Potential influence of phytoplankton density on doliolaria and pentactula appearances in sandfish hatcheries

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Abstract

The present study reports on the influence of phytoplankton food density on the time of appearance of doliolaria and pentactula. 72 h-old auricularia of *Holothuria scabra* were used at the beginning of the experiment. Two different food concentrations were used during the larval rearing: ~1500 cells mL^{-1} day^{-1} (F1) and ~120 cells mL^{-1} day^{-1} (F2). Results show that doliolaria appears significantly – *ca* 7 days – earlier with F1. Pentactula appears from 22 ± 6.6 days with F1, while with F2 they appear after 29.8 ± 9.3 days, but the difference is not significant. The results suggest that when the sea water is saturated with food (as in F1), the density of auricularia does not affect the larval life span (time of appearance of doliolaria). With low food concentration (as in F2), when the larval density is high, the quantity of food in the medium remains low, suggesting that food competition occurs between the larvae and slows down their development into doliolaria and pentactula.

Introduction

In sea cucumber aquaculture and farming, larval development is one of the most sensitive steps: to be able to supply farmers with sea cucumber juveniles, the larval production must be adequate because it regulates the number of juveniles transferred into sea pens. The metamorphosis of auricularia larvae into doliolaria and pentactula represents a major challenge in sandfish hatchery and requires careful control of rearing parameters, including physicochemical parameters and biological parameters such as the quality and quantity of food given to the larvae (James et al. 1994; Battaglene 1999; Asha 2004; Qiu et al. 2015). It is known that the variation of temperature, salinity and pH influences the growth and development of auricularia larvae (Asha and Muthiah 2005). The same is true concerning the amount of phytoplankton and the phytoplankton species given to the larvae (Asha 2004).

Several studies report on the optimal values of all these parameters for the best development of sea cucumber larvae and for different species (e.g. Giraspy and Walsalam 2010). Most of these studies demonstrate the effect of different amounts of food on larval growth, but most show minimal differences in metamorphosis patterns from auricularia larvae to doliolaria and pentactula.

Moreover, although many studies report that the transition from auricularia to doliolaria varies between 7 and 15 days (Qiu et al. 2015), Madagascar Holothurie SA, the Research and Development Unit of Indian Ocean Trepang (Eeckhaut 2008), observed that this can last much longer depending on various parameters like the season and the level of maturity of the parents.

Very few studies report on the influence of parameters (physicochemical and biological) on the time auricularia enter into metamorphosis to give doliolaria and pentactula. The aim of the present research is to demonstrate the influence of phytoplankton density on the time when auricularia enter into metamorphosis.

Materials and methods

The practical experiments were performed in Madagascar Holothurie SA. Auricularia larvae were obtained using *in vitro* fertilisation of eggs from mature broodstock (Leonet et al. 2009; Eeckhaut et al. 2012). From the same batch of broodstock, we selected the biggest auricularia that were >125 μm (filtered on a 125 μm mesh size plankton net) of 3 days of age to start the experiment.

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Rearing density and feeding rate

A total of 5,080,000 auricularia were reared in 18 tanks with a mean density of 0.10 ± 0.04 larvae mL^{-1}. The rearing density varied from one tank to another tank (0.04 larvae mL^{-1} to 0.18 larvae mL^{-1}). We used the phytoplankton species *Thalassiosira pseudonana* for daily feeding of the larvae. Two feeding rates were compared: F1 ~1500 cells mL^{-1} day^{-1} each for 9 tanks with auricularia mean density of 0.09 ± 0.02 larvae mL^{-1} and F2 ~120 cells mL^{-1} day^{-1} each for 9 tanks with auricularia mean density of 0.10 ± 0.06 larvae mL^{-1}. We used a haemocytometer (also known as cell of Malassez) for phytoplankton counting. The mean rearing density and its variation from each tank were the same for F1 and F2 (p = 0.613). During the experiment, the temperature was maintained between 25°C and 27°C, salinity ranged between 35psu and 36psu, and pH averaged 8.2.

Statistical analysis

Statistical analyses were performed using the R software (R Core Team 2017). Statistical difference was determined using t-test, with a level of 5%. Pearson's correlation coefficient was also calculated to determine correlation between larval density and time of appearance of doliolaria and pentactula.

Results

Influence of rearing density and feeding rate on appearance of doliolaria

Doliolaria appeared significantly earlier with F1 (p = 0.029). With the feeding rate F1, they were observed from 17.5 ± 4.5 days, while with F2 they were observed after 24.9 ± 7.8. Within F1, the Pearson's correlation coefficient is r = -0.50, showing no significant correlation between the appearance speed of doliolaria and the rearing density (p = 0.172). Within F2, the higher the density, the longer the appearance of doliolaria, with a significant Pearson's correlation coefficient of r = 0.87 (p = 0.002).

Influence of rearing density and feeding rate on appearance of pentactula

With F1, pentactula appeared from 22 ± 6.6 days, while with F2 they appeared after 29.8 ± 9.3 days. Despite a slight difference, no significant difference was observed between F1 and F2 (p = 0.059). However, the Pearson's correlation coefficient shows negative value with F1, r = -0.76, and a positive value with F2, r = 0.89. With F1, the higher the density, the faster the appearance of pentactula (p = 0.017), while with F2, the higher the density, the longer the appearance of pentactula (p = 0.001).

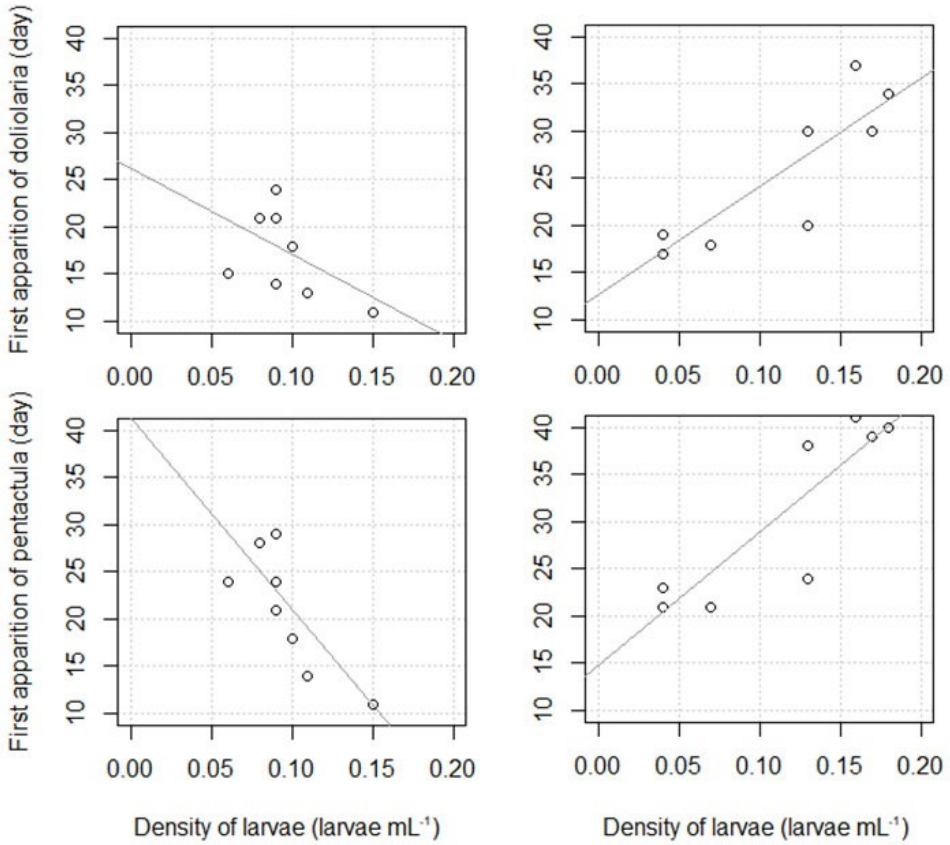

Figure 1. Relationship between the time of appearance of doliolaria (upper graphs) and pentactula (lower graphs), and the larval rearing density. In the left graphs, the phytoplankton concentration was of ~1500 cells mL^{-1} day^{-1}, in the right graphs, it was of ~120 cells mL^{-1} day^{-1}.

Discussion

The present study highlights the potential influence of phytoplankton density on the larval development of *Holothuria scabra*, especially on the doliolaria and pentactula appearance time in hatcheries. With similar rearing parameters (temperature, salinity and pH), larvae of holothurians, in this case *Holothuria scabra,* should grow in a similar way (Asha and Muthiah 2005; Giraspy et al. 2010). Results of the current experiment prove that, despite similar physical rearing parameters, auricularia metamorphose into doliolaria faster when fed with a higher concentration of phytoplankton. Asha and Muthiah (2006) already reported that food concentration has an effect on larval growth, survival and development of the species *Holothuria spinifera*. The optimal feed concentration for this species is 2 x 10^4 cells mL^{-1} of the flagellate *Isochrysis galbana*. Moreover, optimal phytoplankton concentrations differ from one holothurian species to another: 2 to 3 x 10^4 cells mL^{-1} was suggested *for Holothuria scabra* (James et al. 1994), 10^4 to 10^5 cells mL^{-1} for *Actinopyga echinites* (Chen and Chian 1990) and 0.5 to 3 x 10^4 cells mL^{-1} for *Stichopus japonicus* (Ito 1995). The food concentrations that were used in the present study are considerably lower than these references. However, the counting methods, which are usually not provided in detail, may differ from one result to another, and depending on the culture method and culture medium, the size of the phytoplankton can vary. The phytoplankton species used in the present study (*Thalassiosira pseudonana*) is of a bigger size than *Isochrysis galbana*. *Thalassiosira pseudonana* can range in diameter from 2.5–15 μm (Belcher and Swale 1977, 1986; Harris et al. 1995; Hasle 1976; Lange et al. 1983; Lowe and Busch 1975; Muylaert and Sabbe 1996; Price et al. 1987), while *Isochrysis galbana* average size range is less than 7 μm (Cordoba-Matson et al. 2013).

The results show that when phytoplankton concentration is of ~120 cells mL^{-1} day^{-1} the higher the density, the longer the appearance of both doliolaria and pentactula. We suggest that this concentration is not enough to feed all larvae and it induces a competition for food between the larvae. It is probable that metamorphosis is delayed when larvae do not possess enough nutrient reserve in their body. This would explain that the metamorphosis is delayed at higher larval densities. When the phytoplankton concentration is of ~1500 cells mL^{-1} day^{-1}, the sea water is probably saturated with food and the density of auricularia does not affect the time of appearance of doliolaria. We even see a negative correlation between the time of appearance of pentactula and the density of larvae. As doliolaria is a non-feeding pelagic stage, doliolaria could potentially be influenced by a high late auricularia (i.e., those ready to enter into metamorphosis) density: their 'interest' would be to settle fast and to transform into benthic pentactula.

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