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Feeding strategies for striped blenny *Meiacanthus grammistes* larvae

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Abstract

Rotifers and *Artemia salina* nauplii are the most widely used live prey for newly hatched larvae, but they do not always promote optimal survival and growth. Alternative food sources such as copepods, which bypass these inadequacies and promote adequate growth, are needed and they are viewed with considerable interest by the scientific community. The aim of the present study was to test two different diets [rotifers and *A. salina* nauplii (group A) and a mixture (group B) of rotifers/*Tisbe* spp. copepods and *A. salina* nauplii/copepods] during the larval rearing of the striped blenny *Meiacanthus grammistes*. The analysis of the survival rate, size (total length and wet weight) and metamorphosis time during the larval phase of this species showed that *Tisbe* spp. administration can significantly improve larval survival and growth and also reduce the metamorphosis time. The results obtained are related to the fatty acid content of the live prey used and are essential in order to improve the captive production of *M. grammistes* through a closed system and, in turn, to preserve natural stocks.

Keywords: blennies, copepods, ornamental fish, reproduction, rotifers, *Artemia salina*

Introduction

Nowadays, with the increasing popularity of aquariums, ornamental fish have a growing role in the international fish trade. The total value of the wholesale ornamental fish trade is estimated at US$1 billion, and the retail trade at about US$ 3 billion.

Many fish collectors in tropical and subtropical countries still use destructive fishing methods (i.e. cyanide and dynamite), which harm the marine ecosystems and threaten the main food source of the local population. Therefore, in the last few years, a number of scientists have focused their attention on the reproduction of some of the species most commonly used in the aquarium trade with the intent to rear them in captivity (Thresher 1984; Riley & Holt 1993; Brons 1996; Holt 2003; Olivotto, Cardinali, Barbaresi, Maradonna & Carnevali 2003; Olivotto & Carnevali 2004; Olivotto, Yasumasu, Gioacchini, Maradonna, Cionna & Carnevali 2004; Calado Rosa, Morais, Nunes & Narciso 2005; Olivotto, Holt, Carnevali, Holt 2006; Olivotto, Avella, Sampaolesi, Piccinetti, Navarro Ruiz & Carnevali 2008; Olivotto, Buttino, Borroni, Piccinetti, Malzone & Carnevali 2008; Olivotto, Capriotti, Buttino, Avella, Vitiello, Maradonna & Carnevali 2008). The development of a marine ornamental aquaculture is now becoming indispensable not only in order to generate an alternative supply of ornamental marine fish but also to improve the basic knowledge of their life history and thereby lead to a better management of natural stocks.

Meiacanthus grammistes is an attractive and very interesting blenny species, typical of the Indo-Pacific shallow reefs (http://www.fishbase.org). It has alternating black and yellow stripes, which run along the entire length of the body. Once very common in aquarium trade imports, these fish are now becoming sporadic and captive culture of the species may thus be considered a valid alternative to wild-caught specimens.

In larviculture, the main critical bottleneck that scientists need to face is the transition from endogenous to exogenous feeding by the larvae. Most

ornamental species are reared using rotifers (*B. plicatilis*, *B. rotundifornis*) and *Artemia salina* nauplii because these can be cultured in large quantities at high densities. The use of these types of live prey does not always promote optimal larval growth and survival because they usually contain an inadequate fatty acid profile and, in some cases, are of an inappropriate size (Kahan 1981; Sargent, Mcevoy, Estevez, Bell, Bell, Henderson & Tocher 1999; Cahu, Zambonino Infante & Takeuchi 2003; Holt 2003; Olivotto *et al.* 2003; Faulk & Holt 2005). In this regard, there is considerable interest in identifying alternative food sources that do not show such inadequacies and can promote adequate growth and survival (Sun & Fleeger 1995). Copepods, copepodites and nauplii are the food items preferred by fish larvae and, when used as live prey (alone or in combination with rotifers and *A. salina* nauplii), they usually dominate the gut content of the larvae (Holt 2003; Olivotto, Avella *et al.* 2008; Olivotto, Buttino *et al.* 2008; Olivotto, Capriotti *et al.* 2008). Delbare, Dhert and Lavens (1996) summarized the advantages of using copepods in larviculture, which include the wide range in body size between nauplii and adults, their movement and their high content of highly unsaturated fatty acids (HUFAs) [especially eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3)]. Deficiencies in these fatty acids can cause a general decrease in larval health, poor growth, low feed efficiency, anaemia and high mortality (Sargent, Mcevoy & Bell 1997; Sargent *et al.* 1999; Bell, Mcevoy, Estevez, Shields & Sargent 2003; Olivotto *et al.* 2003; Faulk & Holt 2005; Olivotto, Rollo, Sulpizio, Avella, Tosti & Carnevali 2006) and are therefore essential in aquaculture.

Harpacticoid copepods may be considered to be good candidates for mass production because they have a high reproductive potential, a fast population growth rate, a short turnover time, a flexible diet and tolerance to a wide range of chemical and physical environmental factors. They also provide a broad spectrum of prey sizes suitable for the developing fish larvae and are particularly rich in essential fatty acids (Kahan 1981; Sun & Fleeger 1995; Støttrup & Norsker 1997; Sargent *et al.* 1999; Olivotto, Avella *et al.* 2008; Olivotto, Buttino *et al.* 2008). Several studies have demonstrated that harpacticoid copepods should be administered to fish larvae preferably as a supplemental food for the traditional diet based on rotifers and *A. salina*. In fact, larvae fed on harpacticoid copepods alone showed very low survival rates (Støttrup & Norsker 1997; Olivotto, Avella *et al.* 2008; Olivotto, Buttino *et al.* 2008).

The aim of the present study is to optimize the breeding and rearing conditions of *M. grammistes*. The fatty acid profiles of the two different diets (rotifers and *A. salina* nauplii and a mixture of rotifers/copepod nauplii and *A. salina* nauplii/copepod copepodites) were analysed and the live prey was tested during the larval rearing of this species. Survival rate, size (total length and wet weight) and metamorphosis time were recorded and compared.

Materials and methods

Animals

Four sexually mature fish, approximately 10 cm each, were bought in a pet shop (CIP Acquari, Bologna, Italy) in January 2009. Broodstock was kept in a 200 L tank. The temperature in the breeding tank was maintained at 28 °C, salinity 30 ppt, pH 8.2 and NO_2 and NH_3 <0.03 ppm. A photoperiod of 13 h of light and 11 h of darkness was provided exclusively by two 30 W incandescent lights suspended 20 cm above the water surface. PVC pipes were placed in the tank as a surface on which the fish could spawn.

The fish were fed twice a day using *Artemia* sp, frozen plankton and chopped fish and shrimps (Eschematteo, Italy). Under these conditions, the fish spawned every 8–10 days and the egg clutches, each of about 150–200 embryos, were left to parental care throughout embryo development. Hatching occurred, at 28 °C, 8 days post fecundation (DPF). It took place in the breeding tank after the first 60–100 min of darkness. Because larvae were phototactic, they were collected in a 500 mL beaker using a flash light and immediately transferred to the rearing tank. The 20 L larval rearing tank was characterized by the same chemical and physical characteristics as the breeding tanks. The water in the 20 L larval tank was gently replaced 10 times a day by a dripping system. The sides of the tank were covered with black panels to reduce light reflection, while the phytoplankton *Nannochloropsis oculata* (introduced at 08:00, 12:00, 20:00, 24:00 hours) was used (50 000 cells/mL) to condition the tank from day 1 to day 12 post hatch.

The average hatch percentage for the different clutches considered for feeding studies was 96 ± 3%.

Behavioural observations

Notes on reproductive behaviour were taken three times a day (08:00–09:00, 12:00–13:00, 17:00–18:00 hours) during the first 2 months of the experiment.

Attention was focused on the males' courtship and parental care behaviour.

Zooplankton culturing

Different species of zooplankton (listed below) were cultured in order to determine their potential as live prey for *M. grammistes* larviculture. Copepod stock cultures of *Tisbe* spp. were maintained in a temperature-controlled room (26 ± 0.5 °C) in four different 100 L tanks with gentle aeration under the following conditions: 30‰, pH 8.2, NO_2 and NH_3 <0.03 mg L^{-1}. A 14:10 light–dark cycle was maintained in the culture room and the water quality was constantly monitored. Water was completely replaced every 2–3 weeks and the cultures were fed daily with the microalgae *Isochrysis galbana* (50 000 cells mL^{-1}). Culture densities were monitored daily and when nauplii reached a concentration of 1 individual (ind.) mL^{-1}, they were used for the feeding experiment. Naupliar stages were separated from copepodites and adult stages using different-sized mesh (300–100–50–30 μm).

Rotifers (*Brachionus plicatilis*) characterized by an average size of 239 μm were cultured on *N. oculata* (50 000 cells mL^{-1}) in 100 L tanks (salinity 30‰, pH 8.2, NO_2 and NH_3 <0.03 mg L^{-1}) and subjected to constant light. Each day, the necessary amount of rotifers was gently transferred to 50 L cone-shaped tanks for enrichment, performed with Algamac 3000® (Aquafauna Bio-Marine, Hawthorne, CA, USA) using 0.5 g million^{-1} rotifers. Algamac 3000® was homogenized in 500 mL salt water for 1 min and then distributed to the rotifers for enrichment. As recommended by the company, enrichment lasted for 8–9 h.

AF 430 *A. salina* cysts (Inve Technologies, Dendermonde, Belgium) were incubated and hatched following Inve instructions. After hatching, the nauplii were separated from the cysts by siphoning and enrichment was performed with Algamac 3000® (0.2 g/100 000 *A. salina* nauplii) in 10 L buckets filled with filtered seawater at a concentration of 200 nauplii mL^{1} for 8 h at 25 °C under continuous aeration and illumination.

Before being fed to the larvae, rotifers and *A. salina* nauplii were concentrated on a 30 μm mesh and rinsed 10 times with clean seawater (salinity 30‰) in order to remove the remaining enrichment.

Experimental design

Immediately after hatching, larvae were divided into two experimental groups (100 ± 5 larvae per group, in three replicates each) as follows:

Group A (control): fed with Algamac 3000®-enriched rotifers (*Brachionus plicatilis*) (10 ind. mL^{-1}) from day 1 to day 12 post hatch (DPH), followed by Algamac 3000®-enriched AF430 *A. salina* nauplii (6 ind. mL^{-1}) introduced from 10 DPH until the end of the experiment.

Group B: fed with Algamac 3000®-enriched rotifers (*B. plicatilis*) (5 ind. mL^{-1}) and *Tisbe* spp. copepods (5 ind. mL^{-1}) from 1 to 12 DPH, followed by Algamac 3000®-enriched AF430 *A. salina* nauplii (3 ind. mL^{-1}) and *Tisbe* spp. copepods (3 ind. mL^{-1}) introduced from 10 DPH until the end of the experiment.

An additional experimental unit (made of three replicates) for the above-mentioned experiment was set up to estimate the survival rate and metamorphosis time for this species. Each day, at 08:00 and 18:00 hours, dead larvae from three replicates were siphoned from this additional unit and counted in order to estimate survival in the different experimental groups. Survival was expressed as the mean of the results of the three replicates. The number of metamorphosed larvae was also checked every day (in the three replicates): larvae were considered to be metamorphosed when they showed uncompleted alternating black and yellow stripes running along the entire length of the body. Results are the mean of the three replicates.

Sampling of larvae

Samples of larvae (10 ± 1, in three replicates), for each experimental group, were collected 1, 5, 11, 19 and 26 DPH. All samplings were performed at 08:00 hours before feeding the larvae. The larvae were used for biometric measurements (total length TL and wet weight WW) using a Stemi 2000 micrometric microscope (Zeiss vision Italia, Castiglione Orona, Varese, Italy) and a microbalance [OHAUS Explorer E11140 (Pine Brook, NJ, USA) accurate to 0.1 mg]. The larvae were anaesthetized (0.1 g L^{-1}) using MS222 (Sigma-Aldrich, Milan, Italy).

Lipid analysis

Free fatty acids were extracted from lyophilized samples of rotifers, *A. salina* and copepods in triplicate, by homogenizing the samples in 10 volumes of chloroform/methanol (2:1, v/v) using a glass/teflon homogenizer.

In accordance with a modified Folch method (Hamilton, Hamilton & Sewell 1992), the biological material was suspended in a chloroform/methanol mixture (2:1 v/v, 0.5 mL) and sonicated for 1 min.

After centrifugation for 1 min at 10 000 *g*, the supernatant was transferred into a new tube and dried under a nitrogen stream. The raw extract thus obtained was dissolved with diethyl ether and methylated with diazomethane (CH_2N_2) for 60 min at room temperature. The reagent in excess was removed under nitrogen and the derivatized residue was resuspended in *n*-hexane (100 μL) for GCMS analysis (Focus GC-Polaris Q, Thermo) with a 30 m × 0.25 mm ID × 0.25 μm film thickness capillary column (5% diphenyl/95% dimethyl polysiloxane). Elution of fatty acid methyl esters required a temperature program starting with 50 °C for 2.5 min, followed by a 10 °C min^{-1} ramp up to 100 °C and then 5 °C min^{-1} up to 290 °C. Samples were directly injected (2 μL) in a split (1:10) mode with a blink window of 3.5 min. The injection temperature was maintained at 270 °C and the transfer line at 280 °C. Full scan spectra were acquired from 60 to 600 *m/z*.

Individual methyl esters were identified by comparison with known standards (Supelco, PUFA-1 marine source and lipid standards:fatty acid methyl ester mixtures C:4–C24:1) and with reference to published data.

Data analysis

Results are expressed as the mean ± SD. The significance of differences was determined using Student's *t*-test packaged in a statistical software, STAT VIEW 512+™ (Brain Power, Fremont, CA, USA). A P value <0.05 was regarded as statistically significant.

Results

Courtship and larval development

The first spawning was recorded 3 weeks after the fish were introduced into the spawning tank and continued regularly every 8–10 days throughout the experimental period. Courtship was always initiated by the female, which, during the morning of the spawning day, was seen swimming back and forth in front of the male's nest site. At this point, the male left the shelter and approached the female, quivering in front of her. At the same time, the male was often observed nudging the female's abdomen and immediately swimming back to his nest. In a short time, the male showed only his head from the spawning site (PVC pipe) and finally approached the female again with the intent to invite her to enter the nest. Once inside, the female and the male alternated in releasing adhesive 1-mm-diameter eggs and sperm. After spawning, the female moved out of the nest and the male took care of the clutch till hatching occurred. At 28 °C, hatching occurred after 7 days, during the first hour of darkness. A single male was often observed taking care of several egg clutches; thus, no pair bond was established.

Newly hatched larvae appeared very active and swam close to the water surface; the yolk sac at this stage was completely absorbed and larvae measured approximately 3.0 mm. Pigmentation was very light and the anterior part of the body was very large and showed some dark scattered stellate melanophores; a finfold was present (Fig. 1). At 5 DPH (Fig. 2), larvae showed a darker pigmentation on the anterior part of the body, while the remaining part was still very light. The eyes, mouth, gut and a finfold were completely developed. At 11 DPH (Fig. 3), larvae presented an orange stomach area, due to *A. salina* administration. At this stage, black melanophores were developing towards the dorsal part of the body.

At 19 DPH (Fig. 4), the body appeared deeper and rounder, especially in the stomach area. Rays were now present on the dorsal, pelvic and caudal fins, and the body was less transparent. Three distinct lines of melanophores, which ran along the entire length of the body, were clearly evident.

At 24–26 DPH (Fig. 5), the larvae appeared on the verge of entering metamorphosis. They showed uncompleted alternating black and yellow stripes running along the entire length of the body. During the same period, the larvae moved from the surface towards the bottom, reaching the tank corners.

All the figures reported and analysed are referred to larvae fed with a standard rotifer –*A. salina* diet (group A). No developmental differences were observed at 1 and 5 DPH in larvae sampled from groups A and B. At 11, 19 and 26 DPH, the development of group B larvae, fed on a mixed standard copepod-

Figure 1 Newly hatched larvae are very active and swim near the water surface and the yolk sac at this stage is completely absorbed and larvae measure approximately 3.0 mm.

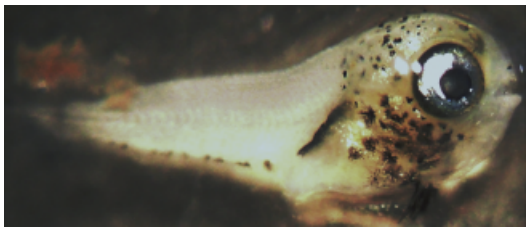

Figure 2 5 DPH larvae present a darker pigmentation on the anterior part of the body while the remaining is still very light. The eyes, mouth and gut and a fin fold are well developed. DPH, day post hatch.

Figure 3 11 DPH larvae present an orange stomach area due to *Artemia salina* administration. The black melanophores have now started developing on the dorsal part of the body. DPH, day post hatch.

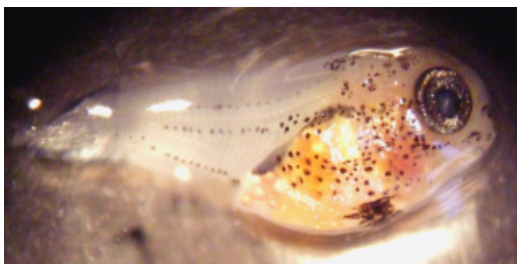

Figure 4 19 DPH, the body is deeper and rounder, and three distinct lines of melanophores that run all along the entire length of the body are now evident. DPH, day post hatch.

based diet, was 2–3 days faster with respect to group A larvae fed on a standard diet.

Survival

Differences ($P < 0.05$) in the survival rate between groups A and B were already evident at 5 DPH. From this time until the end of the experiment (26 DPH), group B larvae, fed on a mixed rotifer/copepod – *A. salina* nauplii/copepod diet, always showed better survival than larvae fed on the standard rotifer/

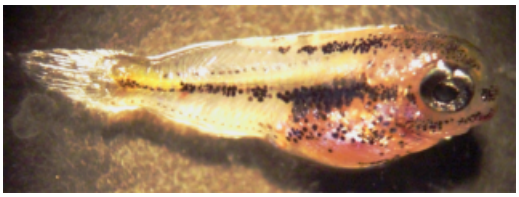

Figure 5 26 DPH, the larvae are on the verge of entering metamorphosis. They show uncompleted alternating black and yellow stripes, which run all along the entire length of the body. Fin rays are now evident. DPH, day post hatch.

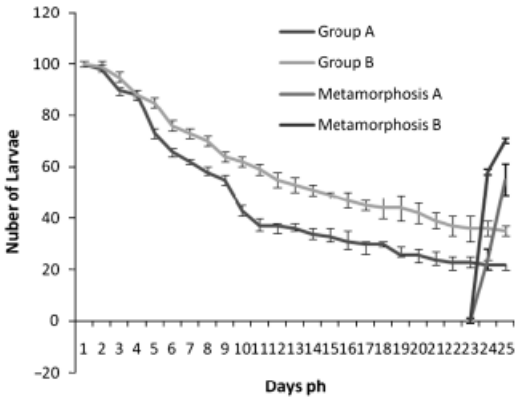

Figure 6 Survival rate and metamorphosis of *Meiacanthus grammistes* larvae fed different diets. Group B larvae fed a mixed rotifers/copepods – *Artemia salina* nauplii/copepods diet, always showed better survival with respect to larvae fed standard rotifers/*A. salina* nauplii diet (group A). Metamorphosis was significantly faster in group B larvae fed a mixed diet with respect to group A larvae fed a standard rotifers – *A. salina* diet.

A. salina nauplii diet (group A). Higher mortality in group A was particularly evident between 9 and 11 DPH when *A. salina* nauplii were first introduced (Fig. 6).

At 26 DPH, the group A larvae (control), fed on rotifers, followed by *A. salina* nauplii, showed 22 ± 2% survival, while the group B larvae, fed on a mixed diet, showed 35 ± 2% survival (Fig. 6). Moreover, at 26 DPH, 70 ± 8% of the group B larvae completed metamorphosis compared with 55 ± 6% of metamorphosed specimens observed in group A.

Biometric results

At 1 and 5 DPH, no significant difference ($P > 0.05$), either in TL or body weight (BW), was observed between group A and B larvae (Fig. 7a and b). In con-

trast, at 11, 19 and 26 DPH, group B larvae fed on copepods showed better growth both in terms of TL and BW compared with group A larvae fed on a standard rotifer/*A. salina* diet (5 ± 0.3 vs. 6, 1 ± 0.2 mm, 7 ± 0.2 vs. 8.3 ± 0.3 mm, 9 ± 0.2 vs 11.0 ± 0.2 mm; 19.6 ± 0.3 vs. 21.1 ± 0.2 mg, 23.5 ± 0.2 vs. 25.6 ± 0.3 mg, 27. 9 ± 0.6 vs. 29.4 ± 0.2 mg respectively) (Fig. 7a and b).

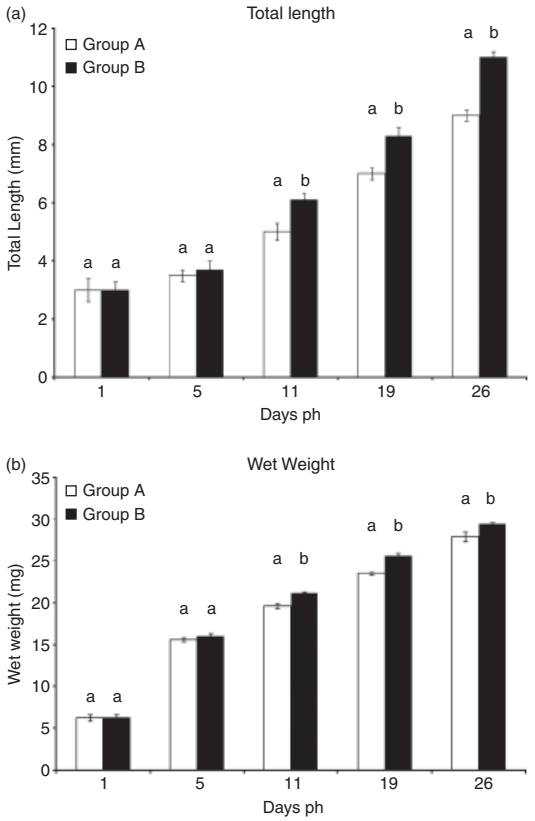

Figure 7 Size (mm) (a) and wet weight (mg) (b) of *Meiacanthus grammistes* larvae fed different diets. Different letters indicate statistical significance between groups A and B ($P < 0.05$).

Lipid analysis

Table 1 reports the percentage of fatty acid in the different diets, based on GC–MS data and expressed as the relative area percentage on total free fatty acids. In rotifers and *A. salina* nauplii, both enriched with Algamac 3000®, the relative percentage of EPA and DHA ranged between 6.90% and 12.8% with respect to the other free fatty acids, with a DHA/EPA ratio below 2; the percentage of total ω3 is < 20% (Table 1). On the contrary, in *Tisbe* spp. Copepods, the DHA content was > 20% and the DHA/EPA ratio was > 3. Furthermore, in this species, the percentage of ω3 fatty acids is very high (27.34 ± 2.3) (Table 1).

Discussion

It is well established that the number of marine ornamental species that can be economically produced in commercial farms today is still limited (Holt 2003). Unlike freshwater aquaria species, where 90% of fish species are currently farmed, the huge majority of marine species are stocked from the wild and the future of marine ornamental aquaculture depends on the ability to produce good-quality gametes and raise large numbers of larvae that successfully metamorphose to juveniles (Holt 2003).

In recent years, there has been an increased focus on supplying aquarium fish through closed system culturing: the development of reliable and sustainable hatchery procedures for the captive propagation of reef fish is now becoming essential not only to reduce pressure on wild populations but also because rearing fish in closed systems is likely to lead to the production of species that survive better and longer in captivity (Holt 2003; Olivotto, Zenobi, Rollo, Migliarini, Avella & Carnevali 2005; Wittenrich, 2007).

At present, even if it is hoped that much of the market demand for the majority of ornamentals may be satisfied through captive production, most marine

Table 1 Percentage of relative fatty acid content in rotifers and *Artemia salina* nauplii enriched with Algamac 3000® and in *Tisbe* spp. copepods

% Relative total free fatty acid	Rotifers + Algamac 3000®	*A. salina* nauplii + Algamac 3000®	*Tisbe* spp. copepods
EPA	6.90 ± 1.6	7.4 ± 0.7	6.5 ± 1.3
DHA	12.8 ± 0.8	9.2 ± 0.9	20.62 ± 2.0
DHA/EPA	1.85 ± 0.2	1.24 ± 0.3	3.17 ± 0.4
%ω3	19.6 ± 1.7	18.7 ± 1.1	27.34 ± 2.3

Fatty acid content was based on GC–MS data and expressed as the relative area percentage on the total free fatty acid area measured by peak integration. The best fatty acid profiles are *Tisbe* spp. copepods with respect to rotifers and *A. salina*.
EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

ornamental aquaculture procedures remain problematic.

Larviculture protocols for marine ornamentals still need to be optimized in order to produce organisms that commercially compete with less expensive specimens collected from the wild and the main critical bottleneck that scientists have to face in rearing ornamental fish larvae is the transition from endogenous to exogenous feeding by the larvae (Holt 2003; Olivotto *et al.* 2003, 2004, 2005; Wittenrich, 2007; Olivotto, Avella *et al.* 2008; Olivotto, Buttino *et al.* 2008; Olivotto, Capriotti *et al.* 2008).

Rotifers and *A. salina* nauplii have been widely used as live prey for newly hatched larvae, but they do not always promote optimal growth (Sun & Fleeger 1995). Problems related to the use of these food items include nutritional deficiencies and inappropriate sizes (Kahan 1981; Leger, Bengston, Simpson & Sorgeloos 1986; Evjemo, Danielsen & Olsen 2001; Holt 2003; Olivotto *et al.* 2006; Olivotto, Avella *et al.* 2008; Olivotto, Buttino *et al.* 2008; Olivotto, Capriotti *et al.* 2008) and thus alternative food sources that bypass these inadequacies and promote adequate growth are needed and are viewed with great interest by the scientific community (Sun & Fleeger 1995).

Recently, it has been demonstrated that the harpacticoid copepod *Tisbe* spp. may be considered to be a suitable live prey for marine fish larviculture when used as a supplement to the traditional diet based on rotifers and *A. salina* nauplii (Støttrup & Norsker 1997; Olivotto, Avella *et al.* 2008; Olivotto, Buttino *et al.* 2008). Because *M. grammistes* is an attractive and commercially requested blenny, which was once very common and appreciated in the aquarium trade but that is now becoming sporadic, the aim of the present study was to optimize the breeding and rearing conditions for this species with an emphasis on courtship, larval development and larval feeding.

While spawning in the striped blenny was fairly straightforward, the food offered was found to be crucial in the early rearing of this species. This is consistent with several studies that have demonstrated the inadequacies of rotifers and *A. salina* in marine larviculture and the positive effect of copepod administration on growth performance in several marine teleost species (Støttrup & Norsker 1997; Cutts 2002; Holt 2003; Drillet, Jørgensen, Sørensen, Ramløv & Hansen 2006; Olivotto, Avella *et al.* 2008; Olivotto, Buttino *et al.* 2008; Olivotto, Capriotti *et al.* 2008). In particular, the two long-chain polyunsaturated fatty acids, EPA (20:5n-3) and DHA (22:6n-3), are required in order to obtain normal growth and development, through maintaining the structural and functional integrity of cell membranes, and as precursors of eicosanoids (Sargent *et al.* 1999). Several studies have demonstrated that HUFAs are essential in larval diets because deficiencies may result in a general decrease in animal health, growth and stress tolerance (Sargent *et al.* 1999; Bell *et al.* 2003; Van der Meer, Olsen, Hamre & Fyhn 2008).

From the lipid analysis carried out on live prey, we observed that *Tisbe* spp. are rich in these two fatty acids and that the DHA/EPA ratio is particularly high, in agreement with the observations made by Sargent *et al.* (1999). A higher and better-balanced content of HUFAs in the copepod with respect to rotifers and *A. salina* nauplii may thus result in a more efficient lipid oxidation and consequent larval growth; this is in accordance with the biometric results obtained in this study (weight and length). In addition to being rich in essential fatty acids (HUFAs) that are extremely important for larval fish survival and growth, harpacticoid copepods not only provide a broad spectrum of prey sizes suitable for developing fish larvae (Gee 1989) but are also able to clean up the tank walls (Delbare *et al.* 1996) and, in more general terms, they are considered to be more suitable for mass cultivation than calanoids (Fukusho 1980; Kahan, Uhlig, Schwenzer & Horowitz 1982; Sun & Fleeger 1995; Støttrup & Norsker 1997; Olivotto, Avella *et al.* 2008; Olivotto, Buttino *et al.* 2008). The better survival rate observed in group B larvae fed on a mixed diet with respect to group A larvae given a standard rotifer/*A. salina* nauplii feed may thus be related to the broad spectrum of size of *Tisbe* spp. copepods and to the fact that they act as a supplemental source of essential fatty acids. Considering the WW and the TL of the larvae, significant differences ($P < 0.05$) between groups A and B are evident from day 11 DPH. Because these copepods are usually found on the tank walls rather than in the water column (Olivotto, Avella *et al.* 2008; Olivotto, Buttino *et al.* 2008), they may be considered to be less available to the fish larvae for predation. This hypothesis, in addition to the fact that newly hatched larvae have poorly developed predatory skills during early larval development, may explain the fact that size differences between the two experimental groups became evident from day 11 DPH. At this stage, the larvae had improved their predatory skills because they were also observed searching for copepods on the tank walls.

Finally, the time to metamorphosis was also significantly reduced by copepod administration. The

importance of HUFAs for a correct development and functionality of the central nervous system (CNS) is well established (Vaidyanathan, Raja Rao & Sastry 1994; Sargent *et al.* 1997) and we can thus assume that the improvement in the larval diet with a supplemental amount of HUFAs derived from copepods may result in an adequate development of the CNS and, hence, in a fully functional hypothalamus–pituitary–thyroid axis, which has a key role in metamorphosis regulation. This is supported by the fatty acid analysis carried out in this study and may thus explain the higher number of metamorphosed larvae in group B at the end of the experiment.

Conclusions

The harpacticoid copepod *Tisbe* spp. may be considered to be a valuable live prey for marine fish larvae. This study showed that this copepod may be cultured at reasonable concentrations and can improve *M. grammistes* larval survival and growth. The breeding and rearing protocol developed in this study may represent a further improvement for the commercial production of this species, thereby helping to preserve natural stocks from overexploitation.

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