13	32.15	32.67
16:0	7.63	36.54	28.30	67.84	140.47	141.79
16:1n-7	4.91	59.15	2.19	18.66	3.89	41.16
18:0	0.69	9.02	0.76	17.17	8.75	38.87
18:1n-7	1.65	0.87	0.24	9.74	1.75	23.53
18:1n-9	14.68	14.60	1.11	56.06	23.96	165.41
18:2n-6	2.51	2.26	9.58	17.30	31.96	80.21
18:3n-3	6.15	0.95	-	3.90	1.50	18.13
18:3n-6	0.23	0.75	12.73	0.00	1.15	0.00
18:4n-3	16.07	0.12	-	4.32	1.81	8.15
20:1n-9	0.12	0.22	-	7.59	0.67	16.09
20:4n-3	0.21	0.48	-	2.61	2.63	6.34
20:4n-6 (ARA)	0.56	0.82	-	7.62	5.66	12.22
20:5n-3 (EPA)	0.79	35.55	-	31.19	8.55	55.20
22:5n-3	0.30	0.14	-	7.45	2.08	21.64
22:6n-3 (DHA)	11.73	0.77	-	86.48	120.6	152.18
Total FA	81.90	178.57	56.0	353.05	387.6	813.6
Saturates	22.00	61.89	30.13	100.15	181.4	213.3
Monounsaturates	21.36	74.84	3.54	92.05	30.3	246.2
Polyunsaturates	38.54	41.84	22.31	160.86	176.0	354.1
n-3 HUFA	13.02	36.94	-	127.72	133.8	235.4
n-3	35.23	38.01	-	135.94	137.2	261.6
n-6	3.30	3.82	22.31	24.92	38.8	92.4
n-3 / n-6	10.70	9.95	0.00	5.45	3.54	2.83
DHA / EPA	14.85	0.02	-	2.77	14.12	2.76
DHA / ARA	20.95	0.94	-	11.35	21.30	12.45
Lipids (%)	26.95	33.72	11.81	45.25	58.40	100

Table 6.

Fatty acid composition (mg g⁻¹) and total lipids (dry weight percentage) in adult *Artemia* cultivated on diets A, B, C and D. Different letters (a-c) between treatments indicate significant differences (ANOVA and post-hoc SNK Test, $p < 0.05$). Data are given as mean $\pm$ sd (Two replicates)

Fatty Acids	Diet							
	A		B		C		D	
14:0	4.37	$\pm$ 0.52 ^a	5.18	$\pm$ 1.32 ^a	5.24	$\pm$ 0.01 ^a	7.08	$\pm$ 0.22 ^a
16:0	26.58	$\pm$ 2.76 ^a	28.71	$\pm$ 1.56 ^a	26.12	$\pm$ 0.92 ^a	32.48	$\pm$ 0.52 ^a
16:1n-7	8.58	$\pm$ 1.13 ^a	9.95	$\pm$ 0.94 ^a	25.09	$\pm$ 0.31 ^b	23.33	$\pm$ 0.78 ^b
18:0	8.96	$\pm$ 0.19 ^a	9.24	$\pm$ 0.06 ^a	11.34	$\pm$ 1.31 ^b	11.26	$\pm$ 0.18 ^b
18:1n-7	7.59	$\pm$ 0.46 ^{ab}	8.27	$\pm$ 0.00 ^b	7.87	$\pm$ 0.09 ^{ab}	7.03	$\pm$ 0.13 ^a
18:1n-9	26.32	$\pm$ 2.62 ^a	28.88	$\pm$ 1.16 ^a	21.26	$\pm$ 3.77 ^a	24.51	$\pm$ 0.39 ^a
18:2n-6	16.95	$\pm$ 1.74 ^b	18.07	$\pm$ 0.50 ^b	11.03	$\pm$ 1.28 ^a	16.73	$\pm$ 0.30 ^b
18:3n-3	3.44	$\pm$ 0.04 ^{bc}	3.95	$\pm$ 1.18 ^c	1.83	$\pm$ 0.39 ^a	2.17	$\pm$ 0.02 ^{ab}
18:3n-6	6.46	$\pm$ 2.01 ^a	7.86	$\pm$ 0.60 ^a	4.84	$\pm$ 0.03 ^a	3.93	$\pm$ 0.15 ^a
18:4n-3	2.70	$\pm$ 0.33 ^b	3.15	$\pm$ 1.50 ^b	1.04	$\pm$ 0.11 ^a	1.55	$\pm$ 0.02 ^{ab}
20:1n-9	0.94	$\pm$ 0.31 ^a	1.16	$\pm$ 0.08 ^a	0.92	$\pm$ 0.08 ^a	0.77	$\pm$ 0.02 ^a
20:4n-3	0.53	$\pm$ 0.06 ^a	0.61	$\pm$ 0.11 ^a	0.53	$\pm$ 0.16 ^a	1.02	$\pm$ 0.00 ^b
20:4n-6 (ARA)	2.94	$\pm$ 0.08 ^b	2.89	$\pm$ 0.19 ^b	1.71	$\pm$ 0.31 ^a	5.16	$\pm$ 0.19 ^c
20:5n-3 (EPA)	14.22	$\pm$ 0.88 ^a	14.62	$\pm$ 0.99 ^a	21.18	$\pm$ 1.44 ^b	28.64	$\pm$ 0.21 ^c
22:5n-3	0.74	$\pm$ 0.11 ^a	0.86	$\pm$ 0.12 ^a	0.85	$\pm$ 0.44 ^a	5.65	$\pm$ 0.06 ^b
22:6n-3 (DHA)	7.88	$\pm$ 0.37 ^{ab}	8.83	$\pm$ 1.89 ^b	4.37	$\pm$ 2.00 ^a	18.84	$\pm$ 0.32 ^c
Total FA	139.20	$\pm$ 11.0 ^a	152.20	$\pm$ 12.20 ^a	145.24	$\pm$ 9.61 ^a	190.10	$\pm$ 2.32 ^b
Saturates	39.91	$\pm$ 3.46 ^a	43.13	$\pm$ 2.94 ^a	42.71	$\pm$ 0.40 ^a	50.81	$\pm$ 0.91 ^b
Monounsaturates	43.43	$\pm$ 4.53 ^a	48.27	$\pm$ 2.17 ^a	55.15	$\pm$ 3.91 ^a	55.64	$\pm$ 1.32 ^a
Polyunsaturates	55.87	$\pm$ 3.05 ^a	60.85	$\pm$ 7.08 ^a	47.38	$\pm$ 6.11 ^a	83.69	$\pm$ 0.09 ^b
n-3 HUFA	23.38	$\pm$ 0.34 ^a	24.92	$\pm$ 3.11 ^a	26.93	$\pm$ 4.04 ^a	54.15	$\pm$ 0.17 ^b
n-3	29.52	$\pm$ 0.63 ^a	32.02	$\pm$ 5.79 ^a	29.80	$\pm$ 4.55 ^a	57.87	$\pm$ 0.17 ^a
n-6	26.36	$\pm$ 3.67 ^b	28.83	$\pm$ 1.29 ^b	17.58	$\pm$ 1.56 ^a	25.82	$\pm$ 0.26 ^b
n-3 / n-6	1.12	$\pm$ 0.18 ^a	1.11	$\pm$ 0.15 ^a	1.69	$\pm$ 0.11 ^b	2.24	$\pm$ 0.03 ^c
DHA / EPA	0.55	$\pm$ 0.06 ^b	0.60	$\pm$ 0.09 ^b	0.20	$\pm$ 0.08 ^a	0.66	$\pm$ 0.02 ^b
DHA / ARA	2.68	$\pm$ 0.20 ^a	3.06	$\pm$ 0.46 ^a	2.50	$\pm$ 0.72 ^a	3.65	$\pm$ 0.07 ^a
Lipids (%)	20.61	$\pm$ 0.89 ^a	21.03	$\pm$ 1.30 ^a	21.44	$\pm$ 2.44 ^a	26.85	$\pm$ 0.06 ^b

Table 7.

Fatty acid composition (mg g⁻¹) and total lipids (dry weight percentage) in adult *Artemia* submitted to STE (A1 to D10) (Letters A, B, C and D indicate the type of ongrowing diet). Different letters (a-g) between treatments indicate significant differences (ANOVA and post-hoc SNK Test, $p < 0.05$). Standard deviations are not shown for clarity (n=2).

Fatty Acids	<i>Artemia</i> enrichment									
	A1	B2	C3	C4	C5	C6	C7	D8	D9	D10
14:0	3.94 ^a	3.81 ^a	7.47 ^d	5.14 ^b	5.24 ^b	5.16 ^b	5.04 ^b	6.34 ^c	7.68 ^d	7.35 ^d
16:0	14.18 ^a	15.16 ^a	20.99 ^b	27.96 ^c	27.42 ^c	27.02 ^c	26.34 ^c	31.69 ^d	37.09 ^f	35.59 ^e
16:1n-7	5.93 ^a	6.36 ^a	13.45 ^b	21.33 ^{cd}	22.27 ^d	22.10 ^{cd}	21.58 ^{cd}	20.71 ^c	23.78 ^e	22.27 ^d
18:0	6.50 ^a	6.93 ^a	7.20 ^a	12.00 ^d	10.24 ^{bc}	10.38 ^{bc}	9.91 ^b	10.95 ^{cd}	11.91 ^e	11.34 ^{de}
18:1n-7	6.11 ^a	6.40 ^a	7.61 ^c	8.29 ^d	7.60 ^c	7.47 ^c	7.28 ^{bc}	6.94 ^b	7.54 ^c	7.21 ^{bc}
18:1n-9	22.52 ^a	23.49 ^a	25.92 ^b	27.30 ^{bc}	27.00 ^{bc}	26.38 ^{bc}	25.81 ^b	26.85 ^{bc}	28.00 ^c	27.79 ^c
18:2n-6	9.56 ^a	10.55 ^b	14.00 ^b	13.75 ^b	14.21 ^b	13.99 ^b	13.70 ^b	18.15 ^c	19.69 ^d	19.68 ^d
18:3n-3	4.51 ^c	4.58 ^c	6.04 ^d	2.57 ^{ab}	2.56 ^{ab}	2.54 ^{ab}	2.45 ^{ab}	2.64 ^b	2.38 ^a	2.48 ^{ab}
18:3n-6	3.24 ^a	3.37 ^a	5.02 ^d	4.08 ^c	3.81 ^{bc}	3.86 ^{bc}	3.73 ^b	3.24 ^a	3.86 ^{bc}	3.56 ^{ab}
18:4n-3	8.20 ^{de}	7.63 ^d	8.68 ^e	1.47 ^b	1.25 ^a	1.24 ^a	1.21 ^a	1.50 ^{bc}	1.64 ^c	1.53 ^{bc}
20:1n-9	0.71 ^{ab}	0.78 ^{bc}	0.62 ^a	1.65 ^e	1.05 ^d	1.08 ^d	1.05 ^d	0.99 ^d	0.82 ^c	0.79 ^{bc}
20:4n-3	1.18 ^d	1.21 ^d	1.14 ^d	0.87 ^{ab}	0.78 ^a	0.78 ^a	0.73 ^a	0.98 ^{bc}	1.10 ^{cd}	1.06 ^{cd}
20:4n-6 (ARA)	2.08 ^{ab}	2.27 ^{bc}	2.66 ^d	2.47 ^{cd}	2.13 ^{ab}	1.95 ^a	1.95 ^a	4.73 ^e	5.94 ^g	5.51 ^f
20:5n-3 (EPA)	10.00 ^a	11.41 ^a	13.51 ^b	23.99 ^d	22.37 ^{cd}	22.45 ^{cd}	21.64 ^c	27.59 ^e	29.77 ^f	28.46 ^{ef}
22:5n-3	0.53 ^a	0.69 ^a	0.51 ^a	1.83 ^c	1.38 ^{bc}	1.38 ^{bc}	1.37 ^b	4.45 ^d	6.56 ^e	6.07 ^{de}
22:6n-3 (DHA)	4.68 ^a	4.94 ^a	5.12 ^a	11.96 ^c	8.65 ^{bc}	8.86 ^{bc}	8.46 ^b	17.04 ^d	20.59 ^e	20.23 ^e
Total FA	103.88 ^a	109.57 ^a	139.94 ^b	166.67 ^d	157.96 ^{cd}	156.65 ^{cd}	152.23 ^c	184.78 ^e	208.37 ^g	200.91 ^f
Saturates	24.62 ^a	25.90 ^a	35.65 ^b	45.10 ^d	42.89 ^{cd}	42.56 ^{cd}	41.28 ^c	48.98 ^e	56.68 ^g	54.28 ^f
Monounsaturates	35.27 ^a	37.03 ^a	47.61 ^b	58.56 ^{cd}	57.92 ^{cd}	57.04 ^{cd}	55.71 ^c	55.48 ^c	60.14 ^d	58.05 ^d
Polyunsaturates	43.99 ^a	46.64 ^a	56.68 ^b	63.01 ^c	57.15 ^b	57.05 ^b	55.23 ^b	80.33 ^d	91.55 ^f	88.58 ^e
n-3 HUFA	16.40 ^a	18.25 ^{ab}	20.29 ^b	38.66 ^d	33.18 ^c	33.46 ^c	32.20 ^c	50.05 ^e	58.03 ^g	55.83 ^f
n-3	29.11 ^a	30.46 ^a	35.01 ^b	42.70 ^c	36.99 ^b	37.24 ^b	35.85 ^b	54.20 ^d	62.05 ^f	59.83 ^e
n-6	14.88 ^a	16.19 ^b	21.68 ^d	20.31 ^c	20.16 ^c	19.81 ^c	19.38 ^c	26.13 ^e	29.50 ^g	28.75 ^f
n-3 / n-6	1.96 ^c	1.88 ^{bc}	1.61 ^a	2.10 ^d	1.83 ^b	1.88 ^{bc}	1.85 ^b	2.07 ^d	2.10 ^d	2.08 ^d
DHA / EPA	0.47 ^{cd}	0.43 ^{bc}	0.38 ^a	0.50 ^d	0.39 ^{ab}	0.39 ^{ab}	0.39 ^{ab}	0.62 ^e	0.69 ^f	0.71 ^f
DHA / ARA	2.25 ^a	2.18 ^a	1.92 ^a	4.86 ^e	4.06 ^{bcd}	4.54 ^{de}	4.34 ^{cde}	3.60 ^b	3.47 ^b	3.67 ^{bc}
Lipids (%)	16.33 ^a	16.71 ^a	20.24 ^b	23.50 ^c	23.43 ^c	24.01 ^c	22.46 ^c	26.94 ^d	26.98 ^d	27.34 ^d

FIGURE CAPTIONS

Fig. 1 Fatty acids content (mg g^{-1}) in ongrownn *Artemia* submitted to LTE for 3, 6 and 9 days (Days 9, 12, 15 and 18 of culture) and a subsequent STE on Prolon (D8), Red Pepper (D9) and Prolon + Red Pepper (D10).

Fig.2 Principal component analysis on fatty acids content in ongrownn (rectangles) and enriched (STE and LTE) adult *Artemia*: 2a and 2c: Scores plot (ongrowing and LTE and STE enrichment diets); 2b and 2d: Correlation loading plots (Fatty acids content). S: Saturates. M: Monounsaturates; P: Polyunsaturates.

FIGURE 1

FIGURE 2