Aquaculture 482 (2018) 137–145

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

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

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ARTICLE INFO

Keywords:
Bluefin tuna larvae
Docosahexaenoic acid
Larval vision
Taurine
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 constituent fatty acids 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

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http://dx.doi.org/10.1016/j.aquaculture.2017.09.039
Received 3 March 2017; Received in revised form 25 September 2017; Accepted 26 September 2017
Available online 28 September 2017
0044-8486/

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 (Fitzgibbon 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 (Mourente 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 intra-membrane 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*) (Mourente and Tocher, 1992; Bell et al., 1995; Mourente, 2003), European sea bass (*Dicentrarchus labrax*) (Navarro et al., 1997), gilthead sea bream (*Sparus aurata*) (Mourente and Tocher, 1993; Mourente, 2003), and turbot (*Psetta maximus*) (Mourente 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 Idrissi 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 (Altshuler 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 quinqueradiata*; 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 sulfinate decarboxylase (CSD), a key enzyme catalyzing the decarboxylation of cysteine sulfinate 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), totoaba (*Totoaba macdonaldi*; López et al., 2015) and white grouper (*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 8 l (200 μm mesh) cages floating in a 400 l V-tank where filtered (10 μm), UV-treated (160 mJoules cm^{-1}), 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 l^{-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, 349 ± 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 l^{-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