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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.

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. 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 (Carcamo 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 50x50 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 20x18 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 digital camera. Image Processing Analysis in Java (ImageJ 1.49V) was calibrated appropriately for image analysis and measurement. A length of 1 mm was measured via a ruler, saved and then used as the standard for individual growth measurement, in terms of test diameter; the widest part of the sea urchin body was measured. To simplify these operations, a solution of KCl 1% was used to induce paralysis and detachment of juveniles from the tanks, as tested by Hagen (2003) for *S. droebachiensis*.

2.2 Experiment 2: Effects of substrates and presence of conspecifics on larval settlement and post-larvae survival and growth

In a second experiment we tested the effectiveness of two substrates on the induction of metamorphosis in competent larvae. Larvae were exposed to polycarbonate plastic layers coated with either *U. lens* or a natural biofilm. *U. lens* was cultivated in laboratory under controlled conditions according to the method described by Daume *et al*. (2004). Natural biofilm, instead, was naturally cultivated submerging the layers in tanks containing estuarine water for 30 days (Cárcamo, 2004). Natural biofilm is widely

employed as larval settlement substrate for various species of sea urchins (Mos et al., 2011) and other invertebrates (Daume et al., 2004; Knauer et al., 1996; Leighton, 1989; Searcy-Bernal et al., 1992).

Furthermore, we tested the effects of the presence/absence of conspecifics on metamorphosis, post-settlement survival and growth of settled juveniles. This trial, therefore, consisted of four treatments: *U. lens* + conspecifics; *U. lens*; natural biofilm + conspecifics; natural biofilm. A total of 28 tanks (7 replicates by four treatments) were employed for this experiment.

Larvae used for this experiment were reared as previously described in experiment 1 and fed with a 50% mixture of *C. gracilis* and *D. tertiolecta*. Once competence was achieved metamorphosis test was conducted as described above and larvae were then randomly distributed in equal concentration (1 larvae/ml) between the four treatments.

Importantly, larvae were never in direct physical contact with adult conspecifics but, instead, the water used for the two conspecific treatments was previously passed through a 20 L tank hosting 5 wild-harvest *P. lividus* adults (diameter larger than 45 mm) and then used for the trial. Conspecifics were starved and hosted for a week in previously filtered (1 μm) NSW, in static condition and with gentle aeration.

The number of larvae undergoing metamorphosis was counted after 24, 48, and 72 h. Survival was assessed at 10 DPS by observing the movement of spines and tube feet of the settled individuals under a Leica MZ8 Stereomicroscope (60× enlargement); growth was assessed at 10 DPS by measuring the individuals' test diameter with a Leica DMC2900 digital camera connected to a Leica MZ8 Stereomicroscope (30× enlargement) and using an image analysis software (Leica Application Suite LAS V4.5).

2.3 Statistical analysis

Data were analyzed by Statistica 6.1 StatSoft, Inc. (2004). The normality and homogeneity of the data distribution were assessed with Shapiro Wilk's W and Levene's tests, respectively. Where required, data on larval development and survival were analyzed by the non-parametric Kruskal-Wallis test; otherwise, one-way analysis of variance (ANOVA) was employed. Post-larvae survival was analyzed using repeated-measures ANOVA with survival as a factor and DPS as a repeated factor. Tukey's honestly-significant difference (HSD) test was used to evaluate all pair-wise treatment comparisons ($p<0.05$). For comparison of the juvenile growth, a size-class distribution was constructed. Size-classes were determined as 1 mm increments in diameter; Kolmogorov-Smirnov test was used to compare size-class distributions and to test the prediction that all size-classes occurred in similar proportions among dietary treatments.

Metamorphosis percentage was analyzed using repeated-measures ANOVA with inducing factor and seawater as factors and time as a repeated factor, while the effects on post-larvae survival and test diameter were assessed by a two-way ANOVA. Tukey's honestly-significant difference (HSD) test was used to evaluate all pair-wise treatment comparisons ($p<0.05$).

3 Results

3.1 Experiment 1: Effect of microalgae diets on larvae and juveniles, development, growth and survival

Larval development was significantly influenced by microalgae diets. ICD resulted in a faster ($p<0.01$) development than all other diets, and at 10 days post-fertilization (DPF) 79.9±7.8% of the larvae fed ICD achieved the competent stage, whilst only 7.9±3.4%,

6.1±3.7% and 30.1±13.4% of the larvae fed Duna, ID and CD, respectively, achieved this stage. Nonetheless, larvae in all treatments reached the competence by 22 DPF and no significant difference were observed after 10 DPF (Table 1). No significant differences between treatments were recorded in larval survival up until the competent stage (Table 2). However, larvae fed the ICD diet presented a significantly lower (p<0.001) larval survival at metamorphosis (4.2±3.5%) than other treatments, Duna (65.0±14.9%), ID (61.8±16.5%) and CD (78.4±10.2%) (Table 2).

All post-larvae in ICD treatment died before 10 days post settlement. Repeated measures ANOVA showed a gradual decreasing of post-settlement survival from 10 to 100 DPS (p<0.001) in all other treatments with no significant difference between treatments. Post-settlers resulting from the ID treatment did not survive past 100 DPS; at the end of the experiment, 180 DPS, a survival of 1.5±1.5% and 3.0±2.0%, respectively for Duna and CD, was recorded (Fig. 1).

At 180 DPS, survivors from both, Duna and CD treatments, had a test diameter from 1.1 mm to 10+ mm, but no significant differences were highlighted by Kolmogorov-Smirnov test on the size-classes distribution, as well as by ANOVA on the mean test diameter, 6.4±0.2 mm and 6.6±0.2 mm for Duna and CD treatment, respectively (Fig. 2).

3.2 Experiment 2: Effects of substrates and presence of conspecifics on larval settlement and post-larvae survival and growth

Metamorphosis was significantly influenced by the presence of conspecifics. Indeed, individuals exposed to seawater treated with conspecifics showed a significantly higher metamorphosis rate throughout the observation period (p<0.01), whilst no significant differences were recorded between substrates (Fig. 3).

At 10 DPS survival rate did not differed between treatments. Significant differences (p<0.05) in the juveniles' test diameter, however, were observed between substrates and a significantly larger test diameter was, in fact, measured for juveniles feeding on natural biofilm (Fig. 4).

4 Discussion

4.1 Experiment 1: Effect of microalgae diets on larvae and juveniles, development, growth and survival

All dietary treatments tested in this study resulted in a better larval development and survival at competence, in comparison with monospecific diets of T-Iso (competence at 29 DPF and 88.5±4.4% survival) and *C. gracilis* (competence at 23 DPF and 43.8±10.1% survival), and a 50% mixture of the same species (competence at 20 DPF and 73.7±8.7% survival) previously tested (Brundu et al., 2016). The survival rate at competence obtained in this study by using *D. tertiolecta* (82.8±10.6%) is higher than previously reported in the literature (Azad et al., 2011; Carboni et al., 2012; George et al., 2004; Kelly et al., 2000; Jimmy et al., 2003; Liu et al., 2007), suggesting that this species can indeed support larval development of *P. lividus*.

Liu et al. (2007) obtained a 65% survival and a normal development of *P. lividus* larvae fed with *D. tertiolecta*; moreover, a greater final body width and a faster development resulted with this species (settlement by day 18 post-fertilization) than microencapsulated formulated feeds (settlement by day 20 post-fertilization). Normal development of larvae fed *D. tertiolecta* was also obtained by Carboni et al. (2012), even though with a much lower survival rate. The difference between this and previous studies could be explained by the smaller tank volumes and lower larval densities

adopted. Nonetheless, our results on the larval survival of *P. lividus* are comparable with those obtained for other sea urchin species fed *D. tertiolecta* monospecific diet.

As previously reported by Brundu et al. (2016), a delay between competence and metamorphosis completion was also observed in this study, potentially causing the unexpectedly mortality observed in larvae fed ICD. It is well known that mixed-species algal diets provide a more balanced nutrient profile than a single-algal diet (Azad et al., 2011; Pechenik, 1987) and they promote better results than single-algal diets on the growth of echinoderm larvae (Azad et al., 2011; Basch, 1996; Cárcamo et al., 2005; Gonzalez et al., 1987) and other marine invertebrates (Marshall et al., 2010). On the contrary, other studies observed allelopathy processes among phytoplankton species, which refers to the release of secondary metabolites by plants, microorganisms, viruses and fungi, which could result in competition among organisms (Gross, 2003). It has been observed, for instance, that *Prymnesium parvum* and *Alexandrium* spp. influence negatively the growth rate of other microalgae species, causing lysis of algal cells, and they can change the natural community structure (Fistarol et al., 2004; Fistarol et al., 2003). Nonetheless, this phenomenon has never been described for the microalgae species used in this study which are, instead, commonly used in combination for aquaculture operations.

The lower larval survival rate of larvae fed the ICD treatment was also reflected in the population crash at 10 DPS, when none of the individuals survived. ICD tertiary combination, therefore, can be considered as an appropriate diet during the larval development stages up to the competence, but it failed to support survival during settlement and post-settlement stages. On the contrary, larvae fed Duna and CD showed significantly higher survival rates during early development and post-metamorphosis. The survival rates at 10 DPS of post-larvae treated with *D. tertiolecta* alone and mixed

with *C. gracilis* are similar to the values reported in the literature for *P. lividus* (Liu et al., 2007) and other sea urchin species (Jimmy et al., 2003; Kelly et al., 2000). The observed differences between dietary treatments are probably due to a different proximal composition of the phytoplankton, as well as to the different cell size. It has been previously reported that *D. tertiolecta* is capable of producing healthy larvae because it has an appropriate cell size (Basch, 1996; Cameron and Hinegardner, 1974; Strathmann, 1971) and fatty acid profile for the larval growth of *P. lividus* (Carboni et al., 2012). On the contrary, T-Iso and *C. gracilis* have a smaller cell size and dry weight in comparison with *D. tertiolecta* (FAO, 2004, 1996), and contain a lower amount of protein, carbohydrate and lipid (FAO, 1996).

Larvae fed Duna, ID and CD showed a gradual decrease in the survival of post-larvae from 10 DPS onwards; ID failed to survive past 100 DPS, while Duna and CD showed a similar survival at 100 and 180 DPS, as well as in the test diameter at the end of the experiment. This suggests that larval dietary treatment influences the survival for the first part of post-metamorphic life, but does not influence the growth of juveniles, as observed for *E. esculentus* by Jimmy et al. (2003). Moreover, the average test diameters at 180 DPS reported by this author, 6.83±1.6 mm with *D. tertiolecta* and 6.55±1.9 mm with a mixture of *D. tertiolecta* and *P. tricornutum*, are similar to our results, 6.4±0.2 mm and 6.6±0.2 mm with Duna and CD, respectively.

The growth of juveniles at 180 DPS was very heterogeneous regardless to the treatment; the large variation recorded in test diameter (from 1.1 mm to 10+ mm) was probably due to competition between individuals of different sizes, as previously observed in other studies (Ebert, 1973; Grosjean et al., 1996; Guillou and Michel, 1993; McCarron et al., 2009).

In a population of 133 juveniles of *L. albus* (76 days old), Gonzalez et al. (1987) recorded a size ranging between 0.5-2.7 mm. In another experiment, the same authors compared two populations (62 days old) treated with *D. tertiolecta* and a mixture of *D. tertiolecta* and T-Iso, obtaining a larger size-class distribution for the population fed the mixture than *D. tertiolecta*.

4.2 Experiment 2: Effects of substrates and presence of conspecifics on larval settlement and post-larvae survival and growth

Echinoderm species, such as sea cucumber *Psolus chitonoides* (Young and Chia, 1982) and sand dollars *D. excentricus* (Burke, 1984) and *Echinarachnius parma* (Pearce and Scheibling, 1990b) are known to settle in response to conspecifics, as well as other marine invertebrate larvae (Crisp, 1974; Hadfield and Paul, 2001; Pawlik, 1992). Among echinoids, only *T. gratilla* was observed to settle in response to the presence of adults and particularly of their faeces, maybe because of a conspecific bacteria mediated signal (Dworjanyn and Pirozzi, 2008; Mos et al., 2011). Our results show, for the first time, that settlement of *P. lividus*, regardless of the employed substrate, is improved by the presence of adult conspecifics. The underlying mechanisms (e.g. bacteria, chemical signals) for the improved metamorphosis rate are however unclear.

Although it has been shown that *U. lens* improves larval settlement of *P. lividus* (Hannon et al., 2014; 2015) and abalones (Daume et al., 2000; Krisinich et al., 2000; Takahashi and Koganezawa, 1988), our study did not confirm that *U. lens* is better than natural biofilm as metamorphosis inducing factor. Before the metamorphosis tests, the polycarbonate plastic layers coated by *U. lens* were carefully washed and wiped clean to remove any unwanted biofilm, which could play an important role for the settlement

cue, as previously hypothesized by Daume et al. (2004). In this way, we excluded a potential effect on metamorphosis of biofilm developed on the *U. lens* substrate.

Hannon et al. (2015) reported a higher larval settlement of *P. lividus* on *U. lens* than *Amphora* spp., a commonly used diatom for abalones settlement (Daume et al., 2000; Gordon et al., 2006). The mean settlement obtained by Hannon et al. (2015) on *U. lens* (50%) is higher than our results on the same substrate, but it is similar to our result on natural biofilm + conspecifics (46.2±3.2%), suggesting that both substrates, *U. lens* and natural biofilm, are good inducing factor for metamorphosis in *P. lividus*.

Similarly to our results, Daume et al. (2000) found that settlement of abalone *H. rubra* is higher on *U. lens* than diatoms, but growth rates were higher on diatoms than *U. lens*. Seki (1997) reported that *U. lens* sustained growth of post-larval *H. discus hannai*, but growth rates were improved by the inoculation of cultured diatoms. According to Kawamura et al. (1983), *U. lens* has been used in aquaculture centers in Japan for the production of the sea urchin *S. intermedius*, as they suggest that newly metamorphosed juveniles feed on diatoms first to then switch to *U. lens*.

Although no differences in survival at 10 DPS was recorded between settlement substrates, the higher diameter of post-larvae settled on the natural biofilm compared to *U. lens* strengthens the importance of diatoms for the growth and survival of *P. lividus* juveniles. This is particularly true as larger settlers are less susceptible to post-settlement mortality (Meidel et al., 1999).

5 Conclusions

This study could represent an improvement in the rearing methods of *P. lividus*. All diets tested in this study supported the larval development of *P. lividus*, but only

juveniles resulting from the Duna and CD treatments survived to 180 DPS. This indicates that it is important to consider the survival of post-larvae and juveniles to establish the efficiency of the dietary treatment on the production of *P. lividus* juveniles.

Seawater previously "conditioned" by the presence of conspecifics increases the larval settlement rate of *P. lividus.* Furthermore, although *U. lens* provides an appropriate settlement substrate for competent larvae, higher growth rate of post-metamorphic individuals was achieved on a natural biofilm. Nevertheless, considering that dominant species in a natural biofilm change throughout the season and between locations, *U. lens* could represents an efficient and more reliable alternative to natural biofilms as metamorphosis inducing factor and first feeding item, but further investigations are required to ascertain its nutritional value for *P. lividus* post larvae.

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Table 1: *Paracentrotus lividus* larvae (%) at competence stage (Cp). Days post fertilization (DPF), *Dunaliella tertiolecta* (Duna), mixture of T-Iso and *D. tertiolecta* (ID), mixture of *Chaetoceros gracilis* and *D. tertiolecta* (CD), mixture of T-Iso, *C. gracilis* and *D. tertiolecta* (ICD). The different letters indicate significant differences among diets (=$p<0.01$). Values are expressed as mean ± SE (n=5).**

Table 2: Survival (%) of *Paracentrotus lividus* larvae at competence and metamorphosis. *Dunaliella tertiolecta* (Duna), mixture of T-Iso and *D. tertiolecta* (ID), mixture of *Chaetoceros gracilis* and *D. tertiolecta* (CD), mixture of T-Iso, *C. gracilis* and *D. tertiolecta* (ICD). The different letters indicate significant differences among diets (*=$p<0.001$). Values are expressed as mean ± SE (n=5).**

Fig. 1: Survival rate of *Paracentrotus lividus* post-larvae at 10, 20, 30, 100 and 180 days post-settlement (DPS). *Dunaliella tertiolecta* (Duna), mixture of T-Iso and *D. tertiolecta* (ID), mixture of *Chaetoceros gracilis* and *D. tertiolecta* (CD). Superscripts indicate significant differences among DPS ($p<0.001$). Values are expressed as mean ± SE (n=5).

Fig. 2: Size-class distributions (diameter in 1 mm intervals) of *Paracentrotus lividus* post-larvae resulting from the Duna and CD treatments, at 180 days post-settlement (DPS).

Fig. 3: Metamorphosis rate of competent larvae of *Paracentrotus lividus* exposed to two substrate types, *Ulvella lens* and natural biofilm, and to the presence or absence of adult conspecifics. Superscripts indicate significant differences between substrates ($p<0.01$). Values are expressed as mean ± SE (n=7).

Fig. 4: Test diameter and survival of *Paracentrotus lividus* post-larvae at 10 days post-settlement. Superscripts indicate significant differences between treatments ($p<0.05$). Values are expressed as mean ± SE (diameter, n=28; survival, n=7).

Table 1

Diet	10 DPF **	13 DPF	16 DPF	19 DPF	22 DPF
Duna	7.9 ± 3.4 [b]	88.3 ± 8.3	90.3 ± 6.3	93.9 ± 4.0	100
ID	6.1 ± 3.7 [b]	62.8 ± 18.1	70.9 ± 17.5	71.7 ± 16.4	78.8 ± 16.2
CD	30.1 ± 13.4 [b]	95.3 ± 4.7	98.1 ± 1.9	100	100
ICD	79.9 ± 7.8 [a]	88.8 ± 3.2	97.0 ± 3.0	100	100

Table 2

Diet	Competence (Cp)	Metamorphosis (Mt) ***
Duna	82.8 ± 10.6	65.0 ± 14.9 [a]
ID	82.0 ± 14.8	61.8 ± 16.5 [a]
CD	84.6 ± 9.9	78.4 ± 10.2 [a]
ICD	72.8 ± 10.6	4.2 ± 3.5 [b]

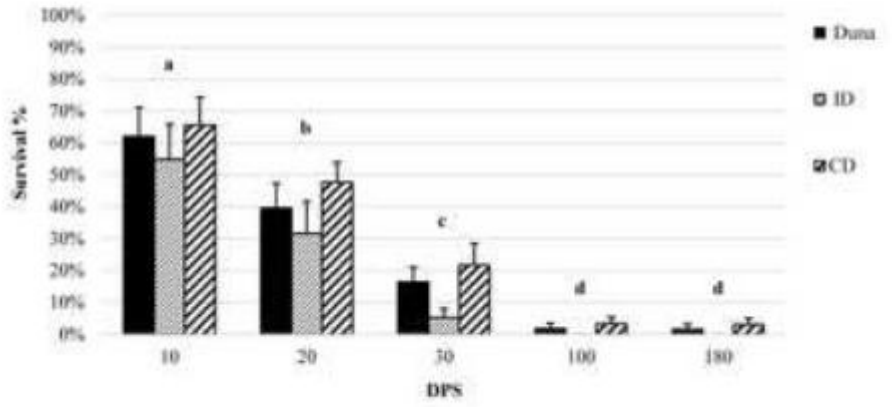
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Survival %
a
b
c
d
d
10
20
30
100
180
DPS
Duna
ID
CD

Figure 1

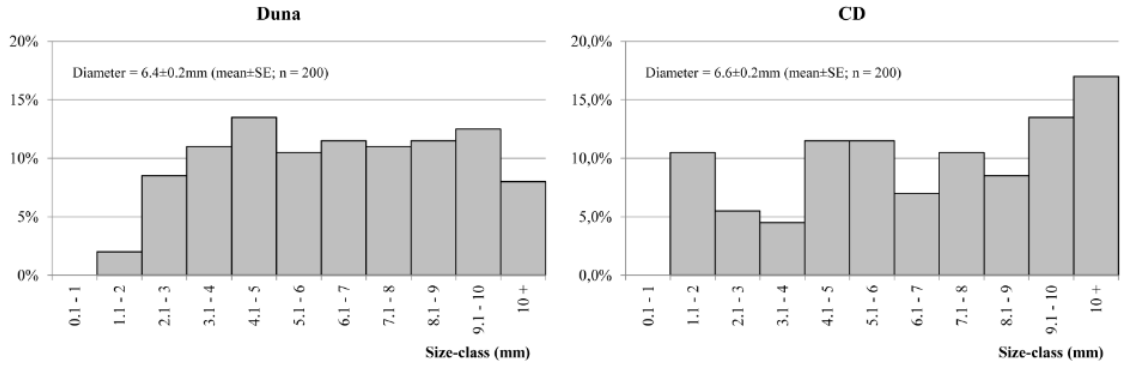
Duna
20%
15%
10%
5%
0%
Diameter = 6.4±0.2mm (mean±SE; n = 200)
0.1 - 1
1.1 - 2
2.1 - 3
3.1 - 4
4.1 - 5
5.1 - 6
6.1 - 7
7.1 - 8
8.1 - 9
9.1 - 10
10 +
Size-class (mm)
CD
20,0%
15,0%
10,0%
5,0%
0,0%
Diameter = 6.6±0.2mm (mean±SE; n = 200)
0.1 - 1
1.1 - 2
2.1 - 3
3.1 - 4
4.1 - 5
5.1 - 6
6.1 - 7
7.1 - 8
8.1 - 9
9.1 - 10
10 +
Size-class (mm)

Figure 2

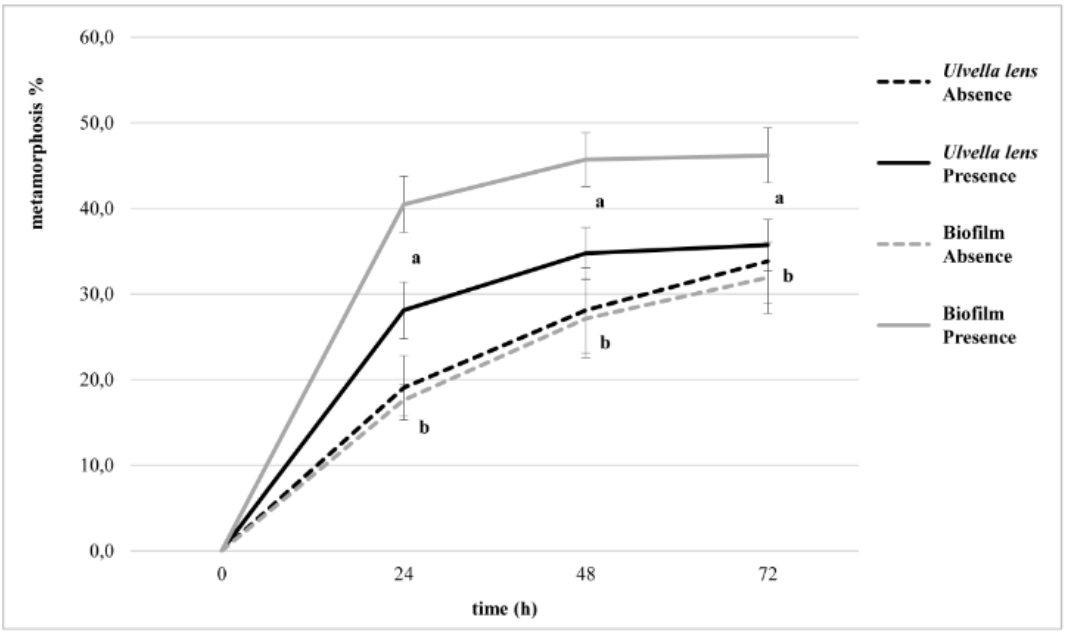
60,0
50,0
40,0
30,0
20,0
10,0
0,0
metamorphosis %
0
24
48
72
time (h)
a
a
a
b
b
b
Ulvella lens
Absence
Ulvella lens
Presence
Biofilm
Absence
Biofilm
Presence

Figure 3

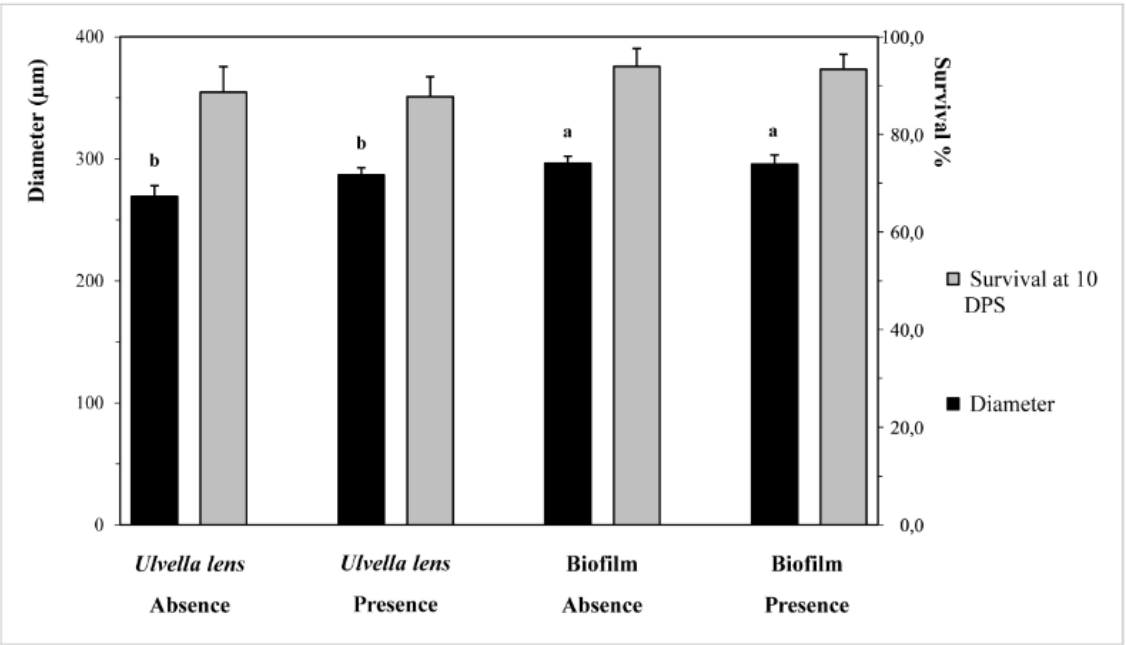
Diameter (µm)
400
300
200
100
0
100,0
80,0
60,0
40,0
20,0
0,0
Survival %
b
b
a
a
Survival at 10 DPS
Diameter
Ulvella lens
Absence
Ulvella lens
Presence
Biofilm
Absence
Biofilm
Presence

Figure 4

Statement of relevance

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

Highlights of this study:

1. This study tests the effects of the presence of adult conspecifics on the larval settlement of *Paracentrotus lividus*; results demonstrate that seawater previously "conditioned" by the presence of conspecifics increases the larval settlement rate.
2. This study demonstrates that the macroalgae *Ulvella lens* could represents an efficient alternative to a natural biofilm of diatoms as metamorphosis inducing factor and first feeding item, although a higher growth rate of post-larvae was achieved on a natural biofilm than on *U. lens*.