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Effects of larval diet and metamorphosis cue on survival and growth of sea urchin post-larvae (*Paracentrotus lividus*; Lamarck, 1816)

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ABSTRACT

In this study, we present the results of two experiments; in the first one we evaluated the effects of four larval dietary treatments on the survival and growth of the sea urchin *Paracentrotus lividus*, larvae and post-larvae. In the second experiment we have measured the effects of two different settlement substrates, combined with the presence of conspecifics, on metamorphosis, survival and growth of post-larvae. The microalgae dietary treatments consisted in: *Dunaliella tertiolecta* (Duna); 50% mixture of *Isochrysis galbana* and *D. tertiolecta* (ID); 50% mixture of *Chaetoceros gracilis* and *D. tertiolecta* (CD); 33% mixture of *I. galbana*, *C. gracilis* and *D. tertiolecta* (ICD). Although all dietary treatments resulted in a good survival at competence, significant difference in post-larval survival was observed between treatments, and indeed, only larvae fed Duna and CD survived to 180 days post settlement (DPS).

In the second experiment, the settlement substrates consisted in a film of cultured *Ulvella lens* or a naturally developing biofilm of diatoms, and the employed rearing water was either natural seawater or seawater previously exposed to *P. lividus* adults. At 10 DPS, larger ($p < 0.05$) post-larvae were observed in the natural biofilm treatment, whilst the presence of conspecifics significantly increased larval settlement in both substrates ($p < 0.01$). These results indicate that it is important to consider the survival of post-larvae and juveniles to establish the efficiency of the dietary treatment on the hatchery production of *P. lividus*. Furthermore, it suggests that improved settlement protocols, such as the use of conspecifics, could contribute to increase hatchery outputs. Finally, it confirms the suitability of *U. lens* as settlement cue but also highlights that further research is required to establish its effectiveness for post-larvae first feeding.

Statement of relevance: Our work contributes to improving hatchery rearing methods of larvae and post-larvae of the sea urchin *Paracentrotus lividus*.

1. Introduction

Paracentrotus lividus (Lamarck, 1816) is the most consumed sea urchin species in Europe (Carboni et al., 2012). Due to the high market demand for its gonads, natural populations are exposed to overfishing in many Mediterranean and non-Mediterranean coastal areas (Pais et al., 2007), causing a sharp decline of the stock (Boudouresque and Verlaque, 2007; Pais et al., 2007; Addis et al., 2009).

This decrease is driving the development of echinoculture methods that started with *Pseudocentrotus depressus* by Yamabe (1962). These culture methodologies could represent a solution to limit the damages caused by wild stock overfishing and to protect natural populations (Mos et al., 2011; Carboni, 2013).

In the culture of sea urchins the transition from planktonic larvae to benthic juveniles represents a critical phase. Indeed, laboratory experiments report variable larval settlement and metamorphosis rates from 0 to 90% (Buitrago et al., 2005; Gosselin and Jangoux, 1996; Grosjean et al., 1998; Huggett et al., 2006; Pearce and Scheibling, 1991; Rahim et al., 2004) with post-settlement periods characterized by mortality rates higher than 90% within the first weeks of *P. lividus* benthic life (Buitrago et al., 2005; Grosjean et al., 1998; Rahim et al., 2004; Shimabukuro, 1991). The echinoculture production could therefore be increased by improving settlement rates and post-larvae survival, which currently represent the main bottleneck limiting this activity (Mos et al., 2011).

Several studies focused on microalgae diets and feed ration (Azad et al., 2011; Carboni et al., 2012; Cárcamo et al., 2005; Kelly et al., 2000; Liu et al., 2007; Pedrotti and Fenaux, 1993) and have identified several microalgae species, such as *Dunaliella tertiolecta*, which supports the rearing cycle and improve the larval survival and development of *P.*

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lividus (Carboni et al., 2012; Liu et al., 2007) and other echinoid species (Azad et al., 2011; George et al., 2004; Hart and Scheibling, 1988; Hinegardner, 1969; Kelly et al., 2000; Pearce and Scheibling, 1990a, 1991, 1994; Sewell et al., 2004). Many authors reported that *D. tertiolecta* is capable of producing healthy larvae because it is easily ingested (i.e. it has an appropriate cell size) and it is quickly digested (Basch, 1996; Cameron and Hinegardner, 1974; Strathmann, 1971). Moreover, it has an appropriate fatty acid profile for the larval growth (Carboni et al., 2012).

Although the effects of dietary treatments on *P. lividus* larval survival and development have been investigated, and it is well known that larval diet and feed ration influence survival and test diameter of various species of sea urchin post-larvae (Hart and Strathmann, 1994; Jimmy et al., 2003; Kelly et al., 2000; Liu et al., 2007; Meidel et al., 1999). However, their effects on determining the survival of juveniles has received little attention. Indeed, few studies focused on the survival and test diameter of various species of sea urchin post-larvae, but these investigations has been carried out just within 10 days post-settlement (Hart and Strathmann, 1994; Kelly et al., 2000; Liu et al., 2007; Meidel et al., 1999). Only Jimmy et al. (2003) evaluated the influence of three microalgal diets on the test diameter of *Echinus esculentus* at 6 months post-settlement.

In aquaculture settings, the transition from planktonic to benthic period is typically promoted by plates colonized with diatoms, which are believed to provide a good settlement cue and represent the initial feed for the juveniles (Cárcamo et al., 2005; Harris et al., 2003; McBride, 2005 Shimabukuro, 1991). However, laboratory experiments demonstrate that the settlement of sea urchin larvae is improved by a wide range of cues, among which the presence of conspecifics (Dworjanyn and Pirozzi, 2008; Mos et al., 2011). Indeed, Dworjanyn and Pirozzi (2008) reported for the first time that the sea urchin *Tripneustes gratilla* preferentially settled in response to the presence of conspecifics and seawater previously exposed to conspecifics and their faeces.

Recently, plates colonized by the green macroalgae *Ulvella lens* have been shown to improve larval settlement and represent the initial feed for juveniles sea urchin (Hannon et al., 2014, 2015; Takahashi et al., 2002) and sea cucumber (Matsuura et al., 2009). However, it has been recognized that some sea urchin species such as *S. intermedius* prefers to feed on diatoms rather than *U. lens* (Kawamura et al., 1983).

In the present study, we reported results of two experiments; in the first one we evaluated the effects of four phytoplankton diets on survival of *P. lividus* larvae at competence and post-larvae at different days post-settlement. In the second experiment, we compared two different settlement substrates, *U. lens* or a natural biofilm, and, although this topic has been investigated in other sea urchin species, such as *T. gratilla* (Dworjanyn and Pirozzi, 2008; Mos et al., 2011), we evaluated for the first time the effects of the presence of conspecifics on larval settlement and post-larval survival and growth of the sea urchin *P. lividus*.

2. Materials and methods

For both experiments, embryos of *P. lividus* were produced in the International Marine Centre — IMC laboratory (Oristano, Sardinia, Italy) from adult sea urchins (diameter larger than 45 mm) following published methods (Liu et al., 2007). Broodstock was collected from 5 m depth at the "Penisola del Sinis-Isola di Mal di Ventre" Marine Protected Area (39°89′N 8°41′W). Ten specimens (5 male, 5 female) were used for the gametes production.

The presence of the fertilization membrane was used to verify the fertilization rate, observed by using a tubular plankton chamber and a Leica DMRB Microscope (100× enlargement) (Azad et al., 2011; Grosjean et al., 1998; Liu et al., 2007). Embryos were stocked at a density of 20/mL until they reached the echinopluteus stage, about 40 h after fertilization took place. Subsequently, the echinoplutei were stocked at density 1.5/mL into 5 L cylindrical white plastic tanks. Cultures were constantly kept in motion by motor-driven rotation. Both embryos and echinopluteus were reared in filtered (0.47 μm) natural seawater (NSW), with a salinity of 36.5 ± 1.0 ppt, without aeration, in continuous light at 31 μmol photons/m^2/s and at a temperature of 19.0 ± 2.0 °C.

2.1. Experiment 1: effect of microalgae diets on larvae and juveniles, development, growth and survival

Four microalgae diets were tested during larval rearing: a single species diet of *D. tertiolecta* (Duna), a two species mixed diet (50% number of cells) of *Isochrysis* aff. *galbana* (T-Iso) and *D. tertiolecta* (ID), a two species mixed diet (50%) of *Chaetoceros gracilis* and *D. tertiolecta* (CD), a three species mixed diet (33%) of T-Iso, *C. gracilis* and *D. tertiolecta* (ICD). Although the three phytoplankton species tested in this study have a different cell size (T-Iso 40–50 μm^3, *C. gracilis* 80 μm^3, *D. tertiolecta* 170 μm^3, FAO, 2004) and dry weight (T-Iso 29.7 pg/cell, *C. gracilis* 74.8 pg/cell, *D. tertiolecta* pg/cell, FAO, 1996), we administered an equal number of microalgae cells to the larvae. Adopting the rearing method tested by Brundu et al. (2016), every three days we restored the amount of phytoplankton consumed by the larvae, guaranteeing constant ad-libitum feeding.

Phytoplankton cultures were maintained in batch lines at 25 °C, exposed to a 16/8 h (L/D) photoperiod at 63 μmol photons/m^2/s and supplied with gentle aeration. The 30 ppt salinity seawater was pre-filtered (1 μm filter paper), enriched with modified Guillard f/2 and autoclaved at 121 °C for 30 min.

The larvae were fed with microalgae cultures in their exponential growth phase, T-Iso 3.7 ± 0.2 million cells/mL, *C. gracilis* 3.3 ± 0.2 million cells/mL, *D. tertiolecta* 4 ± 0.4 million cells/mL. Larvae were reared in a total of 20 tanks, 5 replicates for each dietary treatment. Larval development was assessed every three days by observation of larval structures (number of arms, presence and size of the rudiment), according to previous studies (Carboni et al., 2012; Liu et al., 2007). For these purposes, a minimum of 10 randomly sampled larvae from each replicate were placed in a tubular plankton chamber and they were observed under a Leica MZ8 Stereomicroscope (15× enlargement). Competence of the culture was considered achieved when at least 75% of the sampled larvae were considered to be at this stage.

Larval survival was assessed volumetrically in each replicate and the mean value of each measurement was used to calculate the number of larvae in the tanks. Survival was expressed as percentage of the initial number of larvae stocked.

Metamorphosis tests were conducted when larvae reached competence for settlement. 50 larvae, from each replicate of each treatment, were transferred into shading beakers containing 50 mL of filtered NSW and a 50 × 50 mm polycarbonate layer colonized by the macroalgae *U. lens*, according to the methods described in Daume et al. (2004). The number of larvae undergoing metamorphosis was counted after 24, 48, and 72 h. When at least 75% of the larvae were metamorphosed, the entire larval culture was considered ready to settle and was transferred to 20 rectangular tanks with a volume of 20 L, maintaining the same experimental design adopted for the larval rearing (five replicates by four dietary treatments). Each tank contained NSW and a 20 × 18 cm polycarbonate layer colonized by the macroalgae *U. lens*. The animals were kept in a seawater static system for a month, with 36.5 ± 1.0 ppt salinity, without aeration and a 50% seawater exchange was performed twice per week. Recorded temperature was 19.0 ± 2.0 °C during the trial period and 14 h light photoperiod at 22 μmol photons/m^2/s was applied using fluorescent lamps. After the one month, the tanks were connected to a recirculating system, provided with biological and mechanical filtration (10 μm).

The number of post-larvae in each replicate was recorded at 10, 20, 30, 100 and 180 days post-settlement (DPS) and survival rate was calculated as percentage of the initially stocked competent larvae. Moreover, at 180 DPS, 200 randomly sampled juveniles were placed on a water proof graph paper and photographed with a Canon PowerShot G15