The effect of dietary DHA and taurine on rotifer capture success, growth, survival and vision in the larvae of Atlantic bluefin tuna (Thunnus thynnus)


Malta Aquaculture Research Center, Fort San Lucian, Marsaxlokk, Malta
Laboratoire Aragó, Banyuls/Mer, Cedex, France

ARTICLE INFO

Keywords:
Bluefin tuna larvae
Docosahexaenoic acid
Retinal opsin

ABSTRACT

The severe to complete mortality that occurs during the larviculture of Atlantic bluefin tuna (ABFT; Thunnus thynnus) may be due, in part, to sub-optimal neural and eye development. The adult and larval ABFT eyes are rich in docosahexaenoic acid (DHA; 22:6n-3), which facilitates key intra-membrane reactions in the photoreceptors of the retina. Another critical nutrient is taurine, which plays vital roles that include bile salt conjugation as well as development and function of visual, neural and muscular systems. The objectives of the present study were to (1) determine the pattern of conservation and loss of fatty acid groups and their conjugation during egg and pre-larval development as well as in food deprivation. (2) Determine the effect of rotifer (Brachionus rotundiformis) DHA on hunting success, growth, and retinal opsin abundance in 2–14 dph ABFT larvae. (3) Evaluate the effect of supplemented taurine in rotifers enriched on the most effective DHA level from objective (2) on larval survival and growth.

During the egg and yolk sac larval stages, there was a significant decrease (P < 0.05) in SAT, MONO and PUFA that can be expressed as 46.7%, 50.3% and 57.1%, respectively. Similarly, the levels of DHA, EPA, and ArA were markedly (P < 0.05) reduced that can be expressed as 59.8%, 52.5% and 59.5%, respectively. In the DHA study, there was a rotifer DHA dose dependent (P < 0.05) effect on prey consumption by 3–7 dph ABFT larvae where the highest DHA level (11 mg g⁻¹ DW rotifer) elicited significantly (P < 0.05) higher rotifer consumption compared to the control and moderate DHA diets (2 mg g⁻¹ DW and 5 mg g⁻¹ DW rotifer, respectively). Moreover, larvae with the highest DHA level (7.01 mg g⁻¹ DW) exhibited a significantly (P < 0.05) higher opsin protein concentration (25.27 unit area⁻¹) compared to the 2.83 mg DHA g⁻¹ DW and 1.26 mg DHA g⁻¹ DW fish (20.32 and 16.33 opsin protein unit area⁻¹, respectively). Although there was a significant (P < 0.05) taurine modulated increase in larval length in 10 dph fish, there was a non-significant (P > 0.05) growth advantage, in terms of dry weight, as a result of moderate dietary taurine supplementation at the end of the study. Nevertheless, the moderate 6.44 mg taurine g⁻¹ DW larvae exhibited markedly (P = 0.024) better survival and > 4 times higher (P = 0.0018) average tank biomass (273.6 mg) than the low (1.97 mg g⁻¹ DW) and high (12.62 mg g⁻¹ DW) taurine fish (62.14 and 56.90 mg, respectively). Overall, the data suggests that supplementing effective levels of DHA and taurine contributes to an array of physiological processes resulting in enhanced vision and prey acquisition to markedly improve ABFT larval performance during early development.

1. Introduction

The Atlantic bluefin tuna (ABFT; Thunnus thynnus, Linnaeus 1758) is arguably one of the most prized fish in the sea due to its recreational and commercial importance. In recent years, the expansion of the sushi-sashimi market in Japan has greatly increased demand and profitability resulting in substantial fishing pressure on the Mediterranean population (Fromentin and Powers, 2005). This together with questionable stock assessment and enforcement practices (Webster, 2011) has raised real concerns about the future of this iconic species (Sumaila and Huang, 2012; Webster, 2011). Attempts to understand the biological cycle and grow ABFT in captivity, as a potentially sustainable
alternative to over exploited natural stocks, have been on-going for a number of years particularly within the framework of European grant projects such as Reprodott (Q5RS-2002-0135), Selfdott (212797) and Transdott (311904). These research efforts have paid dividends recently as there is now a concerted effort to establish ABFT aquaculture in a number of countries around the Mediterranean basin. However, there remains a number of major bottlenecks severely hampering the advancement of this nascent industry.

One such obstacle is the exceedingly high mortality characterizing different developmental stages during larval and juvenile rearing (Sawada et al., 2005). In particular, the first 10 days in the larviculture of a variety of tuna species, are plagued by poor survival (Woolley et al., 2013; Kurata et al., 2011; Sawada et al., 2005), which has been associated with numerous factors such as poor environmental conditions, absence of swim bladder inflation, the sinking of larvae during the night and suboptimal prey capture at the beginning of exogenous feeding (Tanaka et al., 2009). After the absorption of the yolk sac, which is coincident with the pigmentation of the eyes and the opening of the mouth and anus, marine fish larvae are able to feed on an exogenous food source and must quickly learn to hunt efficiently and effectively before reaching the point of no return (PNR). Larvae exceeding the PNR in a compromised nutritional state results in the progressive deterioration of the digestive tract leading to certain mortality (Yúfera and Darias, 2007). In fact, due to the warm rearing temperature (ca. 25 °C) of ABFT, yolk sac depletion occurs very quickly compelling the larvae to successfully feed by 2 days post hatching (dph) (Yúfera et al., 2014). The energy maintenance demands of ABFT juveniles and adults were shown to be markedly higher than other cultured teleosts (FitZigibbon et al., 2008; Takii et al., 2005). This is also suggested by their rapid triacylglycerol decrease during embryonic development that further emphasizes the importance of first feeding (Moureute and Tocher, 2009; Takii et al., 2005).

As in other pelagic fish, tuna larvae are visual predators (Benítez-Santana et al., 2007) voraciously consuming mainly instar stages of copepods in nature (Uotani et al., 1990). Similarly to larvae of other marine teleosts, the retinal membranes of the larval ABFT eye are very rich in the essential long chain polyunsaturated fatty acid (LCPUFA); docosahexaenoic acid (DHA; 22:6n-3), which facilitates key intracellular reactions. This is primarily due to its role in membrane fluidity and direct interaction with opsin proteins (Izquierdo and Koven, 2011). The importance of DHA for the proper development of neural tissues has been demonstrated in larval Atlantic herring (Clupea harengus) (Moureute and Tocher, 1992; Bell et al., 1995; Mourente, 2003), European sea bass (Dicentrarchus labrax) (Navarro et al., 1997), gilthead sea bream (Sparus aurata) (Moureute and Tocher, 1993; Mourente, 2003), and turbot (Psetta maxima) (Moureute et al., 1991; Mourente and Tocher, 1992; Mourente, 2003). A number of authors have reported that increased dietary DHA or n-3 HUFA improved feeding behavior in herring larvae (Bell et al., 1995) and prey consumption in gilthead sea bream (Koven et al., 2012) when fed under low light intensity.

In fact tuna species, in particular ABFT, have exceedingly higher levels of DHA and DHA/EPA ratios than other teleosts (Sargent et al., 2002; Tocher, 2003; Mourente, 2003). It is now widely believed that a high concentration of n-3 LCPUFA in neural tissues would be crucial for effective prey capture from the time of first feeding (Bell et al., 1995; Mourente and Tocher, 2009). However, it has not been shown that DHA directly improves vision and prey hunting success in ABFT larvae at first feeding.

Another key nutrient that is found in copepods but is lacking or at trace levels in rotifers, is taurine (Van der Meeren et al., 2008). Taurine (2-aminoethane sulfonic acid) is a β-amino acid that lacks a carboxyl group and therefore, cannot be incorporated into proteins (Li et al., 2009). However, taurine is one of the most abundant, low molecular weight organic constituents of animal tissues and plays vital roles in bile salt conjugation (Kim et al., 2007), osmoregulation, membrane stabilization (Huxtable, 1992), modulation of neurotransmitters (El Idriessi and Trenkner, 2004), as an antioxidant, although its effectiveness remains controversial (Oliveira et al., 2010; Schaffer et al., 2010), and in heart and muscular systems (Salze and Davis, 2015; Oliveira et al., 2010) as well as retinal development and function (Militante and Lombardini, 2002). Taurine has been associated with improved larval vision and performance in the red sea bream and Japanese flounder (Matsunari et al., 2008; Kim et al., 2005) and is known to promote differentiation of retinal cells in vertebrates (Ahlhuier et al., 1993) as well as accumulating in the outer segments of retinal rods where they play a role in shielding the photoreceptor from damaging light exposure (Pasantes-Morales and Cruz, 1985). A taurine requirement has been indicated in marine fish such as juvenile yellowtail (Seriola quinquenadiata; Takagi et al., 2008), bluefin (Thunnus thynnus; Yokoyama et al., 2001) and skipjack (Katsuwonus pelamis; Yokoyama et al., 2001) tunas, Japanese flounder (Paralichthys olivaceus; Kim et al., 2005) and red sea bream (Pagrus major; Matsunari et al., 2008). This was assumed due to a deficiency in cysteine sulfate decarboxylase (CSD), a key enzyme catalyzing the decarboxylation of cysteine sulfate to hypotaurine in the main taurine biosynthesis pathway (Yokoyama et al., 2001; Goto et al., 2003; Chen et al., 2004). The requirement for dietary taurine was further emphasized in studies testing the efficacy of replacing fish meals with taurine poor plant-based proteins in marine species such as juvenile white seabass (Atractoscion nobilis; Jirsa et al., 2014), golden pompano (Trachinotus ovatus; Wu et al., 2015), toaoba (Totaoba macdonaldi; López et al., 2015) and white groupper (Epinephelus aeneus; Koven et al., 2016). On the other hand, freshwater herbivorous species such as the common carp (Cyprinus carpio) do not appear to have a taurine requirement (Fontagné et al., 2000; Carvalho et al., 2004; Kim et al., 2008) although they markedly accumulate tissue taurine while expressing low CSD activity (Yokoyama et al., 2001). This suggests another taurine biosynthesis pathway may be active.

The objectives of the present study were to (1) evaluate the level of DHA available in first feeding ABFT larvae by determining the pattern of conservation and loss of this essential fatty acid as well as other fatty acids during egg and pre-larval development as well as in food deprivation. (2) Determine the effect of rotifer (Brachionus rotundiformis) DHA on food ingestion, growth, survival and retinal opsin abundance in 2–14 dph ABFT larvae. (3) Evaluate the effect of supplemented taurine in rotifers enriched on the most effective DHA level from objective (2) on vision, food acquisition, survival and growth.

2. Methods and materials

2.1. Food deprivation study

The food deprivation experiment was carried out in four replicate 81 (200 μm mesh) cages floating in a 4001 V-tank where filtered (10 μm), UV-treated (160 mJoules cm–2), 40% seawater entered from the bottom of the tank and exited through a 150 μm filter near the surface at a tank exchange rate of 200% day–1. There was no direct lighting over the tank so as to not stimulate a prey searching response after yolk sac depletion. ABFT eggs, that were collected from a cage facility in Malta and transported in 3 cubitainers (ca 15,000 eggs 1–1), reached the IOLR/NCM facility after ca. 35 h in transit. The eggs arrived with an average temperature, oxygen and pH levels of 21.2 ± 0.2 °C, 549 ± 73.1% and 6.96 ± 0.25, respectively, (pH raised to ca 7.9 with titrating 1 N NaOH) and demonstrated a hatching rate of 87.5 ± 5% and survival at the end of the day of hatching (0 dph) of 84.6 ± 12% using three plastic well plates, where each plate had 12 wells (5 ml). The eggs were stocked (50 eggs 1–1) in the mesh cages at secondary organ formation stage (SO1-SO2) while egg samples were immediately taken, after rinsing in fresh and double distilled water (DDW) and placed in 1.5 ml Eppendorf tubes and stored at −32 °C until fatty acid analysis. No zooplankton or phytoplankton were offered to the larvae and in the morning of 1, 2, 5 and 6 dph up to
30 larvae were taken from each cage and identically treated as the previous samples. All samples after one day at −32 °C were freeze dried and stored at −32 °C until fatty acid analysis. The temperature of the seawater in the tank was computer controlled (Gavish, Israel) and rose from ca. 22.5 °C to stocking at a rate of ca. 1 °C day −1 to 25 °C.

2.2. DHA study

The DHA study was carried out in a fifteen 1500 l V-tank flow through system where filtered (10 μm), UV-treated (160 μJ cm −2), 40% seawater entered from the bottom of the tank and UV treated through a 150 μm filter near the surface at a tank exchange rate of 200% day −1 allowing the testing of 3 rotifer (B. rotundiformis) DHA treatments in 5 replicate tanks treatment −1. Eggs collected from a cage facility in Cartegena, Spain reached the IOLR/NCM facility in excellent condition (oxygen > 250%, pH ca. 6.5, temperature 22−23 °C) ca. 26 h after collection at sea and were stocked in the tanks at 30 eggs l −1 and demonstrated a hatching rate of 95.8 ± 4.8% and survival to the end of the day of hatching of 99.8 ± 0.1%.

The temperature of the seawater in the tank was computer controlled (Gavish, Israel) and rose from 22.5 °C at stocking, at a rate of 1 °C day −1, to 25 °C and then maintained at this temperature until the end of the experiment at 14 dph. This end point was chosen as after this age, larvae grow more slowly in this water volume but are too fragile to transfer to another tank. Moreover, 15 dph larvae also begin to feed on a larger Artemia nauplii, whose natural taurine reserves would be a confounding factor.

Following eye pigmentation and mouth opening, 2 dph ABFT larvae were fed the three rotifer DHA treatments, which had been enriched previously for 8 h on the low, medium and high DHA preparations (21.7, 36.5, 63.7 mg DHA g −1 DW, respectively; Bernaqua, Belgium). These enrichment preparations resulted in three significantly (P < 0.05) different DHA levels in the rotifers (2.0, 5.0 and 11.0 mg DHA g −1 DW) and were fed to the larvae at 10 rotifers ml −1 together with the addition of the algae Nanocorophius oculata twice daily at 0.5 × 10 6 cells ml −1 until 14 dph. The illumination of the tanks was measured at the water surface at 500 lx and the light-dark photoperiod was 18:6.

Enrichment preparations and treatment rotifers were collected for fatty acid analysis at least 3 times during the course of the experiment. Larval samples (ca. 10 fish) for length (ocular micrometer) and mastax measurements were taken from 2 to 7 dph while remaining 14 dph larvae at the end of the study were hand counted to calculate survival and samples (50–100 fish per sample) freeze dried for dry weight (DW) and fatty acid determination (described below). Although lipophilization does not completely remove all water, the freeze dried samples were considered as completely dried.

To determine the DHA effect on retinal opsin abundance, 14 dph larvae (3–5 larvae tank −1) were sampled at the end of the experiment for immunocytochemistry (ICC) and were first fixed in 4% paraformaldehyde (PFA) in a 0.1 M phosphate buffer solution followed by dehydration and embedding in methacrylate resin LWR. Ultrathin sections were incubated with primary antibody: monoclonal anti-opsin IgG1 (Sigma-Aldrich) and a second incubation with secondary antibody: immune-gold conjugate 15 nm (Aurion). After being contrasted with uranyl acetate, sections were observed on a Hitachi 7500 electron microscope.

Rotifer consumption was evaluated by counting the number of mastax in the larval gut 90 min after the morning feeding of 3–7 dph fish (5–10 larvae tank −1). This number was considered equal to the number of rotifers consumed. After 7 dph, larval body pigmentation had sufficiently increased to interfere with accurate mastax counting. In brief, sampled larvae were first placed in 1.5 ml Eppendorf vials with buffered formalin (4%) until analysis. Under a fume hood, larvae were removed from the buffered formalin solution and briefly rinsed in DDW where after their total length was measured using an ocular micrometer. The larvae are then placed on a microscope slide with a coverslip on top. The coverslip is gently pressed until gut contents have been extruded and the mastaxes counted under a microscope.

2.3. Taurine study

The taurine study was carried out in a twelve 1500 l conical tank flow-through experimental system allowing the testing of three rotifer (B. rotundiformis) taurine treatments (0, 400 and 600 mg taurine −1 −1 enrichment water) in 4 replicate tanks treatment −1 with environmental conditions described above. ABFT eggs, that were collected from a cage facility in Malta and transported in 3 cubitainers (ca 15,000 eggs l −1), reached the IOLR/NCM facility after ca. 35 h of transit time. The eggs arrived with an average temperature, oxygen and pH levels of 21.2 ± 0.2 °C, 349 ± 73.1% and 6.96 ± 0.25, respectively, (pH raised to ca. 7.9 with slowly dripping 1 N NaOH) and demonstrated a hatching rate of 87.5 ± 5% and survival to the end of the day of hatching of 84.6 ± 12% (tested in three plastic plates, where each plate contained 12 five ml wells). Only viable, floating eggs (1040 ± 55 μm) were stocked in the twelve tanks (20 eggs l −1). Following eye pigmentation and mouth opening ABFT larvae, in their respective treatment tanks, were fed one of the rotifer taurine treatments at a concentration of 10 rotifers ml −1 and the tanks greened with N. oculata twice daily at 0.5 × 10 5 cells ml −1. Each of the rotifer taurine treatments, had been previously enriched (1 × 10 6 rotifers l −1) for 21 h at 25 °C. In addition, all rotifer treatments were enriched on the 32% DHA (63.7 mg DHA g −1 DW) enrichment preparation for 8 h (13 h after taurine enrichment), which was the best performing enrichment in the DHA experiment described above. Enriched rotifers were sampled at least 3 times during the course of the experiment for taurine level while larvae at the end of the study were counted for survival and sampled for dry weight (DW), total length (TL), taurine and fatty acid composition. Larvae for gut mastax measurements were collected and determined as described above in the DHA study. Due to visually poorer larval survival and the concern that it would not be possible to sample enough larvae at 14 dph, the taurine study was terminated at 12 dph.

2.4. Fatty acid analysis

In fatty acid analysis, the following procedure was carried out. The total lipid in freeze dried tissue samples (20 mg) was chloroform-methanol (2:1) extracted (Folch et al., 1957). The lipid-containing chloroform upper phase was removed and evaporated to dryness under a stream of nitrogen and total lipid weighed (GH-120 analytical balance, A & D, San Jose, CA,USA). This was followed by the addition of the internal standard 17:0 (heptadecanoic acid) to each of the samples. The samples were then transmethylated to their fatty acid methyl esters (FAME) by adding 1 ml mg −1 lipid of a 14% solution of boron trifluoride methanol (BF3) and sonicated for 1 h at 50 °C. To the samples were added 0.5 ml double distilled water (DDW) and 1 ml mg −1 lipid of analytical grade hexane (99%), which was vortexed and then centrifuged (2300 rpm) for 2 min. The top hexane layer containing FAME was transferred to a vial and stored at −20 °C until analysis in a Varian 450–220 GC/MS/MS (Agilent technologies, California, USA). Injected FAME samples (1 μl) were separated on a Varian WCOT fused silica column (50 M × 0.32 mm) at a flow rate of 1.5 ml min −1 and identified by known purified standards and quantified using a response factor to the internal standard (17:0; Sigma, St. Louis, MO, USA). The 30 min oven temperature program began at 70 °C for 4 min following injection and then increased to 300 °C at 10 °C min −1 for 3 min.

2.5. Taurine analysis

Freeze dried samples of 2–5 mg for Varian 325–410 HPLC (Agilent
technologies, California, USA) taurine analysis were prepared by adding 3 ml of 6 M HCl and 0.5% phenol. The samples were flushed with nitrogen and placed in a heating block for 24 h at 108–110 °C. After cooling samples to room temperature and filtering (0.45 μm; cellulose nitrate), 0.5 ml carbonate buffer (pH 9), 0.5 ml DMSO (dimethyl sulfoxide) and 0.1 ml DNFB (1-fluoro-2,4-dinitrobenzene) were added and the samples mixed well followed by heating for 15 min at 40 °C then cooled for 10 min. To the samples were added 6.5 ml of 0.01 M of buffered phosphate, vortexed for 30 s and then left to stand for 5 min. The samples were then transferred to HPLC vials and injected (10 μl) into an Acclaim®120 C18 (5 μm, 4.6 × 150 mm) HPLC column (Thermo Scientific, USA). Column flow rate was 1.5 ml min−1 where specific ratios of buffer phosphate 0.01 M (pH 6) and acetoni-trite (90:10, 10:90, 90:10) were introduced into the column at different times (0, 10, 11, 11.01, 18 min), respectively.

2.6. Statistical analysis

One way ANOVA, two way ANOVA and regression (linear and non-linear) analyses, using all replicate values, were carried out using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com). Regression data sets employed Akaike’s Information Criteria (AIC) to compare linear, second order polynomial and other models to determine which most likely generated the data. ANOVA analyses and Barlett’s test for equal variances were carried out simultaneously. If significance (P < 0.05) was found for ANOVA while Barlett’s test was not significant (P > 0.05), then testing differences between groups was carried out by Newman-Keuls Multiple Comparison test. In cases where ANOVA and Barlett’s test were both significant (P < 0.05), then the non-parametric Kruskal Wallis Test was applied followed by Dunn’s multiple Comparison test to determine significant (P < 0.05) differences among treatments. All data are presented as mean ± SEM. Outliers were identified by calculation of the Z value using the Grubbs test (Rousseeuw and Leroy, 2003) and removed if calculated Z value was higher than tabulated value.

3. Results

The level of the saturated (SAT) and monounsaturated (MONO) fatty acids increased marginally from egg to yolk-sac larvae while this was significant (P < 0.05) in the polyunsaturated (PUFA) fatty acids. This was due to the loss of the weight of the lipid free egg chorion (Fig. 1). Nevertheless, this was followed by a significant decrease (P < 0.05) in SAT, MONO and PUFA from 0 to 2 dpf, when ABFT larvae would be offered live food, that can be expressed as 46.7%, 50.3% and 57.1%, respectively. PUFA in food deprived larvae (3 to 6 dpf) continued to drastically (P < 0.05) decrease from 2 to 6 dpf exhausting 78.21% of the stores of this fatty acid group at hatching (Fig. 1), while there were no marked decreases in MONO and SAT groups after 2 dpf. In the PUFA group, the level of the essential fatty acids (EFA); docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (ArA) significantly decreased (P < 0.05) from 0 to 2 dpf, which can be expressed as 59.8%, 52.5% and 59.5%, respectively (Fig. 2). DHA in food deprived larvae continued to drastically (P < 0.05) decrease from 2 to 6 dpf exhausting 95.3% of the stores of this EFA at hatching (Fig. 2). Regression analyses of DHA, EPA and ArA showed that the pattern of decrease (2nd order polynomial) in these long chain PUFA from 0 to 6 dpf were significantly (P < 0.0001) different from each other while DHA exhibited the strongest response. In Fig. 3, there was a significant (P < 0.05) enrichment DHA dose dependent response on the levels of this essential fatty acid in the rotifers and the larvae. Rotifer DHA enrichment in the 21.7, 36.5 and 63.7 mg DHA g−1 preparations significantly (P < 0.05) increased in linear fashion to 2.58, 4.99 and 11.05 mg DHA g−1, respectively. Similarly, larvae consuming these rotifers significantly (P < 0.05) increased their DHA levels, although the slope was less than that of rotifers, to 1.26, 2.83 and 7.01 mg DHA g−1, respectively. The slopes of these lines or the rate of increase of DHA in larvae and rotifers (0.1119 and 0.2042, respectively) were significantly (P < 0.05) different.

In Fig. 4, there was a marked rotifer DHA dose dependent (P < 0.05) effect on prey consumption in 3–7 dpf in ABFT larvae. Different curves for each data set were found (P = 0.0001). Linear regression of the 2 mg DHA g−1 DW rotifer treatment with avg. mastax no. larva−1 was the preferred model according to AIC while second order polynomials were the preferred models (AIC) regressing the 21.7, 36.5 and 63.7 mg DHA g−1 preparations significantly (P < 0.05) increased their DHA levels, although the slope was less than that of rotifers, to 1.26, 2.83 and 7.01 mg DHA g−1, respectively. The slopes of these lines or the rate of increase of DHA in larvae and rotifers (0.1119 and 0.2042, respectively) were significantly (P < 0.05) different. However, the DHA diet fish did not significantly (P > 0.05) consume more rotifers than the control fish (Fig. 4). Two way ANOVA analysis demonstrated that both larval age (dpf) and rotifer DHA had a very significant (P < 0.0001) effect on the variability of rotifer (mastax) consumption (51.75 and 17.63%), respectively as well as their interaction (P = 0.0002; 9.82%). These results are still considered valid despite the fact that p-values do not strictly represent polynomial regressions.

In Fig. 5A there was a larval DHA dose dependent (P < 0.05) effect on opsin protein per unit area in 14 dpf ABFT larvae where the highest DHA level (11 mg g−1 DW rotifer) elicited significantly (P < 0.05) higher rotifer consumption on each day throughout this period compared to the control (2 mg g−1 DW rotifer) and from 5 to 7 dpf compared to the moderate DHA diet (5 mg g−1 DW). Apart from 4 dpf, the moderate DHA diet fish did not significantly (P > 0.05) consume more rotifers than the control fish (Fig. 4). Two way ANOVA analysis demonstrated that both larval age (dpf) and rotifer DHA had a very significant (P < 0.0001) effect on the variability of rotifer (mastax) consumption (51.75 and 17.63%), respectively as well as their interaction (P = 0.0002; 9.82%). These results are still considered valid despite the fact that p-values do not strictly represent polynomial regressions.

Fig. 1. (A) Larval levels of the fatty acid groups; saturated (SAT), monounsaturated (MONO) and polyunsaturated (PUFA) fatty acids (mg g−1 DW larva) in the egg (e), post-hatching and yolk-sac stages (0 and 1 dpf, respectively), larval age when live food would be offered (2 dpf) and during food deprivation (3-6 dpf). Values within a fatty acid group having different letters were significantly (P < 0.05) different. (B) Regression analysis of the decrease (mg g−1 DW larva) of SAT, MONO and PUFA from after hatching (0 dpf) to the end of food deprivation at 6 dpf expressed as second order polynomials, which was the preferred model according to AIC. Different curves for each data set were found (P < 0.0001).
The slopes of the rotifers and larvae linear regressions were significantly different. Mastax values between treatments at a specific DHA enrichment treatment (mg g$^{-1}$ DW) on rotifer and larval DHA levels. Two way ANOVA table lists the P value associated with source of variation. The slopes of the rotifers and larvae linear regressions were significantly different (P = 0.020). Two way ANOVA table lists the P value associated with source of variation. Mastax values between treatments at a specific larval age (dph) having different letters were significantly different (P < 0.05) different.

consistently consumed significantly (P < 0.05) more prey than the other DHA treatments but this was reflected in superior (P < 0.05) growth (total length; TL) only at 4 and 5 dph. After this, increasing larval DHA no longer promoted TL. In fact, at the end of the feeding trial at 14 dph, there was no significant (P > 0.05) difference in TL or DW (data not shown) between the DHA treatments (Fig. 6).

In Fig. 7A, there was a significant (P < 0.05) taurine enrichment dose-dependent response in rotifers that were enriched on taurine levels of 0, 400 and 600 mg l$^{-1}$, which exhibited 2.5, 5.1 and 7.9 mg taurine g$^{-1}$ DW, respectively. Different curves for each data set were found (P = 0.0001). Two way ANOVA table lists the P value associated with source of variation. Mastax values between treatments at a specific larval age (dph) having different letters were significantly different (P < 0.05).

(7.9 mg g$^{-1}$ DW) taurine treatment. In older 12 dph larvae, there was a significant (P < 0.05) rotifer taurine dose dependent accumulation of this nutrient in these fish. In fact, the rate of taurine assimilation was significantly (P = 0.0002) higher in the older 12 dph larvae compared to the 7 dph fish. Nevertheless, dietary taurine level did not have a significant (P > 0.05) effect on larval rotifer consumption (results not presented here). However, in Fig. 8A, 10 dph larvae feeding on 5.1 and 7.9 mg taurine g$^{-1}$ DW rotifers, which were also enriched on the high DHA (11.0 mg DHA g$^{-1}$ DW rotifer) treatment, were significantly (P = 0.01) longer than the control treatment fish (2.5 mg taurine g$^{-1}$ DW). This contrasts with similar age larvae (8 dph) in the previous study that exhibited no dietary DHA effect, when fed taurine deficient DHA enriched rotifers, on larval length (Fig. 6). Larvae consuming rotifers containing 5.1 mg taurine g$^{-1}$ DW exhibited markedly (P = 0.024) better survival compared to fish feeding on low and high dietary taurine values (Fig. 8B). Although, there was a non-significant (P > 0.05) but clear growth advantage in fish fed the 5.1 mg taurine g$^{-1}$ DW rotifers (Fig. 8C), these larvae demonstrated a significantly higher average (273.6 mg) larval tank biomass (no. of larvae × average DW treatment$^{-1}$), which was > 4 times (P = 0.0018) the average tank biomass shown in larvae fed the low and high taurine treatments (62.14 and 56.90 mg, respectively) (Fig. 8D).

4. Discussion

The eggs of ABFT are one of the richest sources of n-3 LCPUFA in the marine environment, particularly DHA which exceeds 19% of the total fatty acids (Takii et al., 1997; Ortega and Mourente, 2010). The DHA/EPA ratio, as well, is substantially higher than most other species (Morais et al., 2011). The present study found a sharp decline in total lipid saturated (SAT), monounsaturated (MONO) and polyunsaturated fatty acids (Takii et al., 1997; Ortega and Mourente, 2010). The DHA/PUFA ratios of neutral lipid as the main energy source involved in gene expression and building blocks for lipid based hormones such as eicosanoids (Sargent, 1995; Wiegand, 1996; Sargent et al., 1989, 2002). The catabolism of neutral lipid as the main energy source can be mobilized for energy metabolism as well as contributing to essential components of cell membranes, ligands involved in gene expression and building blocks for lipid based hormones such as eicosanoids (Sargent, 1995; Wiegand, 1996; Sargent et al., 1989, 2002). The catabolism of neutral lipid as the main energy source involved in gene expression and building blocks for lipid based hormones such as eicosanoids (Sargent, 1995; Wiegand, 1996; Sargent et al., 1989, 2002).
et al., 1999; Sargent et al., 2002; Ortega and Mourente, 2010). DHA is highly representative in neural tissue, particularly in the retina and consequently plays an essential role in vision, the main sense employed during prey hunting in fish larvae (Bell et al., 1995; Mourente and Tocher, 2009). On the other hand, a number of studies reported that the catabolism of DHA as an energy source can occur in Atlantic halibut (Hippoglossus hippoglossus), plaice (Pleuronectes platessa), cod (Gadus morhua), turbot (Scophthalmus maximus) and European sea bass (Dicentrarchus labrax) (Rainuzzo et al., 1992; Rønnestad et al., 1998) while EPA was less metabolized. In contrast to the results of our study, Morais et al. (2011) found in ABFT that the level of DHA was maintained while EPA decreased during yolk sac utilization, which is a period characterized by generally high fatty acid oxidation and utilization. These authors argued that the coincident large increase in desaturase and elongase activity maintained adequate DHA levels in order to contribute to neural membrane synthesis. However, they expressed their fatty acid results in percent (%) of total fatty acids (TFA), a relative term that might be misleading when calculating quantitatively conservation and loss of n-3 LCPUFA of the whole larvae. In contrast, the fatty acid levels in the present study were expressed as mg g$^{-1}$ DW larva. Studies have shown that it is possible to stimulate Δ6 desaturation gene expression and enzymatic activity when fish are fed vegetable oil diets deficient in LCPUFA (Vagner and Santigosa, 2011), which is more closely aligned with the present study reporting a DHA deficit. Nevertheless, the magnitude of these increases in fatty acid synthesis was not great enough to fully satisfy the fish’s requirement. A severe decline in stores of available DHA for tissue deposition during early development would compromise larval performance and indicate a
pressing need for substantial levels of DHA in the zooplankton prey at first feeding.

There was a DHA dose dependent (P < 0.05) linear accumulation of this essential fatty acid in the treatment rotifers and the larvae feeding on them (Fig. 3). This led to a larval DHA dose dependent response in the number of rotifers successfully hunted and consumed (Fig. 4). Larvae feeding on the high DHA containing rotifers markedly (P < 0.05) ingested more prey than the control from 3 to 7 dph and the moderate DHA treatment from 5 to 7 dph. In fact, the association between prey DHA content and prey consumption is strikingly similar to larval DHA and retinal opsin accumulation (Fig. 5). This suggests that enhanced prey hunting success by increasing DHA in the larvae was due to improved vision. Bell et al. (1995) demonstrated that dietary DHA increased the number of strikes at prey by juvenile herring (Clupea harengus) under low light intensity while Koven et al. (2012) reported that dietary DHA increased 23 dph gilthead sea bream (Sparus aurata) hunting success under subdued illumination.

In the retina, visual pigments in the rod and cone photoreceptors are comprised of a trans-membrane opsin protein covalently linked to 11-cis-retinal, an aldehyde derivative of vitamin A (Sharpe et al., 1980; Chinen et al., 2003). When a photopigment absorbs a photon of light, 11-cis-retinal isomerizes to its trans form, which initiates a cascade of intramembrane events. These begin with the activation of the G protein transducin, which in turn activates phosphodiesterase, an enzyme that converts the nucleotide cyclic guanosine 3′-5′ monophosphate (cGMP) to 5′GMP. Under dark conditions, cGMP is a Na+ gated channel protein, allowing an inward flow of Na+ and depolarization of the rod cell. Once phototransduction begins, the resulting lower levels of cGMP close the gated ion channel, which subsequently blocks the influx of Na+ into the cell. This leads to the hyperpolarization of the neural membrane, which ultimately causes the propagation of a signal down the optic nerve to the brain. When returning to dark conditions, cGMP increases resulting in a return to the inward flow of Na+ and Ca2+ ions and the depolarization of the photoreceptor cell (Baylor, 1996). It is generally argued that the DHA-modulated fluidity of the photoreceptor membranes facilitates the critical conformational change in the photopigment when it is light stimulated, leading to a more efficient processing of the visual stimulus and improved prey detection. It was demonstrated that photopigment conformational change and binding to the G-protein is greatly facilitated when in DHA-containing bilayers (Mitchell et al., 1998; Mitchell et al., 2003). In contrast, when the bilayer acyl chains become less unsaturated, this process is delayed. (Litman et al., 2001).

In addition to DHA-driven fluidity, there may be DHA-opsin interactions taking place at specific regions of the protein that interfere with membrane packing as well as contributing to photocycle kinetics (Farrens et al., 1996 Grossfield et al., 2006) resulting in faster response times to photon stimulation (Brown, 1994). DHA promoted opsin synthesis in the ABFT eye in the present study which was also shown in the rat (Rotstein et al., 1996, 1999; Politi et al., 2001). Gaon (2016) recently found that DHA upregulated gene expression of five different opsins (RH1, SW51, RH2a, RH2b and LWS) in gilthead sea bream photoreceptors throughout larval development.

There are a number of pathways potentially tying retinal DHA to opsin synthesis. These include initiating the production of proteins required for the assembly of disc membranes of the photoreceptors (Davis-Bruno and Tassinari, 2011; De Rodriguez Turco et al., 1997), mediating the opsin and rhodopsin transport to the apical process of the photoreceptor membrane (De Rodriguez Turco et al., 1997; Rotstein et al., 1998) or promoting differentiation and postponing apoptosis of photoreceptors (German et al., 2013). Garelli et al. (2006) found in mice that DHA promoted gene expression of CRX (cone-rod homeobox), which is an important transcription factor and regulator of photoreceptor cell development and differentiation suggesting that this process may also be active in the developing ABFT eyes.

The DHA modulated increased consumption of rotifers significantly (P < 0.05) improved early larval growth, in terms of total length up to 5 dph, where after this effect diminished completely (Fig. 6). In order to address this, taurine was considered as a potential factor limiting the growth promoting benefit of DHA and was subsequently studied. Taurine was found to improve larval growth in red sea bream (Pagrus major) (Chen et al., 2004), Japanese flounder (Paralichthys olivaceus), (Chen et al., 2005) and amberjack (Seriola dumerili) (Matsumura et al., 2013). In the present taurine study, there was a significant (P < 0.05) rotifer taurine dose dependent response on taurine accumulation in 7 and 12 dph larvae, where the rate of taurine tissue deposition in the larvae (mg taurine in larvae per mg taurine in the rotifer) markedly (P = 0.0002) increased with age (Fig. 7). Indeed, there was a significant dietary taurine effect on larval length in 10 dph fish where the rotifers also had a high level of DHA (11 mg g⁻¹ DW). This contrasts with similarly age larvae fed taurine deficient rotifers having varying levels of DHA, that showed no dietary effect on larval length. On the other hand, at the end of the experiment there was a non-significant (P > 0.05), moderate rotifer taurine effect on larval weight gain. Nevertheless, the most effective (P < 0.05) larval tissue taurine level,
in terms of average tank biomass and survival treatment−1, was 6.44 mg taurine g−1 DW (Fig. 8). Survival in this study ranged from ca. 0.8 to 2% among the treatments from egg stocking to 12 dph. This percent larval survival is similar to reported levels in other tuna species. Woolley et al. (2013) achieved ca. 0.67% in 11 dph southern bluefin tuna (Thunnus maccocyti), while Takashi et al. (2006) found survival rates <1% for Pacific bluefin tuna (Thunnus orientalis) at 10 dph. These authors claimed that the vast majority of this mortality was due to sinking behavior which has been improved by implementing a 24 h photoperiod (Partridge et al., 2011) and/or an upwelling system to increase swim bladder inflation (Nakagawa et al., 2011).

Taken together, these results indicate that moderate dietary taurine supplementation (5.09 mg g−1 DW) is a limiting factor that improved the DHA promoting effect on larval performance. Taurine plays vital roles in bile salt conjugation, osmoregulation, membrane stabilization, modulation of neurotransmitters, antioxidation and early development of visual, neural and muscular systems (Huxtable, 1992). Consequently, it is not surprising that this nutrient contributes to larval performance and survival. Moreover, taurine may be reinforcing the DHA benefit in vision (Matsunari et al., 2008; Kim et al., 2005) as this nutrient is known to promote differentiation of retinal cells in vertebrates (Altschuler et al., 1993) as well as accumulating in the outer segments of vision (Matsunari et al., 2008; Kim et al., 2005) as this nutrient is known to promote differentiation of a vertebrate retinal cell type (Jirsa, Davis, Da.S., Salze, G.P., Rhodes, M., Drawbridge, M., 2014). Taurine requirement for juvenile white seabass (Atractoscion nobilis) fed soy-based diets. Aquaculture 423, 36–41.

In summary, the present results suggest that dietary DHA fed to 2–14 dph ABFT larvae contributed to retinal opsin synthesis in a dose dependent manner that enhanced the developing fish’s vision and led to improved prey hunting success and ingestion. On the other hand, the DHA modulated increased rotifer consumption did not have a sustained effect on larval growth. This may have been due to a deficiency in dietary taurine, which if provided together with high levels of DHA in the diet, can contribute to an array of physiological processes including vision, to markedly improve ABFT larval performance during early development.

Acknowledgements

These studies were funded within the framework of 7 Framework EU projects Reprodot (QIS5-2002-0135), SelfDott (212797) and Transdot (SE11904).
development of unfed larvae of Atlantic bluefin tuna (Thunnus thynnus L.).

Aquaculture 313, 129–139.


