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Vincent Turpin, Priscilla Decottignies

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**Optimising microalgae diets in sea urchin *Paracentrotus lividus* larviculture to promote aquaculture diversification**

Marta Castilla-Gavilán <sup>a</sup>, Florence Buzin <sup>b</sup>, Bruno Cognie <sup>a</sup>, Justine Dumay <sup>a</sup>, Vincent Turpin <sup>a</sup>, Priscilla Decottignies <sup>a,\*</sup>

<sup>a</sup> Université de Nantes, Institut Universitaire Mer et Littoral, EA 2160 Mer Molécules Santé, 2 rue de la Houssinière BP 92208, 44322 Nantes cedex 3 (France)

<sup>b</sup> SARL Benth'Ostrea Prod, 17 rue du Pays de Monts, 85230 Bouin (France)

\*Corresponding author at: Université de Nantes, Institut Universitaire Mer et Littoral, EA 2160 Mer Molécules Santé, 2 rue de la Houssinière BP 92208, 44322 Nantes cedex 3 (France). *E-mail address:* Priscilla.decottignies@univ-nantes.fr

**Abstract**

The objective of this study was to optimise the rearing of the sea urchin *Paracentrotus lividus* (Lamarck, 1816) larvae through the choice of an optimal diet. The effects on larvae reared in oyster facilities and using different microalgae species were analysed. Four experimental diets were tested: 1) *Isochrysis aff. galbana* (Clone T-ISO), 2) *Dunaliella tertiolecta*, 3) *Rhodomonas* sp. and 4) a combined diet of these three species (1:1:1). The biometrics of larvae were carried out every two days. Post-larval survival was assessed when competence for settlement was achieved. Induction of settlement was carried out by contact between larvae and oyster shell particles. This method, adapted from oyster farming, was used for the first time in sea urchin culture. After 9 days post-settlement, metamorphosed juveniles were sampled and post-settlement survival was assessed. The biochemical composition (proteins, carbohydrates, lipids) of microalgae and larvae was measured.

Age at competence was reached at 15 days post-fertilisation for all diets. Larval survival rate and growth in length and width were higher for larvae fed with *Rhodomonas* sp. These larvae also had the highest lipid content. Post-settlement survival was higher in larvae fed with the combined diet. Larvae in all treatments demonstrated normal growth and development, but *Rhodomonas* sp. presented nutritional advantages for sea urchin larvae: a more rapid development and a higher lipid content.

The use of *Rhodomonas* sp., already widely used in oyster aquaculture, as a diet in *Paracentrotus lividus* culture could be a factor in enabling oyster farmers to diversify towards “echinoculture” and increase their revenue.

### **Keywords**

*Paracentrotus lividus*, development, survival, *Rhodomonas* sp., *Dunaliella tertiolecta*, *Isochrysis aff. galbana* (clone T-ISO).

### **1. Introduction**

Edible sea urchins are traditionally consumed in many cultures, particularly in Asia, Polynesia, Chile and the Mediterranean basin. In recent years, world production reached 73,000 metric tons with an estimated value of 208 million US\$ (FAO, 2016a). More than 80% of this production is absorbed by the Japanese market (Sonu, 1995), where sea urchin roe is a premium food, used in sashimi or sushi. France, where sea urchin fishing is an old tradition (Le Gall, 1987), is the second largest consumer of sea urchins in the world with around 1,000 metric tons per year (Andrew et al., 2002) and the third major importer of sea urchins with around 200 metric tons in 2015 (FAO, 2016b).

As a result of this high demand, world sea urchin fisheries are in decline due to overfishing since 1995 (Andrew et al., 2002; Conand and Sloan, 1989; Kalvass and Hendrix, 1997). Aquaculture is probably the best way to satisfy increasing demand and

consumption, as, for example, with salmon or prawns (Failler, 2007). Sea urchin aquaculture has been successfully developed worldwide, e.g. in Japan (Kayaba et al., 2012), China (Liu et al., 2010), New Zealand (Barker et al., 1998), Australia (Huggett et al., 2006), Scotland (Cook and Kelly, 2009), North America (Eddy et al., 2012) and Chile (Chow et al., 2001). However, in Europe, sea urchin aquaculture remains anecdotal with the FAO reporting only a low production in Ireland (0.5 metric tons in 2013).

In Europe, aquaculture is largely dominated by shellfish, mainly mussel and oyster farming (FAO, 2016a), but this activity has faced a crisis since 2008 due to exceptional mortality events (up to 90% of young oysters) in all farming sites (EFSA AHAW Panel, 2010; Travers et al., 2014).

To deal with the economic consequences of such a crisis and to meet the increasing demand for seafood in Europe, one solution is the diversification of aquaculture activities. One of the keys to sustainable diversification is the identification of native species, taking into account market expectations (Fontaine, 2004).

In France, the Purple Sea Urchin *Paracentrotus lividus* is a native species traditionally consumed in Brittany and on the Mediterranean coast (Le Gall, 1989). Despite earlier studies of this species (Allain, 1972, 1978; Boudouresque, 1987; Le Gall and Bucaille, 1987), its production on an industrial scale remains marginal (only one farm throughout the country), due to farming methods not being economically sustainable for the producers. Thus, this research focuses on the optimisation of the different stages of the production cycle of *P. lividus* by using available local food resources for better economic and environmental sustainability and societal acceptance.

The main site for oyster hatchery and nursery activities in France is the Bay of Bourgneuf. These activities rely on the use of a silicate-rich ground water as an

optimum culture medium for the on-site production of marine phytoplankton (Hatt et al., 2015), the main feed for oyster and sea urchin larvae. In this area, abundant biomasses of red and brown seaweeds (e.g. *Palmaria palmata*, *Grateloupia turuturu*, *Laminaria digitata* or *Saccharina latissima*), the main feed for sea urchin juveniles and adults, can easily be harvested or even produced in integrated systems. Therefore, this estuarine bay may be a potential area for the development of land-based “echinoculture”.

*Paracentrotus lividus* production can be divided into different stages: broodstock conditioning, fertilisation, rearing of larvae, metamorphosis, growth of juveniles, growth of subadults and conditioning for the market (Grosjean, 2001). The aim of this paper is to optimise the larvae rearing phase through the choice of an optimal diet. Growth, development, morphology and survival rate were analysed for sea urchin larvae and post-larvae fed and reared with microalgae species, the materials and methods usually used in oyster hatcheries.

## 2. Materials and methods

### 2.1. Hatchery and rearing methods

*Paracentrotus lividus* adults harvested in Port Manec’h, 47°48’04.0”N 3°44’44.5”W, (Brittany, France) in November 2014, were raised in the Benth’Ostrea Prod aquaculture farm (Bouin, Vendée, France) until gonadal maturation. They were fed *ad libitum* on the red algae *Palmaria palmata* with a photoperiod of 16 h light/8 h dark at 20°C. Seawater used during the spawning, hatching and larval rearing process was filtered (5 µm), UV treated and maintained at 20°C, pH 8 and salinity of 30 ppt.

Spawning of four females and three males ( $33.86 \pm 13.99$  g/whole animal) was chemically induced in April 2015 by injection of a KCl solution (0.5 M; 1 ml per

individual) into the coelom via the peristomial membrane. Eggs were diluted in 5 L of seawater and fertilised by adding 200 ml of diluted sperm in an egg/sperm ratio of 1:4. Fertilisation success (100%) was checked after 1 h. The fertilised eggs were kept in the dark with aeration until they hatched.

The larval culture methods were adapted from Kelly et al. (2000) (Fig. 1). Larvae were cultivated in triplicate tanks with aerated seawater in the dark at a density of 1 individual per ml, using 50-L conical PVC tanks with a water tap in the base. A complete manual water exchange and thorough cleaning of the tanks were carried out daily. Tanks were disinfected using an oxidative disinfectant for 5 minutes and rinsing abundantly with clear water. During this cleaning, larvae were maintained in sieves placed in small containers of seawater.

While a variety of definitions of the age at competence have been suggested, this paper will use the one suggested by Carboni et al. (2012) and Kelly et al. (2000), i.e. the number of days required for at least 75% of the larvae fed each treatment to reach competence for settlement. Competence was considered achieved when the rudiment was equal to or larger than the stomach. At this stage, some tube feet and pedicellariae were also observed.

## 2.2. *Microalgae diets*

Experiments were performed using three microalgae species. *Isochrysis aff. galbana* (clone T-ISO) (diet T) and *Rhodomonas* sp. (diet R) are usually used in oyster aquaculture in the area of the study and they were obtained from the Ifremer Collection (Argenton, France) (McCausland et al., 1999). The third species, *Dunaliella tertiolecta* (NCC WDCM 856 from the Nantes Culture Collection, Mer-Molécules-Santé Laboratory, Nantes, France) (diet D), is one of the most common live feeds used in the larviculture of echinoids (Carboni et al., 2014). Four treatments were tested: three

monospecific diets (diets T, R, and D) and a combination of the three microalgae (diet C).

Microalgae were cultivated in 300-L polyethylene bags filled with ground water and enriched with 1 ml/L of Walne's medium (Walne, 1970). The biometric characteristics of each species of microalgae were obtained using a Beckman Coulter Counter<sup>®</sup> (Table 1).

Microalgae harvested during their exponential phase of growth were fed to the larvae every day. The daily ration was calculated according to the larval stage of development: larvae with two, three and four pairs of arms were fed with  $2.7 \times 10^5 \mu\text{m}^3/\text{ml}$ ,  $8.1 \times 10^5 \mu\text{m}^3/\text{ml}$  and  $13.5 \times 10^5 \mu\text{m}^3/\text{ml}$ , respectively (Carboni et al., 2012). These rations were standardised between treatments according to the microalgae species cell volume (see Table 1 for equivalence between cell volume and number of cells for each treatment). Microalgae cell counting was carried out daily using a haemocytometer.

### 2.3. *Larval growth, morphology and survival*

Between 30 and 50 larvae were randomly sampled from each rearing tank every two days until 14 days post-fertilisation (DPF) and fixed by glutaraldehyde 2.5% in a 0.1 M cacodylate buffer solution. Larvae were observed and photographed using an Olympus AX-70<sup>®</sup> research microscope equipped with a Nikon DXM1200F<sup>®</sup> digital camera. The major larval body features (length, width, post-oral arm length and rudiment length) were measured from photographs using ImageJ software (Schindelin et al., 2012), following the method of Kelly et al. (2000) (Fig. 2), in order to characterise growth and morphology of larvae fed different diets. The day of the appearance of new pairs of arms and rudiment were also recorded.

It is important to note that a manipulation error (fixation dosing) caused the loss of growth and morphology data for treatment D on day 12 post-fertilisation.

Larval survival was assessed at the end of the trial, when competence for settlement was achieved, by counting larvae in five 100 ml samples of each rearing tank:

$$\text{larval survival \%} = \frac{\text{larvae in the rearing tanks}}{\text{larvae initially dispensed into the tanks}} \times 100$$

#### 2.4. *Microalgae total protein, carbohydrate and lipid analyses*

Microalgae were sampled the first and last day of their exponential growth phase from each culture bag. The content of proteins, lipids and carbohydrates in these two samples were measured and averaged to characterize each diet composition. Samples ( $50 \times 10^6$  cells for proteins and carbohydrates, and  $300 \times 10^6$  cells for lipids) were collected in triplicate (6 samples per culture bag). They were filtered on GF/F fibreglass filters (Whatman® 47 mm diameter). The filters were rinsed with ammonium formate (6.8%), frozen at  $-80^\circ\text{C}$ , freeze-dried and ground under liquid nitrogen prior to analyses.

Proteins were extracted following the protocol of Barbarino and Lourenço (2005) modified with a 30-minute precipitation in an ice bath using  $(\text{NH}_4)_2\text{SO}_4$  at 80% saturation. Precipitated proteins were resuspended in 0.5 ml of 1.0 N NaOH and quantified as BSA proteins by the Bradford method (Bradford, 1976).

Carbohydrate content was analysed according to the method of Dubois et al. (1956).

Lipids were extracted following the method of Bligh and Dyer (1959). The lipid content was determined gravimetrically.

#### 2.5. *Larvae total protein, carbohydrate and lipid analyses*

Triplicates of about 1000 sea urchin larvae were sampled from each rearing tank at the end of their larval stage (15 DPF) and after 24 h of starvation, allowing the removal of the gut contents.

They were centrifuged to remove seawater (Moran and Manahan, 2004), frozen at -80°C and freeze-dried.

The extraction of the biochemical components was adapted from Mann and Gallager (1985). Proteins, carbohydrates and lipids were quantified by the Bradford (1976), Dubois et al. (1956) and Bligh and Dyer (1959) methods, respectively, as described above.

#### 2.6. *Settlement method and assessment of post-settlement larval survival*

As usually used in oyster aquaculture, settlement was induced by contact between larvae and detrital shell particles. Competent larvae (4 000, 9 500, 8 000 and 3 500 in average for T, R, D and C diets respectively) were thus gently transferred from tanks to 12 sieves (180 µm pore size, 1 sieve per tank) of 20 cm depth and 40 cm diameter conditioned by the addition of detrital oyster shell particles (200 µm – 300 µm particle size). These sieves were placed in 0.5 m<sup>3</sup> tanks supplied with sea water at 20°C. Water exchange in this phase was carried out every 48 h. After 9 days, larvae were sampled from each sieve and fixed in glutaraldehyde 2.5% in a 0.1 M cacodylate buffer solution. Survival was assessed by counting living juveniles in Dollfus counting chambers as:

$$\text{post-settlement survival \%} = \frac{\text{living juveniles in the sieves}}{\text{larvae initially dispensed into the sieves}} \times 100$$

#### 2.7. *Statistical analyses*

Statistical analyses were performed using the SigmaPlot® 9.0 software. One-way ANOVAs were carried out with diet as a fixed factor. If the normality test failed (Kolmogorov-Smirnov test results), either a log-transformation or a Kruskal-Wallis one-way analysis of variance on ranks was performed. Since proportions were compared, an arcsine transformation was carried out prior to ANOVAs (Sokal and

Rohlf, 1995). *A posteriori* multiple comparison tests were realised in all pairwise between diets when significant differences ( $P < 0.05$ ) were found.

For each diet, linear regressions were established between body width and total larval length and between post-oral arm and larval length. Statistical analyses and slope and intercept comparisons were carried out between diets.

### 3. Results

#### 3.1. Age at competence and survival rate

At 14 DPF, the percentage of larvae with a larger rudiment than stomach was 56%, 72%, 72% and 63% for treatments T, R, D and C, respectively. In all treatments, sea urchin larvae reached competence at 15 DPF and some of them were already metamorphosed at the bottom of the tanks. Larvae fed with diet R and diet D showed significantly higher survival ( $df = 3, n = 15, F = 9.753, P < 0.001$ ) compared to diets T and C (25%, 22%, 14% and 13%, respectively for diets R, D, T and C).

Post-settlement survival was significantly higher ( $P < 0.05$ ) in treatment C (59%) compared to treatments T (20%) and R (20%), while no significant differences were found between treatments C and D (26%) (Fig. 3).

#### 3.2. Larval development and growth

The rudiment was observed for the first time on day 8 post-fertilisation in a few larvae (< 10%), whereas by day 10 the rudiment was present in more than 35% of the larvae in all treatment groups (Fig. 4). In treatment T, a larger percentage of larvae presented a rudiment at 10 DPF (Fig. 4), however rudiment length in this treatment was significantly shorter at day 14 ( $P < 0.05$ ; Fig. 5) compared to other diets. The other three treatments showed a gradient with rudiment size significantly higher in treatment C than in treatment D. The third and fourth pairs of arms (Fig. 4) appeared at days 8 and 12,

respectively. The proportion of larvae with a third pair of arms on day 8 was significantly lower in treatment T ( $P < 0.05$ ), whereas the proportion of larvae with a fourth pair of arms on day 12 was significantly higher in treatment R ( $P \leq 0.001$ ). By day 14 post-fertilisation, almost all larvae presented the rudiment and the fourth pair of arms for all treatments (even treatment D, despite the absence of data for day 12).

For all treatments, the biometric parameters showed the same pattern. The total length of larvae and post-oral arm length (Fig. 6a, 6b) increased until day 8 for all treatments and then decreased. At 12 or 14 DPF, total length increased again until the age at competence. Body length and width showed a continuous increase with a plateau between days 8 and 10 for body length only (Fig. 6c, 6d).

At the end of the larval rearing period (14 DPF), post-oral arm length was significantly longer for larvae fed diet D, when compared with all other treatment groups. There was no significant difference in post-oral arm length between larvae fed diets T, R and C at 14 DPF ( $df = 3$ ,  $n = 86$ ,  $F = 15.517$ ,  $P < 0.001$ ; Fig. 6b). Larvae fed with diets C and R had a significantly longer body length ( $P < 0.05$ ; Fig. 6c) and were significantly wider compared to larvae fed with diets T and D ( $P < 0.05$ ; Fig. 6d).

Significant relationships were found between body width and total larval length (Fig. 7; Table 2) and between post-oral arm length and total larval length (Fig. 8; Table 2) for all treatments. Comparison of slopes and intercepts showed that treatment T presented the significantly lowest changes in larval width with increase in larval length (Coeff = 0.469;  $P < 0.05$ ). However, the change in larval length for treatment R resulted in the significantly lowest change in post-oral arm length (Coeff = 0.271) compared to other diets ( $P < 0.05$ ).

### 3.3. Protein, carbohydrate and lipid content of microalgae

Total protein content (Fig. 9a) was higher in diet R (1,186  $\text{pg}/\mu\text{m}^3$ ). Significant differences ( $P < 0.001$ ) were found between proteins in diet R and diets T, D and C (79  $\text{pg}/\mu\text{m}^3$  and 380  $\text{pg}/\mu\text{m}^3$ , 436  $\text{pg}/\mu\text{m}^3$  respectively). Total carbohydrate content (Fig. 9b) was higher in diet R (34,469  $\text{pg}/\mu\text{m}^3$ ). Significant differences ( $df = 3, n = 6, F = 342.151, P < 0.001$ ) were found between the carbohydrate content in diet R and diets T, D and C (638  $\text{pg}/\mu\text{m}^3$ , 9,691  $\text{pg}/\mu\text{m}^3$  and 11,092  $\text{pg}/\mu\text{m}^3$ , respectively). Lipid content (Fig. 9c) was significantly lower ( $df = 3, n = 6, F = 19.489, P < 0.001$ ) in diet T (447  $\text{pg}/\mu\text{m}^3$ ) than in diets R, D and C (6,252  $\text{pg}/\mu\text{m}^3$ , 2,998  $\text{pg}/\mu\text{m}^3$  and 1,706  $\text{pg}/\mu\text{m}^3$ , respectively).

#### 3.4. Protein, carbohydrate and lipid content of *Paracentrotus lividus* larvae

No differences were found between total protein content and total carbohydrate content for all treatments (Fig. 10). However, total lipid content was significantly ( $P < 0.01$ ) higher in larvae from treatment R (18,115  $\text{pg}/\mu\text{g}$  larvae).

## 4. Discussion

### 4.1. Age at competence

For all dietary treatments tested, larvae reached the age at competence at 15 DPF, which represents a shorter larval stage duration than in previous studies (Brundu et al., 2016a, 2016b; Carboni et al., 2012; Gosselin and Jangoux, 1996; Liu et al., 2007). This may be explained by the faster growth of larvae when they are reared at low density (1 larva/ml, present study) as highlighted by Buitrago et al. (2005) and Kalam Azad et al. (2012).

A higher water temperature used in this work (20°C) compared to studies cited above (17 – 19 °C) could also have an influence in development, allowing the larvae to reach competence earlier.

#### 4.2. Larval survival

Larval survival rates obtained in the present work (13% - 25%) were higher than those observed by Carboni et al. (2012) (5% - 14%). These differences can be explained by the higher density used in their study (4 larvae/ml), given that it is well-known that low initial stocking densities improve larval survival (Brundu et al., 2016a). On the contrary, the studies of Kelly et al. (2000) and Liu et al. (2007), using stocking densities similar to ours (1 and 1.5 larvae/ml, respectively) obtained higher survival rates (55.2% and 67.8-75.6%, respectively). These differences may be explained by the lower feeding and total water exchange rates used in their studies. A low feeding rate could reduce the amount of non-consumed phytoplankton at the bottom of the tanks and consequently the requirement for water exchange, which is crucial to maintain water quality but can affect survival rate (Cárcamo et al., 2005; Kalam Azad et al., 2010). Likewise, a high manual water exchange rate is time-consuming for producers and stressful for larvae (Brundu et al., 2016b; Carboni et al., 2014).

#### 4.3. Post-settlement survival

Although the post-settlement survival rate observed for the combined diet (C) was in the range found in the literature (50% - 80%) (Brundu et al., 2016a; Kelly et al., 2000; Liu et al., 2007), the results for treatments T, R and D were considerably lower than those reported in previous studies.

The success of metamorphosis and settlement in a variety of benthic marine invertebrate larvae is strongly linked to a metamorphosis stimulating factor (Hadfield and Paul, 2001). In sea urchins, it has been reported that this induction could occur by a chemical cue in response to different organic metabolites coming from macrophytes or bacterial biofilms (Dworjany and Pirozzi, 2008; Kitamura et al., 1993; Pearce and Scheibling,

1990; Williamson et al., 2000). In the context of oyster farming diversification, an inorganic inducer of metamorphosis and settlement was used for the first time in sea urchin culture, adapting this method from oyster aquaculture zootechnique. The low post-settlement survival rate obtained in the present work indicates that this oyster farming method is not optimal for echinoculture. Thus, further experiments must be carried out to optimise post-settlement survival of sea urchins in oyster facilities that are intending to diversify into echinoculture. It would be interesting to incorporate small pieces of macroalgae in the sieves. These macroalgae and/or the biofilms associated to its surface could promote the settlement induction (Dworjanyn and Pirozzi, 2008; Hadfield and Paul, 2001) and be a first food for juveniles. Moreover, juveniles fixed on macroalgae could be transferred easily.

#### 4.4. *Development and growth*

The lower stocking density and the higher water temperature used in the present study could enable an earlier appearance of the rudiment and new pairs of arms than in previous works (Carboni et al., 2012; Kelly et al., 2000; Liu et al., 2007). This could explain the shorter larval stage duration described in section 4.1.

The present study confirms that the shortening of arms is a sign of normal larval development as reported by Liu et al. (2007). Larvae continue to feed but could interrupt the growth of arms to store energy for metamorphosis (Fenaux et al., 1994). Moreover, data for the total length are very irregular, because they take arm size into account. This is why, in agreement with Carboni et al. (2012), arm size and total body length were not considered good indicators of growth and only body length and width were used to evaluate the correct larval growth. For these two body features, this work observed higher values before competence than other studies (Carboni et al., 2012; Liu et al., 2007).

#### 4.5. Comparison between treatments

Larvae fed with *Isochrysis aff. galbana* (clone T-ISO) (T) showed the lowest survival rate and presented a delayed development with a smaller rudiment and body (length and width). This was confirmed by the relationship found between body width and total larval length, which showed that *I. aff. galbana* (clone T-ISO) (T) did not provide fast growth. These results agree with previous studies in which this species appeared unsuitable compared to other microalgae for echinoderm larval culture (Brundu et al., 2016b; Fenaux et al., 1988; Pechenik, 1987; Schiopu et al., 2006).

Post-settlement survival rate was higher for larvae fed with the combined diet (C) compared to other treatments. This fact could be explained by a lower density (and consequently a low intraspecific spatial competition (Grosjean et al., 2003) in the settlement sieves for this treatment due to the low larval survival (13%) after rearing. Therefore, although the final output in terms of juveniles was higher for diet C, it cannot be affirmed that this combined diet leads to success in settlement. Further research is needed at this stage.

Several studies consider that an algal mixture mimics the natural planktonic food conditions for echinoids and generally allows better larval development compared with monospecific diets (Basch, 1996; Cárcamo et al., 2005; Marshall et al., 2010). In contrast, Brundu et al. (2016a) and Pedrotti and Fenaux (1993) obtained improvements in growth when feeding larvae with a monospecific diet.

In our study, *Rhodomonas* sp. (R) gave better performances (larval survival, appearance of all pairs of arms and larval length and width at 14 DPF) compared to the combined (C) and *D. tertiolecta* (D) diets, which led, however, to a normal development (without malformations, see (Lawrence, 2013) and a high PS-survival rate in treatment C. Moreover, the ratio of post-oral arm length to larval length confirms the faster

development of larvae fed with *Rhodomonas* sp. (R) (see Fig. 8 and Table 2). Indeed, the phenotypic plasticity of the post-oral arm size make it a good indicator of the morphological development of larvae in response to the food type and availability (Soars et al., 2009; Strathmann et al., 1992). In this way, treatment D, presenting the longest arm larvae at day 14 post-fertilisation, appear as a poor quality food.

Larvae fed with *Rhodomonas* sp. (R) also had the highest lipid content. Gallager et al. (1986) suggested that this could be an indicator of good physiological condition and the capacity for successful metamorphosis.

The biochemical content of the *Rhodomonas* sp. (R) diet could explain these results. In fact, this alga is known to be a good diet for juvenile oysters because of its high carbohydrate levels (Enright et al., 1986), which were also observed in this study. As proposed for the Pacific oyster *Crassostrea gigas* (Brown et al., 1998), the specific growth of sea urchin larvae could be best correlated with the carbohydrates ingested. The high carbohydrate level of this diet could also explain the high lipid content of larvae through their utilisation in lipid synthesis. Moreover, *Rhodomonas* sp. (R) has a high PUFA content (22:6n-3 and 20:5n-3) which are essential for bivalve larvae development (Volkman et al., 1989). Basch (1996) suggests that this could explain the better performance of asteroid larvae when they are fed with *Rhodomonas* sp. (R). The present work confirms his hypothesis that *Rhodomonas* sp. (R) alone may provide the critical nutrients or energy for echinoderm larvae development.

The better performances in sea urchin larviculture obtained with the *Rhodomonas* sp. (R) diet may also be explained by a higher digestibility of these microalgae. For example, the larger surface area/volume ratio of *Rhodomonas* sp. (R) could reduce the cell wall surface to be digested by larvae to have access to the same cell volume compared to the other diets.

## 5. Conclusions

In general, the results show a higher growth, development and survival rate when larvae were fed with *Rhodomonas* sp. (R) or a combined diet (C) but *Rhodomonas* sp. (R) presented nutritional advantages for sea urchin larvae: a rapid development, a higher lipid content and a lower investment for production.

However, to choose between these two diets it is important to consider microalgae production costs in the sea urchin industry. As suggested by Kalam Azad et al. (2010), it is economically advantageous if larval nutritional requirements are met using a monospecific microalgae diet, especially if this algae can feed several invertebrate species in the same hatchery. In this way, the cost of maintaining and cultivating new phytoplankton species/strains and the risk of cross-contamination between species are reduced for commercial oyster hatcheries. A short production cycle can be achieved when *Rhodomonas* sp. (R) is used as the larval diet, enabling a potential reduction in the production costs. Therefore, local resources currently used by oyster farmers could provide a profitable aquaculture diversification, but further investigation is needed in order to optimise post-settlement survival of sea urchins in oyster facilities.

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**Fig. 4** Appearance of 3<sup>rd</sup> and 4<sup>th</sup> pairs of arms and rudiment in *P. lividus* larvae fed different diets: d = day, T = T-ISO, R = *Rhodomonas* sp., D = *D. tertiolecta*, C = combined diet. Error bars represent standard deviations. There are no data for treatment D at 12 DPF.

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**Fig. 7** Linear regressions describing relationships between body width and total larval length for *P. lividus* larvae fed different diets: T = T-ISO, R = *Rhodomonas* sp., D = *D. tertiolecta*, C = combined diet.

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**Fig. 9** Biochemical profiles of microalgae live feeds. Error bars represent confidence intervals. T = T-ISO; R = *Rhodomonas* sp. D = *Dunaliella tertiolecta*; C = combined diet. a = total protein content; b = total carbohydrate content; c = total lipid content.

**Fig. 10** Biochemical profiles of larvae fed different diets. Error bars represent confidence intervals. T = T-ISO; R = *Rhodomonas* sp.; D = *Dunaliella tertiolecta*; C = combined diet.

**Table 1** Biometric characteristics of the three tested species of microalgae. Cell number of the four diets used at the different stages of larval development.

	<i>Isochrysis aff. galbana</i>	<i>Rhodomonas</i> sp.	<i>Dunaliella tertiolecta</i>
<b>Diameter (<math>\mu\text{m}</math>)</b>	3	7	5
<b>Cell volume (<math>\mu\text{m}^3</math>)</b>	14	180	65
<i>Cell number for mono-specific diets (cell day<sup>-1</sup>)</i>			
2 pairs of arms-larvae	955 x 10 <sup>6</sup>	75 x 10 <sup>6</sup>	205 x 10 <sup>6</sup>
3 pairs of arms-larvae	2 850 x 10 <sup>6</sup>	225 x 10 <sup>6</sup>	620 x 10 <sup>6</sup>
4 pairs of arms-larvae	4 800 x 10 <sup>6</sup>	375 x 10 <sup>6</sup>	1 030 x 10 <sup>6</sup>
<i>Cell number for combined diet (cell day<sup>-1</sup>)</i>			
2 pairs of arms-larvae	318 x 10 <sup>6</sup>	25 x 10 <sup>6</sup>	68 x 10 <sup>6</sup>
3 pairs of arms-larvae	950 x 10 <sup>6</sup>	75 x 10 <sup>6</sup>	207 x 10 <sup>6</sup>
4 pairs of arms-larvae	1 600 x 10 <sup>6</sup>	125 x 10 <sup>6</sup>	343 x 10 <sup>6</sup>

**Table 2** Linear regressions describing relationships between (a) body width and total body length, (b) post-oral arm length and total body length for *P. lividus* larvae fed different diets. T = T-ISO, R = *Rhodomonas* sp., D = *D. tertiolecta*, C = combined diet.

	Equation	R <sup>2</sup>	F	P
<i>(a) Linear regressions between body width and total body length</i>				
T diet	$y = 0.4696x + 86.345$	0.1549	50.209	<0.001
R diet	$y = 0.8682x - 116.07$	0.3386	157.652	<0.001
D diet	$y = 0.7772x - 183.18$	0.4756	250.331	<0.001
C diet	$y = 0.6083x - 3.5198$	0.1663	57.037	<0.001
<i>(b) Linear regressions between post-oral arm length and total body length</i>				
T diet	$y = 0.4274x + 19.029$	0.3769	151.827	<0.001
R diet	$y = 0.271x + 131.39$	0.1552	56.034	<0.001
D diet	$y = 0.365x + 96.504$	0.294	114.929	<0.001
C diet	$y = 0.4708x + 5.1327$	0.3428	141.382	<0.001

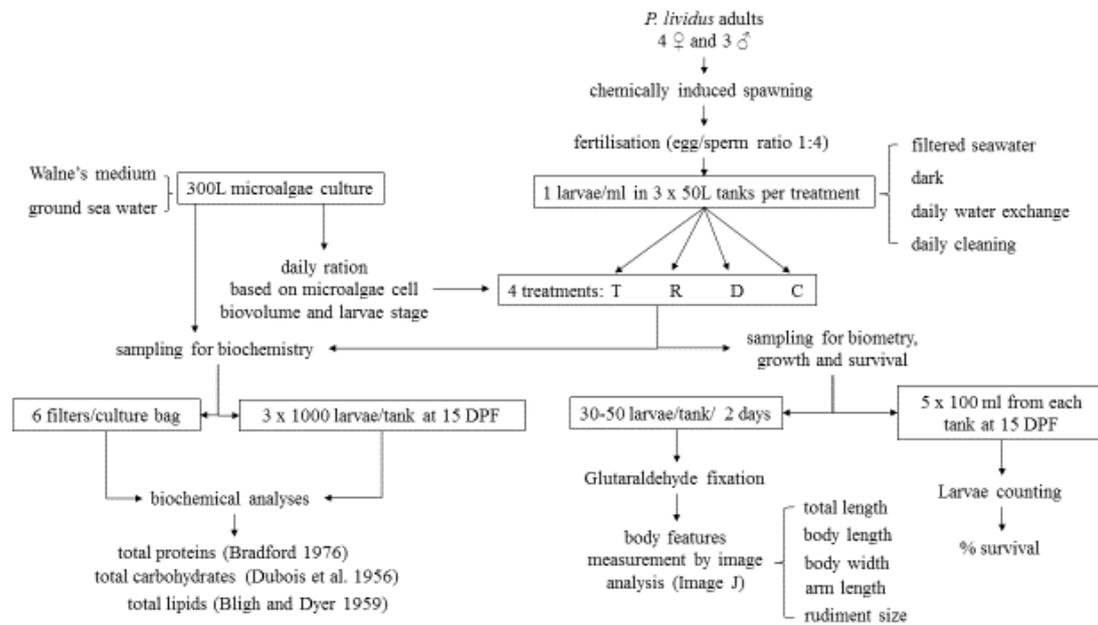
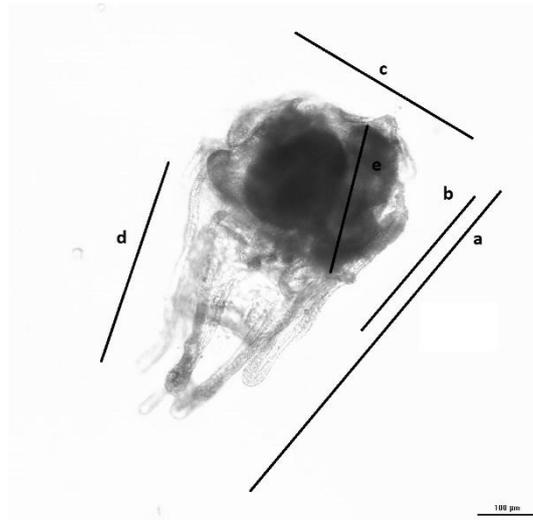
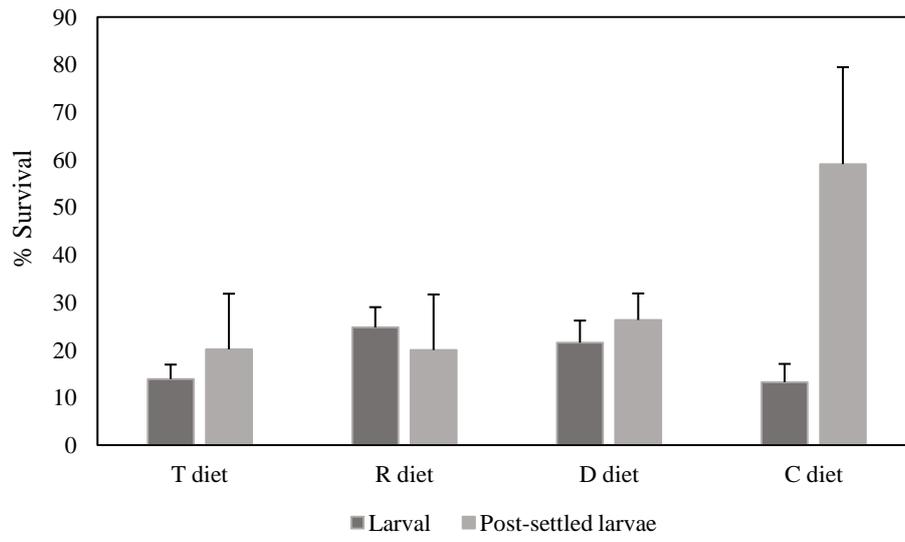


Fig. 1



**Fig. 2**

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**Fig. 3**

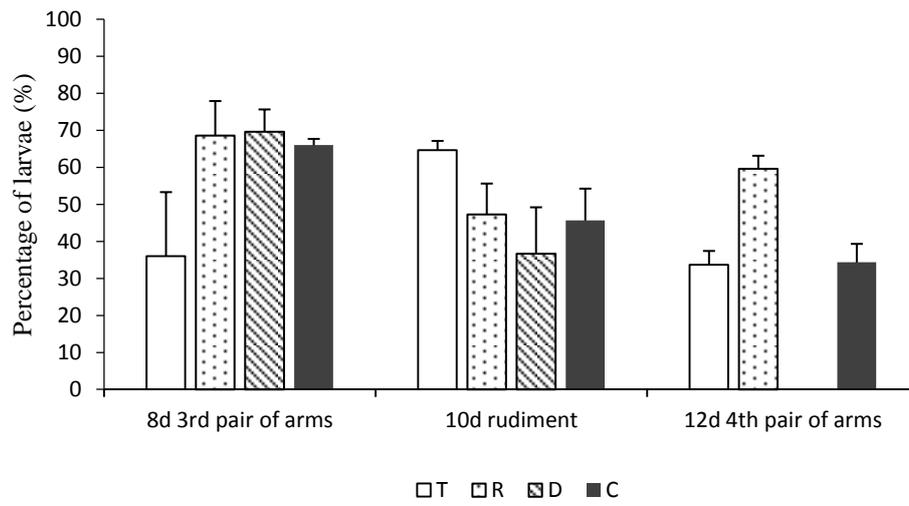


Fig. 4

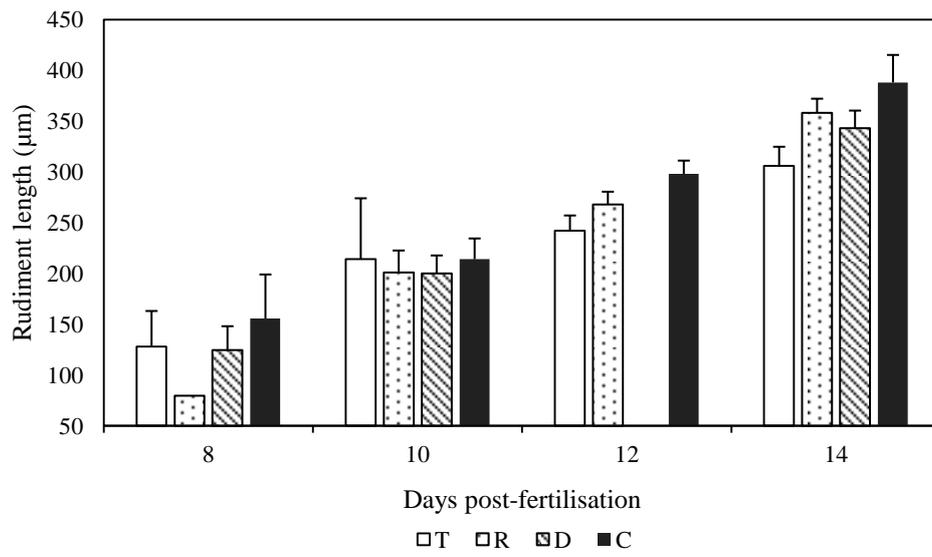


Fig. 5

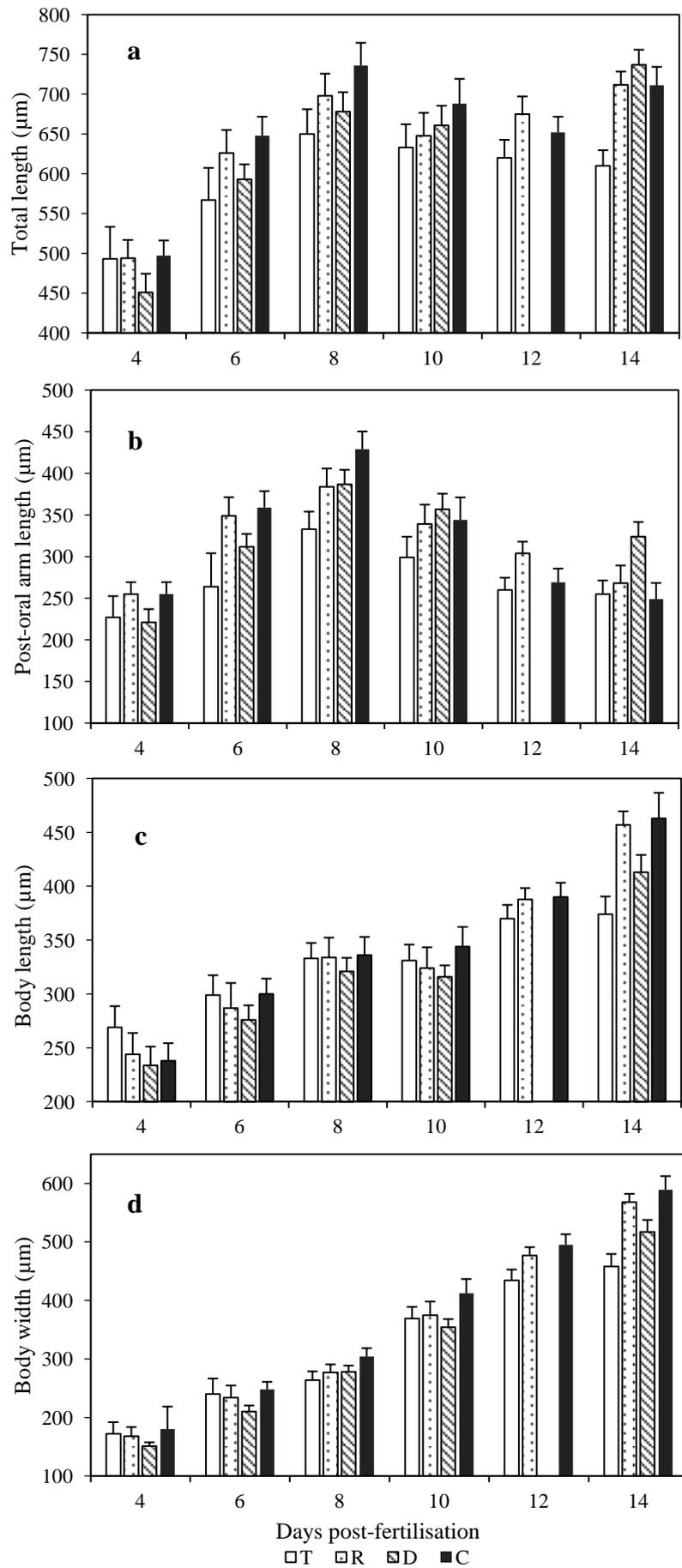


Fig. 6

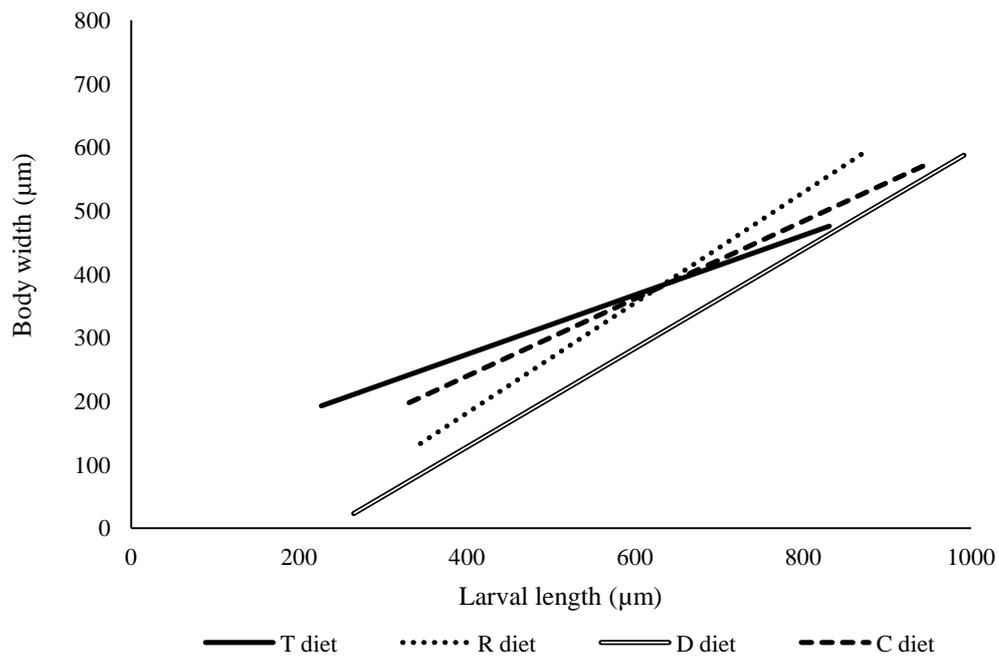


Fig. 7

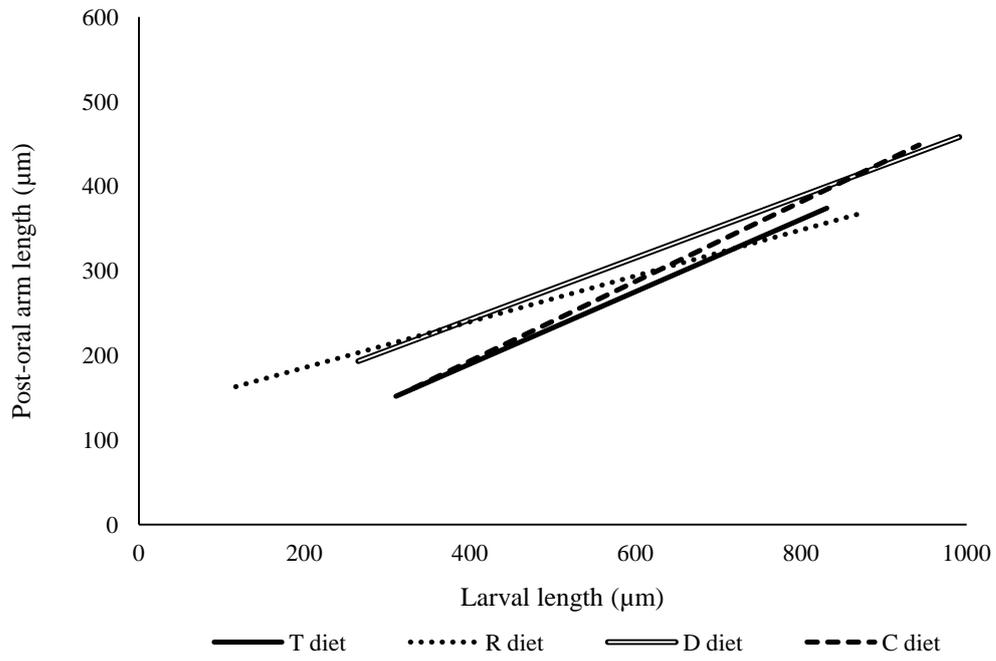


Fig. 8

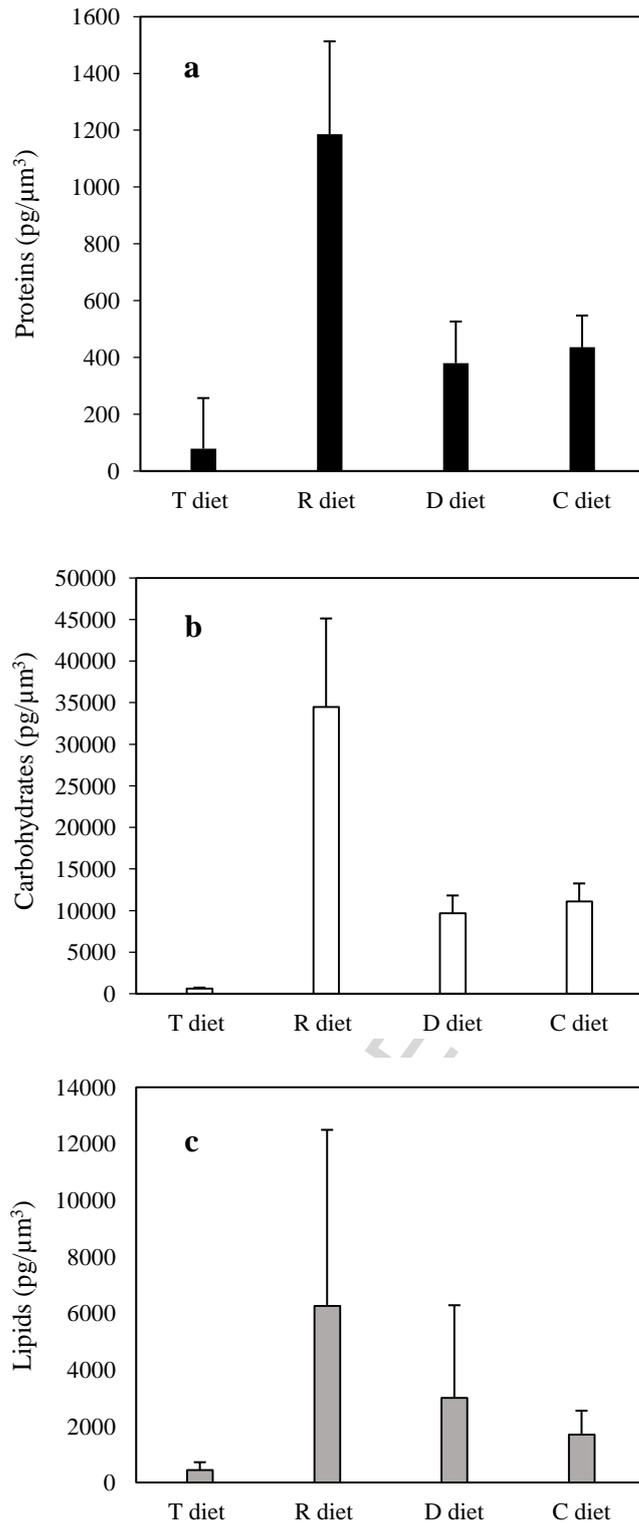


Fig. 9

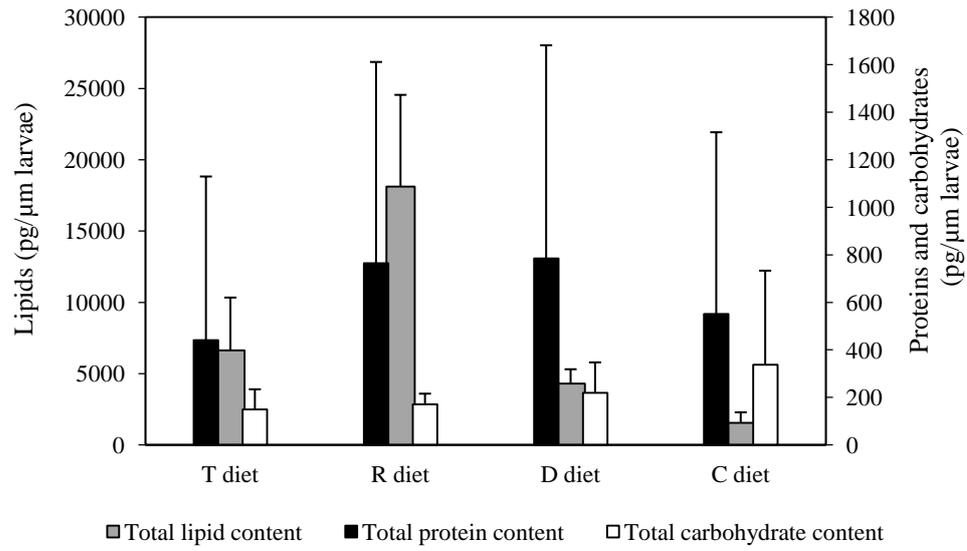


Fig. 10

**Highlights**

- Four experimental microalgal diets were tested in *Paracentrotus lividus* larvae feeding.
- Feeding larvae with a monospecific *Rhodomonas sp.* diet can meet larval nutritional requirements and reduce producing costs.
- Optimization of sea urchin metamorphosis and settlement in oyster facilities needs further investigation.
- Echinoculture can be a potential activity for developing profitable oyster aquaculture diversification.