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Rotifers enriched with taurine by microparticulate and dissolved enrichment methods influence the growth and metamorphic development of northern rock sole (*Lepidopsetta polyxystra*) larvae

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ABSTRACT

Naturally-occurring taurine concentrations in rotifers may not meet the nutritional requirements for species of cultured marine fish during the larval stages. Traditional methods for the enrichment of rotifers by immersion in a nutrient solution may be inefficient and may promote the growth of pathogenic bacteria. Microparticles, specifically wax spray beads (WSBs), have the potential to efficiently enrich live prey with water-soluble substances while maintaining optimal water quality for larviculture. The objectives of this study were to 1) compare the efficiency of enriching rotifers using wax spray beads (WSBs) versus methods whereby rotifers were immersed in solutions of taurine dissolved in the enrichment water (hereon referred to as the 'dissolved method') and 2) determine if northern rock sole (*Lepidopsetta polyxystra*) larvae show increased growth and development as a result of elevated concentrations of taurine in enriched rotifers. Leaching trials and rotifer enrichment trials were conducted to address Objective 1, and a seven-week larval growth trial was conducted to address Objective 2. Results indicated that taurine-WSB enrichment was highly efficient, in that rotifers had higher taurine concentrations (by dry weight) and less taurine was used to enrich rotifers compared to the dissolved method. At the end of the seven-week feeding trial, larvae fed taurine-WSB enriched rotifers (Taurine-WSB) as well as those fed rotifers enriched in 4000 mg taurine l^{-1} (Dissolved 4000 mg l^{-1}) were larger than larvae fed rotifers that had not been enriched with taurine (Control). However, larvae fed rotifers enriched in 50 mg taurine l^{-1} (Dissolved 50 mg l^{-1}) were not significantly larger than larvae in the Control treatment. We conclude that northern rock sole larvae benefit from taurine-enriched rotifers and that enrichment via WSBs is an effective and efficient method for delivering water-soluble nutrients to cold-water fish larvae.

1. Introduction

Traditional methods for determining the nutritional requirements of fish, which utilize purified particulate diets, cannot be used in most larval marine fish studies because of variable food intake and poor growth of larvae when fed particulate diets (Dabrowski, 1984). As a result, the amino acid requirements of marine fish larvae are poorly understood (Conceição et al., 2003). It has been observed that marine fish larvae cultured in semi-extensive systems tend to grow faster and have lower deformity rates when compared to larvae grown in intensive systems (Hamre et al., 2008; Mæhre et al., 2012; Mæland et al., 2000). One explanation for this difference in larval performance is that copepods, the primary prey for larvae both in semi-extensive systems and the wild, better meet the nutritional requirements of marine fish larvae than cultured prey species, such as rotifers. Compared with copepods, rotifers possess much lower concentrations of highly unsaturated fatty acids (HUFA) as well as several micronutrients, most notably of which are iodine, selenium and taurine (Hamre et al., 2008; Mæhre et al., 2012; Mæland et al., 2000). There has been a great deal of research addressing the enrichment of HUFA and other dietary lipids in rotifers, and as a result, several commercial products, such as Algamac (Bio-Marine Inc., Hawthorne, CA, USA), Selco® (Inve Technologies, Dendermonde, Belgium), and Ori-Green (Skretting, Stavanger, Norway) are currently available to address these deficiencies. However, far less research has been directed towards understanding nutritional deficiencies of water-soluble substances, such as taurine, particularly on how live prey can be efficiently supplemented with such substances in intensive larviculture.

The enrichment of live prey with water-soluble nutrients presents a unique set of challenges because these nutrients rapidly leach from typical microparticle types suspended in seawater (Langdon, 2003). Live prey organisms are usually enriched by immersion in solutions of water-soluble nutrients to facilitate uptake via drinking or adsorption, hereafter referred to as the 'dissolved method'. While arguably simple, such approaches tend to result in wastage of large amounts of nutrients due to low uptake efficiencies by live prey organisms. A potentially more efficient way to deliver water-soluble nutrients is to encapsulate them in

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microparticles (e.g., wax spray beads; WSBs) and feed them either directly to the larvae or indirectly by way of WSB-enriched live prey. In a previous study, wax spray beads containing potassium iodide (KI WSB) were shown to elevate the iodine concentrations of *Artemia* more efficiently than when KI was dissolved directly into the seawater (Hawkyard et al., 2011). In the same study, it was shown that zebrafish larvae fed KI WSB-enriched *Artemia* survived at higher rates and had increased whole-body iodine concentrations when compared with zebrafish fed unenriched *Artemia*.

Taurine (2-aminoethanesulfonic acid) has been reported to be 10–400 times more concentrated in copepods compared to rotifers used for aquaculture (Mæhre et al., 2012; van der Meeren et al., 2008). Lacking a carboxyl group, taurine cannot be used for protein synthesis (Conceição et al., 2011). However, taurine has been shown to be important for many biological functions in fish, such as lipid digestion, osmoregulation, neurological functions and visual acuity (Fang et al., 2002; Li et al., 2009; Omura and Inagaki, 2000). Many animals are able to synthesize taurine from methionine and cysteine, and to a lesser extent 4′-phosphopantetheine, through a series of enzymatic conversions (Griffith, 1987). Two of the key enzymes needed in these processes, cysteamine dioxygenase and cysteinesulfinate decarboxylase, have lower activities in fish when compared to mammals and vary greatly among fishes (Goto et al., 2001; Goto et al., 2003; Yokoyama et al., 2001), suggesting that there is a large degree of variability among fishes in their abilities to synthesize taurine. For instance, Kim et al. (2008) found that dietary taurine was required by juvenile Japanese flounder (*Paralichthys olivaceus*) but not by common carp (*Cyprinus carpio*) for growth and survival. Therefore, the essentiality of taurine for marine fish requires assessment for each specific species and perhaps life-stage. Currently, enrichment of rotifers with taurine has resulted in increased growth when fed to several species of marine fish larvae, including Pacific cod (*Gadus macrocephalus*), red sea bream (*Pagrus major*), Japanese flounder (*P. olivaceus*) Senegalese Sole (*Solea senegalensis*), cobia (*Rachycentron canadum*) and amberjack (*Seriola dumerili*) (Chen et al., 2005; Fang et al., 2002; Li et al., 2009; Matsunari et al., 2005; Matsunari et al., 2013; Omura and Inagaki, 2000; Salze et al., 2011; Salze et al., 2012).

In this study, we investigate the means and effects of delivering taurine-enriched rotifers on larval growth and development in northern rock sole (*Lepidopsetta polyxystra*). Northern rock sole is a commercially important species in the capture fisheries of the northeast Pacific and may have potential for aquaculture. In captivity, northern rock sole are reared at temperatures between 2 and 12 °C and undergo a relatively long (>2 months) larval pelagic stage. Northern rock sole larvae are generally fed rotifers through the majority of the larval stage but may be transferred to diets of *Artemia* or artificial particulate diets prior to settlement. We chose northern rock sole as a model species to examine the effects of taurine because: 1) they are cold-water marine fish that feed on taurine-rich wild zooplankton during their pelagic larval stage; and 2) like other Pleuronectids, northern rock sole undergo a distinct metamorphosis at the end of the larval stage that is energetically and nutritionally demanding (Laurel et al., in review). The objectives of this study were to 1) compare the efficacy of wax spray beads for taurine enrichment of rotifers with "dissolved" methods whereby taurine is dissolved in the enrichment water and 2) determine if northern rock sole larvae show increased growth and development as a result of elevated concentrations of taurine in enriched rotifers.

2. Material and methods

2.1. Production of WSB

Taurine (T-0625; Sigma-Aldrich, St. Louis, MO, USA) was ground for 1 h using a jar mill (U.S. Stoneware, NJ, USA) to obtain particle sizes smaller than 0.5 μm. Five grams of powdered taurine was added to 11.67 ml distilled H_2O and resulted in a taurine–water slurry. The slurry was preheated to 75 °C immediately prior to emulsification with 33 g of melted beeswax (refined, Sigma-Aldrich, St. Louis, MO, USA). To facilitate emulsification and to promote bead dispersion in seawater, 0.33 g sorbitan tristearate (Sigma-Aldrich, St. Louis, MO, USA) was added to the molten-lipid mixture, representing 1% of the lipid-fraction by formula weight. The taurine–water slurry was added to the molten-lipid mixture and sonicated with a Labsonic L sonifier (B. Braun Biotech Inc., Allentown, PA, USA) until a stable emulsion was achieved. The emulsion was then added to a preheated (75 °C) spray apparatus described in detail in Hawkyard et al. (2011). The lipid mixture was atomized using nitrogen gas (20 psi) and WSBs were collected in a steel cone, cooled (approx. −80 °C) with liquid nitrogen. WSBs without taurine ("empty WSBs") were produced as described without the addition of taurine.

2.2. WSB leaching and particle size analysis

For leaching trials, a total of 100 mg of WSBs were weighed into a 50 ml polypropylene centrifuge tube and suspended in 25 ml 0.5% sodium dodecyl sulfate. Sodium dodecyl sulfate was used to disrupt WSB aggregates and promote even dispersion of particles in suspension as described in Langdon et al. (2008) and Hawkyard et al. (2011). WSB suspensions were sonicated gently for 10 s to disrupt WSB clumps, capped and placed on a culture rotator at 20 rpm. After the allotted time (0, 5, 15, 30 or 60 min), WSBs were collected on a 0.2 μm Durapore membrane filter (EMD Millipore, Billerica, MA, USA). The filter was placed in a clean test tube with a perforated cap and freeze dried for 72 h in a Labconco Freezone freeze drier (Labconco Inc., Kansas City, MO, USA). Freeze-dried WSBs were scraped from the filter with a metal spatula and stored under an atmosphere of nitrogen at −20 °C for later taurine analysis. Particle sizes were determined by suspending 10–20 mg WSB in 5 ml 0.5% sodium dodecyl sulfate. Particle clumps were disrupted with brief (1–2 s) sonication and 50 μl of the resulting suspension was placed on a glass slide with cover slip. Digital images were taken with a Leica DM 1000 microscope (Leica Microsystems, Wetzlar, Germany) fitted with a Leica DFC 400 camera (Leica Microsystems, Wetzlar, Germany). Particle diameters were measured using Image-J software (National Institute of Health, Bethesda, MD, USA).

2.3. Rotifer culture and enrichments

Rotifers were cultured on a diet of RotiGrow® algae paste (Reed Mariculture, Campbell, CA, USA) using methods described in Copeman and Laurel (2010). Rotifer enrichments were carried out in 15 l polycarbonate enrichment cones (Aquatic Habitats, Apopka, FL, USA) filled with 2 l filtered seawater (32 ppt, 26 °C) at a density of 500 rotifers ml^{-1}. Experimental enrichments are shown in Table 1. Briefly, rotifers were enriched with: 1) 500 g empty WSB l^{-1} (Control); 2) 50 mg dissolved taurine l^{-1} in addition to 500 g empty WSB l^{-1} (Dissolved 50 mg l^{-1}); 3) 500 g empty WSB l^{-1} and 4000 mg dissolved taurine l^{-1} (Dissolved 4000 mg l^{-1}); or 4) 500 g taurine WSB l^{-1} (Taurine-WSB). Enrichment with 50 mg l^{-1} dissolved taurine (Dissolved 50 mg l^{-1}) was chosen because this was estimated to be equivalent to the quantity of taurine provided to the rotifers in the Taurine-WSB treatment. Enrichment with 4000 mg l^{-1} (Dissolved 4000 mg l^{-1}) was chosen because this was the estimated quantity of taurine required for dissolved enrichment to obtain equivalent taurine concentrations in rotifers enriched with taurine-WSB. "Empty" wax spray beads were added to the Dissolved and Control treatments to ensure that enrichments for rotifers in all treatments were equivalent in both energy and lipid quality. In order to disrupt clumps of beads, WSBs were sonicated using a Labsonic L sonifier in 200 ml 1% w/v gum Arabic (Sigma-Aldrich, St. Louis, MO, USA) solution prior to addition to the enrichment water. In the Dissolved treatments, taurine (T-0625; Sigma-Aldrich, St. Louis, MO, USA) was ground into a powder for 1 h using a jar mill (U.S. Stoneware, NJ, USA) and dissolved in filtered