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Fatty acid utilization during the early larval stages of Florida pompano (*Trachinotus carolinus*) and Common snook (*Centropomus undecimalis*)

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Abstract

The pattern of conservation and loss of fatty acids from the yolk sac during the endogenous feeding period and subsequent starvation was studied in pompano and snook larvae. Fundamental information on the early fatty acid dynamic and mobilization of pompano and snook larvae was collected. In both species, fatty acids were utilized as an energy source after hatching. Mono-unsaturated fatty acids were catabolized, while saturated and poly-unsaturated fatty acids were conserved. High levels of arachidonic acid (ARA) in pompano and snook eggs, as well as selective retention in the unfed larvae suggest a high dietary requirement for this fatty acid during the early stages of larval development. The effect of an ARA supplementation was therefore investigated in snook larvae at the rotifer feeding stage. The fatty acid profile of the larvae was successfully influenced to match that of wild eggs; however, no significant improvement in growth or survival was observed. Future research should be carried out over a longer period of time and include factors related to stress resistance.

Keywords: fatty acid, starvation, fish, larvae, arachidonic acid, enrichment

Introduction

In the wild, fish larvae feed mainly on copepods (Hunter 1981), which provide adequate nutrition and a large variety of prey sizes essential for successful development (Van der Meeren, Olsen, Hamre & Fyhn 2008). Nevertheless, more research is needed before mass copepod production can be economically viable and hatcheries have to rely on the traditional live preys: rotifers and Artemia (Stottrup 2000). Despite the convenience of their production, rotifers and Artemia have a main drawback which resides in their poor nutritional profile (Conceição, Yúfera, Makridis, Morais & Dinis 2010). Both prey types are deficient in essential polyunsaturated fatty acids (PUFAs), which have long been identified as critical nutritional factors in marine fish larval development and survival (Watanabe, Kitajima & Fujita 1983; Sargent, McEvoy & Bell 1997). Indeed, due to their inability to elongate and desaturate linolenic acid (18:3n-3) and linoleic acid (18:2n-6) to the PUFAs eicosapentaenoic acid (20:5n-3, EPA), docosahexaenoic acid (22:6n-3, DHA) and arachidonic acid (20:4n-6, ARA), marine fish depend solely on their diet to fulfil their requirements (Sargent, McEvoy, Estévez, Bell, Bell, Henderson & Tocher 1999; Tocher 2003).

Polyunsaturated fatty acid deficiencies strongly impact growth and normal development of fish larvae (Mourente, Rodriguez, Tocher & Sargent 1993; Watanabe 1993). DHA is highly concentrated the neural and visual cell membrane and a deficiency has been shown to impact visual development (Neuringer, Anderson & Connor 1988; Bell, Batty, Dick, Fretwell, Navarro & Sargent 1995; Benítez-Santana, Masuda, Juárez Carrillo, Ganuza, Valencia, Hernández-Cruz & Izquierdo 2007), skeletal development (Cahu, Zambonino Infante & Takeuchi 2003; Roo, Hernández-Cruz, Socorro, Fernández-Palacios, Montero & Izquierdo

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2009) and stress and immune responses (Montero, Kalinowski, Obach, Robaina, Tort, Caballero & Izquierdo 2003; Ganga, Tort, Acerete, Montero & Izquierdo 2006). EPA and ARA are both precursors of eicosanoids, and compete for the cyclo-oxygenases and lipoxygenases that generate the local hormones prostaglandins, thromboxanes and leukotrienes that are involved in the modulation of neural transmission, hypothalamic functions and many immune functions (Bell, Ashton, Secombes, Weitzel, Dick & Sargent 1996; Tocher, Bell & Sargent 1996; Sargent, McEvoy *et al.* 1999). Even though EPA is the main C20 PUFA in fish tissues, ARA seems to be the preferred substrate for eicosanoid production and the metabolites produced are of higher biological activity (Tocher *et al.* 1996). EPA competitively inhibits the production of eicosanoids from ARA therefore eicosanoid actions are determined by the ARA/EPA ratio in the tissues (Sargent, Bell, McEvoy, Tocher & Estévez 1999). Similarly, DHA and EPA compete for the formation of phospholipid structures with a higher biological value for DHA than EPA (Rodriguez, Pérez, Badía, Izquierdo, Fernández-Palacios & Lorenzo Hernandez 1998; Sargent, McEvoy *et al.* 1999; Glencross 2009). Determining the optimal DHA:EPA:ARA ratio has proven difficult, since changing the percentage of one PUFA alters the ratio of all three (Sargent, Bell *et al.* 1999). Considerable research has been carried out on cold and temperate water species, where a DHA/EPA ratio equal or greater than 2 has been considered adequate (Sargent, Bell *et al.* 1999). An ARA/EPA ratio close to 0.25 was found necessary for good growth and survival of gilthead sea bream *Sparus aurata* and European sea bass larvae *Dicentrarchus labrax* (Atalah, Hernández-Cruz, Ganuza, Benítez-Santana, Ganga, Roo, Montero & Izquierdo 2011; Atalah, Maria Hernandez-Cruz, Benitez-Santana, Ganga, Roo & Izquierdo 2011). It has been shown that an excess of ARA can cause malpigmentation in flatfish and an ARA/EPA ratio of 0.25 for turbot *Scophthalmus maximus*, Atlantic halibut *Hippoglossus hippoglossus* and Japanese flounder *Paralichthys olivaceus* and 0.5 for Senegalese sole *Solea senegalensis* gave the best percentage of normal pigmentation (McEvoy, Estevez, Bell, Shields, Gara & Sargent 1998; Estevez, Kaneko, Seikai, Tagawa & Tanaka 2001; Bell 2003; Villalta, Estévez, Bransden & Bell 2008). An ARA supplementation has proven beneficial in improving growth and survival in turbot (Bell, Castell *et al.* 1995; Bell, Batty *et al.* 1995; Castell, Bell, Tocher & Sargent 1994), gilthead sea bream (Koven, Barr, Lutzky, Ben-Atia, Weiss, Harel, Behrens & Tandler 2001) and striped bass *Morone saxatilis* (Harel, Gavasso, Leshin, Gubernatis & Place 2001). Bessonart, Izquierdo, Salhi, Hernández-Cruz, González and Fernández-Palacios (1999) have shown that, in gilthead sea bream, the effect of an ARA supplementation is enhanced if associated with a high DHA/EPA ratio, which highlights the necessity to consider FA proportions in addition to absolute amount (Sargent, McEvoy *et al.* 1999; Izquierdo, Socorro, Arantzamendi & Hernández-Cruz 2000).

On the other hand, little is known about the PUFA requirement of marine finfish from subtropical/tropical waters, as most of them are new emerging species in aquaculture. As a general trend, these species tend to have intermediate to high DHA and ARA levels and low EPA levels, leading to high DHA/EPA and ARA/EPA ratios (Gibson 1983; Fogerty, Evans, Ford & Kennett 1986; Faulk & Holt 2003; Ogata, Emata, Garibay & Furuita 2004; Yanes-Roca, Rhody, Nystrom & Main 2009). This higher ARA content suggests that larvae from tropical species may require more ARA than cold/temperate species to ensure normal development (Ogata *et al.* 2004; Faulk & Holt 2005).

Common snook and Florida pompano are species commonly found in the tropical and subtropical western Atlantic Ocean including the Gulf of Mexico and previous work has shown that eggs of both species have a high ARA/EPA ratio (Yanes-Roca *et al.* 2009; Main, Resley, Rhody, Nystrom, Stevens & Adams 2010). Since fish eggs contain all the essential nutrients required for the successful development of the embryo and the yolk-sac larvae, it is believed that their composition reflects the optimal first feeding diet (Heming & Buddington 1988). The study of the pattern of conservation and loss of fatty acids (FAs) from the yolk sac during the endogenous feeding period has proven a useful approach to investigate the requirements of fish larvae with the most critical FAs spared to the detriment of others catabolized as an energy source (Izquierdo 1996; Hamre, Yúfera, Rønnestad, Boglione, Conceição & Izquierdo 2013).

The aim of this study was to examine the changes occurring in the FA profiles of common snook and pompano larvae deprived of food after hatching to gain a better understanding of their FA requirements. In addition, an experiment was

carried out to investigate the effectiveness of an ARA supplementation in snook larvae at the rotifer feeding stage, to produce larvae with PUFA ratios close to that of natural eggs and to study the impact on early larval development.

Materials and methods

Starvation trial

Pompano broodstock were captured off of Florida's west coast and held inland in a zero-discharge recirculating system at the Mote Marine Laboratory Center for Marine and Freshwater Aquaculture Research, in Sarasota, Florida. Spontaneous spawning was obtained after photo-thermal conditioning in a 25 m^3 tank, and hormonal induction of mature females with sGnRHa (50 μg kg^{-1}). Fish were fed daily a 40% thread herring, 30% shrimp, 30% squid diet at 6.5% body weight during the conditioning period. Snook broodstock were collected in estuarine waters around Sarasota, Florida with seines in mid-July during the ambient spawning window. Capture and strip spawning procedures were similar to those described in Yanes-Roca *et al.* 2009.

Collected eggs from the captive pompano (fertilization rate = 54.3%) and wild snook (fertilization rate = 68.2%) broodstocks were placed into small conical tanks with aeration. Once the aeration was stopped, the buoyant fertilized eggs accumulated at the surface and the poor, unfertilized eggs were drained from the hatchers. The aeration was then turned back on and three 5 ml samples were taken and counted to estimate the egg concentration. Approximately 750 eggs were volumetrically stocked in sieves made of a 100 mm diameter PVC pipe sealed at one end with a 330 μm mesh. The sieves were set on a grid in a 340 L water table (salinity 35 g L^{-1} ± 1, dissolved oxygen 5 mg L^{-1} ± 1, pH 8.5 ± 0.5, temperature 27.5°C ± 1) equipped with a 25 W UV light and a 50 L min^{-1} pump. In addition, three egg aliquots were sampled from the conical tank, drained on a 100 μm sieve and rinsed with deionized water before storage at −70°C.

Larvae hatched after 24 h for pompano (hatch rate = 73.2%) and 18 h for snook (hatch rate = 96%). Three sieves were removed each day and the larvae anaesthetized with MS222 and carefully separated from egg casings and any dead eggs or larvae. The larvae from each sieve were pooled and rinsed with deionized water then stored at −70°C. The trials were stopped when no living larvae were observed in the sieves, at 5 days post hatch (DPH) for pompano, and 6 DPH for snook.

Live food trial

Two trials were run to study the effect of an ARA supplementation on snook larvae. Eggs for the first trial were obtained from captive broodstock captured off the west coast of Florida and held in a 45 m^3 tank at the Mote Marine Laboratory Center for Marine and Freshwater Aquaculture Research. Spawning was induced by photo-thermal conditioning and sGnRHa implantation (female only, 50 μg kg^{-1}). Fish were fed a 50% shrimp, 50% thread herring diet at 2.5% body weight every other day during the conditioning period. Fish spawned spontaneously (fertilization rate = 65.8%). Eggs for the second trial were collected in the wild in mid-August, using the technique described above (fertilization rate = 49.2%). In both trials, the eggs were stocked at 150 eggs L^{-1} in 130 L tanks in a recirculating system equipped with drum filtration, biological filtration, ozonation, carbon filtration, UV lights and an inline heater unit (temperature 27.5 ± 1°C, salinity 35 ± 1 g L^{-1}, dissolved oxygen of 6 ± 1 mg L^{-1} and pH of 8 ± 0.5).

From 3 days after hatching (hatching rate: trial 1 = 69.8%, trial 2 = 47.6%), larvae were fed twice a day with enriched rotifers (5 mL^{-1}) after shading with RotiGrow *plus* (Reed Mariculture Inc, Campbell, CA, USA) at 500 000 cells per litre.

During the first trial, three enrichments were tested in quadruplicate: Algamac 3050 (A1) (Aquafauna Bio-Marine Inc, Hawthorne, CA, USA), and two experimental formulations (Fa and Fb) (Reed Mariculture Inc) while during the second trial only two enrichments were tested in quadruplicate due to limited egg availability: Algamac 3050 (A2) and one experimental formulation (Fc) (Reed Mariculture Inc). The Algamac 3050 (flake form) and experimental formulations (oil-based) were emulsified prior to rotifers enrichment. Rotifers (*Brachionus plicatilis*) were batch cultured in six 200 L tanks at 27°C (salinity 35 g L^{-1}) and fed concentrated *Nannochloropsis* (Nanno 3600, Reed Mariculture Inc). One tank was harvested daily and the rotifers were rinsed and transferred to 18 L buckets (one per enrichment) and enriched for 7 h, then rinsed and fed to the larvae or stored at 8°C overnight for the following morn-

ing feeding. Samples of enriched rotifers were preserved for FA analysis by sieving on a 55 μm mesh and rinsing with deionized water before storage at −70°C.

At 1, 7, 12 and 15 DPH (end of the rotifer feeding period) for the first trial and 1, 5, 9 and 14 DPH for the second trial, 10 larvae per tank were photographed and standard length and eye diameter were measured using ImageJ (U.S. National Institutes of Health, Bethesda, Maryland, USA). At the end of each trial, all larvae were counted to determine survival and 50 larvae per tank were preserved for FA analysis. In addition to standard length and eye diameter, swim bladder inflation was also assessed at that point.

Fatty acid analyses

Lipids were extracted according to Folch, Lees and Sloane Stanley (1957) and the FA composition was determined by gas-liquid chromatography after preparation of fatty acid methyl esters (FAMEs) according to Morrison and Smith (1964). FAMEs were analysed on a gas chromatograph (Shimadzu GC-2014, Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with a flame ionization detector using a Phenomenex ZB-WAX plus capillary column (30 m long, 0.53 mm internal diameter, 1.0 μm thickness; Phenomenex, Torrance, CA, USA). The flow rate of the carrier gas helium was 4 mL min^{-1}. Temperature was held at 160°C for 5 min then increased up to 220°C at 3°C per minute and maintained at this temperature for 30 min. Injector and detector temperatures were 250 and 260°C respectively. FAMEs peaks were identified by comparison with known standards (Supelco, Inc., Bellefonte, PA, USA). During lipid extraction, tricosanoic acid (23:0) was added as an internal standard for subsequent quantification of FAs.

Statistical analyses

Statistical analysis was performed with MINITAB® version 16.0 (Minitab Ltd., Coventry, UK). Growth and eye diameter data were compared using a General Linear Model (GLM) analysing all time and treatment interactions. Significant differences between treatment means were tested by Tukey's test ($P < 0.05$). Non-homogeneous data (survival, swim bladder inflation and FA composition) were arcsine square root transformed before a one-way ANOVA followed by a Tukey *post hoc* test ($P < 0.05$). All data are presented as mean ± SE of the mean.

Results

Fatty acid utilization

FA data are presented in Table 1 for pompano and Table 2 for snook. In addition the change in concentration of DHA, EPA, ARA, total saturated, total mono-saturated and total poly-unsaturated from the egg to the end of the starvation period are represented in Fig. 1 for pompano and Fig. 2 for snook.

In both pompano and snook, the four predominant FAs in the eggs and newly hatched larvae (0 DPH) were DHA, 16:0, 18:1n-9 and 16:1n-7, contributing to over 50% of total FA. For pompano DHA was the main FA throughout the experiment (4 DPH), while for snook 16:0 was the main FA in the egg and larvae up to 2 DPH when its proportion decreases, while DHA increases. At the end of the trial, DHA, 16:0 and 18:1n-9 were the predominant FAs, followed by 18:0 then ARA for pompano and ARA followed by 18:0 for snook.

In pompano, from hatching to the end of the trial period, proportions of DHA and ARA increased from 33.7 ± 0.5% up to 35.2 ± 0.9%, and 3.2 ± 0.1% up to 5.5 ± 0.2% respectively, while EPA decreased from 4.7 ± 0.2% down to 3.0 ± 0.0%. In snook, these FAs followed a similar trend with the proportion of DHA and ARA increasing from 16.2 ± 0.2% up to 25.8 ± 0.5%, and from 6.5 ± 0.2% up to 9.4 ± 0.2% respectively, while EPA decreased from 3.0 ± 0.2% down to 2.3 ± 0.0%. Accordingly, DHA/EPA and ARA/EPA ratios increased from 7.1 ± 0.1 up to 11.9 ± 0.4, and 0.7 ± 0.0 up to 1.9 ± 0.1 respectively for pompano; and from 5.4 ± 0.2 up to 11.1 ± 01, and 2.2 ± 0.1 up to 4.0 ± 0.1 respectively for snook.

Overall, the proportion of saturated FAs increased in both species between hatching and the end of the experiment, from 22.5 ± 0.8% up to 27.7 ± 0.7% in pompano larvae and from 28.4 ± 0.4% up to 32.4 ± 0.4% in snook larvae. In addition, the total proportion of n-6 increased from 6.2 ± 0.0% up to 8.5 ± 0.1% in pompano and from 11.8 ± 0.3% up to 15.6 ± 0.9% in snook. In contrast, the proportion of mono-unsaturated FAs decreased from 22.8 ± 0.7% down to 15.1 ± 0.4% in pompano and from 28.9 ± 0.2% down to 17.1 ± 0.8% in

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Table 1 Fatty acid profile of fertilized eggs and unfed pompano larvae up to 4 days post hatch (DPH). Only fatty acids contributing to at least 1% at one point are reported, all fatty acids are included in totals and ratios. Values are ±SEM, $n = 3$. Letters indicate significant differences within a same row (Tukey test, $p < 0.05$)

% total FA	Fertilized egg	0 DPH	1 DPH	2 DPH	3 DPH	4 DPH
14:0	1.8 ± 0.0^{c}	2.0 ± 0.1^{c}	1.6 ± 0.1^{bc}	1.1 ± 0.1^{ab}	1.1 ± 0.0^{a}	0.8 ± 0.1^{a}
16:0	21.5 ± 0.1^{b}	17.1 ± 0.8^{a}	18.2 ± 0.1^{a}	17.7 ± 0.7^{a}	18.3 ± 0.1^{a}	17.5 ± 0.6^{a}
18:0	2.9 ± 0.0^{a}	2.5 ± 0.1^{a}	4.2 ± 0.4^{b}	5.8 ± 0.1^{c}	7.5 ± 0.2^{d}	8.3 ± 0.4^{d}
Total saturated	27.1 ± 0.1^{bc}	22.5 ± 0.8^{a}	24.9 ± 0.3^{a}b	25.7 ± 0.8^{bc}	28.1 ± 0.4^{c}	27.7 ± 0.7^{bc}
16:1n-7	4.4 ± 0.0^{d}	5.0 ± 0.1^{d}	4.8 ± 0.0^{d}	3.8 ± 0.2^{c}	3.0 ± 0.1^{b}	2.1 ± 0.1^{a}
18:1n-9	17.9 ± 0.2^{d}	15.0 ± 0.5^{c}	14.4 ± 0.5^{c}	13.7 ± 0.6^{bc}	12.0 ± 0.1^{ab}	10.3 ± 0.4^{a}
18:1n-7	2.7 ± 0.0^{c}	2.4 ± 0.0^{bc}	2.4 ± 0.1^{bc}	2.3 ± 0.1^{b}	2.1 ± 0.1^{ab}	1.8 ± 0.1^{a}
Total monounsaturated	25.6 ± 0.1^{d}	22.8 ± 0.7^{c}	22.0 ± 0.5^{c}	20.3 ± 0.9^{c}	17.8 ± 0.2^{b}	15.1 ± 0.4^{a}
18:2n-6	1.2 ± 0.0^{b}	1.3 ± 0.0^{b}	1.3 ± 0.1^{b}	1.0 ± 0.0^{a}	1.3 ± 0.4^{b}	0.9 ± 0.1^{a}
20:4n-6	2.4 ± 0.1^{a}	3.2 ± 0.1^{b}	3.7 ± 0.1^{c}	3.9 ± 0.1^{c}	4.7 ± 0.1^{d}	5.5 ± 0.2^{e}
20:5n-3	3.2 ± 0.0^{a}	4.7 ± 0.2^{c}	4.6 ± 0.2^{c}	3.9 ± 0.2^{b}	3.3 ± 0.1^{a}	3.0 ± 0.0^{a}
22:5n-6	0.9 ± 0.0^{a}	1.2 ± 0.1^{ab}	1.2 ± 0.1^{ab}	1.4 ± 0.1^{b}	1.4 ± 0.1^{b}	1.3 ± 0.0^{b}
22:5n-3	3.4 ± 0.0^{c}	3.4 ± 0.2^{c}	2.7 ± 0.0^{b}	2.6 ± 0.1^{b}	2.0 ± 0.0^{a}	1.8 ± 0.0^{a}
22:6n-3	28.8 ± 0.2^{a}	33.7 ± 0.5^{b}	32.0 ± 0.2^{ab}	33.1 ± 0.3^{b}	33.4 ± 0.6^{b}	35.2 ± 0.9^{b}
Total polyunsaturated	43.4 ± 0.3^{a}	51.1 ± 0.8^{b}	48.8 ± 0.5^{b}	49.1 ± 0.5^{b}	48.8 ± 0.7^{b}	50.4 ± 0.8^{b}
Total n-3	36.8 ± 0.2^{a}	43.2 ± 0.9^{b}	40.4 ± 0.4^{ab}	40.5 ± 0.5^{ab}	39.3 ± 0.8^{ab}	40.4 ± 0.8^{ab}
Total n-6	5.0 ± 0.1^{a}	6.2 ± 0.0^{b}	6.7 ± 0.2^{c}	7.0 ± 0.1^{c}	8.0 ± 0.1^{d}	8.5 ± 0.1^{d}
n-3/n-6 ratio	7.4 ± 0.1^{c}	7.0 ± 0.3^{c}	6.0 ± 0.1^{b}	5.8 ± 0.2^{b}	5.0 ± 0.2^{a}	4.8 ± 0.1^{a}
ARA/EPA	0.8 ± 0.0^{a}	0.7 ± 0.0^{a}	0.8 ± 0.0^{a}	1.0 ± 0.0^{b}	1.4 ± 0.1^{c}	1.9 ± 0.1^{d}
DHA/EPA	8.9 ± 0.1^{b}	7.1 ± 0.1^{a}	7.0 ± 0.3^{a}	8.5 ± 0.2^{b}	10.1 ± 0.2^{c}	11.9 ± 0.4^{d}
Total FA (mg FA g^{-1} dry weight)	190.7 ± 3.0^{c}	181.5 ± 1.1^{bc}	179.4 ± 2.3^{b}	173.5 ± 1.2^{b}	147.5 ± 2.7^{a}	146.3 ± 0.7^{a}

snook larvae. The proportion of poly-unsaturated FAs and total proportion of n-3 increased from 37.4 ± 0.4% up to 48.7 ± 0.7%, and 23.3 ± 0.3% up to 31.4 ± 0.9% respectively in snook larvae, while contrastingly they did not vary significantly in pompano larvae with an average of 49.7 ± 0.2% and 40.8 ± 0.7% respectively. Consequently, the n-3/n-6 ratio decreased from 7.0 ± 0.3 down to 4.8 ± 0.1 in pompano, while it did not vary significantly in snook larvae with an average of 1.9 ± 0.8.

The total amount of FA decreased by 23% in pompano larvae with 190.7 ± 3 mg in the eggs and 146.3 ± 0.7 in the larvae at the end of the trial, and by 43% in snook larvae with 193.3 ± 3.5 mg in the eggs and 110.1 ± 2.2 mg at the end of the trial.

Live feed trials

At the end of the trials, survival rates from hatching for the A1 and A2 larvae and the Fc larvae were similar (ranging from 5.2 ± 1.2% to 6.9 ± 0.9%), significantly higher than that of Fa larvae with 2.4 ± 0.5% and Fb larvae with 2.1 ± 0.4% (Table 3). No significant difference was observed in the proportion of functional swim bladder in A1 and A2 larvae (80.2 ± 2.1% and 77.1 ± 3.1% respectively); however, it was significantly higher compared to the other treatments that ranged from 50.9 ± 2.8% to 58.4 ± 5.2% (Table 3).

At the end of the first trial, standard length was significantly higher for the Fa and Fb larvae compared to the A1 larvae (4.26 ± 0.12, 4.29 ± 0.11 and 3.98 ± 0.09 mm respectively), while there was no significant difference in eye diameter with an average of 0.38 ± 0.08 mm (Fig. 3). At the end of the second trial, there were no significant differences in standard length and eye diameter between treatments (Fig. 3).

FA composition of eggs, enrichment formulations, enriched rotifers and larvae at the end of the trial are presented in Table 4 for the first trial and Table 5 for the second trial. In addition, the PUFAs content and ratios of the enrichment formulations, enriched rotifers and larvae at the end of the trial are represented in Fig. 4 for trial 1 and Fig. 5 for trial 2.

In the first trial, there was no significant difference in total PUFA between the different enrichments with an average of 59.1 ± 1% of total FA. However, total PUFA differed in the rotifers with the greatest amount in A1 rotifers (66.2 ± 1.0% of total FA) followed by Fa rotifers (57.6 ± 1.0% of total FA) and Fb rotifers (51.5 ± 0.2% of total FA). At the end of the trial, total PUFA in the larvae was

Table 2 Fatty acid profile of fertilized eggs and unfed snook larvae up to 5 days post hatch (DPH). Only fatty acids contributing to at least 1% at one point are reported, all fatty acids are included in totals and ratios. Values are ±SEM, $n = 3$. Letters indicate significant differences within a same row (Tukey test, $P < 0.05$)

% total FA	Fertilized egg	0 DPH	1 DPH	2 DPH	3 DPH	4 DPH	5 DPH
14:0	1.9 ± 0.1^{bc}	2.4 ± 0.0^{c}	1.9 ± 0.1^{bc}	1.9 ± 0.2^{bc}	0.9 ± 0.2^{a}	1.3 ± 0.1^{a}	1.6 ± 0.1^{b}
16:0	21.7 ± 0.7	19.7 ± 0.2	19.4 ± 0.1	19.9 ± 0.3	20.2 ± 0.5	20.3 ± 0.2	20.6 ± 0.4
17:0	1.0 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.1	0.9 ± 0.1
18:0	5.2 ± 0.1^{a}	4.5 ± 0.1^{a}	5.3 ± 0.3^{a}	6.8 ± 0.5^{a}	9.2 ± 0.7^{b}	9.1 ± 0.1^{b}	8.5 ± 0.1^{b}
Total saturated	30.6 ± 0.6^{b}	28.4 ± 0.4^{a}	28.4 ± 0.3^{a}	30.3 ± 0.3^{b}	31.8 ± 0.4^{bc}	32.5 ± 0.8^{bc}	32.4 ± 0.4^{c}
16:1n-7	7.6 ± 0.3^{b}	9.0 ± 0.1^{b}	7.4 ± 0.5^{b}	5.6 ± 0.4^{b}	3.0 ± 0.4^{a}	3.8 ± 0.2^{a}	4.1 ± 0.4^{a}
18:1n-9	16.9 ± 0.3^{b}	15.1 ± 0.2^{b}	13.9 ± 0.2^{b}	11.8 ± 0.3^{b}	9.2 ± 0.3^{a}	9.4 ± 1.0^{a}	9.7 ± 1.4^{a}
18:1n-7	4.6 ± 0.2^{b}	4.3 ± 0.1^{b}	3.9 ± 0.1^{b}	3.0 ± 0.1^{b}	2.4 ± 0.1^{a}	2.5 ± 0.2^{a}	2.6 ± 0.3^{a}
Total monounsaturated	29.5 ± 0.2^{b}	28.9 ± 0.2^{b}	25.7 ± 0.1^{b}	20.9 ± 0.6^{b}	15.0 ± 0.8^{a}	16.3 ± 0.8^{a}	17.1 ± 0.8^{a}
16:3n-4	1.4 ± 0.0^{b}	1.4 ± 0.0^{b}	1.4 ± 0.0^{b}	1.1 ± 0.0^{a}	1.1 ± 0.1^{a}	1.0 ± 0.6^{a}	1.0 ± 0.1^{a}
18:2n-6	2.2 ± 0.5^{b}	2.7 ± 0.2^{c}	2.7 ± 0.0^{c}	2.0 ± 0.0^{b}	1.4 ± 0.1^{a}	2.1 ± 0.2^{b}	2.1 ± 0.8^{b}
20:4n-6	5.4 ± 0.3^{a}	6.5 ± 0.2^{ab}	7.7 ± 0.1^{b}	8.4 ± 0.1^{b}	9.5 ± 0.1^{c}	9.5 ± 0.1^{c}	9.4 ± 0.2^{c}
20:5n-3	2.4 ± 0.4^{b}	3.0 ± 0.1^{a}	2.3 ± 0.2^{b}	2.3 ± 0.1^{b}	2.4 ± 0.1^{b}	2.4 ± 0.0^{b}	2.3 ± 0.0^{b}
22:5n-6	2.1 ± 0.2^{ab}	1.8 ± 0.0^{a}	2.2 ± 0.1^{ab}	2.8 ± 0.2^{ab}	3.0 ± 0.3^{bc}	3.2 ± 0.1^{bc}	3.3 ± 0.2^{c}
22:5n-3	2.7 ± 0.1^{c}	2.6 ± 0.0^{c}	2.4 ± 0.0^{b}	2.0 ± 0.1^{a}	2.0 ± 0.1^{a}	2.1 ± 0.3^{ab}	2.2 ± 0.7^{b}
22:6n-3	14.5 ± 0.2^{a}	16.2 ± 0.2^{b}	17.5 ± 0.2^{b}	20.0 ± 0.3^{b}	26.1 ± 0.6^{c}	26.0 ± 0.3^{c}	25.8 ± 0.5^{c}
Total polyunsaturated	33.6 ± 0.5^{a}	37.4 ± 0.4^{b}	39.3 ± 0.4^{b}	41.4 ± 0.2^{b}	47.7 ± 0.4^{c}	49.4 ± 0.9^{c}	48.7 ± 0.7^{c}
Total n-3	20.8 ± 0.6^{a}	23.3 ± 0.3^{a}	23.3 ± 0.4^{a}	25.3 ± 0.4^{a}	31.1 ± 0.6^{b}	31.4 ± 0.8^{b}	31.4 ± 0.9^{b}
Total n-6	10.6 ± 0.5^{a}	11.8 ± 0.3^{ab}	13.7 ± 0.2^{ab}	14.1 ± 0.3^{ab}	14.7 ± 0.4^{b}	15.5 ± 0.4^{b}	15.6 ± 0.9^{b}
n-3/ n-6 ratio	2.1 ± 0.3^{b}	2.0 ± 0.1^{ab}	1.7 ± 0.1^{a}	1.8 ± 0.1^{a}	2.1 ± 0.1^{b}	2.0 ± 0.0^{ab}	2.0 ± 0.1^{ab}
ARA/EPA ratio	2.8 ± 0.6^{b}	2.2 ± 0.1^{a}	3.4 ± 0.3^{c}	3.6 ± 0.2^{c}	3.9 ± 0.2^{d}	4.0 ± 0.1^{d}	4.0 ± 0.1^{d}
DHA/EPA ratio	7.1 ± 0.7^{ab}	5.4 ± 0.2^{a}	7.7 ± 0.6^{ab}	8.6 ± 0.2^{ab}	10.8 ± 0.1^{b}	10.9 ± 0.0^{b}	11.1 ± 0.1^{b}
Total FA (mg FA g^{-1} dry weight)	193.3 ± 3.5^{e}	187.3 ± 4.1^{de}	163.4 ± 2.2^{d}	147.7 ± 1.9^{cd}	134.1 ± 1.2^{bc}	119.6 ± 2.3^{ab}	110.1 ± 2.2^{a}

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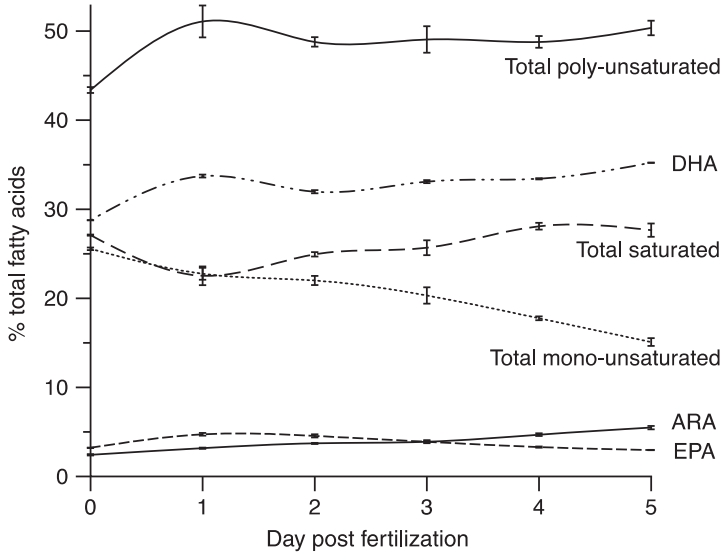

Figure 1 Evolution of selected fatty acids in eggs (0 days post fertilization) and unfed pompano larvae (1–5 day post fertilization). Values are ±SEM, $n = 3$.

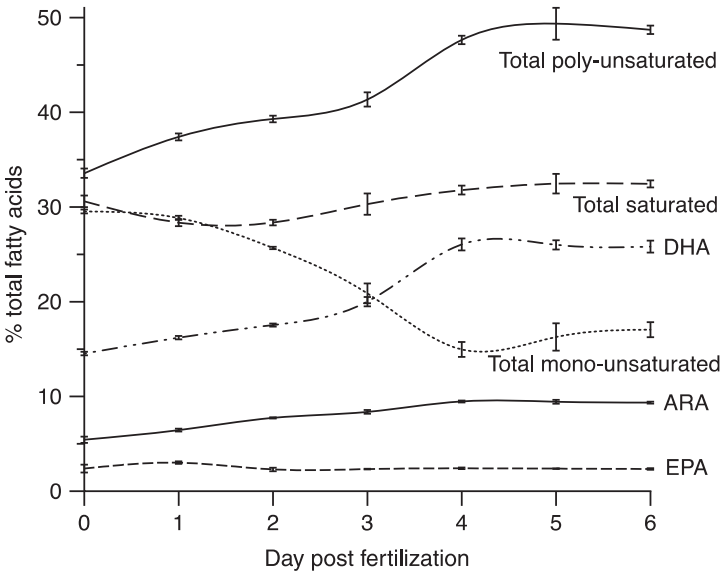

Figure 2 Evolution of selected fatty acids in eggs (0 days post fertilization) and unfed snook larvae (1–5 day post fertilization). Values are ±SEM, $n = 3$.

significantly higher for A1 and Fa larvae with an average of 47.4 ± 0.5% of total FA compared to Fb larvae (43.1 ± 0.7% of total FA). In trial 2, no significant differences were observed in PUFA contents between treatments with an average of 63.6 ± 1.0% of total FA in the enrichment, 54.0 ± 0.5% of total FA in the rotifers and 43.5 ± 0.4% of total FA in the larvae.

DHA content in trial 1 was greatest in the A1 enrichment and rotifers (40.8 ± 0.8% and 38.2 ± 0.8% of total FA), followed by the Fa enrichment and rotifers (29.8 ± 0.7% and 25.0 ± 0.6% of total FA) then the Fb enrichment and rotifers (28.0 ± 0.4% and 20.6 ± 0.3% of total FA). However, at the end of the trial there was no significant difference in larvae DHA content with an average of 24.3 ± 0.4% of total FA. In trial 2, DHA content was significantly higher for the A2 enrichment, rotifers and larvae (40.8 ± 0.6%, 27.3 ± 0.4% and 22.7 ± 0.1% of total FA respectively), than in their equivalent from the Fc treatment (22.9 ± 0.2%, 15.3 ± 0.4% and 19.4 ± 0.3% of total FA respectively).

ARA content in trial 1was the greatest in the Fb enrichment (17.5 ± 0.1% of total FA) then in the Fa enrichment (14.7 ± 0.1% of total FA) followed by the A1 enrichment (1.6 ± 0.3% of total FA). Likewise, highest ARA content was observed

Table 3 Per cent survival and per cent of functional swim bladder at the end of live feed trials with snook larvae fed rotifers enriched with Algamac 3050 (A1), Formulation a (Fa) or Formulation b (Fb) during the first trial, and Algamac 3050 (A2) or Formulation c (Fc) during the second trial. Values are ±SEM, $n = 4$ in trial 1 and trial 2, 10 larvae per tank. Superscript letters indicate significant differences within a same column (Tukey test, $P < 0.05$)

		Survival(%)	Functional swim bladder(%)
Trial I	A1	6.9 ± 0.9^{b}	80.2 ± 2.1^{b}
	Fa	2.4 ± 0.5^{a}	50.9 ± 2.8^{a}
	Fb	2.1 ± 0.4^{a}	52.1 ± 3.4^{a}
Trial 2	A2	5.2 ± 1.2^{b}	77.1 ± 3.1^{b}
	Fc	5.4 ± 0.9^{b}	58.4 ± 5.2^{a}

in the Fb rotifers (11.1 ± 0.6% of total FA) then in the Fa rotifers (9.3 ± 0.3% of total FA) followed by the A1 rotifers (2.8 ± 0.1% of total FA). At the end of the trial, the ARA content was significantly lower in the A1 larvae (4.4 ± 0.9% of total FA) than in the other treatments (average of 7.1 ± 0.4% of total FA). In trial 2, ARA content was significantly higher in the Fc enrichment, rotifers and larvae (20.8 ± 0.2%, 14.0 ± 0.7% and 8.7 ± 0.3% of total FA respectively) than in their equivalent from the A2 treatment (1.6 ± 0.3%, 3.3 ± 0.2% and 5.2 ± 0.2% of total FA respectively).

At the end of the first trial, the highest DHA/EPA ratio was observed in the A1 larvae (8.9 ± 0.1) and the lowest ratio was observed in the Fa larvae (7.0 ± 0.3) while the Fb ratio was not significantly different from the other treatments (7.9 ± 0.6). In the second trial, the DHA/EPA ratio in the A2 larvae (6.5 ± 0.1) was significantly higher than that of the Fc larvae (5.1 ± 0.3). The highest ARA/EPA ratio in trial 1 was observed in the Fb larvae (2.4 ± 0.1), the lowest ratio in the A1 larvae (1.6 ± 0.2) while the ratio in the Fa larvae was not significantly different from the other treatments (2.0 ± 0.1). In the second trial, the Fb larvae presented an ARA/EPA ratio significantly higher (2.3 ± 0.2) than that of the A2 larvae (1.5 ± 0.2).

Discussion

The study of FA utilization in pompano and snook unfed larvae revealed clear patterns of lipid utilization and mobilization. Eggs from both species contained an oil globule and are classified as being high in lipids (>19% of dry weight) (Sargent, Henderson & Tocher 1989). After hatching the decline in total FA reflected the utilization of lipids as an energy source. This is in accordance with previous studies in species such as red drum *Sciaenops ocellatus* (Vetter, Hodson & Arnold 1983), red sea bream *Pagrus major* (Tandler, Watanabe, Satoh & Fukusho 1989), gilthead sea bream (Koven, Kissil & Tandler 1989; Rønnestad, Koven, Tandler, Harel & Fyhn 1994), turbot (Rainuzzo, Reitan, Jorgensen, Olsen & Jørgensen 1994), Senegal sole (Mourente & Vázquez 1996), common dentex *Dentex dentex* (Mourente, Rodriguez, Grau & Pastor 1999), white sea bream *Diplodus sargus* (Cejas, Almansa, Jérez, Bolaños, Felipe & Lorenzo 2004) and Atlantic bluefin tuna *Thunnus thynnus* (Morais, Mourente, Ortega, Tocher & Tocher 2011) indicating that fast developing eggs from temperate and warm waters, with high lipid content and an oil globule, incorporate a large fraction of neutral lipids in the oil droplet which is utilized for energy soon after hatching. Contrastingly, cold water species with slower egg development such as Atlantic herring *Clupea harengus* (Tocher, Fraser, Sargent & Gamble 1985a) Atlantic cod *Gadus morhua* and plaice *Pleuronectes platessa* (Rainuzzo, Reitan & Jorgensen 1992) or Atlantic halibut *Hippoglossus hippoglossus* (Rainuzzo *et al.* 1992; Ronnestad, Finn, Lein & Lie 1995) mainly catabolize free amino acid after hatching (Rønnestad 1999).

The neutral lipid fraction is generally rich in mono-unsaturated FAs that are preferentially used for energy (Kamler 2007). It seems to be the case in this study as, even though no lipid class analyses were performed, a strong decrease in mono-unsaturated FAs and no increase in their elongation products were observed in both species after hatching. The selective retention of certain fatty acids has been described as a biochemical strategy allowing the preservation of the most essential components of biological membranes during starvation periods (Izquierdo 1996). It has been recently demonstrated in turbot that the expression profile of some genes involved in lipid metabolism (Hepatic lipase, FA synthetase and Diacylglycerol O-acyltransferase homologue 1) responded solely to starvation (Cunha, Galante-Oliveira, Rocha, Planas, Urbatzka & Castro 2013). In the present study saturated fatty acids (SFAs) and PUFAs were preferentially retained. SFAs and PUFAs play important roles in respectively the sn-1 and sn-2 position of structural phospholipids with phosphatidylcholine containing high levels of 16:0, phosphatidylserine incorporating high levels of 18:0 associated with C22 PUFAs,

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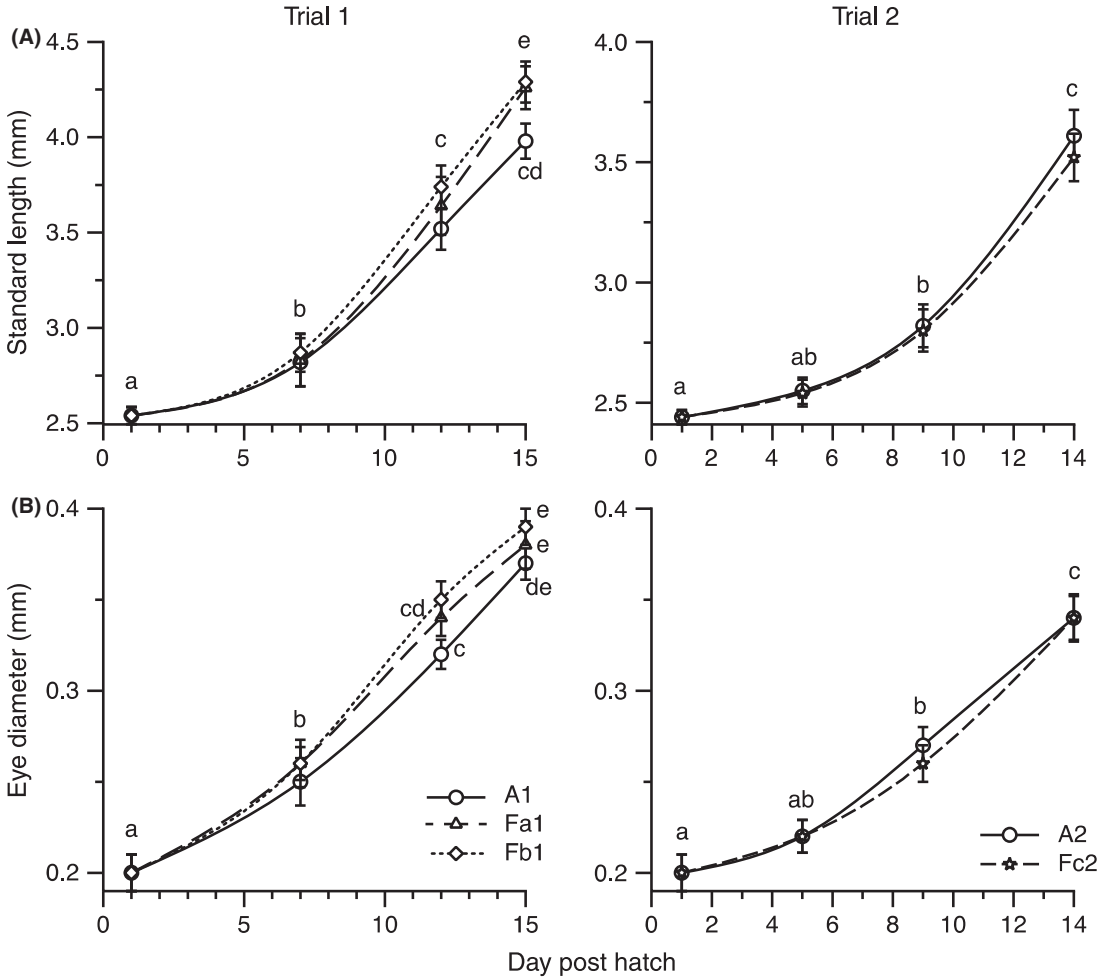

Figure 3 Standard length (A) and eye diameter (B) of snook larvae fed rotifers enriched with Algamac 3050 (A1), Formulation a (Fa1) or Formulation b (Fb1) during the first trial, and Algamac 3050 (A2) or Formulation c (Fc2) during the second trial. Values are ±SE of the mean, $n = 4$ in trial 1 and trial 2, 10 larvae per tank and sampling point. Letters indicate significant differences between treatments and time points (Tukey test, $P < 0.05$).

and phosphatidylinositol including high levels of 18:0 associated with C20 PUFAs and more particularly ARA (Tocher 1995; Sargent *et al.* 2002). In both pompano and snook, the main PUFAs selectively retained were DHA and ARA, while low levels of EPA were observed. DHA is selectively retained in unfed larvae of several species, including Atlantic herring (Tocher, Fraser, Sargent & Gamble 1985b), gilthead sea bream (Koven *et al.* 1989), red sea bream (Tandler *et al.* 1989), cod (Van der Meeren, Wilhelmsen, Klungsosyr & Kvenseth 1993), turbot (Rainuzzo *et al.* 1994), Senegal sole (Mourente & Vázquez 1996) common dentex (Mourente *et al.* 1999) and Atlantic bluefin tuna (Morais *et al.* 2011). This reflects the importance of DHA in larvae structural development and in particular on neural and visual functions (Bell, Castell *et al.* 1995; Bell, Batty *et al.* 1995; Sargent, Bell *et al.* 1999). ARA is selectively retained along with DHA in fewer species, including turbot (Rainuzzo *et al.* 1994), Senegal sole (Mourente & Vázquez 1996) and bluefin tuna (Morais *et al.* 2011). In addition, in Atlantic halibut, cod, plaice (Rainuzzo *et al.* 1992) and white sea bream (Cejas *et al.* 2004), ARA solely was conserved, while utilization of DHA was observed, indicating a special role of ARA in the early larval development of these species. Concurrently to the retention of ARA, a decrease in EPA level is observed throughout the starvation period in pompano while a significant decrease is only observed between 0 and 1 dph in snook. In the absence of information on bioconversion rates in

Table 4 Fatty acid profile of fertilized eggs, Algamac 3050 (A1), Formulation a (Fa) and Formulation b (Fb) and their enriched rotifers during the trial (6 sampling times) as well as their resulting snook larvae at the end of the trial. Only fatty acids contributing to at least 1% in one of the treatments are reported, all fatty acids are included in totals and ratios. Values are ±SEM, $n = 3$ for the eggs and enrichments, $n = 4$ for larvae (50 larvae per tank), $n = 6$ for enriched rotifers. Superscripted letters indicate significant differences within a same row (Tukey test, $P < 0.05$). nd means not detected

		A1			Fa			Fb		
% total FA	Fertilized Eggs	Enrichment	Enriched Rotifers	Larvae	Enrichment	Enriched Rotifers	Larvae	Enrichment	Enriched Rotifers	Larvae
12:0	0.0 ± 0.0^{a}	0.3 ± 0.1^{b}	0.1 ± 0.0a^{b}	0.2 ± 0.1^{b}	0.9 ± 0.0^{c}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	1.1 ± 0.0^{c}	0.3 ± 0.0^{b}	0.3 ± 0.1^{b}
14:0	3.1 ± 0.1^{b}	9.8 ± 0.6^{e}	4.4 ± 0.2^{c}	1.3 ± 0.1^{a}	6.0 ± 0.2^{d}	3.2 ± 0.1^{b}	0.9 ± 0.2^{a}	6.0 ± 0.1^{d}	3.4 ± 0.2^{bc}	0.9 ± 0.1^{a}
16:0	17.9 ± 0.8^{c}	25.9 ± 0.7^{d}	15.2 ± 0.8^{bc}	16.5 ± 1.2^{c}	11.7 ± 0.3^{ab}	12.0 ± 0.3^{ab}	14.6 ± 0.3^{bc}	11.8 ± 0.2^{ab}	11.0 ± 0.5^{a}	16.2 ± 0.6^{c}
17:0	0.7 ± 0.0^{cd}	nd	0.5 ± 0.0^{c}	1.2 ± 0.2^{ef}	0.1 ± 0.0^{b}	0.9 ± 0.0^{de}	1.2 ± 0.1^{ef}	0.1 ± 0.0^{b}	0.7 ± 0.1^{cd}	1.3 ± 0.1^{f}
18:0	4.8 ± 0.1^{c}	0.5 ± 0.0^{a}	1.5 ± 0.1^{b}	8.4 ± 0.4^{d}	1.7 ± 0.6^{b}	2.7 ± 0.1^{b}	7.9 ± 0.5^{d}	2.2 ± 0.6^{b}	2.6 ± 0.2^{b}	9.3 ± 0.7^{d}
Total saturated	27.1 ± 0.8^{c}	37.0 ± 0.6^{d}	22.0 ± 0.8^{ab}	27.9 ± 0.7^{c}	20.4 ± 0.2^{a}	19.2 ± 0.4^{a}	25.0 ± 0.7^{bc}	21.3 ± 0.9^{ab}	18.2 ± 0.8^{a}	28.4 ± 1.5^{c}
16:1n-7	8.0 ± 0.2^{f}	nd	3.2 ± 0.2^{c}	3.0 ± 0.2^{c}	2.2 ± 0.0^{b}	5.9 ± 0.2^{e}	3.2 ± 0.4^{c}	1.7 ± 0.0^{b}	4.8 ± 0.4^{d}	2.9 ± 0.5^{c}
18:1n-9	11.1 ± 0.4^{f}	0.1 ± 0.0^{a}	0.6 ± 0.1^{b}	4.9 ± 0.3^{c}	14.5 ± 0.2^{g}	7.7 ± 0.3^{de}	6.9 ± 0.3^{d}	13.9 ± 0.3^{g}	8.4 ± 0.3^{e}	7.1 ± 0.3^{de}
18:1n-7	6.6 ± 0.1^{d}	nd	1.8 ± 0.1^{b}	3.2 ± 0.2^{c}	nd	3.8 ± 0.2=	3.7 ± 0.1^{c}	nd	3.1 ± 0.3^{c}	2.9 ± 0.3^{bc}
20:1n-9	0.5 ± 0.0^{bc}	nd	0.5 ± 0.1^{bc}	0.7 ± 0.1^{c}	0.3 ± 0.0^{bc}	1.4 ± 0.2^{d}	1.4 ± 0.1^{d}	0.2 ± 0.0^{b}	1.4 ± 0.1^{d}	1.3 ± 0.1^{d}
Total monounsaturated	26.4 ± 0.5^{f}	0.1 ± 0.0^{a}	6.2 ± 0.4^{b}	11.9 ± 0.7^{c}	17.0 ± 0.3^{de}	18.9 ± 0.4^{e}	15.3 ± 0.4^{d}	15.9 ± 0.3^{d}	17.8 ± 0.4^{de}	14.6 ± 0.6^{d}
18:2n-6	1.8 ± 0.0^{c}	0.1 ± 0.0^{a}	0.9 ± 0.1^{b}	1.5 ± 0.1^{c}	4.6 ± 0.1^{e}	4.4 ± 0.1^{e}	2.4 ± 0.1^{d}	4.9 ± 0.1^{e}	4.6 ± 0.1^{e}	2.6 ± 0.2^{d}
18:3n-6	0.2 ± 0.1^{a}	0.2 ± 0.0^{a}	0.3 ± 0.0^{a}	0.5 ± 0.0^{b}	0.8 ± 0.0^{c}	0.7 ± 0.0^{c}	0.5 ± 0.1^{b}	1.1 ± 0.1^{d}	0.8 ± 0.0^{c}	0.6 ± 0.1^{b}
18:4n-3	0.5 ± 0.1^{a}	0.3 ± 0.1^{a}	0.4 ± 0.1^{a}	1.1 ± 0.8^{ab}	2.4 ± 0.1^{c}	1.7 ± 0.2^{bc}	0.4 ± 0.1^{a}	0.7 ± 0.0^{ab}	0.8 ± 0.1^{ab}	0.4 ± 0.1^{a}
20:3n-6	0.6 ± 0.1^{bc}	0.4 ± 0.0^{a}	0.8 ± 0.1^{c}	0.8 ± 0.1^{c}	0.5 ± 0.2^{b}	1.6 ± 0.1^{e}	1.0 ± 0.1^{d}	1.1 ± 0.2^{d}	1.7 ± 0.2^{e}	0.8 ± 0.1^{c}
20:4n-6	5.1 ± 0.0^{c}	1.6 ± 0.3^{a}	2.8 ± 0.1^{b}	4.4 ± 0.9^{c}	14.7 ± 0.1^{g}	9.3 ± 0.3^{e}	7.1 ± 0.2^{d}	17.5 ± 0.1^{h}	11.1 ± 0.6^{f}	7.0 ± 0.6^{d}
20:4n-3	0.4 ± 0.0^{bc}	0.8 ± 0.0^{cd}	1.0 ± 0.1^{d}	0.4 ± 0.1^{bc}	nd	0.7 ± 0.1^{cd}	0.3 ± 0.1^{bc}	nd	0.5 ± 0.0^{c}	0.2 ± 0.1^{b}
20:5n-3	4.5 ± 0.1^{c}	1.0 ± 0.5^{a}	5.8 ± 0.5^{d}	2.8 ± 0.3^{b}	1.0 ± 0.0=	7.7 ± 0.4^{e}	3.6 ± 0.3^{bc}	1.2 ± 0.0^{a}	6.4 ± 0.2^{d}	3.0 ± 0.3^{b}
22:5n-6	1.5 ± 0.1^{c}	14.6 ± 0.5^{e}	12.8 ± 0.3^{e}	8.8 ± 0.2^{d}	0.2 ± 0.0^{ab}	0.6 ± 0.1^{b}	1.1 ± 0.4^{bc}	0.1 ± 0.0^{a}	0.5 ± 0.1^{b}	1.3 ± 0.9^{bc}
22:5n-3	3.3 ± 0.1^{cd}	0.3 ± 0.0^{a}	2.7 ± 0.3^{c}	1.9 ± 0.1^{b}	2.5 ± 0.1^{bc}	4.4 ± 0.2^{d}	3.2 ± 0.3^{cd}	3.0 ± 0.0^{cd}	3.5 ± 0.4^{cd}	2.3 ± 0.4^{bc}
22:6n-3	22.3 ± 0.8^{ab}	40.8 ± 0.8^{e}	38.2 ± 0.8^{e}	24.5 ± 0.4^{b}	29.8 ± 0.7$_{d}$	25.0 ± 1.0^{b}	25.1 ± 0.6^{b}	28.0 ± 0.4^{c}	20.6 ± 0.3^{a}	23.4 ± 0.3^{ab}
Total polyunsaturated	42.6 ± 0.9^{a}	60.4 ± 1.4^{d}	66.2 ± 1.0^{e}	48.2 ± 0.7^{b}	58.2 ± 1.0^{d}	57.6 ± 1.0^{d}	46.7 ± 0.4^{b}	58.7 ± 0.5^{d}	51.5 ± 0.2^{c}	43.1 ± 0.7^{a}
Total n-3	31.8 ± 0.6^{b}	43.4 ± 0.6^{cd}	48.4 ± 0.9^{d}	31.2 ± 3.2^{b}	37.1 ± 0.8^{c}	35.8 ± 1.0^{bc}	31.2 ± 1.2^{b}	33.8 ± 0.5^{b}	36.8 ± 0.6^{c}	26.4 ± 1.5^{a}
Total n-6	9.4 ± 0.1^{a}	16.8 ± 0.4^{c}	17.7 ± 0.3^{cd}	16.2 ± 1.1^{c}	21.0 ± 0.2^{d}	17.0 ± 0.4^{c}	12.6 ± 0.3^{b}	24.9 ± 0.0^{e}	19.0 ± 0.5^{cd}	12.5 ± 1.6^{b}
n-3/n-6	3.4 ± 0.2^{f}	2.6 ± 0.1^{e}	2.7 ± 0.0^{e}	1.9 ± 0.2^{c}	1.8 ± 0.0^{b}	2.1 ± 0.1^{d}	2.5 ± 0.2^{de}	1.4 ± 0.0^{a}	1.9 ± 0.1^{c}	2.1 ± 0.0^{d}
ARA/EPA	1.1 ± 0.0^{b}	1.5 ± 0.^{2c}	0.5 ± 0.0^{a}	1.6 ± 0.2^{cd}	14.9 ± 0.1^{f}	1.2 ± 0.1^{b}	2.0 ± 0.1^{de}	14.7 ± 0.6^{f}	1.8 ± 0.2^{d}	2.4 ± 0.1^{e}
DHA/EPA	5.0 ± 0.2^{ab}	39.4 ± 1.5^{f}	6.6 ± 0.8^{b}	8.9 ± 0.1^{c}	30.2 ± 0.5^{e}	3.3 ± 0.2^{a}	7.0 ± 0.3^{b}	23.5 ± 0.4^{d}	3.2 ± 0.2^{a}	7.9 ± 0.6^{bc}
Total FA (mg FA g^{-1} dry weight)	151.4 ± 5.0^{d}	398.3 ± 5.0^{g}	153.9 ± 6.3^{d}	88.5 ± 6.3^{b}	254.8 ± 1.2^{e}	82.7 ± 3.9^{b}	64.2 ± 3.5^{a}	328.7 ± 4.1^{f}	106.2 ± 8.4^{c}	94.5 ± 2.2^{b}

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Table 5 Fatty acid profile of fertilized eggs, Algamac 3050 (A2) and Formulation c (Fc), and their enriched rotifers during the trial (six sampling times) as well as their resulting snook larvae at the end of the trial. Only fatty acids contributing to at least 1% in one of the treatments are reported, all fatty acids are included in totals and ratios. Values are ±SEM, n = 3 for the eggs and enrichments, $n = 4$ for larvae (50 larvae per tank), $n = 6$ for enriched rotifers. Superscripted letters indicate significant differences within a same row (Tukey test, $P < 0.05$). nd means not detected

		A2			Fc		
% total FA	**Fertilized Eggs**	**Enrichment**	**Enriched Rotifers**	**Larvae**	**Enrichment**	**Enriched Rotifers**	**Larvae**
14:0	2.2 ± 0.1^{b}	9.8 ± 0.6^{d}	5.1 ± 0.1^{c}	1.2 ± 0.0^{a}	5.8 ± 0.3^{c}	3.0 ± 0.1^{b}	1.2 ± 0.1^{a}
16:0	22.9 ± 0.5^{b}	25.9 ± 0.7^{c}	18.0 ± 0.3^{a}	23.8 ± 0.1^{bc}	16.0 ± 0.4^{a}	15.8 ± 0.3^{a}	22.3 ± 0.3^{b}
18:0	5.5 ± 0.1^{d}	0.5 ± 0.1^{a}	1.7 ± 0.0^{b}	8.9 ± 0.1^{e}	3.0 ± 0.1^{c}	2.6 ± 0.1^{c}	8.0 ± 0.2^{e}
Total saturated	32.4 ± 0.5^{c}	37.0 ± 1.1^{d}	25.6 ± 0.4^{b}	34.9 ± 0.6^{c}	25.5 ± 0.5^{b}	21.9 ± 0.3^{a}	32.5 ± 0.3^{c}
16:1n7	8.0 ± 0.4^{c}	nd	7.7 ± 0.2^{c}	4.0 ± 0.1^{b}	0.6 ± 0.1^{a}	8.2 ± 0.2^{c}	5.1 ± 0.2^{bc}
18:1n9	14.4 ± 0.8^{e}	0.1 ± 0.0^{a}	2.7 ± 0.1^{b}	5.6 ± 0.2^{c}	2.0 ± 1.1^{b}	6.3 ± 0.2^{c}	7.1 ± 0.3^{d}
18:1n7	5.3 ± 0.1^{d}	nd	2.3 ± 0.1^{b}	2.9 ± 0.2^{b}	3.9 ± 1.1^{c}	2.3 ± 0.1^{b}	2.8 ± 0.1^{b}
20:1n9	0.2 ± 0.0^{b}	nd	1.2 ± 0.0^{c}	1.0 ± 0.0^{c}	0.2 ± 0.0^{b}	1.4 ± 0.0^{c}	1.2 ± 0.1^{c}
Total monounsaturated	28.2 ± 0.6^{e}	0.1 ± 0.0^{a}	13.9 ± 0.3^{c}	13.6 ± 0.1^{c}	6.7 ± 0.3^{b}	18.2 ± 0.3^{d}	16.3 ± 0.3^{d}
16:3n4	1.5 ± 0.1^{d}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.3 ± 0.0^{c}	nd	0.3 ± 0.0^{c}	0.3 ± 0.0^{c}
18:2n6	1.9 ± 0.1^{bc}	0.1 ± 0.0^{a}	1.6 ± 0.1^{b}	1.7 ± 0.1^{b}	4.7 ± 0.1^{d}	4.8 ± 0.1^{d}	2.6 ± 0.2^{c}
18:3n6	0.6 ± 0.0^{c}	0.2 ± 0.0^{a}	0.4 ± 0.1^{bc}	0.3 ± 0.0^{ab}	1.1 ± 0.0^{d}	1.0 ± 0.0^{d}	0.5 ± 0.0^{c}
18:3n3	1.1 ± 0.1^{c}	0.1 ± 0.1^{ab}	0.2 ± 0.0^{ab}	0.1 ± 0.0^{a}	0.2 ± 0.0^{ab}	0.3 ± 0.0^{b}	0.1 ± 0.0^{a}
20:3n6	0.5 ± 0.0^{b}	0.4 ± 0.0^{a}	0.7 ± 0.1^{bc}	0.6 ± 0.0^{b}	1.8 ± 0.1^{d}	1.6 ± 0.0^{d}	0.9 ± 0.1^{c}
20:4n6	4.7 ± 0.2^{bc}	1.6 ± 0.3^{a}	3.3 ± 0.2^{b}	5.2 ± 0.2^{c}	20.8 ± 0.2^{f}	14.0 ± 0.7^{e}	8.7 ± 0.3^{d}
20:5n3	2.4 ± 0.1^{b}	1.0 ± 0.5^{a}	7.3 ± 0.3^{d}	3.5 ± 0.1^{c}	1.4 ± 0.1^{a}	7.1 ± 0.4^{d}	3.8 ± 0.2^{c}
22:5n6	1.3 ± 0.1^{a}	14.6 ± 0.5^{e}	8.9 ± 0.2^{d}	5.5 ± 0.2^{c}	5.8 ± 0.2^{c}	3.4 ± 0.1^{b}	3.1 ± 0.1^{b}
22:5n3	2.9 ± 0.1^{b}	0.3 ± 0.0^{a}	2.5 ± 0.1^{b}	2.5 ± 0.1^{b}	4.6 ± 0.3^{d}	3.7 ± 0.1^{c}	3.1 ± 0.0^{bc}
22:6n3	9.5 ± 0.4^{a}	40.8 ± 0.6^{f}	27.3 ± 0.4^{e}	22.7 ± 0.1^{d}	22.9 ± 0.2^{d}	15.3 ± 0.4^{b}	19.4 ± 0.3^{c}
Total polyunsaturated	29.3 ± 0.6^{a}	62.4 ± 1.4^{d}	54.4 ± 0.4^{c}	43.4 ± 0.1^{b}	64.77 ± 0.5^{d}	53.5 ± 0.6^{c}	43.7 ± 0.6^{b}
Total ω3	17.5 ± 0.4^{a}	43.4 ± 1.4^{d}	38.8 ± 0.3^{d}	29.3 ± 0.1^{c}	30.26 ± 0.9^{c}	27.6 ± 0.2^{bc}	26.9 ± 0.1^{b}
Total ω6	9.3 ± 0.2^{a}	16.8 ± 0.4^{c}	15.0 ± 0.1^{b}	13.4 ± 0.1^{b}	34.44 ± 0.3^{e}	25.1 ± 0.7^{d}	16.1 ± 0.6^{c}
ω3/ω6	1.9 ± 0.2^{cd}	2.6 ± 0.1^{e}	2.6 ± 0.2^{e}	2.2 ± 0.4^{d}	0.88 ± 0.0^{a}	1.1 ± 0.1^{b}	1.7 ± 0.1^{c}
ARA/EPA	2.0 ± 0.1^{c}	1.5 ± 0.2^{b}	0.5 ± 0.1^{a}	1.5 ± 0.2^{b}	14.53 ± 0.3^{d}	2.0 ± 0.1^{c}	2.3 ± 0.2^{c}
DHA/EPA	4.0 ± 0.3^{b}	39.4 ± 0.5^{f}	3.7 ± 0.2^{b}	6.5 ± 0.1^{d}	15.95 ± 0.4^{e}	2.2 ± 0.1^{a}	5.1 ± 0.3^{c}
Total FA (mg FA g^{-1} dry weight)	173.4 ± 2.9^{c}	398.3 ± 5.0^{d}	174.8 ± 4.1^{c}	110.0 ± 5.5^{a}	392.0 ± 2.0^{d}	141.5 ± 3.1^{b}	120.3 ± 2.9^{ab}

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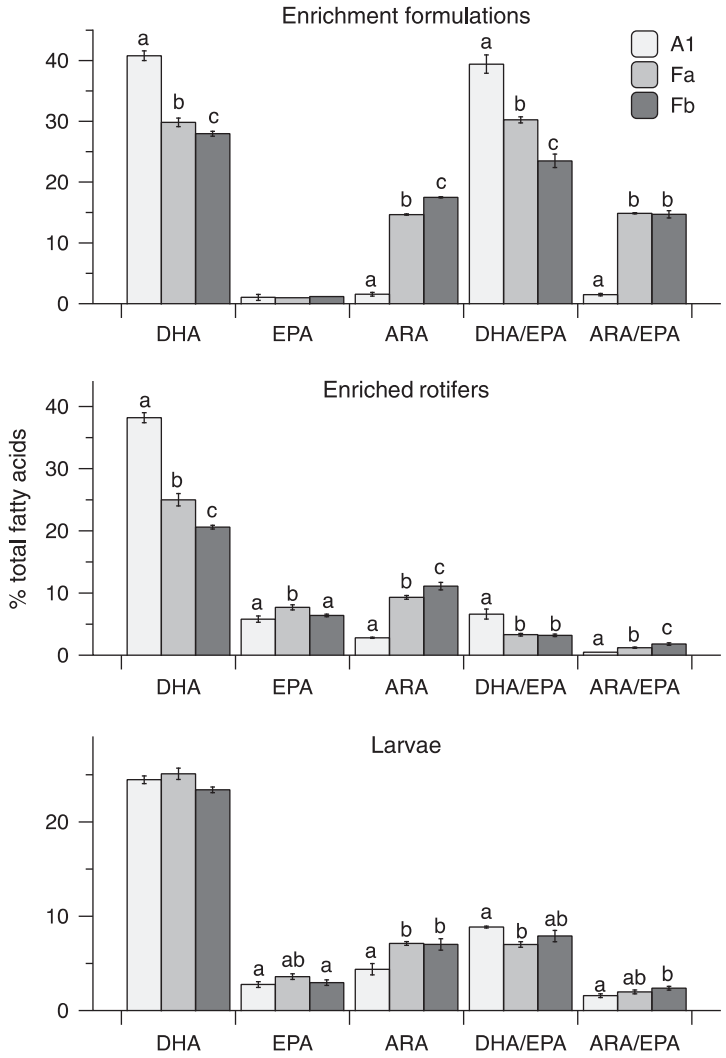

Figure 4 DHA, EPA, ARA, DHA/EPA and ARA/EPA ratios in the enrichment formulations, enriched rotifers and larvae at the end of the first trial. Values are ±SEM, $n = 3$ for the enrichment formulations, $n = 4$ for larvae (50 larvae per tank) and $n = 6$ for enriched rotifers. Letters indicate significant differences between treatments.

these species, the decrease could be due to either catabolism or bioconversion to DHA.

Among the enrichment formulations tested in the second part of the study, the ARA supplementation did not increase survival or eye diameter. An increase in growth was observed in the first enrichment trial, yet, considering the lower survival and swim bladder inflation rates in the supplemented treatments, the difference can probably be attributed to the lower density in these treatments rather than the supplementation. In the second trial, no significant difference was observed in growth or survival between treatments even though the rate of functional swim bladder was significantly lower for the supplemented larvae. The oil-based formulation of the supplemented treatments seemed to negatively impact the swim bladder inflation success of the larvae compared to the flake formulation of Algamac 3050 despite efforts to reduce the oil film at the surface of the tanks. Future studies could benefit from supplementing the enrichment with ARA powder rather than oil.

Large differences were observed in the fatty acid profile of the larvae at the end of the trials. The main challenge in achieving a high ARA/EPA ratio is the relatively high natural EPA content of rotifers. Indeed, even when the proportion of EPA in the enrichment formulation was as low as 1%, the proportion reached over 5% in the enriched rotifers. At the end of the first trial, Fa and Fb larvae successfully incorporated ARA and EPA at a 2:1 ratio; however, the low 22:5n6 (Docosapentaenoic acid, DPA) content in these treatments compared to that of the Algamac treatment was a concern. In the second trial, a higher level of DPA was provided in the Fc formulation, which might

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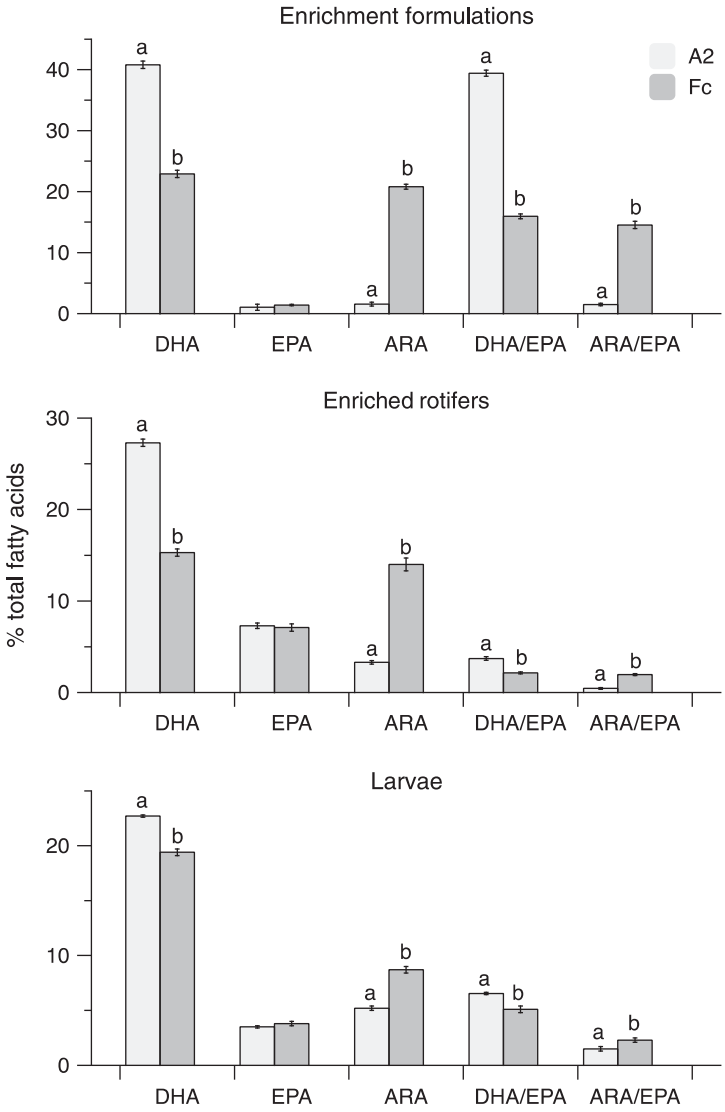

Figure 5 DHA, EPA, ARA, DHA/EPA and ARA/EPA ratios in the enrichment formulations, enriched rotifers and larvae at the end of the second trial. Values are ±SEM, $n = 3$ for the enrichment formulations, $n = 4$ for larvae (50 larvae per tank) and $n = 6$ for enriched rotifers. Letters indicate significant differences between treatments.

have played a role in the increase in survival from the Fa and Fb larvae. The Fc formulation appears to be adequate in providing the necessary amount and proportions of fatty acid to snook larvae in reference to the wild eggs; however, even though growth and survival were not different than that of Algamac 3050, the rate of functional swim bladder development was significantly lower. Additional studies should therefore investigate the use of ARA powder rather than oil as mentioned previously, or improve the stability of the oil emulsion. Bessonart *et al.* (1999) did not observe a significant improvement in growth after supplementing ARA to gilthead sea bream larvae for 2 weeks, but a difference was observed after 3 weeks. In addition, Koven *et al.* (2001) did not observe growth or survival improvement either after supplementing larvae from the same species for 2 weeks; nevertheless, a significant increase in stress resistance was observed in the supplemented larvae exposed to handling stress potentially suggesting more robust larvae. Therefore, future studies should investigate the effect of an ARA supplementation over a longer period of time and include the study of additional parameters such as cortisol production.

In conclusion, these studies bring fundamental information on the early fatty acid requirements of pompano and snook larvae. Findings also provided the first insight of snook larvae fatty acid incorporation during the live food period and the influ-

ence of an ARA supplementation on final fatty acid incorporation and ratios in snook larvae.

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