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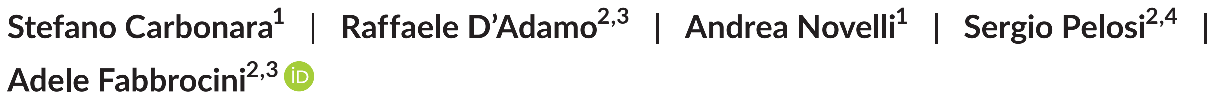
Increasing the stocking density in *Paracentrotus lividus* larviculture: Effects on survival and metamorphosis rates



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Abstract

The development of sustainable methods for sea urchin juvenile production is currently constrained by high mortality rates during larval growth and the high costs of larval rearing systems management. With the aim of developing a method for the production of juveniles of the purple sea urchin *Paracentrotus lividus* in a medium-scale recirculating system, the present study focused on the effects of high stocking densities on larval growth. Plutei larvae were reared at three different densities (up to 7 ind/ml) in a semi-static culture system. Larval survival and metamorphosis success were evaluated in order to identify the most effective density range. The highest metamorphosis rates (80%–95%) were obtained at 4 and 7 larvae/ml. These results are comparable with (and in some cases higher than) those reported for the same species at much lower larval densities. In conclusion, the rearing conditions tested here show for the first time that a significantly higher (4 ind/ml) stocking density than those of traditional *P. lividus* rearing methods (based on large volumes and low densities) can be adopted, thus supporting the feasibility of an increase in the final output of competent larvae with no increase in rearing volumes.

KEYWORDS
larval density, larviculture, metamorphosis, *Paracentrotus lividus*, sea urchin

1 | INTRODUCTION

Sea urchin roe are considered a delicacy in many parts of the world. In Mediterranean countries, almost all roe consumed derive from *Paracentrotus lividus* and a serious alteration in wild population dynamics has been observed (Guidetti, Terlizzi, & Boero, 2004; Pais, Serra, Meloni, Saba, & Ceccherelli, 2012).

The increasing difficulty in meeting market demand through fishing has led in recent decades to investment in research supporting echinoderm aquaculture (Brown & Eddy, 2015; Parisi et al., 2012; Vizzini, Miccichè, & Vaccaro, 2015; Volpe et al., 2018).

Currently, the main bottleneck in *P. lividus* aquaculture is juvenile availability, whose production at a hatchery scale still need to be improved (Azad, McKinley, & Pearce, 2010; Brundu, Monleón, Vallainc, & Carboni, 2016; Hannon, Officer, & Chamberlain, 2017). Infact, the high mortality rates occurring during larval growth, together with the high running costs of larval rearing systems, are the main constraints that still need to be resolved for sustainable juvenile production methods to be developed (Azad, Pearce, & McKinley, 2011; Carboni, Vignier, Chiantore, Tocher, & Migaud, 2012; Dworjanyn & Pirozzi, 2008).

Sea urchins, notably *P. lividus*, are widely used as model species in laboratory research. All phases of their biological cycle, especially the larval development stages, have thus been studied for a long time (Fenaux, Cellario, & Etienne, 1985). Nonetheless, most of the literature still involves small-to-medium scale studies (Azad, Pearce, & McKinley, 2011, 2012; Brundu et al.., 2016; Carcamo, Candia, & Chaparro, 2005), while few attempts have been made to upscale the developed protocols (Buitrago et al., 2005; De La Uz, Carrasco, Rodríguez, & Anadón, 2013).

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Therefore, there is a need for improved species-specific protocols for larval production, rearing, settlement induction and post-metamorphic endotrophic juvenile management, taking into account and optimizing the main factors that may increase efficiency in medium-to-large scale systems (Azad et al., 2010).

Several authors have investigated the effects on sea urchin larval growth of factors such as temperature, pH and feeding regime (Brennand, Soars, Dworjanyn, Davis, & Byrne, 2010; Brundu et al., 2016; Carcamo et al., 2005; Hardy et al., 2014). In contrast, few studies have focused on the effect of stocking density during larval development (Azad, Pearce, & McKinley, 2012; Buitrago et al., 2005), especially regarding the Mediterranean species *P. lividus*.

Within a wider research program aimed at the optimization of methods for the production of juveniles of the Mediterranean sea urchin *P. lividus* in medium-scale recirculating rearing systems, the present study focused on the effects of stocking density on larval growth. Specifically, plutei larvae obtained from sea urchins matured in a recirculating system were reared at three different stocking densities (from 0.25 to 7 ind/ml); larval survival on reaching competence and metamorphosis rates were evaluated in order to identify the effects of stocking density on the quality of the competent larvae.

2 | MATERIALS AND METHODS

2.1 | Ethic statements

The present study did not involved vertebrate, protected or endangered species. All experimental procedures on sea urchins were in compliance with the Directive 2010/63/EU.

2.2 | Broodstock rearing

Adult *P. lividus* specimens (test diameter without spines 35–45 mm) were reared at the Ittica Caldoli Fish Farm, at a density of 1 sea urchin per 5 L in a recirculating aquaculture system filled with 1,500 L of natural filtered (45 μm) sea water (FSW). Sea urchins were kept in a natural photoperiod, at constant salinity (36 PSU ± 1), temperature (18 ± 1°C), pH (7.9–8.4) and oxygen concentration (6–10 ppm). These rearing conditions were found to be optimal in previous *P. lividus* rearing trials (Fabbrocini & D'Adamo, 2010; Fabbrocini & D'Adamo, 2011). Sea urchins were fed every 2 days on a mixed diet composed of whole corn seeds (*Zea mays*) and chunked defrosted sardines (*Sardina pilchardus*) at a ratio of 70:30 (W/W), corresponding to 2% of the total biomass. This feeding regime was found to support *P. lividus* gonad maturation in a preliminary rearing trial (Carbonara, Maurizio, Novelli, & Fabbrocini, 2015). At each feeding, all residual material from the previous distribution was removed.

2.3 | Gamete collection and fertilization protocol

In accordance with the protocol developed for sea urchin gonad conditioning, before performing fertilization for embryo production, six specimens were randomly collected from the tank in order to confirm by means of histological evaluation that the gametes are from mature (not premature or post-spawned) specimens, with reference to the classification scheme of Byrne (1990). Following positive gonad evaluation, 15 sea urchins were randomly sampled from the tank and induced to spawn by injecting 1 ml of 0.5 M KCl through the peristomial membrane surrounding the mouth using a 1 ml insulin syringe. An aliquot of gametes from each sea urchin was immediately diluted at a rate of 1:100 in FSW and observed by microscope to confirm the gender. Gametes from each animal were then separately collected: sperm samples were dry-collected by micropipette directly from gonopores and stored undiluted in 10 ml polypropylene tubes at 4°C until use; eggs were collected by placing spawning females on 100 ml beakers containing FSW; eggs were then washed twice in FSW and stored at 18°C until use. Egg morphology and sperm motility were evaluated as described in Fabbrocini and D'Adamo (2011); then, egg samples showing good morphology were pooled (n = 7), as were sperm samples (n = 6) whose motility parameters fell within the range recorded for mature and in-spawning *P. lividus* specimens collected in the field (Fabbrocini, Maurizio, & D'Adamo, 2016).

Paracentrotus lividus embryos were obtained as described in Carbonara, D'Adamo, Novelli, Pelosi, and Fabbrocini (2018). Briefly, fertilization was carried out in a 20 L plastic bucket containing 15 L of FSW (sperm-egg ratio 15,000:1; 200 eggs/ml). The percentage of fertilized eggs was verified to be higher than 98% by observing the fertilization membrane in 200 eggs. The rearing bucket was then kept in the dark, at 18 ± 1°C, under gentle aeration, until the embryos reached the gastrula stage.

2.4 | Larval rearing system

On reaching the gastrula stage, the embryos were transferred to the larval rearing vessels, which consisted of 50 L transparent polyethylene bags. For each experimental bag the volume of embryo solution containing the appropriate gastrulae amount was carefully poured from the rearing bucket taking care not to shake the bucket bottom, where unfertilized eggs and not-developed embryos were deposited. 35 PSU FSW was then slowly added to each bag to a final volume of 50 L.

The larvae were reared at three densities: 0.25, 4 and 7 ind/ml, in 35 PSU FSW. The first two tested densities were selected with reference to Grosjean, Spirlet, Gosselin, Vaitilington, and Jangoux (1998) and Carboni et al. (2012), respectively; the highest was chosen on the basis of positive results obtained in a preliminary trial conducted to evaluate the feasibility of more intensive *P. lividus* larval rearing conditions.

Three replicate bags for each tested density were run.

Larvae were kept at a salinity of 35 PSU ± 1, a water temperature of 18–22°C and a photoperiod of 12/12 D/L. Water oxygenation and gentle movement were provided by bubbling air from the bottom of the bags (Grosjean et al., 1998).

Starting from the third day post-fertilization (dpf), larvae were fed laboratory-cultured live algae twice a day. A mix of *Dunaliella tertiolecta* and *Phaeodactylum tricornutum* was used, as in Carboni et al. (2012) and Grosjean et al. (1998) respectively. *Phaeodactylum tricornutum* and *D. tertiolecta* were batch-cultured in 20 L transparent polyethylene bags containing Guillard f/2 medium (Guillard, 1975), and used for the experiment at the exponential growth phase. Microalgal concentrations were evaluated by haemocytometer, and a mix of the two cultures (50/50 by number of cells) was provided each time. The feeding regime varied in accordance with the density of the larvae and the developmental stages. At each feeding the amount of microalgae supplied was calculated to obtain for each experimental density the final microalgal concentrations (cells/ml) reported in Table 1.

The feeding regime was defined on the basis of preliminary experiments in which observations of larval stomach contents were performed twice a day for each larval density, assuring an ad-libitum feeding provision for all the tested conditions.

Since our final aim was the definition of a rearing protocol that could easily be upscaled for the production of *P. lividus* juveniles, we used a low water exchange rate: every 2 days, 33% of the culture water was siphoned off, and an equal volume of new FSW was added. The low flow rate and the use of a 40 μm mesh to screen out larvae prevented them from being lost or damaged during the procedure.

The progression of the developmental stage was monitored by microscope each day, together with larval gut contents (to detect any food deficiency), considering 10–15 larvae from each replicate. No evident differences in feeding activity among stocking densities were observed.

Larvae were considered competent when rudiment development could be observed, and swimming behaviour was directed towards the walls or the bottoms of the containers (Gosselin & Jangoux, 1998). Sixteen days post fertilization, before larvae being collected for the first time for the settlement trials, larval survival at the three tested densities was assessed and expressed as percentage of the initial number of larvae stocked for each experimental bag.

TABLE 1 Feeding regime of *Paracentrotus lividus* larvae during the rearing trial. The reported microalgal amounts represent the final concentration to be obtained in the experimental vessels after each food provision. (D = *D. tertiolecta;* P = *P. tricornutum*)

Larval concentration (ind/ml)	Microalgae amount (cells/ml) Developmental stage	
	4-arms	6-arms to competence
0.25	D 1.5×10^4	D 1.5×10^4
	P 1.5×10^4	P 1.5×10^4
4	D 1.5×10^4	D 3×10^4
	P 1.5×10^4	P 3×10^4
7	D 1.5×10^4	D 3×10^4
	P 1.5×10^4	P 3×10^4

2.5 | Settlement assay

Starting from 16 dpf, settlement competence was tested each day.

Ground *Ulva* solution (GUS) was used as a metamorphosis-inducing factor, having been found to give similar metamorphosis rates to *Corallina*-conditioned sea water (Carbonara et al., 2018), widely employed as a settlement inducer for *P. lividus* larvae (Grosjean et al., 1998; De La Uz et al., 2013; Vaïtilingon, Morgan, Grosjean, Gosselin, & Jangoux, 2001).

Ground *Ulva* solution was prepared as described in Carbonara et al. (2018). Briefly, samples of *Ulva* sp. were hand-collected in shallow waters along the southern Adriatic coast (Lesina Marina, 41°54'55.0"N 15°20'37.0"E), and transferred to the laboratory in refrigerated bins containing sea water from the collection site. In the laboratory, samples were carefully checked and washed, in order to eliminate undesired material and fragments of other algal species. GUS was prepared by grinding fragments of *Ulva* sp. in a mortar together with FSW at a rate of 1/2.5 (W/V). The extract was sieved and then filtered (0.7 μm). Erythromycin (7.5 μg/ml) was added to reduce microbial contamination (Feuerbacher, Bonar, & Barrett, 2017).

Every day, larvae were randomly collected from each experimental bag and carefully pipetted into sterile 10 ml polystyrene multiwell dishes containing the metamorphosis-inducing solution (25 larvae/well). Six wells for each experimental bag were run. FSW was used as a control for the measurement of spontaneous metamorphosis, i.e. not depending on the presence of the inducing factor but on the FWS itself or as a consequence of a drastic reduction in larval selectivity (Swanson et al., 2012; Swanson, Marshall, & Steinberg, 2007; De La Uz et al., 2013).

The multi-well dishes were incubated at 22°C in the dark. Larval settlement was checked by microscope 24 hr after the addition of the metamorphosis-inducing solution. Attached larvae with well-developed spines and tube feet were considered to be properly settled. The results were expressed as the percentage of settled larvae of the total exposed to metamorphosis-inducing solution.

Tests of metamorphosis induction were carried out every day from 16 to 25 dpf, when a declining reaction to the inducer was observed.

2.6 | Statistical analyses

Differences in survival rates as a function of larval density were evaluated by one-way ANOVA. Metamorphosis rates were analysed statistically using repeated-measures ANOVA with larval density as a categorical factor and dpf as a repeated factor. Significant differences between densities were explored by HSD Tukey's test. Prior to analysis, data were arcsine-transformed and tested for normality by Kolmogorov–Smirnov test and for homogeneity of variance by Cochran's test. $p < 0.01$ was considered significant.

3 | RESULTS

Survival rates on reaching competence were 47.6% for larvae reared at a density of 0.25 ind/ml, 64.5% for those reared at 4 ind/ml, and 57.9% for those reared at 7 ind/ml. Although a higher survival rate was recorded at a density of 4 ind/ml, no significant differences as a function of larval density were found (Figure 1).

Larval metamorphosis rates obtained in the settlement trials carried out from 16 to 25 dpf are shown in Figure 2. A significant effect of larval density on the ability to undergo metamorphosis as a function of days post fertilization was observed. Indeed, the positive response to the inducing solution was observed first in larvae reared at 0.25 ind/ml, but they retained this ability for a shorter time than those reared at the two higher concentrations tested. In detail, at 18 dpf, larvae reared at the lowest concentration showed a metamorphosis rate of around 50%, significantly higher than that of larvae reared at a density of 4 ind/ml (20%). Larvae reared at 7 ind/ml did not undergo metamorphosis. At 19 dpf, similar percentages (around 70%) were observed for 0.25 and 4 ind/ml, while a significantly lower value (27%) was obtained for 7 ind/ml. At 20 dpf, the 0.25 ind/ml larvae showed a metamorphosis rate of around 20%, significantly lower than the two higher densities. Intermediate values were obtained with the 7 ind/ml larvae (47%), while significantly higher rates were recorded for larvae reared at 4 ind/ml (72%). In the following 2 days, larvae reared at both 4 and 7 ind/ml showed metamorphosis percentages ranging from 80% to 95%, with no significant difference between them, while significantly lower values (around 20%) were again observed in the 0.25 ind/ml larvae. Starting from 23 dpf, larvae reared at 0.25 ind/ml did not respond to the inducing solution, while declining values (around 60%) were also observed for the two higher densities, with no significant difference between them. In our experimental conditions, no spontaneous metamorphosis occurred: control larvae diluted in FSW, i.e. in absence of the inducing factor (GUS), never underwent metamorphosis, irrespective of larval age and rearing density.

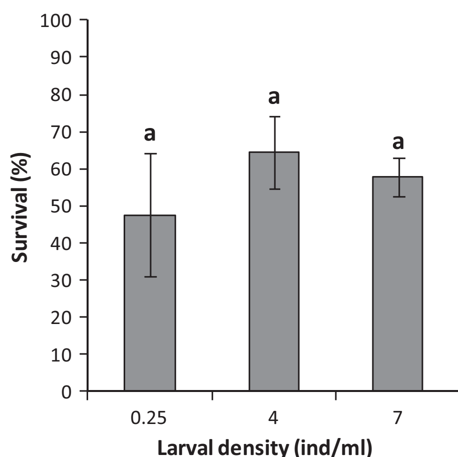

FIGURE 1 Survival rate (%) of *Paracentrotus lividus* larvae reared at the three tested stocking densities. Evaluations were made at the achievement of competence (16 days post-fertilization). Values are expressed as mean ± *SD* ($n = 3$). Different superscripts indicate significant differences ($p < 0.01$)

4 | DISCUSSION

The rearing density of the larval stages is one of the most frequently manipulated parameters in aquaculture, since increasing it reduces the production costs of juveniles. However, if larval concentrations are too high, this may lead to larval damage during swimming, reducing access to food and increasing wastage in the rearing system (Azad et al., 2012). Therefore, its effect on larval development and survival has been extensively studied in many species of interest to aquaculture, to identify the optimum range for each species (Azad et al., 2012; Buitrago et al., 2005; Lagos, Herrera, Sánchez-Lazo, & Martínez-Pita, 2015).

In this article, we evaluated the effect of stocking density on the survival and metamorphosis rates of *P. lividus* larvae. We obtained survival rates ranging from 47.6% to 63.5%, comparable and in some cases higher than those reported for the same species in other studies (Brundu et al., 2016; Carboni et al., 2012; De La Uz et al., 2013; Grosjean et al., 1998; Liu et al., 2007; Paredes, Bellas, & Costa, 2015; see Table 2).

In our experiments, three larval densities were tested (0.25, 4 and 7 ind/ml), and although higher survival rates were recorded with larvae reared at 4 and 7 ind/ml than at 0.25 ind/ml, the differences were not significant. In contrast, rates of successful metamorphosis were observed to be significantly affected by density, the best results being obtained at the higher stocking densities. In previous studies of *P. lividus* larvae, the highest survival and metamorphosis rates were obtained at densities ranging from 0.25 to 1.5 ind/ml (Brundu et al., 2016; Grosjean et al., 1998; Liu et al., 2007; Paredes et al., 2015; De La Uz et al., 2013), while increasing the density to 4 ind/ml resulted in lower larval survival (Carboni et al., 2012). It should be pointed out that in these studies *P. lividus* larvae were

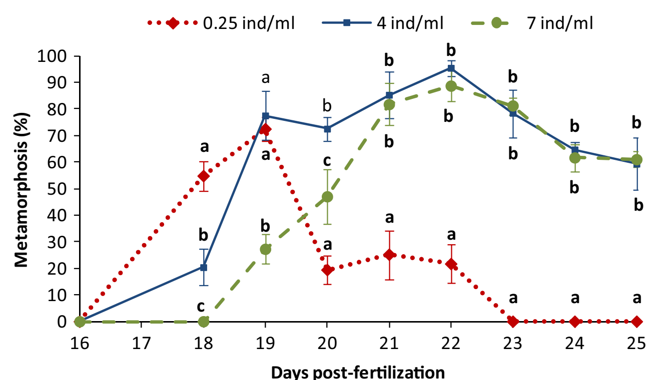

FIGURE 2 Metamorphosis rate (%) of *Paracentrotus lividus* larvae reared at the three tested stocking densities. Evaluations were made from the 16th to the 25th day post-fertilization (dpf). Values are expressed as mean ± *SD* ($n = 3$). Different superscripts indicate significant differences among densities at the same dpf ($p < 0.01$) [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Survival at competence and metamorphosis rates reported in literature for *Paracentrotus lividus* larvae reared in different volumes and at different stocking densities in comparison with those obtained in the present study. Details regarding the other experimental conditions were not reported in the present Table

Larval concentration (ind/ml)	Volume (L)	Survival (%)	Metamorphosis (%)	Reference
0.25	200	56	80.4	Grosjean et al. (1998)
0.25	200	–	11–73	De La Uz et al. (2013)
0.25	50	47.6	19.4–72.5	This study
1	100	–	28.2	Paredes et al. (2015)
1.5	60	67.8–75.6	10–50	Liu et al. (2007)
1.5	5	72.8–84.6	15–45	Brundu et al. (2016)
4	80	5.2–14.4	–	Carboni et al. (2012)
4	50	64.5	20.3–95.4	This study
7	50	57.8	27.2–88.7	This study

grown under a wide range of conditions, impeding comparison of the obtained results. On the other hand, similar results have been found for other sea urchin species: Buitrago et al. (2005) tested densities from 0.25 to 1 ind/ml for *Lytechinus variegatus* larvae, finding no significant differences in terms of survival. The effects of a wider range of stocking densities, from 0.5 to 4.0 ind/ml, were evaluated for *Strongylocentrotus purpuratus* larvae by Azad et al. (2012), finding the optimal culture density for its static system to be 1.0 ind/ml, and observing increased mortality at higher larval concentrations.

The effect of increasing larval density observed in our study may be explained first of all by considering that we provided a higher number of algal cells per individual larva than other protocols (Azad et al., 2012; Buitrago et al., 2005; Carboni et al., 2012), our feeding ratio having been adapted following observation of larval stomach contents twice a day in preliminary tests. Consistent with this hypothesis, Fenaux, Strathmann, and Strathmann (1994) also found a positive effect of increased food availability on larval development, while similar survival and metamorphosis rates were obtained with the same feeding rate as our study (Grosjean et al., 1998) and with ad-libitum feeding (Brundu et al., 2016).

In addition to feed ratio, diet type is also a key factor in successful larval development (Azad et al., 2010 and Azad et al., 2011; Brundu et al., 2016; Carboni et al., 2012). Both *D. tertiolecta* and *P. tricornutum* have been successfully used for *P. lividus* larval rearing (Brundu et al., 2016; Carboni et al., 2012; Grosjean et al., 1998; Liu et al., 2007; Vaïtilingon et al., 2001). They also achieved good larval development and metamorphosis in our case, when mixed and supplied in large quantities.

In our study, larvae reached competence from 18 to 22 dpf, in accordance with what has previously been reported for *P. lividus* (Gosselin & Jangoux, 1998; Grosjean et al., 1998; Liu et al., 2007). However, differences in the time needed to achieve competence and the ability to complete metamorphosis were observed as a function of density (Figure 2). Indeed, the positive response to the inducing solution was first obtained (18 dpf) in larvae reared at 0.25 ind/ml. However, they retained this ability for a shorter time than those reared at the two higher densities, showing metamorphosis rates of around 70% but never reaching the 90% recorded for the two higher densities. Faster achievement of competence as a result of greater food availability has been reported in sea urchins (Fenaux et al., 1994; Sewell, Cameron, & McArdle, 2004). On the other hand, Vaïtilingon et al. (2001) demonstrated that overfed competent larvae may allocate the energy acquired from the food to non-metamorphic processes, thus reducing metamorphosis success rates. It may thus be supposed that the high feeding rate adopted in our rearing trials could account for the faster development of the larvae reared at 0.25 ind/ml and for their early loss of competence, as well as for the absence of a density-dependent delay in competence achievement in the larvae reared at 4 and 7 ind/ml, which has been reported in previous studies of echinoderm larvae (Azad et al., 2012; Buitrago et al., 2005). Moreover, a shortening of the post-oral arms in *Psammechinus miliaris* larvae was observed as a consequence of over-feeding (Kelly, Hunter, Scholfield, & McKenzie, 2000). Even if in this study larval growth was not considered as evaluation parameter, at the same time of the survival rates assessment (16 dpf), the overall larval length (Liu et al., 2007) was measured in 30 competent larvae for each experimental density. Consistently with Kelly et al. (2000), smaller larval lengths were observed in larvae reared at 0.25 ind/ml (629 μm ± 53), in respect to those reared at 4 and 7 ind/ml (795 μm ± 80 and 729.52 μm ± 52 respectively).

Rearing tank volume and geometry are of crucial importance to both adult and larval performance. Indeed, despite the higher handling and management costs of large-scale rearing systems, a positive correlation between vessel volume and larval quality and survival has been reported for many species. Small rearing volumes can negatively affect larvae due to their low buffering capacity, as well as their different hydrodynamics, which may influence the settlement of algae, making them more or less available for larval consumption, while in larvae capable of active movement it could alter feeding activity (Backhurst & Harker, 1988; Lika, Pavlidis, Mitrizakis, Samaras, & Papandroulakis, 2015; Ruttanapornvareesakul, Sakakura, & Hagiwara, 2007). The rearing vessels used in our trials, transparent 50 L polyethylene bags, resulted in survival and metamorphosis performances comparable to those obtained for the same species in both small-scale (Brundu et al., 2016) and large-scale (Grosjean et al., 1998; De La Uz et al., 2013) systems. As a medium-scale rearing system, it represents a good compromise between management effort and production efficiency. Indeed, to our knowledge, it is the first

time that metamorphosis rates of 80%–90% have been obtained for *P. lividus* larvae reared at stocking densities above 2 ind/ml (Table 2). On the other hand, since post-settlement survival rates have been found to depend also on larval rearing conditions, especially on the energy stored during the larval stage (Mos, Cowden, Nielsen, & Dworjanyn, 2011; Vaïtilingon et al., 2001), further studies will seek to evaluate the post-metamorphosis performance of larvae reared under our experimental conditions.

Larval metamorphosis success may be highly variable also depending on the settlement inducers (Mos et al., 2011; De La Uz et al., 2013). Sea urchin larvae have been found to settle and metamorphose in response to a variety of environmental factors, including the presence of macroalgae, microbial or diatom-based biofilms and conspecific adults, as the bioactive compounds triggering metamorphosis may be released from algae or from their epiphytic biofilm (Dworjanyn & Pirozzi, 2008; Mos et al., 2011; Swanson et al., 2012; De La Uz et al., 2013). As sea water conditioned by the presence of conspecific adult specimens fed on *Ulva* spp. has been found to induce metamorphosis in *P. lividus* larvae (Gosselin & Jangoux, 1998), in our previous experiments (Carbonara et al., 2018) we assumed that grazed algal fragments release dissolved chemical cues, in accordance with what reported for other sea urchin species (Swanson et al., 2012). Therefore, in order to mimic the decomposing algal conditions arising from the sea urchins' grazing activity, we ground *Ulva* spp. fragments in a mortar and used the obtained solution (GUS) as metamorphosis inducing factor. The presence of inducing factors in the GUS is also proven by the absence of metamorphosis in control samples (FSW), consistently with what is reported for *P. lividus* and other sea urchin species (Swanson et al., 2012; De La Uz et al., 2013).

Finally, it is well-known that sea urchin larval quality also depends on broodstock rearing conditions (Fabbrocini & D'Adamo, 2011; White, Dworjanyn, Nichols, Mos, & Dempster, 2016). The recirculating system used here to induce gamete maturation in the sea urchin broodstock required relatively low management effort and costs, supporting the production of a considerable amount of good-quality embryos, which in turn enabled the promising results obtained in terms of metamorphosis.

5 | CONCLUSIONS

Summarizing our results, it may be concluded that stocking density has a significant effect on *P. lividus* larval growth. Under the tested rearing conditions, a density of 4 ind/ml can be considered to perform better than 7 ind/ml, as the former saw metamorphosis rates exceed 70% from 19 dpf and the latter from 21 dpf.

The algal diet, a mix of *D. tertiolecta* and *P. tricornutum* provided in abundant quantities, was found to support fast larval development even at the highest tested stocking density.

The medium-scale rearing system used here (50 L polyethylene bags) represents a good compromise between management effort and production efficiency. Moreover, the semi-static rearing conditions entail less larval disturbance than static rearing systems, without huge needs in terms of phytoplankton and water supply.

In conclusion, these rearing conditions allow the production of competent larvae at a stock density significantly higher than the traditional rearing methods developed for *P. lividus* (which entail large volumes and low densities), thus supporting the feasibility of an increase in the final output of competent larvae with no increase in rearing volumes.

DATA ACCESSIBLITY

The authors declare that the data that support the findings of this study are available within the article.

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REFERENCES

Azad, A. K., McKinley, R. S., & Pearce, C. M. (2010). Factors influencing the growth and survival of larval and juvenile echinoids. *Reviews in Aquaculture*, *2*, 121–137. https://doi.org/10.1111/j.1753-5131.2010.01030.x

Azad, A. K., Pearce, C. M., & McKinley, R. S. (2011). Influence of microalgal species and dietary rations on larval development and survival of the purple sea urchin, *Strongylocentrotus purpuratus* (Stimpson, 1857). *Aquaculture*, *322–323*, 210–217. https://doi.org/10.1016/j.aquaculture.2011.09.029

Azad, A. K., Pearce, C. M., & McKinley, R. S. (2012). Influence of stocking density and temperature on early development and survival of the purple sea urchin, *Strongylocentrotus purpuratus* (Stimpson, 1857). *Aquaculture Research*, *43*, 1577–1591. https://doi.org/10.1111./j.1365-2109.2011.02960x

Backhurst, J. R., & Harker, J. H. (1988). The suspension of feed in aerated rearing tanks: The effect of tank geometry and aerator desing. *Aquaculture Engineering*, *7*, 379–395.

Brennand, H. S., Soars, N., Dworjanyn, S., Davis, A. R., & Byrne, M. (2010). Impact of ocean warming and ocean acidification on larval development and calcification in the sea urchin *Tripneustes gratilla*. *PLoS ONE*, *5*(6), e11372. https://doi.org/10.1371/journal.pone.0011372

Brown, N. P., & S. D. Eddy (Eds.) (2015). *Echinoderm aquaculture* (pp. 1–368). Hoboken, NJ: Wiley Blackwell. https://doi.org/10.1002/9781119005810

Brundu, G., Monleón, L. V., Vallainc, D., & Carboni, S.. (2016). Effects of larval diet and metamorphosis cue on survival and growth of sea urchin post-larvae (*Paracentrotus lividus*, Lamarck, 1816). *Aquaculture*, *465*, 265–271. https://doi.org/10.1016/j.aquaculture.2016.09.014

Buitrago, E., Lodeiros, C., Lunar, K., Alvarado, D., Indorf, F., Frontaldo, K., … Vasquez, Z. (2005). Mass production of competent larvae of the sea urchin *Lytechinus variegatus* (Echinodermata: Echinoidea). *Aquaculture International*, *13*, 359–367. https://doi.org/10.1007/s10499-004-6551-y

Byrne, M. (1990). Annual reproductive cycles of the commercial sea urchin *Paracentrotus lividus* from an exposed intertidal and sheltered subtidal habitat on the west coast of Ireland. *Marine Biology*, *104*, 275–289.

Carbonara, S., D'Adamo, R., Novelli, A., Pelosi, S., & Fabbrocini, A. (2018). Ground *Ulva* solution (GUS): A promising metamorphosis cue for *Paracentrotus lividus* larviculture. *Aquaculture*, *491*, 289–294. https://doi.org/10.1016/j.aquaculture.2018.03.044

Carbonara, S., Maurizio, D., Novelli, A., & Fabbrocini, A. (2015). *The gamete maturation and gonad growth control in reared sea urchins using different diets*. Paper presented at the Aquaculture 2015 Conference "Cutting edge science in aquaculture", Montpellier (France), 23–26 August 2015.

Carboni, S., Vignier, J., Chiantore, M., Tocher, D. R., & Migaud, H. (2012). Effect of dietary macroalgae on growth, survival and fatty acid composition of sea urchin *Paracentrotus lividus* throughout larval development. *Aquaculture*, *324–325*, 250–258. https://doi.org/10.1016/j.aquaculture.2011.10.037

Carcamo, P. E., Candia, A. I., & Chaparro, O. R. (2005). Larval development and metamorphosis in the sea urchin *Loxechinus albus* (Echinodermata: Echinoidea): Effects of diet type and feeding frequency. *Aquaculture*, *249*, 375–386. https://doi.org/10.1016/j.aquaculture.2005.03.026

De La Uz, S., Carrasco, J. F., Rodríguez, C., & Anadón, N. (2013). Metamorphosis, growth andsurvival of early juveniles of *Paracentrotus lividus* (Echinodermata: Echinoidea): Effects of larval diet and settlement inducers. *Cahiers de Biologie Marine*, *54*, 691–695.

Dworjanyn, S. A., & Pirozzi, I. (2008). Induction of settlement in the sea urchin *Tripneustes gratilla* by macroalgae, biofilms and conspecifics: A role for bacteria? *Aquaculture*, *274*, 268–274. https://doi.org/10.1016/j.aquaculture.2007.11.030

Fabbrocini, A., & D'Adamo, R. (2010). Gamete maturation and gonad growth in fed and starved sea urchin *Paracentrotus lividus* (Lmk, 1816). *Journal of Shellfish Research*, *29*(4), 1051–1059.

Fabbrocini, A., & D'Adamo, R. (2011). Gamete and embryos of sea urchins (*Paracentrotus lividus*, Lmk 1816) reared in confined conditions: Their use in toxicity bioassays. *Chemistry & Ecology*, *27*(2), 105–115. https://doi.org/10.1080/02757540.2011.625931

Fabbrocini, A., Maurizio, D., & D'Adamo, R. (2016). Sperm motility pattern as a tool to evaluate differences in sperm quality across different gonad development stage in the sea urchin *Paracentrotus lividus*. *Aquaculture*, *452*, 115–119. https://doi.org/10.1016/j.aquaculture.2015.10.033

Fenaux, L., Cellario, C., & Etienne, M. (1985). Croissance de la larve de l'oursin *Paracentrotus lividus*. *Marine Biology*, *86*, 151–157. https://doi.org/10.1007/BF00399021

Fenaux, L., Strathmann, M. F., & Strathmann, R. R. (1994). Five tests of food-limited growth of larvae in coastal waters by comparisons of rates of development and form of echinoplutei. *Limnology and Oceanography*, *39*, 84–98. https://doi.org/10.4319/lo.1994.39.1.0084

Feuerbacher, O., Bonar, S. A., & Barrett, P. J. (2017). Enhancing hatch rate and survival in laboratory-reared hybrid Devils Hole Pupfish through application of antibiotics to eggs and larvae. *North American Journal of Aquaculture*, *79*, 106–114. https://doi.org/10.1080/15222055.2016.1240123

Gosselin, P., & Jangoux, M. (1998). From competent larva to exotrophic juvenile: A morphofunctional study of the perimetamorphic period of *Paracentrotus lividus* (Echinodermata, Echinoidea). *Zoomorphology*, *118*, 31–43.

Grosjean, P., Spirlet, C., Gosselin, P., Vaitilington, D., & Jangoux, M. (1998). Land-based closed-cycle echinicolture of *Paracentrotus lividus* (Lamarck) (Echinoidea: Echinodermata): A long-term experiment at a pilot scale. *Journal of Shellfish Research*, *17*(5), 1523–1531.

Guidetti, P., Terlizzi, A., & Boero, F. (2004). Effects of the edible sea urchin, *P. lividus*, fishery along the Apulian rocky coast (SE Italy, Mediterranean sea). *Fisheries Research*, *66*, 287–297. https://doi.org/10.1016/S0165-7836(03)00206-6

Guillard, R. R. L. (1975). Culture of phytoplankton for feeding marine invertebrates. In W. L. Smith, & M. H. Chanley (Eds.), *Culture of marine invertebrate animals* (pp. 26–60). New York, NY: Plenum Press.

Hannon, C., Officer, R. A., & Chamberlain, J.(2017). Evaluation of the efficacy of algal-conditioned substrates for inducing settlement of *Paracentrotus lividus* larvae. *Aquaculture Research*, *48*, 1968 – 1973. https://doi.org/10.1111/are12959

Hardy, N., Lamare, M., Uthicke, S., Wolfe, K., Doo, S., Dworjanyn, S., & Byrne, M. (2014). Thermal tolerance of early development in tropical and temperate sea urchins: Inferences for the tropicalization of eastern Australia. *Marine Biology*, *161*, 395–409. https://doi.org/10.1007/s00227-013-2344-z

Kelly, M. S., Hunter, A. J., Scholfield, C. L., & McKenzie, J. D. (2000). Morphology and survivorship of larval *Psammechinus miliaris* (Gmelin) (Echinodermata: Echinoidea) in response to varying food quantity and quality. *Aquaculture*, *183*, 223–240. https://doi.org/10.1016/S0044-8486(99)00296-3

Lagos, L., Herrera, M., Sánchez-Lazo, C., & Martínez-Pita, I. (2015). Effect of larval stocking density on growth, survival and whole body cortisol of the Mediterranean mussel *Mytilus galloprovincialis* (Lamarck, 1819) larvae reared under laboratory conditions. *Aquaculture Research*, *46*, 1648–1656. https://doi.org/10.1111/are.12318

Lika, K., Pavlidis, M., Mitrizakis, N., Samaras, A., & Papandroulakis, N. (2015). Do experimental units of different scale affect the biological performance of European sea bass *Dicentrarchus labrax* larvae? *Journal of Fish Biology*, *86*, 1271–1285. https://doi.org/10.1111/jfb.12636

Liu, H., Kelly, M. S., Cook, E. J., Black, K., Orr, H., Zhu, J. X., & Dong, S. L. (2007). The effect of diet type on growth and fatty-acid composition of sea urchin larvae. I. *Paracentrotus lividus* (Lamarck, 1816) (Echinodermata). *Aquaculture*, *264*, 247–262. https://doi.org/10.1016/j.aquaculture.2006.12.021

Mos, B., Cowden, K. L., Nielsen, S. J., & Dworjanyn, S. (2011). Do cues matter? Highly inductive settlement cues don't ensure high post-settlement survival in sea urchin aquaculture. *PLoS ONE*, *6*(12), e28054. https://doi.org/10.1371/journal.pone.0028054

Pais, A., Serra, S., Meloni, G., Saba, S., & Ceccherelli, G. (2012). Harvesting effects on *Paracentrotus lividus* population structure: A case study from northwestern Sardinia, Italy, before and after the fishing season. *Journal of Coastal Research*, *28*, 570–575. https://doi.org/10.2112/JCOASTRES-D-10-00119.1

Paredes, E., Bellas, J., & Costa, D. (2015). Sea urchin (*Paracentrotus lividus*) larval rearing – Culture from cryopreserved embryos. *Aquaculture*, *437*, 366–369. https://doi.org/10.1016/j.aquaculture.2014.12.022

Parisi, G., Centoducati, G., Gasco, L., Gatta, P. P., Moretti, V. M., Piccolo, G., … Pais, A. (2012). Molluscs and echinoderms aquaculture: Biological aspects, current status, technical progress and future perspectives for the most promising species in Italy. *Italian Journal of Animal Science*, *11*, e72. https://doi.org/10.408/ijas.2012.e72

Ruttanapornvareesakul, Y., Sakakura, Y., & Hagiwara, A. (2007). Effect of tank proportion on survival of seven-band grouper *Epinephelus septemfasciatus* (Thunberg) and devil stinger *Inimicus japonicus* (Cuvier) larvae. *Aquaculture Research*, *38*, 193–200. https://doi.org/10.1111/j.1365-2109.2007.01653.x

Sewell, M. A., Cameron, M. J., & McArdle, B. H. (2004). Developmental plasticity in larval development in the echinometrid sea urchin *Evechinus chloroticus* with varying food ration. *Journal of Experimental Marine Biology and Ecology*, *309*, 219–237. https://doi.org/10.1016/j.jembe.2004.03.016

Swanson, R., Byrne, M., Prowse, T. A. A., Mos, B., Dworjanyn, S. A., & Steinberg, P. D. (2012). Dissolved histamine: A potential habitat marker promoting settlement and metamorphosis in sea urchin larvae. *Marine Biology*, *159*, 915–925. https://doi.org/10.1007/s00227-011-1969-2

Swanson, R. L., Marshall, D. J., & Steinberg, P. D. (2007). Larval desperation and histamine: How simple responses can lead to complex changes in larval behaviour. *Journal of Experimental Biology*, *210*, 3228–3225. https://doi.org/10.1242/jeb.004192

Vaïtilingon, D., Morgan, R., Grosjean, P., Gosselin, P., & Jangoux, M. (2001). Effects of delayed metamorphosis and food rations on the perimetamorphic events in the echinoid *Paracentrotus lividus* (Lamarck, 1816) (Echinodermata). *Journal of Experimental Marine Biology and Ecology*, *262*, 41–60. https://doi.org/10.1016/S0022-0981(01)00281-7

Vizzini, S., Miccichè, L., & Vaccaro, A. (2015). Use of fresh vegetables discards as sea urchin diet: Effect on gonad index and quality. *Aquaculture International*, *23*, 127–139. https://doi.org/10.1007/s10499-014-9803-5

Volpe, M. G., Fabbrocini, A., Siano, F., Coccia, E., Scordella, G., Licchetti, E., … Paolucci, M.(2018). Gonad quality of sea urchin *Paracentrotus lividus* cultured in an offshore pilot-scale trial on the south-east Italian coast. *Aquaculture Nutrition*, *24*, 1444 –1455. https://doi.org/10.1111/anu.12681

White, C. A., Dworjanyn, S. A., Nichols, P. D., Mos, B., & Dempster, T. (2016). Future aquafeeds may compromise reproductive fitness in a marine invertebrate. *Marine Environmental Research*, *122*, 67–75. https://doi.org/10.1016/j.marenvres.2016.09.008

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