Aquaculture 422–423 (2014) 115–123

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Performance and skeletal abnormality of striped trumpeter *Latris lineata* larvae and post larvae fed vitamin A enriched *Artemia*

Reham K. Negm [a,*], Jennifer M. Cobcroft [a,b], Malcolm R. Brown [c], Barbara F. Nowak [d], Stephen C. Battaglene [a]

[a] Institute for Marine and Antarctic Studies (IMAS), Fisheries, Aquaculture and Coast Centre, University of Tasmania, Private Bag 49, Hobart, Tasmania 7001, Australia
[b] University of the Sunshine Coast, Locked Bag 4, Maroochydore DC, Queensland 4558, Australia
[c] CSIRO Marine Research, GPO Box 1538, Hobart, Tasmania 7001, Australia
[d] National Centre for Marine Conservation and Resource Sustainability (NCMCRS), AMC, University of Tasmania, Locked Bag 1370, Launceston 7250, Tasmania, Australia

ARTICLE INFO

Article history:
Received 11 July 2013
Received in revised form 7 November 2013
Accepted 9 November 2013
Available online 25 November 2013

Keywords:
Marine fish larvae
Retinyl palmitate
Live feeds
Dose–response
Malformations

ABSTRACT

Several nutritional studies have found that excess or reduced dietary vitamin A (VA) causes skeletal malformations in marine fish larvae. Feeding VA enriched rotifers to striped trumpeter (*Latris lineata*) from 6 to 18 days post hatch (dph) has been shown to affect vertebral column malformations but not jaw malformation. Our study examined the effect of dietary VA during the later *Artemia* feeding period on the prevalence of malformations. Larvae were initially reared in a 3000 l tank and fed a diet of Algamac-3050 enriched rotifers from 2 to 16 dph. At 16 dph, larvae were transferred to 24 × 300 l tanks and fed *Artemia* enriched with one of six doses of retinyl palmitate (VA) four times per day from 19 to 44 dph. Enrichment with emulsions containing 0 (control), 795, 1558, 3174, 4911 and 10,518 ng retinyl palmitate mg^{-1} emulsion resulted in an average VA in *Artemia* of 0, 6, 7, 27, 30 and 55 ng retinyl palmitate mg^{-1} dry weight (DW). The retinoid content of *L. lineata* at 44 dph was positively correlated with the enriched *Artemia* they fed on. Growth in length (15.91 ± 0.31 mm, mean ± SD) and dry weight (5.08 ± 0.32 mg), and survival (26.1 ± 2.9%), were not significantly affected at 44 dph by increasing dietary doses of retinyl palmitate. Retinyl palmitate enrichment in *Artemia* did not affect jaw malformation in *L. lineata*, which is contrary to other studies on a range of marine fish species treated with increased doses of dietary VA. By the end of the study, 54 ± 10% and 18 ± 6% of the post larvae across treatments had short lower and open jaws, respectively. Severe jaw malformations affected 50 ± 11% of the post larvae. Early culture of larvae in a larger tank was likely to reduce overall prevalence of jaw malformations compared to previous studies as interaction with tank walls was reduced. The prevalence of vertebral column malformations in 44 dph post larvae (58 ± 4%) were also not affected by dietary VA. The study suggests that the window of influence for dietary VA is during earlier development of bone structures in *L. lineata*.

1. Introduction

Skeletal malformation is a limiting factor in the commercial scale production of some cultured marine fish species, although the occurrence and types of malformations vary according to species. The most common regions that are affected are the jaw, operculum, vertebral column and fins (Fraser and De Nys, 2011; Koumoundouros, 2010). In a survey of 18 marine fish hatcheries from around Australia, 44% indicated that skeletal malformations were a significant issue, with up to 70% malformation in some batches of cultured yellowtail kingfish, *Seriola lalandi*, and up to 95% in striped trumpeter, *Latris lineata* (Cobcroft and Battaglene, 2013). Malformations add to the cost of farming by reducing survival, adding infrastructure and labour costs due to manual sorting of malformed fish that reduce marketability and decrease fish quality (Cahu et al., 2003; Koumoundouros et al., 1997, 2002). European research reviewed available scientific data and knowledge and identified three probable reasons for malformations: rearing temperature, tank environment such as the dissolved oxygen and water currents or quality, and nutrition (Cahu et al., 2003; Divanach et al., 1997; Haaparanta et al., 1997; Hattori et al., 2004; Lall and Lewis-McCrea, 2007; Sfakianakis et al., 2004). In aquaculture, the knowledge of larval fish nutrition and feeding is still incomplete, due in part to the huge number of aquatic species cultivated and variation in their physiology and behaviour (Teles et al., 2011). Amino acids, oxidised lipids, phospholipids, PUFAs, minerals and vitamins A, C, D and K have previously been linked with malformations that appear both during the larval and juvenile stages (Cahu et al., 2003; Lall and Lewis-McCrea, 2007).

Retinoids, which are vitamin A (VA)-related compounds include all the compounds that possess the same biological activity as retinol and regulate bone metabolism among other functions including morphogenesis, cellular differentiation and tissue homeostasis (Haga et al., 2002b; Ross et al., 2000). Nutritional requirement studies of larval marine fish, and the effect of different nutrients on skeletal development and performance parameters, are usually examined through enrichment of live feed, i.e. either rotifers or *Artemia* (Copeman et al., 2002;

* Corresponding author. Tel.: +61 3 6227 7266; fax: +61 3 6227 8035.
E-mail address: rmknegm@utas.edu.au (R.K. Negm).

0044-8486/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aquaculture.2013.11.008

Giménez et al., 2007; Roo et al., 2009). Enrichment of *Artemia* with graded doses of VA or different VA metabolites is the best way to examine the effects of VA on normal development but is technically challenging and resource intensive, and this approach is reported for relatively few species (Fernández et al., 2009; Takeuchi et al., 1998; Tarui et al., 2006).

Dietary VA has been extensively studied in flatfish where it affects pigmentation and skeletal malformation (Estevez and Kanazawa, 1995; Fernández and Gisbert, 2011; Haga et al., 2002a; Martinez et al., 2007; Suzuki et al., 2000). Skeletal malformations in different vertebral regions, fins, opercular and caudal fin complexes due to improper VA doses have been reported in other marine fish, such as red sea bream, *Pagrus major*, gilthead sea bream, *Sparus aurata* and European sea bass, *Dicentrarchus labrax*, (Fernández et al., 2008; Hernández-H et al., 2006; Mazurais et al., 2009). Cephalic malformations, particularly in the jaw, were reported with imbalanced VA doses in Japanese flounder, *Paralichthys olivaceus*, summer flounder, *P. dentatus* and *D. labrax* (Haga et al., 2002b, 2003; Martinez et al., 2007; Villeneuve et al., 2006). Variation in bone tissue type and embryonic origin, nutritional, and environmental factors may have variable effects on different skeletal elements (Cahu et al., 2003; Haga et al., 2002a; Prestinicola et al., 2013; Smith and Hall, 1990), and our study was focussed on the effect of dietary VA on jaw and vertebral malformations.

The high incidences of jaw malformation remain the largest impediment to the production of high quality *L. lineata* juveniles (Cobcroft and Battaglene, 2009; Cobcroft et al., 2001). *Latris lineata* occurs in southeastern Australia and New Zealand. Intensive research has been undertaken on this species since the early 1990s as a result of its selection as the best candidate for sea cage culture diversification in Tasmania (Battaglene and Cobcroft, 2007). Research has examined the early larval requirements for essential fatty acids during both rotifer and *Artemia* feeding periods (Bransden et al., 2005a, 2005b) and for vitamins C and E (Battaglene et al., unpublished data; Brown et al., 2005a, 2005b). Jaw malformation was linked with walling behaviour that is affected by tank colour (Cobcroft and Battaglene, 2009). Other factors implicated in jaw malformation, via their impact on walling behaviour of *L. lineata*, are green water, tank size and live feed availability (Battaglene and Cobcroft, 2007; Cobcroft et al., 2012). Parasite infection and non-inflation of the swim bladder have been associated with spinal malformations in *L. lineata* post larvae and juveniles (Grossel et al., 2003; Trotter et al., 2005).

We have examined the effect of increasing dietary retinyl palmitate doses during rotifer feeding (first feeding to 18 dph, days post hatch) on *L. lineata* larval performance parameters (growth and survival) and skeletal development, particularly jaws and vertebral column and found there were no significant effects on larval growth or survival to 43 dph and the incidence and severity of jaw malformations were not affected (Negm et al., 2013). However, malformations in the vertebral column were correlated with the VA content of the larvae. While the safe dose of total VA inclusion during the rotifer feeding period was recommended to be more than 123 ng mg^{-1} dry weight (DW) rotifers (Negm et al., 2013), the appropriate dose during *Artemia* feeding is unknown.

The objective of the present research was to evaluate the effect of VA during the *Artemia* feeding period. Six graded doses of retinyl palmitate in *Artemia* were fed to *L. lineata* post larvae, from 17 to 44 dph, and the larval performance and skeletal development examined, with focus on the jaw and vertebral column.

2. Materials and methods

2.1. Egg incubation and rearing of larvae

Gametes were obtained from naturally spawning broodstock held under controlled photoperiod and ambient water temperature at the Institute for Marine and Antarctic Studies, Fisheries Aquaculture and Coasts Centre (IMAS-FACC), Hobart. Eggs from one female were fertilised with sperm from three males. Immediately after fertilisation the embryos were disinfected in ozonated seawater at 0.9 ppm for 65 s, to reduce the risk of infection with pathogens (Battaglene and Morehead, 2006). The seawater used was filtered to 1 μm and ozonated to 700 mV ORP for ≥10 min, treated with UV at 254 nm, and carbon-filtered before distribution to the incubation unit or larvae tanks at 300–350 mV ORP. The embryos were incubated as described in Negm et al. (2013). Yolk sac larvae (4.62 ± 0.09 mm total length) were stocked into a larval culture tank 2 dph at 10 larvae l^{-1}.

Larvae were reared from 2 until 16 dph in a cylindroconical 3000 l tank fitted with a 390 μm central screen. The temperature range was 15.9 to 16.4 °C, salinity range was 32.4 to 33.8 ppt, pH between 8.09 and 8.36, the dissolved oxygen was > 96% and photoperiod was 16 h L: 8 h D (lights on at 09:00 and off at 01:00). The tank was static from 2 dph (stocking) until 6 dph (first feeding) when the incoming flow of sea water was 750 l h^{-1} from 23:00 to 08:00. Surface skimmers were used from 8 until 13 dph to remove oily films from the water surface to facilitate swim bladder inflation (Trotter et al., 2005). The larvae were reared in green water from 6 until 19 dph, where 300 l of temperature-acclimated *Nannochloropsis oculata* was added to the tank at 08:30 (before the lights came on) to achieve a turbidity of 3 NTU. Moderate aeration was used during the light period.

Larvae were fed rotifers *Brachionus plicatilis* (Austria strain) from 6 until 18 dph, once per day at 09:00 at a feeding density of 10 ml^{-1} and then 5 ml^{-1} on 17 and 18 dph. Rotifers were cultured on commercial paste algae (*Nannochloropsis sp*, Reed Mariculture Inc. CA, USA) and enriched with Algamac-3050 (Aquafauna Biomarine Inc. CA, USA), prepared according to manufacturer's instructions, for 8 h at 2 g per million rotifers at 500 rotifers ml^{-1}. Enriched rotifers were rinsed, counted and introduced to the 3000 l tank or the 24 x 300 l tanks at the required density.

The term "larvae" is used throughout to describe *L. lineata* until 37 dph, and the term "post larvae" refers to older larvae that have completed flexion but not metamorphosed to the juvenile (44 dph). Terminology is based on the definition of Leis and Trnski (1989), that "larvae" is the developmental stage between hatching and the completion of full external characters (fins and scales), including the yolk sac, preflexion, flexion and postflexion stages.

2.2. Experiment conditions

On 16 dph, 600 larvae were stocked into each of 24 × 300 l hemispherical tanks, with black base and marble coloured walls to reduce larval walling behaviour (Cobcroft and Battaglene, 2009). Larvae and post larvae were reared in the experimental tanks from 16 until 44 dph where temperature was 16.38 ± 0.17 °C, salinity 32.35 ± 0.23 ppt, pH 8.10 ± 0.05 and dissolved oxygen was 102.18 ± 2.46%. The photoperiod was increased to 20 h L: 4 h D on 18 dph and 24 h L from 19 dph onwards to reduce downward nocturnal migration and associated mortalities (Bransden et al., 2005a). Light intensity was 10.6 ± 1.4 μmol s^{-1} m^{-2} at the water surface. The incoming flow water exchange was 104 ± 1 l h^{-1} for 9 h from 16 to 18 dph and then increased to 24 h from 19 dph. Water was recirculated for each tank through the 390 μm central screens into 63 μm bag screens in an external sump to filter out the uneaten prey, increasing the total flow to 232 ± 10 l h^{-1} for 9 h on 16, 17 and 18 dph and for 24 h from 19 dph until 44 dph.

The effect of dietary VA during the *Artemia* feeding period on the performance, growth, survival and skeletal malformation in larvae and post larvae was determined. *Artemia* were enriched with six graded doses of retinyl palmitate and fed to the larvae from 17 to 44 dph (4 tanks per treatment). *Artemia* were fed four times each day at 09:00, 13:00, 17:00 and 21:00 at an initial density of 0.25 ml^{-1} per feed, except on 17 dph and 18 dph when the larvae were fed *Artemia* at 17:00 and 21:00 only. From 25 dph, the feeding density was adjusted