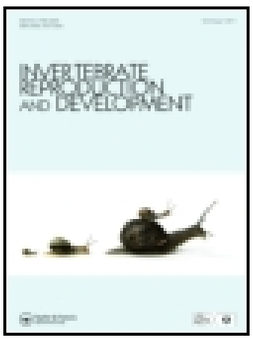

Invertebrate Reproduction & Development

ISSN: 0792-4259 (Print) 2157-0272 (Online) Journal homepage: http://www.tandfonline.com/loi/tinv20

Experimental evaluation of the feeding rate, growth and fertility of the sea urchins *Paracentrotus lividus*

Nadia Ruocco, Valerio Zupo, Davide Caramiello, Francesca Glaviano, Gianluca Polese, Luisa Albarano & Maria Costantini

To cite this article: Nadia Ruocco, Valerio Zupo, Davide Caramiello, Francesca Glaviano, Gianluca Polese, Luisa Albarano & Maria Costantini (2018): Experimental evaluation of the feeding rate, growth and fertility of the sea urchins *Paracentrotus lividus*, Invertebrate Reproduction & Development, DOI: 10.1080/07924259.2018.1504125

To link to this article: https://doi.org/10.1080/07924259.2018.1504125

INVERTEBRATE REPRODUCTION & DEVELOPMENT
https://doi.org/10.1080/07924259.2018.1504125

Taylor & Francis
Taylor & Francis Group

Experimental evaluation of the feeding rate, growth and fertility of the sea urchins *Paracentrotus lividus*

Nadia Ruocco[a,b,c], Valerio Zupo[d], Davide Caramiello[e], Francesca Glaviano[e], Gianluca Polese[b], Luisa Albarano[a,b] and Maria Costantini[a]

[a]Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Napoli, Italy; [b]Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Napoli, Italy; [c]Bio-Organic Chemistry Unit, Institute of Biomolecular Chemistry-CNR, Pozzuoli, Naples, Italy; [d]Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Napoli, Italy; [e]Unit Marine Resources for Research, Stazione Zoologica Anton Dohrn, Naples, Italy

ABSTRACT

The trophic ecology of the sea urchin *Paracentrotus lividus*, a key species in several shallow benthic communities, has been intensively studied, but the role of various foods in the processes of growth and gonadal maturation is still scarcely understood. This research assessed the effects of two fundamental food items for wild specimens of the sea urchin *Paracentrotus lividus*, the tissues of the seagrass *Posidonia oceanic*a and of the green alga *Ulva rigida*, compared to the effect of a commercial compound feed on the somatic growth, gonad development, fertilization success and post-embryonic development. Consumption rates along with the C/N ratios were measured in the feeds and in the faecal pellets. We demonstrated that feeding for three months on *U. rigida* and *P. oceanica* did not affect growth and gonadal index of adults, fertilization processes and first cleavage and development, as well as field-collected animals. In contrast, a diet based on formulated pellets triggered a significant increase of gonadal index, but lack of gamete production, due to a follicular hyperthrophy. Our work will be useful for the definition of optimal diets for the production of mature broodstocks of an ecologically important marine model organism.

Impact statement

– We aim at defining the daily feeding rate of the sea urchin P. lividus
– P. lividus represents a key species in various benthic communities.
– Feeds are important in the processes of growth and gonadal maturation of sea urchins.
– Several factors influence sea urchin feeding rates.

ARTICLE HISTORY
Received 4 April 2018
Accepted 19 July 2018

KEYWORDS
Development; feeding rates; fertility; gonadal index; growth; sea urchin

Introduction

Paracentrotus lividus is a species of sea urchin belonging to the family *Parechinidae* within the large phylum *Echinodermata* (Pawson 2007). This species widely occurs in different marine environment, such as the Mediterranean Sea and the Eastern Atlantic Ocean from western Scotland and Ireland to the Azores, Canary Islands and Morocco (Boudouresque and Verlaque 2001).

In the Mediterranean sea, this sea urchin is considered to be a key species for several coastal communities associated to vegetated ecosystems, thanks to its role in their food webs (Zupo and Fresi 1984). It is an important consumer of plant tissues (Boudouresque et al. 2007), and it is also a well-established model organism for eco-toxicological and physiological studies. In addition, the gonads of *P. lividus* are considered a gastronomic delicacy and consequently its market demand has significantly increased since the early 1970s, causing a depletion of this species in different site of the Mediterranean (Guidetti et al. 2004; Lawrence 2001). In fact, harvesting of *P. lividus* is reflected in population structures from fished and control locations: since humans selectively collect the largest sea urchins (>4 cm), large-sized *P. lividus* were rare at the exploited locations (Guidetti et al. 2004).

Several attempts have been applied to identify effective formulated diets with the aim to promote body growth and gonadal maturation of adults in land based systems (Caltagirone et al. 1992; Fabbrocini et al. 2012). An extensive literature investigates the effect of diets on growth of *P. lividus*, as well as dietary effects on reproductive success (Bayed et al. 2005; Frantzis and Gremare 1993; Carboni et al. 2012; Lawrence 2013). However, some key issues still limit its industrial exploitation.

CONTACT Valerio Zupo vzupo@szn.it; Maria Costantini maria.costantini@szn.it

Yet the wide development of a major commercial urchin-farming industry has been restrained by the lack of a fully developed technology for the cost-effective production of sea urchins with the desired gonad quality (Carboni et al. 2012). Furthermore, it is important to consider that the feeding rates measured in the field may differ from those taken in aquaculture, also according to the composition and quality of its feeds (Bayed et al. 2005). Feeds appear to play a pivotal role in the regulation of the reproductive cycle and it has been proven that gonadic growth is strongly correlated with the availability, quantity and quality of food (Boudouresque and Verlaque, 2001; Boudouresque and Verlaque 2013). Therefore, the measurement of feeding rates according to the quality and quantity of foods can be important to plan correct feeding procedures to finalize the culture of this species and a rapid maturation of gonads (Spirlet et al. 1998).

In this study, we aimed to experimentally define the feeding rates of *P. lividus* reared in test tanks, according to the feeds used, and to measure the effects of feeds on its body growth and the maturation of gonads, as compared to the well-known patterns observed in the environment (Vadas et al. 2000). Adults of the sea urchins *P. lividus* were fed on the green alga *Ulva rigida*, the seagrass *Posidonia oceanica* and a commercial compound feed used in aquaculture for three months. More in details, fresh *U. rigida* and *P. oceanica* represent natural dietetic items, also because both these plants characterized environments usually populated by *P. lividus*. In fact, this sea urchin is one of the major macro-herbivores in the Mediterranean Sea eating a range of red, green and brown algae in addition to seagrass (Boudouresque and Verlaque 2001). *U. rigida* is considered a control food in several feeding experiments on fish and invertebrates (Valente et al. 2006) and it is included in various diets for sea urchins (Frantzis and Gremare 1993), demonstrating that it affected the growth rate of *P. lividus* (Frantzis et al. 1992). Moreover, *P. lividus* represents the dominant grazer for *P. oceanica*, choosing this seagrass because of the greater availability of shelter and food in the seagrass (Pinna et al. 2012). It prefers leaves covered with epibiota and adult, thicker leaves (Vergés et al. 2011), consuming all parts of the seagrass as a 'preferred' species for feeding during spring and summer (Boudouresque and Verlaque 2001). In fact, the sea urchin is one of the main consumers of *P. oceanica* (Verlaque 1987), avoiding other species that synthesize toxic or repellent secondary metabolites (Guerriero et al. 1992; Lemée et al. 1996; Tejada et al. 2013). Moreover, *P. lividus* is a key species that controls the dynamics of seaweeds and seagrasses, by eliminating, when at high densities, the erect stratum of algae and seagrasses (Sala and Zabala 1996). Sea urchins were also fed with a pre-hydrated pelletized formulated feed (Classic K®; Hendrix SpA, Mozzecane-VR, Italy). Previous data demonstrated that this commercial pellets provided rapid fattening of gonads (Fabbrocini and D'Adamo 2010, 2011; Fabbrocini et al. 2012), representing a positive control on the size and quality of gonads. It is also inexpensive, available on the market and producing negligible amounts of wastes (Fabbrocini et al. 2012).

Materials and methods

Ethics statement

Wild individuals of *P. lividus* (Lamarck) were collected from a site in the Bay of Naples that is not privately owned or protected in any way, according to the Italian legislation (DPR 1639/68, 09/19/1980 confirmed on 01/10/2000). Field studies did not include endangered or protected species. All experimental procedures on animals were in compliance with the welfare guidelines of the European Union (Directive 609/86).

Sea urchin collection

Adult sea urchins *Paracentrotus lividus* were collected during in January (corresponding to the start of reproductive cycle; Byrne 1990) by scuba-divers in the site 'Rocce Verdi' in the Gulf of Naples, Italy. Sixty individuals of an average weight of 40 g were collected and immediately transported to the laboratory, using a thermally insulated box containing seawater. Further, they were transferred to plastic tanks with recirculating seawater, prior to start the feeding experiments. Sea urchins were individually measured using a calliper, to record the maximum horizontal diameter of thecae; adult specimens with a diameter between 4 and 5 cm (excluding the spines), that is a typical diameters for mature adults, were selected for the experiments.

Experimental rearing apparatus

A continuous open flow-through (35 l per hour) system was set, consisting of nine rectangular glass tanks (three tanks for each diet used; chamber size 30 × 35 × 40 with 35 l of sea water): seawater was pumped from the sea, collected in an outdoor basin, then filtered twice on gauze filters (200 µm) and moved to an indoor basin, filtered again by means of a protein skimmer, a UV sterilizer, a refrigerator and a mechanical filter, then moved to the experimental tanks. The aeration in the

tanks was provided by airstones. Used water was released through outflow tubes from each tank at a rate of 1 full change per hour. The main abiotic parameters were recorded three times a week using a multiparametric probe (YSI 85, YSI, Incorporated) and kept constant using water chillers, circulation pumps and filters (temperature 18 ± 1°C; salinity 38 ± 1;, dissolved O_2 7 mg/l; pH 8.1). The internal surfaces of tanks were manually cleaned of their epiphytes and fouling organisms three times a week, using synthetic sponges and scrapers.

Feeding experiments

Preliminary experiments were performed to define the daily feeding rates of adult sea urchins. Ten adults of *P. lividus* were reared in each tank of the continuous open flow-through system, and then fed with 10, 20 and 30 g wet weight (WW) per day of the green macroalgae *U. rigida* (often used as a control to feed sea urchins in laboratory experiments) to define their daily feeding rate. *U rigida* was chosen cause it is considered among the most palatable and nutritionally suitable feeds for *P. lividus* (Hiratsuka and Uehara 2007; Cyrus et al. 2015), and therefore, its consumption is close to the maximum feeding rates for this species. The other feeds were provided at the same rate and the presence of small residuals of all the feeds was checked prior to start the experiments. Food consumption was measured after 24, 48 and 72 h to check that residuals were still present, for all the items, after 1 day and that they could be still consumed in further days. Doses were set in order to assure that the residual food was maintained in low abundance, to avoid water pollution and lixiviation (Sartori and Gaion 2016). Once determined this initial dose, guaranteeing an *ad libitum* consumption in 24 h, on all items considered, we started the feeding experiments.

Twenty adult *P. lividus* (10 females and 10 males for each diet treatment, identified according to the morphology of the apical system of females and the different genital plates between females and males under the dissecting microscope; Philip and Foster 1971; Jeffery and Emlet 2003), collected in the field in January, were reared in each experimental tank and fed, alternatively, with 20 g per day of (a) fresh *U. rigida*, (b) fresh *P. oceanica* leaves (including both brown and green tissues) and (c) pelletized (2.5 × 2.5 × 5 mm) pre-hydrated formulated feed (Classic K; HENDRIX SpA, Mozzecane, Verona, Italy pre-hydrated formulated feed (HENDRIX,Verona, Italy; a commercial food characterized by a high protein content 465 g kg^{-1}, proteins of animal origin accounting for <50 g kg^{-1}) as suggested by Fabbrocini and D'Adamo (2010). Before starting our experiments, we considered a three-day time frame of starvation, in according with other previous feeding experiments (Ruocco et al. 2018). The short acclimation and starvation time was chosen because our aim was to study the effects of three different feeds on the maturation gonads and increase of gonadal index (GI), starting from the gonadic state characterizing the experimental sea urchins at the moment of the collection. A longer starvation time would produce auto-digestion of gonadic tissues to sustain the metabolism of starved animals (Sartori et al. 2015) and this was in contrast with our experimental aims.

The residual amount of food in each tank was weighed (fresh weight) every day to calculate the individual daily food consumption. Macroalgae and *Posidonia* tissues were still alive when the residuals were removed; therefore, we should exclude any significant process of degradation and weight reduction within the experimental time considered. However, controls of the feeds were placed into own containers, to exclude the influence of feeding sea urchins, to determine possible change in their fresh weight. Since the feeds were daily replaced and they were mainly ingested by sea urchins in the first hours after the administration we can exclude the influence of lixiviation processes in the formulated feeds. Excess moisture was removed from *U. rigida* by blotting the leaves on paper towels before weighing. The total food daily ingested was calculated as the difference between the feed introduced and that removed in each tank. Food intake was calculated in milligrams of feeds (dry weight) consumed per animal, per day. Since adult sea urchins were grouped in a tank for each diet, an average consumption was also evaluated.

Carbon and nitrogen measurements in feeds and faecal pellets

Additional samples of algae and *Posidonia oceanica* tissues were collected and stored for chemical analyses, in order to define the quality of the fresh feeds provided, as described above. To this end, additional thalli of *U. rigida* and leaves of *P. oceanica* were collected in the Gulf of Naples by scuba-divers, transferred to the laboratory and stored at −20°C. Two gram of three independent samples of algae and three of the seagrass were subsequently dried at 65°C for three days up to constant weight. In parallel, *P. lividus* faecal pellets were collected in the experimental tanks after continuous feeding on fresh tissues of *U. rigida* and *P. oceanica*, as well as on formulated diet pellets. As we used a short starvation period (as described above) and considering

that faecal material is still produced for a large number of days after food intake has ceased, we collected faecal pellets 15 days after the beginning of feeding experiments, in order to be sure that gut retention time could not affect this analysis. The faecal pellets were similarly dried up to constant weight, as described above. Dry samples were homogenized in a grinder in order to obtain a thin dust. Samples were weighed and loaded into a Carbon/Nitrogen (CN) Analyzer (FlashEA 1112 Automatic Elemental Analyzer, Thermo Scientific Waltham, MA, USA), following the procedure described by Hedges and Stern (1984). Acetanilide was used as standard. C/N analyses were conducted in duplicate. The data obtained allowed for an interpretation of results obtained according to the three experimental diets.

Gonadal index and histological preparation

Evaluations of the GI% were performed on 20 field-collected adult specimens of the sea urchin *P. lividus* (t0) as compared with 20 specimens fed one month and three months on each of the three feeds, i.e. *U. rigida, P. oceanica* and formulated pellets. These sea urchins were weighed, sacrificed and dissected; their gonads were extracted and weighed (fw) for the evaluation of the GI as (Sánchez-España et al. 2004; Fabbrocini and D'Adamo 2011; Keshavarz et al. 2017):

1) GI = gonadal wet weight (g)/sea urchin wet weight (g) × 100

The gonads of three males and three females for each treatment were dissected, fixed in Bouin, included in paraffin blocks, sliced and stained by hematoxylin, to evaluate the histological structure of tissues and interpret the results of other data sets (Byrne 1990). The slices, after staining, were enclosed into permanent mountings and observed under the optical microscope.

Gamete collection, embryo culture and morphological analysis

After three months of feeding, as described, adults of *P. lividus* reared in the experimental tanks were injected 2 ml of 2M KCl through the peribuccal membrane to trigger the release of gametes. Eggs were immediately washed with filtered seawater (FSW) and kept in FSW until use. Concentrated sperm was collected and kept at 4°C until use. Eggs were fertilized in FSW, utilizing sperm-to-egg ratios of 100:1 (Romano et al. 2011).

Fertilized eggs were incubated at 20°C in a controlled temperature chamber on a 12h/12h light/dark cycle. These experiments were conducted in triplicates, fertilizing 400 eggs in 3 ml of sea water.

Percentages of fertilization of first cleavage at about 1 h post fertilization (hpf) and normal and malformed embryos (48 hpf) were evaluated for at least 200 plutei from each female (fixed in formaldehyde 4% in FSW) using a light microscope (Zeiss Axiovert 135TV; Carl Zeiss, Jena, Germany).

Statistical analyses

The statistical significance of differences among daily feeding rates recorded according to the three feeds, and C and N concentrations in feeds and faecal pellets were evaluated by the average and variation of the data reported as 'mean ± standard deviation (SD)'. SD bars were plotted in order to allow an immediate perception of the intervals of superimposition of our replicates. Statistical significance of differences between individual treatments was evaluated using *t*-tests (Prism 3.0, GraphPad Prism 4.00 for Windows, GraphPad Software, San Diego California USA). $p < 0.05$ was considered as statistically significant.

Results

Daily feeding rate

Preliminary experiments were performed to define the daily feeding rates of adult sea urchins. Ten adults of sea urchins *P. lividus* were reared in each tank of the continuous open flow-through system and then fed with 10, 20 and 30 g WW per day of the green macroalga *U. rigida* (usually used as control to fed sea urchins in SZN Animal Facility) to approximately determine the daily sea urchin feeding rate. Food consumption was measured after 24, 48 and 72 h. Food consumption was higher in the first 24 h on fasted animals with significant differences among the three quantities (Figure 1; 10g versus 20g p value = 0.047, indicated with a; 20 g vs. 30 g, p value = 0.06, indicated with b; 10 g vs. 30 g, p value = 0.0012, indicated with c). At 24 h the food consumption decreased, but differences were not significant among the three quantities; at 72 h, the daily feeding rates stabilized on an average of about 1 g WW per sea urchin, independently of the initial quantity of *U. rigida* administered.

According to these preliminary experiments, sea urchins were fed 20 g (WW) per day of *U. rigida* and *P. oceanica*. In the case of formulated pellets, *ad libitum* feeding was 1.8 g/day per 20 sea urchins, i.e. about 0.10 g/animal.

After three months of treatment, the daily feeding rate (as dry weight, DW) for a sea urchin corresponded

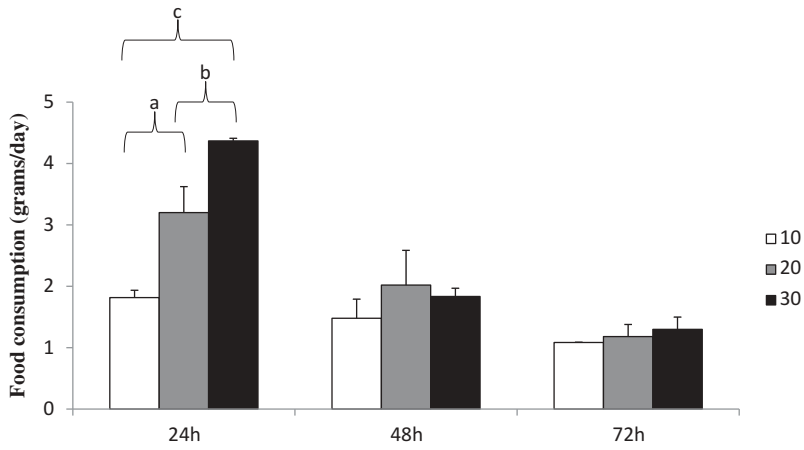

Figure 1. Ten adults of sea urchins *Paracentrotus lividus* were put in a tank of the continuous open flow-through system, fasted for three days and then fed with 10 (white bars), 20 (grey bars) and 30 (black bars) grams wet weight (WW) per day of the green macroalgae *Ulva rigida* (usually used as control to fed sea urchins in SZN Animal Facility). Statistically significant differences have been detected only after 24 h of feeding using 10 and 20 g of *Ulva rigida*: 10 versus 20 g, p value = 0.0471 (a); 10 versus 30 g = 0.0012 (c); 20 versus 30 g, p value = 0.0606 (b). After 48 and 72 h of feeding no significant differences have been detected ($p > 0.05$).

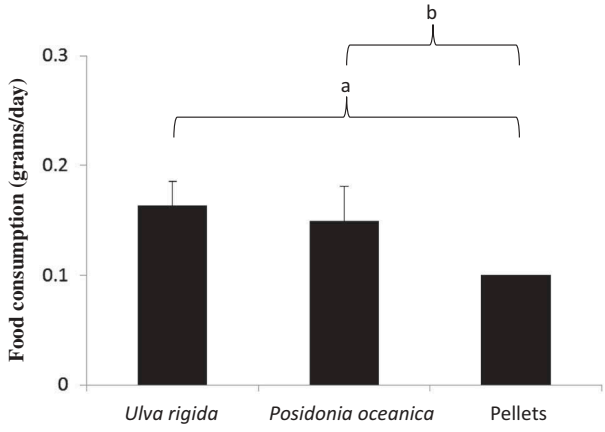

Figure 2. Daily feeding rate. The histogram shows the daily feeding rate per animal after feeding for one month with *Ulva rigida, Posidonia oceanica* and the pre-hydrated formulated feed. *Ulva rigida* versus *Posidonia oceanica, p* value = 0.06 (no significant value $p > 0.05$); formulated pellets versus *Ulva rigida p* value < 0.0001 (a); *P. oceanica* and pellets p value < 0.0001 (b).

to 0.16, 0.15 and 0.10 g for *U. rigida, P. oceanica* and formulated pellets, respectively (Figure 2).

Differences in individual daily feeding rates were not significant (*t*-test) between treatments *U. rigida* and *P. oceanica* (p = 0.06). In contrast, the daily feeding rates obtained for formulated pellets were significantly lower both in comparison of *U. rigida* versus pellets p value <0.0001 (indicated with a); *P. oceanica* and pellets p value <0.0001 (indicated with b).

Table 1. Composition in percentage (%) of nitrogen (N) and carbon (C) of the three feeds, *Ulva rigida, Posidonia oceanica* and formulated pellets, and of the faecal pellets collected from sea urchins fed with the three feeds. C/N ratio has also been reported both in the three feeds and in the correspondent faecal pellets.

	Composition (%)					
	Feed		Faecal pellets		C/N ratio	
	N	C	N	C	Feed	Faecal pellets
Ulva rigida	2.19	26.00	1.55	15.88	11.85	10.27
Posidonia Oceanica	2.41	36.64	1.03	30.95	15.23	30.09
Pellets	4.86	42.18	1.83	22.39	8.67	12.25

Carbon and nitrogen contents of feeds and faecal pellets

The amounts of carbon (C) and nitrogen (N) measured by CN Analyzer in the three feeds (namely, *U. rigida, P. oceanica* and formulated pellets) were compared with those measured in the sea urchin faecal pellets (Table 1).

The C/N ratio was highest in *P. oceanica* (15.2) and it decreased in *U. rigida* (11.9) reaching the lowest value (8.7) in the formulated pellets (Figure 3(a)). Statistically significant differences (*t*-test) in the C/N ratios were found between *U. rigida* and *P. oceanica* (p = 0.03, indicated with a), as well as between *U. rigida* and formulated pellets (p = 0.0274, indicated as c). The difference is highly significant between *P. oceanica* and pellets (p = 0.005, indicated with b). Concerning faecal pellets, *P. oceanica* exhibited a higher C/N ratio (30.1) in respect to *U. rigida*. This value decreased in the

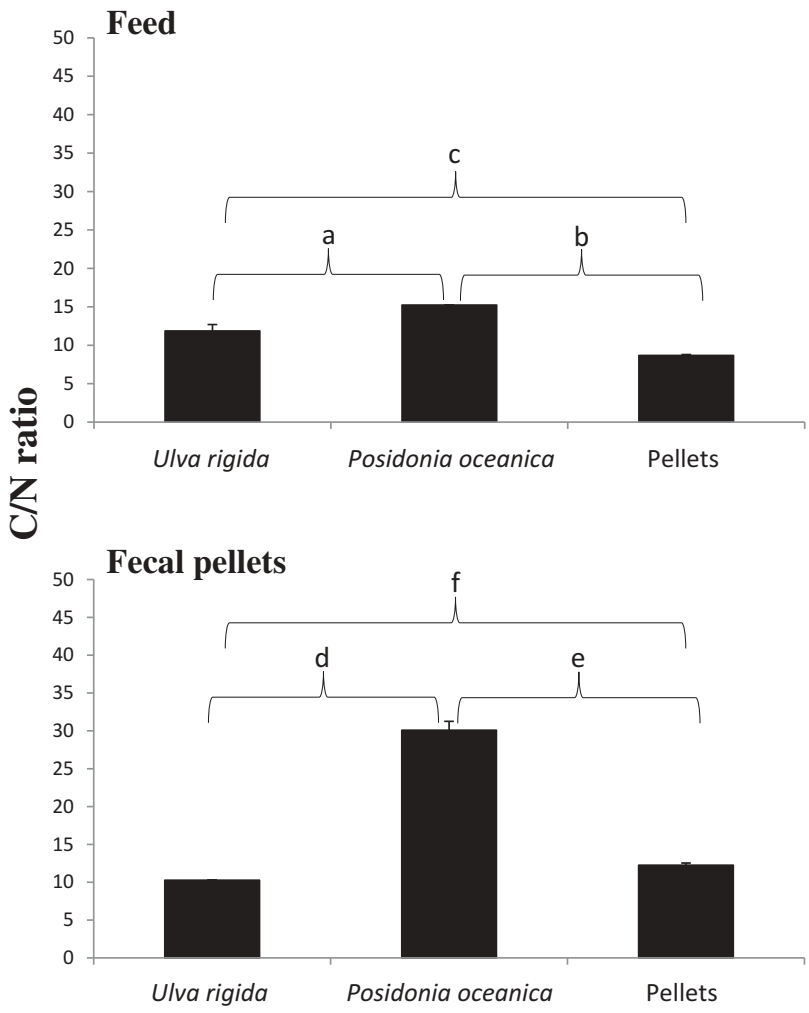

Figure 3. Carbon and nitrogen measurements in feeds and faecal pellets. Carbon (C) and nitrogen (N) ratio (C/N ratio) measured by CN Analyzer (A) in the three feeds, *Ulva rigida, Posidonia oceanica* and formulated pellets, and (B) in the faecal pellets collected from sea urchins fed with these three diets. C/N ratio in feeds: *Ulva rigida* versus *Posidonia oceanica, p* value = 0.03 (a); *Ulva rigida* versus formulated pellets, *p* value = 0.0274 (b); *Posidonia oceanica* and formulated pellets, *p* value = 0.005 (c). C/N ratio in faecal pellets: *Posidonia oceanica* versus both *Ulva rigida, p* value = 0.002 (d) and formulated pellets, *p* value = 0.0024 (e); *Ulva rigida* versus formulated pellets, *p* value = 0.0186 (f).

pellets (12.2) reaching the lowest value in *U. rigida* (10.3) (Figure 3(b)).

The differences in C/N ratios were significant between *P. oceanica* and both *U. rigida* (p = 0.0017, indicated with d) and pellets (p = 0.0024, indicated with e); significant difference were found also between *U. rigida* and pellets (p = 0.0186, indicated with f).

Adult growth and gonadal index

No significant differences in growth rates were found among adult sea urchins fed for one month with *U. rigida, P. oceanica* and pellets in comparison with adults collected in the field at the beginning (t0; Table 2).

Moreover, no significant differences were found among the GI values of sea urchins fed one month on *U. rigida, P. oceanica* and pellets in comparison with those collected in the field at the start of the experiments ($p > 0.05$). Different results have been reached after three months of feeding. In fact, we observed a high significant increase in the GI values in sea urchins fed with formulated pellets, in comparison with those fed with *U. rigida* (p = 0.005, indicated with a) and *P. oceanica* (p = 0.005, indicated with b). In Figure 4, we reported the gonads from sea urchins fed with formulated pellets in comparison with those from adults fed with *U. rigida* and *P. oceanica* after three months of feeding experiments.

Fertility of sea urchins

Gametes were collected from the sea urchins at two distinct periods, i.e. after one and three months of

Table 2. Adult sizes (in millimetres) and gonadal index (GI ± SD, n = 20/group) of adults of sea urchin *Paracentrotus lividus* collected in the field at the beginning (t0) and after one month and three months of the feeding experiments with *Ulva rigida, Posidonia oceanica* and formulated pellets (p value > 0.05 for adult sizes after one and three months of feeding, and GI after one month of feeding; p = 0.005 for GI after three months of feeding in sea urchins fed with formulated pellets, in comparison with those fed with *Ulva rigida* (a) and *Posidonia oceanica* (b).

		Ulva rigida	*Posidonia oceanica*	Formulated pellets
Adult size				
t0	40.2 ± 2.26			
1 month		41.3 ± 2.17	39.9 ± 2.39	40.5 ± 2.65
3 months		41.9 ± 2.45	40.6 ± 1.90	40.9 ± 2.93
GI				
t0	4.0 ± 0.51			
1 month		3.9 ± 0.39	4.2 ± 0.49	4.0 ± 0.60
3 months		4.2 ± 0.44	4.4 ± 0.47	14.7 ± 1.13
			a	
			b	

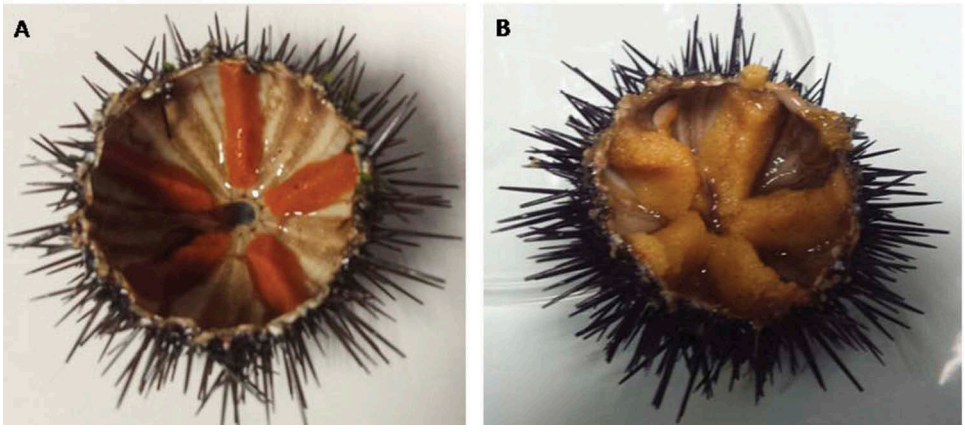

Figure 4. Gonads from (A) adult female fed on *Ulva rigida* and *Posidonia oceanica* and (B) adult female fed on formulated pellets after three months of feeding experiments.

feeding trials (see Materials and Methods Section). Gametes were collected only from those sea urchins in the replicates fed on *U. rigida* and *P. oceanica*. In the case of sea urchins fed on formulated pellets, after one month of feeding gametes were present only in five individuals (three females and two males); after three months gametes were absent in all the individuals under this treatment. As soon as the fertilization occurred, we measured the fertilization success and the first mitotic cleavage (1 hpf) to obtain two blastomeres (Table 3). Then the embryonic development has been followed until the pluteus stage. Fertilization and first cleavage were obtained in 100% of gametes produced by individuals under treatments with *U. rigida* and *P. oceanica*. Morphological observations of the only three females producing gametes revealed that feeding one month on formulated pellets induced significant (p = 0.04) increase in the percentage of abnormal embryos in respect to *U. rigida* and *P. oceanica*.

Histological observations of the gonads for both the treatments with *U. rigida* and formulated pellets demonstrated a stage quite close to a maturity. In fact, *U. rigida*, at the end of the treatment, produced ovaries in pre-mature recovering stage (Figure 5(a)), with oocytes still filling the centre of follicular masses.

The commercial pellet treatment exhibited as well pre-mature ovaries with a lower abundance of oocytes in the centre of follicules and a few mature eggs in theor periferical areas (Figure 5(b)). However, in this case the gonadic tissues appeared hypertrophic and vacuolated in the cortex.

Discussion

The effect of feeding on physiological and reproductive conditions of sea urchins is essential to understand their biology and ecology and to develop novel feeds for echinoculture. However, it is interesting to observe that the feeds ingested vary according to their quality

Table 3. Percentage of fertilization, first cleavage (two blastomeres), normal plutei and malformed plutei in the embryos from sea urchins *Paracentrotus lividus* collected in the field at the beginning (t0) and after three months of feeding with *Ulva rigida, Posidonia oceanica* and formulated pellets. In the case of formulated pellets after one month of feeding only five adults (three females and two males) produced gametes (data reported in the table), whereas after three months of feeding no adults produced gametes.

	t0	*Ulva rigida*	*Posidonia oceanica*	Formulated pellets
Fertilization	100	100	100	100
First cleavage	100	100	100	100
Normal plutei	90	91	90	80
Malformed plutei	10	9	10	20

and this study represents a confirmation of this regulation of their feeding activity. Although considered as an herbivorous species, *P. lividus* has often been classified as an opportunistic omnivore taking advantage of various food sources (Zupo and Fresi 1984; Boudouresque and Verlaque 2001). However, they do have preferences when presented with choice, determined by the chemical and physical characteristic of feeds. Information about nutrition, digestion and digestibility is still limited. Production of sea urchins is the results of ingestion, digestion and absorption, which have important implications for their nutrition (Boudouresque and Verlaque 2013). Even if food is abundantly and continuously available in their own environment, sea urchins do not necessarily feed continuously. In fact, the consumption is high when food is supplied after starvation, in our experimental conditions, and it decreases in conditions of feeding *al libitum* (Bonsdorff 1983). Moreover, food consumption is related to the reproductive stages. Concerning the digestion system, the part corresponding to stomach is the primary site of production of digestive enzymes and that corresponding to intestine is the primary site of uptake of nutrients, although their gut is not structurally differentiated into a stomach and an intestine, but a long digestive tube (Lawrence et al. 2013). Regional differences in digestive enzymatic activity are consistent with regional differences along the intestine tracts. The 'stomach' has much higher amylase activity than the 'intestine'. Almost all studies on digestive enzymes in sea urchins concern carbohydrates. Many studies have shown cellulase activity on the linear, soluble carboxymethylcellulose with minimal cellulase activity on native cellulose. Amylase also occurs in sea urchins, as well as glycogenase and agarose.

Sea urchins play an important role in shaping some coastal shallow benthic communities thanks to their grazing activity in rocky bottoms, also recognized to be able to transform communities dominated by macroalgae into barren areas so reducing biodiversity, altering ecosystem functions and regulating sea urchin population dynamics (Palacín et al. 1998; Sala et al. 1998; Barnes and Crook 2001; Prado et al. 2007; Hereu et al. 2012). Moreover, *P. lividus* plays a central role by directly removing plant biomass (both green and brown tissues), improving nutrient export, and modifying plant production in ecosystems dominated by the seagrass *P. oceanica* (Tomas et al. 2005; Prado et al. 2007; Ruiz et al. 2009; Planes et al. 2011). At the same time, *P. lividus* is intensively exploited in many Mediterranean areas because male and female gonads are considered a delicacy (Guidetti et al. 2004; Furesi et al. 2014).

Our data showed that *P. lividus* daily ingests about the same quantities of *U. rigida* and *P. oceanica* and significantly lower amounts of artificial pellets. This difference in daily feeding rates could be due to a strong preference for *U. rigida* and *P. oceanica* by *P. lividus* or to a different nutritional value of the considered artificial food. In fact, this feed better corresponds to the sea

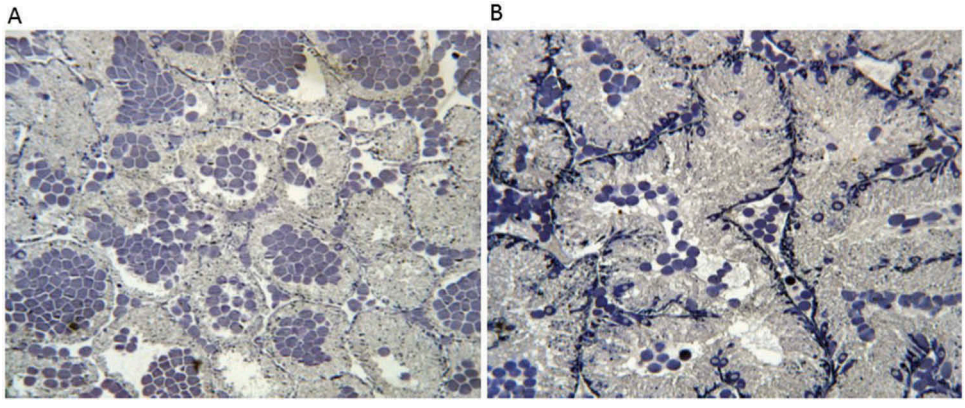

Figure 5. Representative histological sections observed under the optical microscopy of *Paracentrotus lividus* ovaries reared with *Ulva rigida* (left, A) and with formulated pellet (Right, B). The scale bar corresponds to 10 µm.

urchin nutritional needs, probably due to its high protein and lipid contents (Fabbrocini and D'Adamo 2010). In particular, protein content of *U. rigida* is 6.64%, whereas carbohydrate is 22% and total lipid contents 12%, phenol 23% and moisture content 76%, total free amino acid 8.9%, chlorophyll a 13%, chlorophyll b 7.5% and carotenoids 4.5% (Satpati and Pal 2011). In *P. oceanica* the total carbohydrates, proteins and lipids are, respectively, 28.98 mg/g, 607.50 mg/g and 40.50 mg/g (El Din and El-Sherif 2013). The composition of the formulated pellets consists of crude protein 46.5%, crude fat 10.5%, crude fiber 2.4% and ashes 9.5%, proteins of animal origin accounting for less than 5% (Mihranyan 2010).

C/N data revealed that *P. oceanica* had the highest C/N ratio and the faecal pellets of individuals fed on its tissues are as well characterized by high C content. We may hypothesize that these relationships are due to a higher abundance of structural carbohydrates (cellulose) characterizing the seagrass tissues. Cellulose is a complex carbohydrate, representing the structural component of cell walls in both green plants and algae (Baldan et al. 2001). In fact, several green algae have walls containing a cellulosic content up to 70% of their dry weight (Ott and Maurer 1977). According to our hypothesis, sea urchins discharge the excess of carbon due to cellulose through the faecal pellets, as well as it was demonstrated in various studies on invertebrate consumers of *P. oceanica* (Kawamata 1997).

To date, several studies defined the daily feeding rates of different sea urchins in the field. In the case of *Strongylocentrotus nudus* trough mathematical models have been estimated the amount of kelp eaten, using the brown algae *Laminaria* spp., *Eisenia bicyclis* and *Undaria pinnatifida* present in the habitats of this sea urchin (Hiratsuka and Uehara 2007). The predicted feeding rate was 0.5 g wet mass $\times$ d^{-1} $\times$ $animal^{-1}$, considering adults of about 40–50 mm. Feeding rates of four sea urchin species, *Echinometra* sp. A, *E. mathaei, E.* sp. C and *E. oblonga* (belonging to the genus *Echinometra*), were investigated after feeding on a diet prepared from turf algae and agar for a 7-day period (Scheibling and Anthony 2001). In that case, the feeding rates differed significantly among the four species of sea urchins, being between 0.14 and 0.29 g for sea urchins of 30–35 mm. Furthermore, adult *Strongylocentrotus droebachiensis* were fed on two diets, the invasive green alga *Codium fragile* and the brown alga *Laminaria* sp. (Cyrus et al. 2015). The feeding rates declined from June to July and remained low (about 0.1 g DW per urchin of 38–52 mm per day) through September. That these data are comparable with our results, showing that the daily feeding rates (DW) for a sea urchin correspond to 0.16, 0.15 and 0.10 g for *U. rigida, P. oceanica* and formulated pellets, respectively.

Interestingly, the three different diets did not produce size increments of adult sea urchins, but affected their gonad growth and reproduction performance. In fact, we demonstrated that, at the end of feeding experiments (after three months), *U. rigida* and *P. oceanica* didn't produce effects on the gonad growth. Differently, after three months the formulated pellets affected the GI, resulting in a significant growth of the gonads probably due to the high content of crude proteins of this food (as reported above). Our data confirmed its contribution in the production of large gonads (Sartori and Gaion 2016), which represents the major aim of echinoculture practices (Fabbrocini and D'Adamo 2010). Despite the large increase in the volume of their gonads, surprisingly they were not capable of producing gametes. This could be probably correlated to the colour and texture of their gonads, which were very different from those normally observed in sea urchins collected from the field and/or fed with the other two feed used in this work (see Figure 4). Both treatments demonstrated to be sufficient for a rapid increase of the ovary tissues and a maturation of gonads, starting from a spent stage. However, the effects were quite different both from a histological point of view and according to the results of fertilization tests. In fact, *U. rigida* produced a slower maturation and enlargement of ovaries, with the production of several oocytes, while the commercial pellets produced a hypertrophy of the follicular tissues, a diffused vacuolization, and the maturation of a few eggs, which conducted finally to a low fertilization success with compromised post-fertilization embryonic and larval development to pluteus. It is well-known in literature that the nutrients' composition of a diet has a significant effect on the growth (Marsh and Watts 2007). For this reason, optimizing a feed for the best production of gonads in a sea urchin requires a consideration to balance the energy demands with the availability of various protein and non protein dietary principles, including vitamins, carotenoids and fatty acids (Castell et al. 2004; González-Durán et al. 2008). On this line, in the last 20 years, technology for sea urchin culture, including reproduction and diet formulation, has been improved for the supply of sea urchin (Watts et al. 2013). On this line, our data demonstrated that there is strong relationship between GI and dietary contents of proteins, as in the case of commercialized pellets. These data were in agreement with other investigation reported by Pearce et al. (2002c), demonstrating a positive effects of proteins on gonadal increase of *S. droebachiensis* fed with

artificial diets at increasing protein levels. Comparable results have been reported by De Jong-Westman et al. (1995) for adults of *S. droebachiensis* fed with prepared diets at high level of proteins.

In conclusion, this study represents an additional attempt to correlate the daily feeding rate of adult *P. lividus* with the composition of feeds and their effects of growth and reproductive success. Results will be useful for the definition of optimal diets for the production of mature bloodstocks with high quality of eggs and for larval production also in industrial culture of this ecologically important and well-established marine model organism.

Acknowledgments

Nadia Ruocco has been supported by a PhD (PhD in Biology, University of Naples Federico II) fellowship co-funded by the Stazione Zoologica Anton Dohrn and Bio-Organic Chemistry Unit of the Institute of Biomolecular Chemistry-CNR, Pozzuoli, Naples.

The authors thank the Fishery Service of the Stazione Zoologica and the Monitoring and Environmental Data Unit for providing the sea urchins.We thank Alberto Macina from the Marine Resources for Research Unit (Stazione Zoologica) for his technical support in the setting-up of the continuous open flow-through system for feeding experiments. Our special thank for the Unit Monitoring and Environmental Data (Stazione Zoologica) for carbon and nitrogen measurements by C/N Analyzer. We also thank Massimo Perna of the Department of Integrative Marine Ecology for his help in the fixation of embryos.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Valerio Zupo http://orcid.org/0000-0001-9766-8784

References

Baldan B, Andolf P, Navazio L, Tolomio C, Mariani P. 2001. Cellulose in algal cell walls: an 'in situ' localization. European Journal of Histochemistry : EJH. 45:51–56.

Barnes DKA, Crook AC. 2001. Implications of temporal and spatial variability in *Paracentrotus lividus* populations to the associated commercial coastal fisheries. Hydrobiologia. 465:95–102.

Bayed A, Quiniou F, Benrha A, Guillou M. 2005. The *Paracentrotus lividus* populations from the northern Moroccan Atlantic coast: growth, reproduction and health condition. Journal Mar Biologic Association UK. 85:999–1007.

Bonsdorff E. 1983. Appetite and food consumption in the sea urchin *Echinus esculentus*. L. Sarsia. 68:25–27.

Boudouresque CF, Verlaque M. 2013. Paracentrotus lividus. In: Lawrence JM, editor. Sea urchins: biology and ecology. The Netherlands, Amsterdam: Elsevier; p. 297–327.

Boudouresque CF, Bernard G, Bonhomme P, Charbonnel E, Le Diréach L, Ruitton S. 2007. Monitoring methods for *Posidonia oceanica* seagrass meadows in Provence and the French Riviera. Sciences Reports Port-Cros Natl Park. 22:17–38.

Boudouresque CF, Verlaque M. 2001. Ecology of *Paracentrotus lividus*. Developments Aquacult Fisheries Sciences. 32:177–216.

Byrne M. 1990. Annual reproductive cycles of the commercial sea urchin *Paracentrotus lividus* from an exposed intertidal and a sheltered subtidal habitat on the west coast of Ireland. Mar Biologic. 104:275–289.

Caltagirone A, Fernandez C, Francour P. 1992. Formulation of an artificial diet for the rearing of the urchin *Paracentrotus lividus*: I. Comparison of different binding agents. In: Scalera-Liaci L, Canicatti C, editors. Echinoderm research 1991. Rotterdam: Balkema Publisher; p. 115–119.

Carboni S, Vignier J, Chiantore M, Tocher DR, Migaud H. 2012. Effects of dietary microalgae on growth, survival and fatty acid composition of sea urchin *Paracentrotus lividus* throughout larval development. Aquaculture (Amsterdam, Netherlands). 324–325:250–258.

Castell JD, Kennedy EJ, Robinson SMC, Parsons GJ, Blair TJ, Gonzalez-Duran E. 2004. Effect of dietary lipids on fatty acid composition and metabolism in juvenile green sea urchins (*Strongylocentrotus droebachiensis*). Aquaculture (Amsterdam, Netherlands). 242:417–435.

Cyrus MD, Bolton JJ, Scholtz R, Macey BM. 2015. The advantages of *Ulva* (Chlorophyta) as an additive in sea urchin formulated feeds: effects on palatability, consumption and digestibility. Aquacult Nutritional. 21:578–591.

De Jong-Westman M, Qian PY, March BE, Carefoot TH. 1995. Artificial diets in sea urchin culture: effects of dietary protein level and other additives on egg quality, larval morphometries, and larval survival in the green sea urchin, *Strongylocentrotus droebachiensis*. Canadian Journal of Zoology. 73:2080–2090.

El Din NGS, El-Sherif ZM. 2013. Nutritional value of *Cymodocea nodosa* and *Posidonia oceanica* along the western Egyptian Mediterranean coast. Egyptian Journal Aquat Researcher. 39:153–165.

Fabbrocini A, D'Adamo R. 2010. Gametes maturation and gonad growth in fed and starved sea urchin *Paracentrotus lividus* (Lamarck, 1816). Journal Shellfish Researcher. 29:1–9.

Fabbrocini A, D'Adamo R. 2011. Gametes and embryos of sea urchins (*Paracentrotus lividus*, Lmk., 1816) reared in confined conditions: their use in toxicity bioassays. Journal of Chemical Ecology. 27:105–115.

Fabbrocini A, D'Adamo R, Del Prete F, Langellotti AL, Rinna F, Silvestri F, Sorrenti G, Vitiello V, Sansone G. 2012. Cryopreserved semen in ecotoxicological bioassays: sensitivity and reliability of cryopreserved *Sparus aurata* spermatozoa. Ecotoxicology and Environmental Safety. 84:293–298.

Frantzis A, Gremare A. 1993. Ingestion, absorption, and growth rates of *Paracentrotus lividus* (Echinodermata: echinoidea) fed different macrophytes. Marine Ecology Progress Series. 95:169–183.

Frantzis A, Grémare A, Vétion G. 1992. Growth rates and RNA: DNA ratios in *Paracentrotus lividus* (Echinodermata:

echinoidea) fed on benthic macrophytes. Journal of Experimental Marine Biology and Ecology. 156:125–138.
Furesi R, Madau FA, Palomba A, Pulina P. 2014. Stated preferences for consumption of sea urchin: a choice experiment in Sardinia (Italy). Int J Food System Dynamics. 3:111–119.
González-Durán E, Castell JD, Robinson SMC, Blair TJ. 2008. Effects of dietary lipids on the fatty acid composition and lipid metabolism of the green sea urchin *Strongylocentrotus droebachiensis*. Aquaculture (Amsterdam, Netherlands). 276:120–129.
Guerriero A, Meinesz A, D'Ambrosio M, Pietra F. 1992. Isolation of toxic and potentially toxic sesqui- and monoterpenes from the tropical green seaweed *Caulerpa taxifolia* which bas invaded the region of Cap Martin. Helvetica Chimica Acta. 75:689–695.
Guidetti P, Terlizzi A, Boero F. 2004. Effects of the edible sea urchin, *Paracentrotus lividus*, fishery along the Apulian rocky coast (SE Italy, Mediterranean Sea). Fisheries Researcher. 66:287–297.
Hedges JI, Stern JH. 1984. Carbon and nitrogen determination of carbonate-containing solids. Limnology and Oceanography. 29:657–663.
Hereu B, Linares C, Sala E, Garrabou J, Garcia-Rubles A, Diaz D, Zabala M. 2012. Multiple processes regulate long-term population dynamics of sea urchins on Mediterranean Rochy Reefs. PLoS One. 7:e36901.
Hiratsuka H, Uehara T. 2007. Feeding rates and absorption efficiencies of four species of sea urchins (genus Echinometra) fed a prepared diet. Comparative Biochemical Physiological A. 148:223–229.
Jeffery CH, Emlet RB. 2003. Macroevolutionary consequences of developmental mode in temnopleurid echinoids from the Tertiary of southern Australia. Evolution. 57:1031–1048.
Kawamata S. 1997. Modelling the feeding rate of the sea urchin *Strongylocentrotus nudus* (A. Agassiz) on kelp. Journal of Experimental Marine Biology and Ecology. 210:107–127.
Keshavarz M, Kamrani E, Biuki NA, Zamani H. 2017. Study on the gonadosomatic indices of sea urchin *Echinometra mathaei* in Persian Gulf, Iran. Pakistan Journal of Zoology. 49(3):923.
Lawrence J.M. 2001. The edible sea-urchins. Amsterdam: Elsevier.
Lawrence JM, Lawrence AL, Watts SA. 2013. Feeding, digestion and digestibility of sea urhcins. In: Lawrence JM, editor. Sea urchins: biology and ecology, 3rd edition. Developments in aquaculture and fisheries science (Vol. 38). The Netherlands: Elsevier, Amsterdam; p. 135–154.
Lawrence JM. 2013. Sea Urchins: biology and Ecology, 3rd edition. Developments in aquaculture and fisheries science. Vol. 38. The Netherlands: Elsevier, Amsterdam.
Lemée R, Boudouresque CF, Gobert J, Malestroit P, Mari X, Meinesz A, Menager V, Ruitton S. 1996. Feeding behavior of *Paracentrotus lividus* in the presence of *Caulerpa taxifolia* introduced in the Mediterranean Sea. Oceanologica Acta. 19:245–253.
Marsh AG, Watts SA. 2007. Biochemical and energy requirements of gonad development. In: Lawrence JM, editor. Sea Urchins: biology and Ecology, 3rd edition. Developments in aquaculture and fisheries science (Vol. 38). The Netherlands: Elsevier, Amsterdam; p. 35–53.
Mihranyan A. 2010. Cellulose from Cladophorales green algae: from environmental problem to high-tech composite materials. Journal of Applied Polymer Science. 119:2449–2460.
Ott JA, Maurer L. 1977. Strategies of energy transfer from marine macrophytes to consumer levels: the *Posidonia oceanica* example. In: Keegan B, O'Ceidigh P, Boaden P, editors. Biology of benthic organisms. Oxford: Pergamon Press; p. 493–502.
Palacín C, Giribet G, Carner S, Dantart L, Turon X. 1998. Low density of sea urchins influences the structure of algal assemblages in the western Mediterranean. Journal Sea Researcher. 39:281–290.
Pawson DL. 2007. Phylum Echinodermata. Zootaxa. 1668:749–764.
Pearce CM, Daggett TL, Robinson SMC. 2002c. Effect of protein source ratio and protein concentration in prepared diets on gonad yield and quality of the green sea urchin, *Strongylocentrotus droebachiensis*. Aquaculture (Amsterdam, Netherlands). 214:307–332.
Philip GM, Foster RJ. 1971. Marsupiate tertiary echinoids from south-eastern Australia and their zoogeographic significance. Palaeontology. 14:666–695.
Pinna S, Pais A, Campus P, Sechi N, Ceccherelli G. 2012. Habitat preferences of the sea urchin *Paracentrotus lividus*. Marine Ecology Progress Series. 445:173–180.
Planes S, Raventos N, Ferrari B, Alcoverro T. 2011. Fish herbivory leads to shifts in seagrass Posidonia oceanica investments in sexual reproduction. Marine Ecology Progress Series. 431:205–213.
Prado P, Tomas F, Alcoverro T, Romero J. 2007. Extensive direct measurements of *Posidonia oceanica* defoliation confirm the importance of herbivory in temperate seagrass meadows. Marine Ecology Progress Series. 340:63–71.
Romano G, Costantini M, Buttino I, Ianora A, Palumbo A. 2011. Nitric oxide mediates the stress response induced by diatom aldehydes in the sea urchin *Paracentritus lividus*. PloS One. 6:e25980.
Ruiz JM, Pèrez M, Romero J, Tomas F. 2009. Importance of herbivory on seagrass (*Posidonia oceanica*) decline near a fish farm: an experimental approach. Botanic Mar. 52:449–458.
Ruocco N, Costantini S, Zupo V, Lauritano C, Caramiello D, Ianora A, Budillon A, Romano G, Nuzzo G, D'Ippolito G, et al. 2018. Toxigenic effects of two benthic diatoms upon grazing activity of the sea urchin: morphological, metabolomic and de novo transcriptomic analysis. Scientific Reports. 8:5622.
Sala E, Boudouresque CF, Harmelin-Vivien M. 1998. Fishing, trophic cascades, and the structure of algal assemblages: evaluation of an old but untested paradigm. OIKOS. 82:425–439.
Sala E, Zabala M. 1996. Fish predation and the structure of the sea urchin *Paracentrotus lividus* populations in the NW Mediterranean. Marine Ecology Progress Series. 140:71–81.
Sánchez-España AI, Martínez-Pita I, García FJ. 2004. Gonadal growth and reproduction in the commercial sea urchin *Paracentrotus lividus* (Lamarck, 1816) (Echinodermata: echinoidea) from southern Spain. Hydrobiol. 519:61–72.
Sartori D, Gaion A. 2016. Can sea urchins benefit from an artificial diet? Physiological and histological assessment for echinoculture feasibility evaluation. Aquacult Nutritional. 22:1214–1221.
Sartori D, Scuderi A, Sansone G, Gaion A. 2015. Echinoculture: the rearing of *Paracentrotus lividus* in a recirculating aquaculture system experiments of artificial diets for the maintenance of sexual maturation. Aquacult Internat. 23:111–125.
Satpati GG, Pal R. 2011. Biochemical composition and lipid characterization of marine green alga *Ulva rigida*- a nutritional approach. Journal Algal Biomass Utln. 2:10–13.

Scheibling RE, Anthony SX. 2001. Feeding, growth and reproduction of sea urchins (*Strongylocentrotus droebachiensis*) on single and mixed diets of kelp (*Laminaria* spp.) and the invasive alga *Codium fragile* ssp. tomentosoides. Mar Biologic. 139:139–146.

Spirlet C, Grosjean P, Jangoux M. 1998. Reproductive cycle of the echinoid *Paracentrotus lividus*: analysis by means of the maturity index. International Journal Invertebr Repr Developments. 34:69–81.

Tejada S, Deudero S, Box A, Sureda A. 2013. Physiological response of the sea urchin *Paracentrotus lividus* fed with the seagrass *Posidonia oceanica* and the alien algae *Caulerpa racemosa* and *Lophocladia lallemandii*. Marine Environmental Research. 83:48–53.

Tomas F, Turon X, Romero J. 2005. Effects of herbivores on a *Posidonia oceanica* seagrass meadow: importance of epiphytes. Marine Ecology Progress Series. 287:115–125.

Vadas RL, Beal B, Dowling T, Fegley JC. 2000. Experimental field tests of natural algal diets on gonad index and quality in the green sea urchin, *Strongylocentrotus droebachiensis*: a case for rapid summer production in post-spawned animals. Acquaculture. 182:115–135.

Valente LMP, Gouveia A, Rema P, Matos J, Gomes EF, Pinto IS. 2006. Evaluation of three seaweeds *Gracilaria bursa-pastoris, Ulva rigida* and *Gracilaria cornea* as dietary ingredients in European sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture (Amsterdam, Netherlands). 252:85–91.

Vergés A, Alcoverro T, Romero J. 2011. Plant defences and the role of epibiosis in mediating within-plant feeding choices of seagrass consumers. Oecologia. 166:381–390.

Verlaque M. 1987. Relations entre *Paracentrotus lividus* (Lamarck) et le phytobenthos de Méditerranée occidentale. In: Boudouresque CF, editor. Coll intern sur *Paracentrotus lividus* et les Oursins comestibles. Marseille: GIS Posidonie Publ; p. 5–36.

Watts SW, Lawrence AL, Lawrence JM. 2013. Nutrition. In: Lawrence JM, editor. Sea Urchins: biology and Ecology, 3rd edition. Developments in aquaculture and fisheries science (Vol. 38). The Netherlands: Elsevier, Amsterdam; p. 155–170.

Zupo V, Fresi E. 1984. A study on the food web of the *Posidonia oceanica* ecosystem: analysis of the gut contents of Echinoderms. In: Boudouresque CF, Jeudi De Grissac A, Olivier J, editors. 1stInternational workshop on *Posidonia oceanica* beds. Marseilles: GIS Posidonie Publishing; p. 373–379.