Latin American Journal of Aquatic Research
E-ISSN: 0718-560X
lajar@ucv.cl
Pontificia Universidad Católica de Valparaíso
Chile

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Latin American Journal of Aquatic Research, vol. 42, núm. 1, marzo, 2014, pp. 150-159
Pontificia Universidad Católica de Valparaíso
Valparaiso, Chile

Lat. Am. J. Aquat. Res., 42(1): 150-159, 2014
DOI: 103856/vol42-issue1-fulltext-12

Research Article

Growth and survival of *Hippocampus erectus* (Perry, 1810) juveniles fed on *Artemia* with different HUFA levels

Nicolás Vite-Garcia[1,2,4], Nuno Simoes[2], Olivia Arjona[3]
Maite Mascaro[2] & Elena Palacios[3]
[1]Programa de Posgrado en Ciencias del Mar y Limnología
Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyoacán, Ciudad de México
[2]Programa de Investigación en Ecología y Cultivo de Especies Marinas de Ornato (PIECEMO)
Facultad de Ciencias, Universidad Nacional Autónoma de México, Sisal, Yucatán, México
[3] Programa de Acuicultura, Centro de Investigaciones Biológicas del Noroeste
Instituto Politécnico Nacional 195, Playa Palo de Santa Rita Sur, La Paz, BCS, C.P. 23096 México
[4]Universidad Juárez Autónoma de Tabasco, División Académica de Ciencias Agropecuarias
Villahermosa, Tabasco, C.P. 86280, México

ABSTRACT. Survival during first months after birth is one of the bottlenecks for consolidating the seahorse farming industry. In this work, *Artemia* metanauplii enriched with two highly unsaturated fatty acids (HUFA) rich commercial emulsions with different docosahexaenoic acid (DHA) levels (63% and 14% of total lipids), a vegetable oil with no DHA, and non-enriched *Artemia* as control, were used to feed 5-day-old juvenile *Hippocampus erectus* for 60 days. Enriched *Artemia* had similar levels of DHA (13% and 9%), despite great differences of DHA in the emulsions, with traces of DHA in non-enriched and vegetable oil enriched *Artemia*. More than 20% of DHA was found in 24 h starved juveniles fed both DHA-enriched treatments, similar to values in newly born juveniles, but those fed vegetable oil enriched *Artemia* or non-enriched *Artemia* had 5% of DHA. Total lipid and protein levels were similar in juveniles from the four treatments. The n-3/n-6 ratio was almost four-fold higher in seahorses fed DHA-enriched treatments compared to juveniles fed the non-enriched treatments. Survival of seahorses only partially reflected the DHA levels: it was lower in the vegetable oil treatment, similar in the seahorses fed *Artemia* with higher DHA and in the control treatment, and higher in seahorses fed the HUFA-enriched *Artemia* with lower DHA levels, although growth was similar in the two DHA-enriched *Artemia* treatments. Juvenile *H. erectus* seahorses perform better when they have at least 20% of DHA in their tissues, and these levels can be attained with no more than 14% of DHA in emulsions, eliminating the need for more expensive emulsions with higher DHA levels.

Keywords: *Hippocampus erectus,* seahorse, DHA, growth, fatty acids, survival, aquaculture.

Crecimiento y sobrevivencia de juveniles de *Hippocampus erectus* (Perry, 1810) alimentados con *Artemia* con diferentes niveles de HUFA

RESUMEN. La sobrevivencia durante los primeros meses de vida es uno de los cuellos de botella para la consolidación de la industria del cultivo del caballito de mar (*Hippocampus erectus*). En este trabajo se utilizaron metanauplios enriquecidos con dos emulsiones comerciales, con diferentes niveles de ácido docosahexaenóico (DHA) (63% y 14%), con aceite vegetal sin DHA, y metanauplios sin enriquecimiento como control, para alimentar caballitos de mar de 5 días de vida durante 60 días. La *Artemia* enriquecida tuvo niveles similares de DHA (13% y 9%), a pesar de las grandes diferencias en las emulsiones, con trazas de DHA en la *Artemia* no enriquecida y la enriquecida con aceite vegetal. Se obtuvo más de 20% de DHA en juveniles, muestreados con 24 h de ayuno, de los tratamientos con enriquecimiento de DHA, con valores similares a los de caballitos recién nacidos, pero los caballitos que se alimentaron con *Artemia* enriquecida con aceite vegetal y con *Artemia* no enriquecida tuvieron 5% de DHA. Los lípidos y proteínas totales fueron similares en los caballitos de los cuatros tratamientos. La razón n-3/n-6 fue casi cuatro veces mayor en caballitos alimentados con los tratamientos ricos en DHA comparados con los otros dos tratamientos. La sobrevivencia de caballitos de mar estuvo reflejada parcialmente por los niveles de DHA: fue mínima en caballitos del tratamiento de aceite vegetal, intermedia y similar en caballitos del tratamiento control y en los del tratamiento con enriquecimiento de HUFA (ácidos grasos altamente insaturados) con mayores niveles de DHA, y resultó máxima en caballitos del tratamiento enriquecido con HUFA con menores niveles de DHA.

Juveniles de *H. erectus* tienen un mejor desempeño cuando tienen en sus tejidos 20% de DHA, y estos niveles pueden ser alcanzados con no más de 14% de DHA en las emulsiones, eliminado la necesidad de utilizar emulsiones con mayores niveles de este ácido graso, que tienen un precio más elevado.

Palabras clave: *Hippocampus erectus*, caballito de mar, DHA, ácidos grasos, crecimiento, sobrevivencia, acuicultura.

Corresponding author: Nicolás Vite (nic.vite@gmail.com)

INTRODUCTION

Seahorses have low fertility, defined social structures (strict monogamy in many species), low mobility, small home ranges, limited distribution, and close association with habitat, traits that cause their populations to be highly vulnerable to human impact on the coastal zone they inhabit (Foster & Vincent, 2004; Vincent *et al.,* 2005). *Hippocampus erectus* populations in the Gulf of Mexico are affected mainly by-catch (Baum *et al.,* 2003), but also by habitat degradation, and capture for trade. Among the protective measures proposed for seahorse protection are trade restriction of wild specimens (included in Convention on International Trade in Endangered Species of Wild Flora and Fauna CITES appendix II), establishment of protection zones, environmental education for raising awareness of populations in coastal areas, and more recently, implementation of aquaculture programs. Seahorse culture can be a partial solution to replace wild animal capture and provide alternative economic opportunities to fishermen in developing countries (Payne & Rippingale, 2000; Job *et al.,* 2006; Koldewey & Martin-Smith, 2010). Substantial efforts have been made to develop a suitable technology to culture different species of seahorses worldwide (Wilson & Vincent, 1998; Woods, 2000; Job *et al.,* 2006; Lin *et al*., 2008; Planas *et al*., 2008), but one of the biggest bottlenecks for the development of the seahorse culture industry is still low survival of young seahorses, frequently attributed to inadequate nutrition of broodstock and brood.

Seahorses are gestated by the male. When they are born, they are referred to as juveniles and are an exact replica of the adult, which is a considerable advantage, because, contrary to most other cultured fish, newborn seahorses have a relatively large mouth; in the case of *H. erectus,* juveniles can be fed on *Artemia* metanauplii, avoiding the need of other live prey (Vite-García *et al.,* 2009). An additional advantage of *Artemia* metanauplii is that they can be easily enriched using oils and emulsions (Leger *et al.,* 1986). Enrichment of *Artemia* to feed juvenile seahorses is important, because it is not known if juvenile seahorses can synthesize long-chained highly unsaturated fatty acids (HUFA) from shorter precursors (PUFA: polyunsaturated fatty acids). Most marine fish studied so far cannot synthetize HUFA, and freshwater fish that can, are not able to in early stages of larval development (Sargent *et al.,* 2002). *H. hippocampus* broodstock fed a docosahexaenoic acid (DHA, 22:6n-3) depleted diet produce newborns that have 7% of DHA, compared to 16% in broodstock fed mysids with more DHA (Otero-Ferrer *et al*., 2012). Newly born seahorse have high levels of HUFA, particularly DHA with levels above 20% relative to total fatty acids in *H. kuda* (Saavedra *et al*., 2013) and levels of 16-17% for *H. guttulatus* (Faleiro & Narciso, 2011, 2013). These high DHA levels decrease as juveniles grow if given a DHA-depleted diet, as is the case with non-enriched *Artemia* nauplii, and this decrease has been associated to a decrease in survival (Chang & Southgate, 2001; Shapawi & Purser, 2003; Wong & Benzie, 2003), with the exception of juvenile *H. guttulatus*, which did worse when fed with enriched *Artemia* nauplii (Palma *et al*., 2011). Consequently, most studies use enriched *Artemia* metanauplii as a standard for seahorse juvenile culture (Chang & Southgate, 2001; Lin *et al*., 2008; Olivotto *et al*., 2008; Hora & Joyeux, 2009; Otero-Ferrer *et al*., 2010). Woods (2003) found a significant better growth and survival in *H. abdominalis* fed diets containing 13 and 8% of DHA (relative concentration) compared with a diet having 4% of DHA. Chang & Southgate (2001) found juveniles grew more when fed *Artemia* metanauplii enriched with 8% of DHA, compared to 4 or 6% of DHA. No nutritional studies have been done for *H. erectus*, but since apparently the higher the DHA content in the emulsions used to enrich the *Artemia* metanauplii, the better the survival of juvenile seahorses of other species, we here tested an emulsion that has an excess DHA content and compared it to a standard commercial emulsion for fish. We also included an isolipidic treatment with no DHA to test if the positive effect of the emulsion was not a result of an increased caloric intake, and the traditions non-enriched *Artemia* metanauplii for comparison purposes to other studies.

MATERIALS AND METHODS

Origin of juveniles

Three wild pregnant male seahorses (*Hippocampus erectus*) were captured in Laguna de Chelem, Yucatán, México, (21°15´-21°17´N, 89°39´-89°48W) (under scientific license granted in official letter "SGPA/DGVS/03153/10" from SEMARNAT, Mexican Ministry of the Environment and Natural Resources). The seahorses were transferred (no more than 2 h after capture) to Unidad Multidisciplinaria de Docencia e Investigación (UNAM), Sisal, Yucatán, México. Until the time of parturition (1-5 days), pregnant seahorses were kept in 100-L glass aquaria (70H×50L×30W cm) with a recirculation system. Temperature was maintained at 26 ± 2°C, salinity at 33-36, pH at 8.1-8.3, NO_2^- < 0.3 mg L^{-1}, NO_3^- < 5 mg L^{-1}, NH_3/NH_4^+ < 0.1 mg L^{-1}, and 12:12 photoperiod. Aquaria were cleaned daily. Inside the tanks, a polypropylene rope structure was provided for seahorse attachment. Adults were fed *ad libitum* twice a day on live adult *Artemia* with a typical fatty acid composition, high in eicosapentaenoic acid (EPA) (18.4%), 18:1n-7 (15.7%), 16:1n-7 (13.4%), 18:0 (11.0%), 16:0 (10.7%), 18:2n-6 (5.4%), 18:3n-3 (3.4%), and arachidonic acid (ARA) (2.5%), and no DHA.

Three broods (average 370 juveniles per brood) from three males (average height = 141.3 ± 5.75 mm) were used, born with a 3-day difference between the first and the last brood. At the time of birth juveniles were 11.3 ± 0.2 mm (height), and the relative fatty acid composition at birth was (only values above 1% are shown): 16:0 = 11.9 ± 0.82, 18:0 = 9.0 ± 0.6, 16:1n-7 = 2.4 ± 0.3, 18:1n-9 = 6.0 ± 2.9, 18:1n-7 = 4.3 ± 0.3, 18:2n-6 = 1.6 ± 0.3, ARA = 8.8 ± 0.6, EPA = 7.1 ± 1.1, and DHA = 22.9 ± 2.7. Total saturated fatty acids (SFA) = 28.9 ± 1.2, monounsaturated fatty acids (MUFA) = 21.1 ± 2.1, and polyunsaturated fatty acids (PUFA) = 48.7 ± 1.4. The n-3/n-6 ratio was = 2.6 and DHA/EPA ratio was = 3.3. No differences between newly-born juveniles sampled from the three broods were obtained for individual fatty acid composition.

Each brood was placed individually in 100-L tanks with the same conditions as described for the broodstock. From birth until the beginning of the experiment (4-7 days), juveniles were given enriched *Artemia* metanauplii with Selco emulsion (see below) three times a day; survival of the three broods was over 90% from birth until the beginning of the experiment.

Experimental design

For the experiment three dietary treatments and a control were used to test survival and growth of juvenile seahorses. It is well known that although DHA levels were high in the emulsions, they tended to decrease in the enriched metanauplii, probably a due to fatty acid retroconversion (Navarro *et al*., 1999; Han *et al*., 2001; Palacios *et al*., 2004). Since we wanted to further increase DHA levels supplied to juvenile seahorses, we used commercial oil designed for human consumption containing more than 60% of DHA, compared to 10-15% of DHA generally present in the emulsions designed for fish. The control treatment consisted on *Artemia* metanauplii without enrichment. Two high-HUFA commercial emulsions with different DHA levels were used for metanauplii enrichment on treatments, and one more emulsion without HUFA was used: (1) DHA Protein Selco® INVE, Belgium (developed for marine fish), hereinafter referred to as Selco, which had: ARA = 0.7%, EPA = 7.2%, DHA = 14.4%; (2) EPAX1050TG EPAX® Norway, AS (designed to increase omega 3 in humans), hereinafter referred to as EPAX which had: ARA = 9.2%, EPA = 18.2%, DHA = 63.3%; (3) A treatment consisting in canola oil, rich in omega 3 and 6 PUFA, specifically 18:2n-6 = 20% and 18:3n-3 = 9% but no omega 3-HUFA, hereinafter referred to as Canola. Canola oil and EPAX were emulsified in seawater using a blender. *Artemia* metanauplii were enriched twice (24 h after hatching and 24 h later) and harvested at 26 h. This double enrichment was found to be the best in order to retain HUFA in metanauplii in preliminary tests where several enrichment times were compared. *Artemia* samples from the four diets were taken for biochemical and fatty acid analyses every week during the experiment.

For the experiment, two interconnected systems with six-100 L tanks each were used (total 12 tanks). Aquarium conditions were the same as described for the broodstock. From each brood (n = 3), 240 juvenile seahorses were distributed in four tanks (60 seahorses each) with a different dietary treatment; thus, the three broods were taken as replicates of each dietary treatment. Seahorses were fed twice a day at 10:00 and 17:00 h, with 10 metanauplii mL^{-1}. Leftover food and feces were collected daily with a siphon. Dead juveniles were registered daily. A sample of 20 juveniles from each brood was measured (height and weight) prior to the experiment, and then a sample of 20 seahorses were measured after 15 and 30 days; all surviving seahorses were measured at 60 days. Considering measurements provided by Foster & Vincent (2004), height (HT) was chosen to use instead of the standard length to minimize stress on the fishes. The Fulton´s condition index K was calculated as follows: K = 100×WW (g)/HT (cm^3) (Zhang *et al.,* 2011).

For fatty acid and biochemical analyses, 30 juveniles from each brood were sampled prior the experiment, and five juveniles from each treatment and brood were sampled at the end of the experiment (4 diets × 3 broods = 12 individual samples of 5 seahorses), and then kept at -80°C until the biochemical and fatty acid analyses were performed. Before being sampled, seahorses were starved for 24 h to make sure they had their guts empty. The experiment was concluded after 60 days. The initial fatty acid composition (day 0) is shown in Table 3. No differences between juveniles sampled from the three broods were obtained for individual fatty acid composition.

Biochemical and fatty acid analyses

The biochemical and fatty acid analyses of *Artemia* metanauplii and juvenile seahorses were done at CIBNOR-La Paz, México. Both biochemical and fatty acid analyses, juveniles sampled at the start of the experiment were analyzed pooling five juveniles in a sample, in order to attain an adequate weight for the tests; juveniles after 60 days were analyzed individually. Total proteins, carbohydrates, and lipid analyses were performed in lyophilized samples, and data are referred to as mg g^{-1} dw. These analyses were done according with the methodology described in Palacios *et al.* (2000) and Vite-García & Saucedo (2008).

For fatty acid analyses frozen seahorses were opened longitudinally and cut into four transversal segments with a scalpel, placed in a glass vial, 6 mL Chloroform:methanol (2:1 v/v), 10 µL antioxidant (BHT), and 10 µL of 23:0 as internal standard were added to each sample. The segments were macerated directly in the solvent using a glass mortar. All the lipids were transesterified using boron-trifloride methanol (BF3 14% methanol, Supelco, Bellefonte, PA, USA), and analyzed in a Hewllet-Packard CG 6890-N gas chromatography equipped with a DB-23 (30 min length×0.25 mm inner diameter, 0.25 µm-thick-film) fused silica capillary column (J. & WScientific, Folsom, CA, USA), flame ionization detector (FID) at 280°C, helium as a carrier gas and a temperature gradient 110-220°C at 3°C min^{-1}. The identification of FAME was made by comparison to retention time of standards (Supelco, Bellefonte, PA, USA) (Palacios *et al.,* 2007).

Statistical analyses

For final survival (%), growth (mm), weight (mg), biochemical (lipids, proteins, carbohydrates, mg g^{-1} dw) and fatty acid (% of total fatty acids) composition of juveniles and *Artemia* metanauplii, one-way ANOVA was used to test significant differences among the four dietary treatments and a Tukey test for mean comparisons was performed when significant differences were found. All statistical analyses were made using Statistica™ v. 6.0.

RESULTS

Survival and growth of juvenile seahorses

Survival of juvenile seahorses fed the four treatments for 60 days is depicted in Figure 1a. A higher mortality was observed in the Canola treatment from the second week. A more gradual decrease was found for juveniles fed non-enriched metanauplii and EPAX treated metanauplii. The final survival after 60 days was significantly higher ($F = 5.22$; $P < 0.05$) for juveniles fed Selco (82%), followed by EPAX-enriched metanauplii (58%) and juveniles fed non-enriched metanauplii (49%), and the lowest for the Canola-enriched metanauplii (16%).

Condition index was higher in seahorses at the start of the experiment (0.38), ending after 60 days with 0.32 at the EPAX treatment and a minimum of 0.30 in seahorses fed Canola treatment (Fig. 1b). Seahorses' weight (Fig. 2a) at the start of the experiment was 17 ± 5 mg for all treatments, but weight was significantly different ($F = 106.3$; $P < 0.001$) by the end of the experiment with bigger juveniles fed Selco (249 ± 53 mg) and EPAX (231 ± 55 mg), followed by juveniles fed non-enriched metanauplii (141 ± 50 mg), and the smallest juveniles were obtained when fed Canola (109 ± 39 mg).

Height (Fig. 2b) followed a similar behavior with initial values of 16.4 ± 1.8 mm for all treatments, then significantly bigger juveniles were obtained by the end of the experiment when fed Selco (43.4 ± 0.2 mm) or EPAX (41.6 ± 0.6 mm) compared to significantly ($F = 76.45$; $P < 0.001$) smaller juveniles fed non-enriched metanauplii (35.4 ± 0.4 mm) and still smaller juveniles fed Canola (32.9 ± 0.1 mm).

Biochemical and fatty acid composition of metanauplii in the four treatments

Total protein in *Artemia* metanauplii was higher in non-enriched metanauplii and lower in Selco, with intermediate values for EPAX and Canola. Total carbohydrates were also higher in non-enriched metanauplii, with lower levels in EPAX, and intermediate levels in Selco and Canola. There were no significant differences between the total lipid content in Selco, Epax and Canola, but in the non-enriched metanauplii were significantly lower (Table 1).

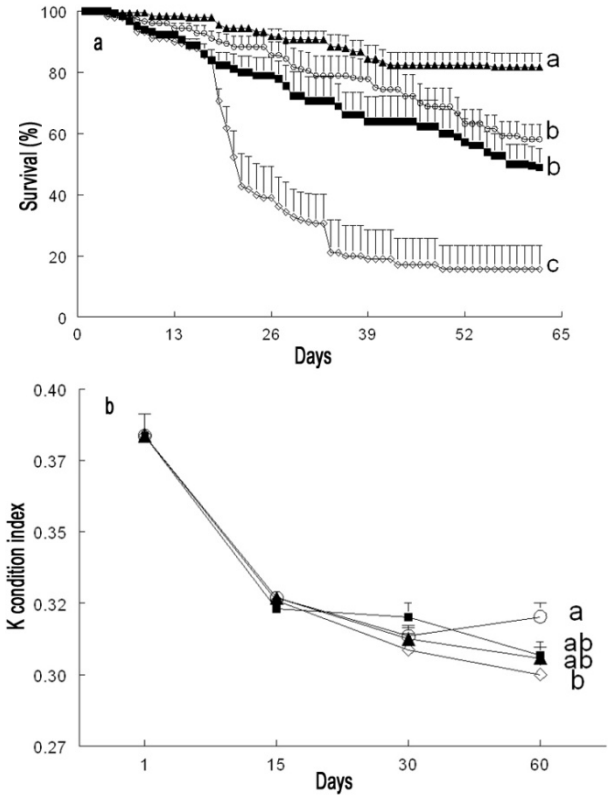

Figure 1. a) Survival (%), b) K condition index of *H. erectus* juveniles during treatment. Black squares: *Artemia* without enrichment; white rectangles: *Artemia* + Canola oil; black triangles: *Artemia* + Selco; white circles: *Artemia* + EPAX. Different letters are significantly different at 60 days ($P < 0.05$).

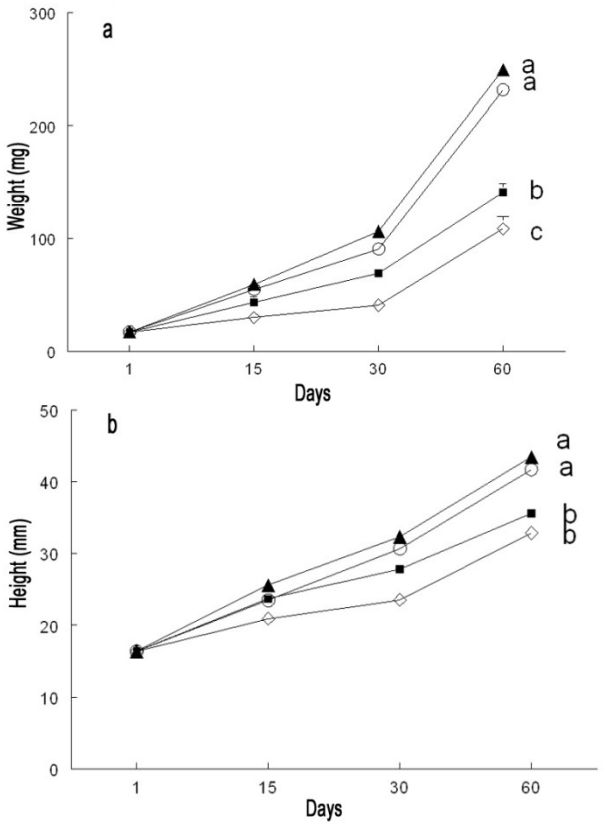

Figure 2. a) Weight (mg), b) height (mm) gain during treatment. Black squares: *Artemia* without enrichment, white rectangles: *Artemia* + Canola oil, black triangles: *Artemia* + Selco, white circles: *Artemia* + EPAX. Different letters are significantly different at 60 days ($P < 0.05$).

One of the work objectives to enrich *Artemia* metanauplii with DHA to levels above those reached with Selco enrichment was attained, as the EPAX had significantly higher levels of DHA (13.2%) compared to Selco (9%). Nevertheless, we expected the enrichment to be more pronounced, considering the very high levels of DHA in the EPAX oil (63% *vs* 14% in Selco). Canola and non-enriched metanauplii had traces of DHA (Table 2).

The EPA level was also higher in the EPAX (10.9%) compared to Selco (7.7%), and both were higher than in Canola (1.7%) or non-enriched metanauplii (1.4%). The level of ARA was significantly higher in Selco (1.6%) than in any other treatment (0.8-1.1%).

We expected the levels of 18:1n-9, 18:2n-6 and 18:3n-3 to be higher in the Canola, which was true for the 18:1n-9 (36.7%), almost double than that of the other treatments. The level of 18:2n-6 was also higher in the Canola and doubled the levels in the EPAX. However, the level of 18:3n-3 was higher in the non-enriched metanauplii (29.8% compared to 19.3% in Canola).

Biochemical and fatty acids composition of juveniles after 60-days treatment

After the 60 day-treatment, total protein and lipid levels in juveniles were not significantly different among treatments regardless of differences among enriched and non-enriched metanauplii (Table 1). However, total carbohydrates were significantly higher in the juveniles fed the Selco followed by the EPAX. The lowest carbohydrate levels were found in juveniles fed the non-enriched and the Canola.

All fatty acids analyzed in juvenile seahorses were affected by treatments at the end of the experimental period (Table 3). The level of DHA was four-fivefold higher in juveniles fed Selco and EPAX, compared to juveniles fed Canola or non-enriched metanauplii. In juveniles fed EPAX, EPA was significantly higher (7%) in comparison to the juveniles fed the Selco

Table 1. Biochemical (mg g^{-1} dw) levels (mean ± SE) in non-enriched and enriched *Artemia* metanauplii with canola oil, Selco, and EPAX (n = 9 samples per treatment), in juveniles at the start of the experiment, and in juveniles at the end of the experiment. Different letters in the same row indicate significant differences ($P < 0.05$) in metanauplii.

	Control	Canola	Selco	EPAX
Artemia metanauplii				
Carbohydrates	31.9 ± 10.9 a	21.8 ± 4.4 ab	20.2 ± 8.5 ab	17.9 ± 6.3 b
Proteins	393 ± 60.7 a	315 ± 37.8 ab	305 ± 54.5 b	338 ± 45.3 ab
Lipids	191 ± 48.4 b	234 ± 71.9 a	278 ± 88.7 a	218 ± 62.8 a
Juveniles initial (0 days)				
Carbohydrates	3.0 ± 0.2			
Proteins	324.3 ± 25.0			
Lipids	70.2 ± 2.3			
Juveniles final (60-days)				
Carbohydrates	4.8 ± 0.5 c	4.2 ± 0.6 c	8.8 ± 1.0 a	5.8 ± 0.5 b
Proteins	222.0 ± 17.3 a	176.6 ± 20.1 a	176.6 ± 12.5 a	191.0 ± 11.8 a
Lipids	63.5 ± 8.3 a	58.6 ± 11.8 a	74.9 ± 8.0 a	63.3 ± 4.3 a

Table 2. Fatty acid (% of total fatty acids) levels (mean ± SE) in non-enriched and enriched *Artemia* metanauplii with canola oil, Selco, and EPAX (n = 9 samples per treatment). Different letters in the same row indicate significant differences ($P < 0.05$) in metanauplii.

	Control	Canola	Selco	EPAX
14:00	0.7 ± 0.01 b	0.5 ± 0.01 c	1.3 ± 0.02 a	0.6 ± 0.01 c
16:00	12.3 ± 0.08 a	9.1 ± 0.20 b	11.9 ± 0.19 a	9.0 ± 0.06 b
17:00	0.8 ± 0.01 a	0.6 ± 0.02 b	0.8 ± 0.01 a	0.8 ± 0.01 a
18:00	7.7 ± 0.24 a	6.4 ± 0.21 b	5.8 ± 0.13 b	6.2 ± 0.21 b
20:00	0.2 ± 0.01 b	0.3 ± 0.02 a	0.2 ± 0.01 b	0.3 ± 0.01 a
22:00	0.2 ± 0.02 b	0.3 ± 0.03 b	0.1 ± 0.02 c	0.4 ± 0.01 a
24:00:00	0.1 ± 0.02 c	0.1 ± 0.02 c	0.7 ± 0.04 b	2.7 ± 0.04 a
16:1n-7	2.4 ± 0.06 b	1.7 ± 0.06 d	4.0 ± 0.04 a	2.0 ± 0.04 c
18:1n-9	22.2 ± 0.23 b	36.7 ± 0.66 a	21.6 ± 0.08 b	16.9 ± 0.15 c
18:1n-7	8.6 ± 0.07 a	7.6 ± 0.21 b	6.2 ± 0.15 d	6.8 ± 0.11 c
20:1n-9	0.9 ± 0.04 d	1.1 ± 0.02 c	2.0 ± 0.07 a	1.3 ± 0.02 b
18:2n-6	6.8 ± 0.04 c	10.5 ± 0.24 a	7.5 ± 0.04 b	4.5 ± 0.07 d
18:3n-6	0.5 ± 0.02 a	0.3 ± 0.02 b	0.3 ± 0.02 b	0.3 ± 0.02 b
18:3n-3	29.8 ± 0.31 a	19.3 ± 0.57 b	15.9 ± 0.44 c	18.5 ± 0.39 b
20:3n-3	1.1 ± 0.02 a	0.7 ± 0.02 c	0.7 ± 0.01 c	0.8 ± 0.01 b
20:4n-6	0.9 ± 0.13 b	0.8 ± 0.14 b	1.6 ± 0.08 a	1.1 ± 0.09 b
20:5n-3	1.7 ± 0.09 c	1.4 ± 0.08 c	7.7 ± 0.17 b	10.9 ± 0.28 a
22:6n-3	0.1 ± 0.01 c	0.1 ± 0.04 c	9.0 ± 0.43 b	13.2 ± 0.17 a
∑ SAT	22.1 ± 0.27 a	17.4 ± 0.44 c	20.8 ± 0.31 b	19.9 ± 0.21 b
∑ MUFA	35.1 ± 0.30 b	48.0 ± 0.45 a	34.7 ± 0.16 b	28.8 ± 0.20 c
∑ PUFA	41.3 ± 0.09 c	33.3 ± 0.43 d	42.8 ± 0.42 b	49.7 ± 0.18 a
n-3/n-6	3.9 ± 0.10 b	1.8 ± 0.05 d	3.5 ± 0.03 c	7.2 ± 0.12 a
DHA/EPA	0.1 ± 0.01 b	0.1 ± 0.02 b	1.2 ± 0.06 a	1.2 ± 0.04 a

(5%), and these levels were significantly higher compared to the juveniles from Canola (2%) or non-enriched metanauplii (3%). Hence, the DHA/EPA ratio was significantly higher in juveniles fed the Selco (4.8), followed by juveniles fed EPAX (3.4), with a much lower ratio for the other two treatments (2.1). The high DHA and EPA levels affected the total PUFA levels, which reached more than half of the total fatty acids, and the n-3/n-6 ratio, which was four-fold higher in juveniles fed the Selco and EPAX compared to the other two treatments.

In all treatments ARA relative concentration decreased at 60 days. Starting with 4.6% the lowest concentration occurred in EPAX (2%) and the highest

Table 3. Fatty acid (% of total fatty acids) levels (mean ± SE) in juvenile seahorses (n = 15 organisms per treatment) fed non-enriched and enriched *Artemia* metanauplii for 60 days. Different letters in the same row indicate significant differences ($P < 0.05$) among treatments.

	Initial (0 day)	Final (60 day-treatment)			
		Control	Canola	Selco	EPAX
14:00	0.32 ± 0.01	0.32 ± 0.03 b	0.30 ± 0.02 b	0.42 ± 0.03 a	0.29 ± 0.01 b
16:00	7.51 ± 0.11	8.67 ± 0.10 a	7.69 ± 0.07 b	6.85 ± 0.06 c	6.78 ± 0.13 c
17:00	0.91 ± 0.05	0.62 ± 0.00 a	0.53 ± 0.01 a	0.14 ± 0.09 b	0.62 ± 0.01 a
18:00	8.55 ± 0.22	9.67 ± 0.30 a	8.20 ± 0.44 b	6.19 ± 0.15 c	6.64 ± 0.19 c
20:00	0.30 ± 0.01	0.42 ± 0.02 a	0.38 ± 0.03 a	0.20 ± 0.01 b	0.23 ± 0.01 b
22:00	0.36 ± 0.02	0.46 ± 0.01 a	0.42 ± 0.01 ab	0.20 ± 0.05 c	0.33 ± 0.01 b
24:00:00	2.35 ± 0.13	1.65 ± 0.15 b	1.41 ± 0.17 b	1.41 ± 0.03 b	2.52 ± 0.04 a
16:1n-7	0.96 ± 0.03	1.07 ± 0.08 b	0.98 ± 0.06 b	1.44 ± 0.09 a	1.10 ± 0.03 b
18:1n-9	12.87 ± 0.37	15.61 ± 0.50 b	20.65 ± 1.06 a	13.28 ± 0.24 bc	12.62 ± 0.22 c
18:1n-7	4.91 ± 0.11	5.66 ± 0.36 a	6.31 ± 0.30 a	4.48 ± 0.11 b	5.51 ± 0.06 a
20:1n-9	0.68 ± 0.04	0.60 ± 0.08 c	0.93 ± 0.03 a	0.87 ± 0.03 ab	0.74 ± 0.02 bc
18:2n-6	4.62 ± 0.20	7.96 ± 0.37 b	9.99 ± 0.36 a	5.55 ± 0.13 c	4.12 ± 0.06 d
18:3n-6	0.22 ± 0.01	0.29 ± 0.03 ab	0.25 ± 0.02 a	0.19 ± 0.01 b	0.18 ± 0.00 b
18:3n-3	6.24 ± 0.43	11.86 ± 1.19 a	10.80 ± 1.16 a	8.42 ± 0.69 a	10.98 ± 0.45 a
20:4n-6	4.46 ± 0.14	3.20 ± 0.24 a	2.41 ± 0.21 bc	3.07 ± 0.08 ab	2.03 ± 0.05 c
20:5n-3	3.64 ± 0.19	2.56 ± 0.07 c	2.05 ± 0.04 c	5.35 ± 0.20 b	6.95 ± 0.17 a
22:6n-3	20.13 ± 0.55	5.29 ± 0.92 b	4.40 ± 0.71 b	25.74 ± 1.04 a	23.24 ± 0.79 a
∑ Sat.	20.62 ± 0.39	22.16 ± 0.39 a	19.24 ± 0.65 b	15.65 ± 0.17 c	17.62 ± 0.35 b
∑ MUFA	28.85 ± 0.25	33.05 ± 0.36 b	37.72 ± 0.45 a	26.03 ± 0.37 c	25.38 ± 0.42 c
∑PUFA	47.54 ± 0.40	41.29 ± 0.28 b	39.65 ± 0.17 b	55.08 ± 0.42 a	53.91 ± 0.69 a
n-3/n-6	22.07 ± 0.62	8.79 ± 0.87 b	8.08 ± 0.47 b	31.89 ± 1.75 a	33.27 ± 6.21 a
DHA/EPA	5.68 ± 0.37	2.09 ± 0.40 c	2.15 ± 0.35 bc	4.84 ± 0.27 a	3.35 ± 0.13 b

in juveniles from non-enriched treatment (3.2%). Juveniles fed the Canola treatment had significantly higher levels of 18:1n-9 and 18:2n-6, compared to the other treatments. The highest values of 18:1n-9 was also reflected in the total MUFA content in this treatment. Juveniles fed the non-enriched metanauplii had significantly higher levels of 16:0 and 18:0 and, therefore, higher levels of total saturated fatty acids (Table 3).

DISCUSSION

The objective of this work was to test the rearing performance (survival and growth) of juvenile seahorses by giving them different HUFA levels, particularly DHA. We usually use Selco emulsion with 14% of DHA (according to our analysis) for *Artemia* metanauplii enrichment, so to increase DHA levels we used EPAX, a commercial oil produced from raw Peruvian fish for human consumption with more than 60% of DHA, which was emulsified and given to *Artemia* metanauplii. Canola oil was used as an isolipidic treatment to enrich metanauplii, but devoid of HUFA. Contrary to our expectations, the DHA level in the metanauplii was not much higher when using EPAX (13%) compared to Selco (9%), although there was a significant difference between both. DHA is particularly difficult to increase in *Artemia* metanauplii as it has been previously reported; for example, Faleiro & Narciso (2011) managed to increase DHA levels in *Artemia* metanauplii to 4% when using Algamac 2000 emulsion containing 27% of DHA. This decrease of DHA in enriched *Artemia* metanauplii can be a result of retroconversion from DHA to EPA, which has been proposed before in *Artemia* (Navarro *et al.,* 1999; Han *et al.,* 2001). However, it could also be a result of the *Artemia* strain used, since different strains might present different retroconversion capacities, and of different enrichment methodologies, such as enriching twice, washing after the enrichment, light and temperature, etc. Using double enrichment and ICES emulsions with 9% DHA, we had previously obtained 13% of DHA in *Artemia* metanauplii (Palacios *et al.,* 2004). Part of the DHA in EPAX could have been lost by oxidation during emulsification and enrichment, since it is not designed as commercial emulsion to be used dissolved in water. In any case, we started with a

4-fold DHA in EPAX compared to Selco, and ended with 50% increase in DHA levels in metanauplii. As expected, Canola-enriched and non-enriched metanauplii had traces of DHA and less than 2% of EPA, but the three treatments with enriched metanauplii had similar lipid levels.

When comparing performance to biochemical and fatty acid composition of juveniles, we found that even if there were differences in the DHA proportion in metanauplii between Selco and EPAX, juveniles had similar DHA levels in their tissues in both treatments (23-26%). Interestingly, 23% of DHA was also what we found the newborn juveniles had before first feeding (see methodology). It is possible that DHA upper levels are regulated in juvenile *H. erectus* seahorse, as DHA is not further accumulated when given extra DHA in the diet. By contrast, in seahorses fed non-enriched metanauplii and Canola, DHA content had a strong decrease (more than 80%), till reaching 4-5% of total fatty acids. Faleiro & Narciso (2011) found similar levels of DHA in *H. guttulatus* juveniles at the beginning of the experiment (17%) compared to our levels in *H. erectus* (20%) in this study; DHA levels in *H. guttulatus* fell to 3-7% feeding Algamac-enriched *Artemia* with 4% of DHA after 30 days of experiment.

Lower levels of n-3 HUFA might be affecting growth, as juveniles fed non-enriched metanauplii or Canola were smaller; this is further supported by a significant correlation between DHA tissue levels of juveniles and weight (r = 0.89) and a lower correlation of EPA in tissues and weight (r = 0.78). Other studies have also concluded that higher DHA content has a positive effect on fish performance during early stages (Mourente *et al.,* 1993; Olivotto *et al.,* 2011; Trushenski *et al.,* 2012). On these stages there is a great energy demand because the rapid growth of fishes. DHA promotes a better development and function of motocondria, and a higher production of enzymes and coenzimes with a central position in the metabolism (Olivotto *et al.,* 2011; Yin & Tang, 2012).

In relation to survival, we found juveniles fed Canola that had 4% DHA started dying by the second week, and end of the experiment had the highest mortality, consistent with the lower condition index. The lowest survival for juveniles fed the Canola treatment could be observed from the second week of culture, which is consistent with the results for *Morone saxatilis*, where a mass mortality after two or three weeks of culture was observed but only in organisms fed low levels of HUFA in diets (Tuncer & Harrell, 1992). However, the juveniles fed non-enriched metanauplii also lacked HUFA, and juveniles were left with 5% of DHA after 60 days of treatment; nonetheless, survival was similar to the juveniles from the EPAX, that had 23% DHA. Therefore it is possible that Canola oil might have anti-nutritional factors that not only hamper growth (Manajan *et al.,* 1997; Ali *et al.,* 2009) but also survival, in spite of the loss of most anti-nutritional factors during canola oil commercial processing.

In contrast to growth, survival was not so strongly associated to DHA levels in juveniles ($r^2 = 0.44$; $F = 7.9$; $P < 0.05$) and no correlation was found to any other fatty acid or to the general biochemical composition of seahorse tissues. Juveniles fed Selco had excellent survival rates in spite of similar DHA levels (25%) with EPAX treated juveniles (23%). It should be pointed out that Selco is not solely composed of fatty acids, it is also enriched in vitamin C, amino acids, and phospholipids (according to the manufacturer), which is not the case of EPAX.

Selco-treated juveniles had more ARA (both in the diet and in the tissues), more carbohydrates and a higher DHA/EPA ratio compared to EPAX-treated juveniles that could account for some differences in survival. In *Sparus aurata* larvae, increasing levels of ARA from 0.1 to 1% in diets significantly improves survival (Bessonart *et al*., 1999). Has been discussed the importance of ARA with DHA and EPA by Sargent *et al*. (2002), who proposed that, in diets, the ratio DHA/EPA should also consider ARA levels, and be on the order of 8:1 EPA/ARA, which is very similar to that occurring in the Selco treatment. ARA can be a precursor for the synthesis of eicosanoids through the COX and LOX enzymes. These enzymes also used EPA as substrate to produce other eicosanoids, therefore, if ARA levels are too low in comparison with those of EPA, the synthesis of certain prostaglandins may be inhibited and biased in favor of eicosanoids derived from EPA, with reduced biological activity (Bransden *et al*., 2004). In this experiment the highest EPA/ARA ratio occurred in EPAX and this may have been detrimental to their survival.

Thus, juvenile *H. erectus* seahorses, which have above 20% of DHA in tissues, are expected to perform better during culture, particularly in reference to growth. This relative concentration in tissues could be attained with approximately 10% of DHA in *Artemia* metanauplii used as diet. Survival is less dependent on DHA levels and less dependent on EPA levels in tissues. In this research, the work was focused on the DHA role, because seems to be the most important HUFA for fish in early stages, further research is recommended regarding the role of EPA/ARA ratio in performance of seahorses rearing.

ACKNOWLEDGMENTS

The authors are grateful to M.Sc. Gemma Martínez M., technician in charge of Area Experimental de Ecología y Conducta, UMDI Sisal, where seahorses were maintained and cultured and to Diana Dorantes for English editing. This research was funded by projects PAPIIT No. IN208009 and SEP-CONACYT No. 101610 granted to M. Mascaró and SEP-CONACYT No. 4081 granted to Elena Palacios. Nicolas Vite-García is a doctoral CONACYT fellowship recipient, No. 42594.

REFERENCES

Ali, S., F. Anwar, S. Ashraf, F. Talpur & M. Ashraf. 2009. Evaluation of canola seeds of different cultivars with special emphasis on the quantification of erucic acid and glucosinolates. Grasas Aceites, 60: 89-95.

Baum, J.K., J. Meeuwig & A. Vincent. 2003. Bycatch of lined seahorses (*Hippocampus erectus*) in a Gulf of Mexico shrimp trawl fishery. Fish. Bull., 101: 121-131.

Bessonart, M., M. Izquierdo, M. Salhi, C. Hernández-Cruz, M. González & H. Fernández-Palacios. 1999. Effect of dietary arachidonic acid levels on growth and survival of gilthead sea bream (*Sparus aurata* L.) larvae. Aquaculture, 179: 265-275.

Bransden, M.P., J. Cobcroft, S. Battaglene, G. Dunstan, P. Nichols & J. Bell. 2004. Dietary arachidonic acid alters tissue fatty acid profile, whole body eicosanoid production and resistance to hypersaline challenge in larvae of the temperate marine fish, striped trumpeter (*Latris lineata*). Fish Physiol. Biochem., 30: 241-256.

Chang, M. & S. Southgate. 2001. Effects of varying dietary fatty acid composition on growth and survival of seahorse, *Hippocampus* spp., juveniles. Aquar. Sci. Conserv., 3: 205-214.

Faleiro, F. & L. Narciso. 2011. Prey-predator dynamics in seahorses (*Hippocampus guttulatus*): deciphering fatty acid clues. Aquacult. Res., 2011: 1-16.

Faleiro, F. & L. Narciso. 2013. The disadvantages of mating outside home: How breeding in captivity affects the reproductive success of seahorses? J. Sea Res., 78: 85-90.

Foster, S.J. & A. Vincent. 2004. Life history and ecology of seahorses: implications for conservation and management. J. Fish Biol., 65: 1-61.

Han, K., I. Geurden & P. Sorgeloos. 2001. Fatty acid changes in enriched and subsequently starved *Artemia franciscana* metanauplii enriched with different essential fatty acids. Aquaculture, 199: 93-105.

Hora, M.S. & J. Joyeux. 2009. Closing the reproductive cycle: growth of the seahorse *Hippocampus reidi* (Teleostei, Syngnathidae) from birth to adulthood under experimental conditions. Aquaculture, 292: 37-41.

Job, S.D., D. Buu & A. Vincent. 2006. Growth and survival of the tiger tail seahorse, *Hippocampus comes*. J. World Aquacult. Soc., 37: 322-327.

Koldewey, H.J. & K. Martin-Smith. 2010. A global review of seahorse aquaculture. Aquaculture, 302: 131-152.

Leger, P., D. Bengtson, K. Simpson & P. Sorgeloos. 1986. The use and nutritional value of *Artemia* as a food source. Oceanogr. Mar. Biol. Ann. Rev., 24: 521-623.

Lin, Q., J. Lin & D. Zhang. 2008. Breeding and juvenile culture of the lined seahorse, *Hippocampus erectus* Perry, 1810. Aquaculture, 277: 287-292.

Manajan, A., S. Otja & S. Bhaidwi. 1997. Imbibition induced changes in antinutritional constituents and functional properties of rapeseed (*Brassica campestris* var. *toria*) meal. FASEB J., 11: 1104.

Mourente, G., A. Rodriguez & J. Sargent. 1993. Effects of dietary docosahexaenoic acid (DHA; 22:6n-3) on lipid and fatty acid composition and growth in gilthead sea bream (*Sparusaurata* L.) larvae during first feeding. Aquaculture, 112: 79-98.

Navarro, J.C., R. Henderson, L. McEvoy, M. Bell & F. Amat. 1999. Lipid conversions during enrichment of *Artemia*. Aquaculture, 174: 155-166.

Olivotto, I., M. Avella, G. Sampaolesi, C. Piccinetti, R. Navarro & O. Carnevali. 2008. Breeding and rearing the longsnout seahorse *Hippocampus reidi*: rearing and feeding studies. Aquaculture, 283: 92-96.

Olivotto, I., M. Di Stefano, S. Rosetti, L. Cossignani, A. Pugnaloni, F. Giantomassi & O. Carnevali. 2011. Live prey enrichment, with particular emphasis on HUFAs, as limiting factor in false percula clownfish (*Amphiprion ocellaris*, Pomacentridae) larval development and metamorphosis: molecular and biochemical implications. Comp. Biochem. Physiol., 159: 207-218.

Otero-Ferrer, F., L. Molina, J. Socorro, R. Herrera, H. Fernández-Palacios & M. Izquierdo. 2010. Live prey first feeding regimes for short-snouted seahorse *Hippocampus hippocampus* (Linnaeus, 1758) juveniles. Aquacult. Res., 41: e8-e19.

Otero-Ferrer, F., L. Molina, J. Socorro, H. Fernández-Palacios & M. Izquierdo. 2012. Effect of different live prey on spawning quality of short-snouted seahorse, *Hippocampus hippocampus* (Linnaeus, 1758). J. World Aquacult. Soc., 43: 174-186.

Palacios, E., A.M. Ibarra & I.S. Racotta. 2000. Tissue biochemical composition in relation to multiple spawning in wild and pond-reared *Penaeus vannamei* broodstock. Aquaculture, 185: 353-371.

Palacios, E., A. Bonilla, A. Pérez, I.S. Racotta & R. Civera. 2004. Influence of highly unsaturated fatty acids on the responses of white shrimp (*Litopenaeus vannamei*) postlarvae to low salinity. J. Exp. Mar. Biol. Ecol., 299: 201-215.

Palacios, E., I.S. Racotta, B. Aparicio, O. Arjona & C. Martínez-Palacios. 2007. Lipid classes and fatty acids during embryogenesis of captive and wild silverside (*Chirostoma estorestor*) from Pátzcuaro Lake. Fish Physiol. Biochem., 33: 81-91.

Palma, J., D. Bureau & J. Andrade. 2011. Effect of different *Artemia* enrichments and feeding protocol for rearing juvenile long snout seahorse, *Hippocampus guttulatus*. Aquaculture, 318: 439-443.

Payne, M.F. & R.J. Rippingale. 2000. Rearing West Australian seahorse, *Hippocampus subelongatus*, and juveniles on copepod metanauplii and enriched *Artemia*. Aquaculture, 188: 353-361.

Planas, M., A. Chamorro, P. Quintas & A. Vilar. 2008. Establishment and maintenance of threatened long-snouted seahorse, *Hippocampus guttulatus*, broodstock in captivity. Aquaculture, 283: 19-28.

Saavedra, M., M. Masdeu, P. Hale & W. Holt. 2013. Dietary fatty acid enrichment increases egg size and quality of yellow seahorse *Hippocampus kuda*. Anim. Reprod. Sci. (in press).

Sargent, J.R., D. Tocher & J. Bell. 2002. The lipids. In: J. Halver & R. Hardy (eds.). Fish nutrition. Elsevier Science, New York, pp. 182-246.

Shapawi, R. & G.J. Purser. 2003. The value of enriched *Artemia* in supporting growth and survival of juvenile pot-bellied seahorses *Hippocampus abdominalis*. J. World Aquacult. Soc., 34: 533-541.

Trushenski, J., M. Schwarz, A. Bergman, A. Rombenso & B. Delbos. 2012. DHA is essential, EPA appears largely expendable, in meeting the n-3 long-chain polyunsaturated fatty acid requirements of juvenile cobia *Rachycentron canadum*. Aquaculture, 326: 81-89.

Received: 1 August 2013; Accepted: 18 December 2013

Tuncer, H. & R. Harrell. 1992. Essential fatty acid nutrition of larval striped bass *Morone saxatilis* and palmetto bass (*M. saxatilis* x *M. chrysops*). Aquaculture, 101: 105-121.

Vincent, A., K. Evans & A. Marsden. 2005. Home range behaviour of the monogamous Australian seahorse, *Hippocampus whitei*. Environ. Biol. Fish., 72: 1-12.

Vite-García, N. & P. Saucedo. 2008. Energy storage and allocation during reproduction of Pacific winged pearl oyster *Pteria sterna* at Bahía de la Paz, Baja California Sur, Mexico. J. Shellf. Res., 27: 375-383.

Vite-García, N., D. Alexandre, G. Martínez, M. Mascaró & N. Simoes. 2009. First feeding captive seahorse *Hippocampus erectus* (Perry, 1810) using *Artemia* and freshwater amphipods. WAS, Book of Abstracts, World Aquaculture. Veracruz, 25-29: 330 pp.

Wilson, M.J. & A. Vincent. 1998. Preliminary success in closing the life cycle of exploited seahorse species, *Hippocampus* spp., in captivity. Aquar. Sci. Conserv, 2: 179-196.

Wong, J.M. & J.A. Benzie. 2003. The effects of temperature, *Artemia* enrichment, stocking density and light on the growth of juvenile seahorses, *Hippocampus whitei* (Bleeker, 1855), from Australia. Aquaculture, 228: 107-121.

Woods, C.M. 2000. Improving initial survival in cultured seahorses, *Hippocampus abdominalis* Leeson, 1827 (Teleostei: Syngnathidae). Aquaculture, 190: 377-388.

Woods, C.M. 2003. Effects of varying *Artemia* enrichment on growth and survival of juvenile seahorses, *Hippocampus abdominalis*. Aquaculture, 220: 537-548.

Yin, F. & B. Tang. 2012. Lipid metabolic response, peroxidation, and antioxidant defence status of juvenile lined seahorse, *Hippocampus erectus*, fed with highly unsaturated fatty acids enriched *Artemia* metanauplii. J. World Aquacult. Soc., 43: 716-726.

Zhang, D., F. Yin & J. Lin. 2011. Criteria for assessing juvenile quality of the lined seahorse, *Hippocampus erectus*. Aquaculture, 322-323: 255-258.