UNIVERSITÀ DEGLI STUDI
DI GENOVA

Scuola di Scienze matematiche, fisiche e naturali

Corso di Laurea Magistrale in Biologia ed Ecologia Marina

Feeding the sea urchins *Paracentrotus lividus* and *Arbacia lixula* different fresh macroalgae: assessing differences in morpho-functional parameters

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Anno accademico 2019/2020

11 FEBBRAIO 2021

Abstract

Fishery of different species of sea urchins as a food resource caused in the last decades a dramatic depletion in populations. Beside the overexploitation of these species, climate changes, as ocean acidification, caused a disappearance of urchins from areas of former abundance, especially along the Mediterranean coasts. Several studies aim to improve echinoculture techniques, analysing rearing strategies and diet formulation for optimizing gonad yield and quality in order to satisfy, at least in part, the growing market request. Limitations are given by the fact that the gonads of reared specimens do not have an acceptable quality, but also by the long time and critical steps required to obtain marketable sized sea urchins (5 cm) and for growing the new broodstock (around 2-3 years).

The present work is a contribution to the BRITEs Project (Byproduct Recycling: Innovative TEchnology from the Sea), that aims to recycle sea urchin waste produced by restaurants to generate valuable by-products as biocarbonates, to use in the production of feed for fish and invertebrates, and collagen, an important protein widely used for the production of human skin substitutes.

We evaluated the role of calcium carbonate and antioxidants in the diet, analysing the effects of different macroalgae with different content of these two components, tested simultaneously on both species of sea urchin present in the Mediterranean Sea, *Paracentrotus lividus* and *Arbacia lixula*. The land-based experiment, located in the Marine Laboratory of Camogli (CNR-IBF, Italy), lasted three months, feeding the organisms exclusively fresh macroalgae, selected according to their different quantity of calcium carbonate and antioxidants. We tested: (1) *Ellissolandia elongata*, a red algae, whose articulated thallus is mainly made up of calcium carbonate, (2) *Padina pavonica*, a brown algae characterised by abundant depositions of calcium carbonate on the surface of the thallus and by the presence of antioxidants, (3) *Dictyota dichotoma,* also a brown algae, known to be rich in antioxidants. At the end of the experiment we measured for each specimen morpho-functional parameters (body, gonads, gut and lantern weight, test and lantern size and test robustness), which allowed us to calculate the Gonado-Somatic Index (GSI), the Jaw/Test ratio (JTR), Repletion Index (RI) and the Lantern Index (LI).

The results confirmed differences in food preference and tolerance to breeding conditions between the two species: *P. lividus* is less selective and more resistant to laboratory conditions compared to *A.lixula*. The algae supplied did not provide enough nourishment to *A. lixula*, which has a higher JTR (i.e. larger jaws compared to test dimension), especially if fed the brown algae. Test robustness of adult organisms did not seem to be influenced by the different macroalgal diets. In *P.lividus* it is possibly due to the fact that they were in the maturation phase of the gonads and invested more energy in that sector, rather than in growth. Therefore, using juvenile organisms is recommended to appreciate differences in the morpho-functional parameters in the short period. Alternatively, it is necessary to plan longer periods of exposure. Lastly, our data demonstrate that, in *P. lividus, E. elongata* diet, the calcified algae rich in calcium carbonate, seems to promote gonadal development. This supports the usefulness of adding biocarbonates to feeds used in aquaculture.

1. Introduction

Harvesting of different species of sea urchins as a food resource has been performed since the beginning of XVII sec., with dramatic ecological consequences worldwide. The world market demand of gonads has significantly increased since the early 70s (especially in Japan), with both the natural growth of the world population and the increasing interest in this delicacy (Williams, 2002; FAO, 2011). Sea urchin roe (gonad), which represents the edible part of the urchins, is a valuable excellence and it is appraised for both size and quality criteria (taste, firmness and colour). Interest in cultivation of sea urchins has increased over the last two decades due to the depletion of wild stocks as a result of overfishing (Sartori & Gaion, 2016). Despite good improvements in echinocolture, the market request is still satisfied primarily through harvesting in nature, since its production on an industrial scale remains marginal due to farming methods not yet economically sustainable for the producers (Ciriminna et al., 2020).

Along Mediterranean coasts, the most intensively exploited species is *Paracentrotus lividus*, whose reddish-orange gonads owe their quality to the high nutritional content, especially for the considerable presence of carotenoids (Sartori et al., 2015).

In Italy, despite the collection of this echinoid is regulated by law, *P. lividus* fishery is common in the southern regions and it is often performed throughout the year by illegal harvesters (Tortonese, 1965; Guidetti et al., 2004; Pais et al., 2012). As a consequence of this uncontrolled uptake, the population has been dramatically exploited in shallow subtidal rocky reefs.

According to the last data released by FAO (2013), the production of *P. lividus* through aquaculture systems is 10 t/year in Europe, and it has to sustain a landing activity of 108 t/year (Sartori et al. 2015). As a consequence, it is clear the urgent need to develop fast and reliable echinoculture strategies aimed to respond to this over-exploitation.

In this background an increasing number of studies aim to improve the echinoculture, analysing feeding strategies and diet formulation for optimizing gonad yield and quality and to fill the gap between the growing market request and the natural supply.

One of the limitations for its development is given by the fact that the gonads of the specimens reared with artificial feed do not have an acceptable quality (Shpigel et al., 2005), but also it requires a long time and critical steps to obtain marketable sized sea urchins (5 cm) and around 2-3 years for growing the new broodstock (Castilla-Gavilán et al, 2018; Aminur Rahman M. et al., 2014). In particular, settlement and metamorphosis (transition from planktonic larvae to benthic juveniles) are the most critical stages in the development and culture of sea urchin larvae (Aminur Rahman M. et al., 2014).

Several studies tested new types of artificial and sustainable food that determined a weight increase in edible tissues and preserved the organoleptic characteristics similar to those of the wild product (Prato et al., 2016, Prato et al., 2018, Vizzini et al., 2019, Sartori et al. 2015, Sartori & Gaion, 2016, Marta Castilla-Gavilán et al., 2019, Ciriminna et al., 2020, Brundu et al., 2016). These encouraging results indicate that food industry discards may be suitable alternative ingredients for the production of sustainable feeds for sea urchin aquaculture (Ciriminna et al., 2020).

Other studies focused on different aspects of *P. lividus* rearing, providing insights useful to develop echinoculture techniques. Grosjean et al. (1998) presented a 7-year experimental rearing method to produce the edible sea urchin *P. lividus* on a pilot scale, separating the whole rearing cycle into seven stages: (1) fertilization; (2) larval culture; (3) metamorphosis; (4) growth of juveniles; (5) growth of subadults and (6) growth of adults, which is further divided into (6a) conditioning for marketing of roe (exploitation), and (6b) providing gametes (broodstock). They performed all activities on land and analysed each stage of the rearing process. The results of this experiment are promising, finding that land-based, closed-cycle echinoculture is a potential viable supplement to fisheries to sustain worldwide sea urchin roe production.

In more recent times, Castilla-Gavilán et al. (2018) analysed growth, development, morphology and survival rate for sea urchin larvae and post-larvae, fed and reared with three microalgae species: *Isochrysis aff. galbana* and Rhodomonas sp. (usually used in oyster aquaculture), *Dunaliella tertiolecta*, one of the most common live feeds used in the larviculture of echinoids (Carboni et al., 2014). Testing four treatments (three monospecific diets and a combination of the three microalgae) their results showed a higher growth, development and survival rate when larvae were fed with *Rhodomonas sp*. or a combined diet, but *Rhodomonas sp*. presented nutritional advantages for sea urchin larvae: a rapid development, a higher lipid content and a lower investment for production. Prato et al. (2018), instead, showed that a diet composed of 50% of a pelletized diet and 50% of fresh Ulva sp. can bring to a significant increase of the gonado-somatic index of *P. lividus*.

All these studies provide quite encouraging insights in the implementation of *P. lividus* aquaculture in Mediterranean waters, to satisfy the increasing food request of the human population.

The present study is a contribution to the BRITEs Project (Byproduct Recycling: Innovative TEchnology from the Sea), funded by the PRIN program (Progetti di Rilevante Interesse Nazionale, MIUR).

The BRITEs project, involving the University of Padova, Milano and Genova, studies the exploitation of the sea urchin's waste from the restaurant/fish industry to generate valuable by-products. The two most important by-products that this project is going to exploit are: 1) biocarbonates to use in the production/supplementation of feed for fish and invertebrates, and 2) the extraction of collagen, an important protein widely used for the production of human skin substitutes. Through the enhancement and use of these by-products a circular economy would be supported, thanks to a better exploitation of the raw product, capable of increasing the commercial value of the exploited species.

The present study aims to improve the knowledge in the field of sea urchin breeding evaluating the role of calcium carbonate and antioxidants in the diet for this organism.

Particularly, we will evaluate possible effects of different macroalgal diets tested simultaneously on both species of sea urchin present in the Mediterranean Sea, naturally coexisting in the superficial rocky shores and in the *Posidonia oceanica* meadows (Chiantore et al., 2008, Privitera et al., 2008):

-*Paracentrotus lividus* (LAMARCK, 1816), commonly called "female sea urchin" or “Purple sea urchin”, is a sea urchin which belongs to the Parechinidae family;

- *Arbacia lixula* (LINNAEUS, 1758), “male sea urchin” or “black sea urchin”, is a sea urchin which belongs to the Arbaciidae family.

We select the macroalgae to use as feed according to their different compositional characteristics, in particular for the different quantity of calcium carbonate and antioxidants. Testing the role of calcium carbonate in sea urchin is really important since it is the chemical compound that mostly constitutes sea urchin test, and as such, fundamental for the growth, survival and welfare of the animal (Asnaghi et al., 2019).

The selected macroalgae are:

• *Ellissolandia elongata*, a red algae of the Corallinaceae family, whose articulated (geniculate) thallus is mainly made up of calcium carbonate.

• *Padina pavonica*, a brown algae of the Dictyotaceae family, is characterised by abundant depositions of calcium carbonate on the surface of the thallus.

• *Dictyota dichotoma,* also a brown algae of the Dictyotaceae family, is known to be rich in antioxidants.

We have chosen these species because they have different important functions, according to their composition, towards sea biota. Calcium carbonate plays a fundamental role in the chemical-physical equilibrium of the sea. The Corallinales are the predominant order of calcified macroalgae found in temperate waters (Williamson et al., 2014). The red *E. elongata* is an articulated calcareous species, whitish-pink to reddish-lilac, it has branching, pinnate flexible fronds and an erect thallus attached to the rocky substrate by a crustose holdfast. The fronds are made of small calcified segments (intergenicula), separated from uncalcified nodes (genicula), which provide flexibility to the erect algal thallus (Marchini et al., 2019).

P. pavonica is the only calcareous genus of the brown algae. As a member of the Dictyotales, it is widely distributed in warm, shallow seas. $CaCO_3$ is precipitated in the form of needle-shaped aragonite crystals. *P. pavonica* presents a biologically induced extracellular calcification, which results in whitish precipitations. These carbonate deposits are arranged in concentric bands on both thallus surfaces with interspaces where reproductive structures, such as tetrasporangia, can develop. The side of the blade facing the sea surface with the enrolled margins represents the “upper” surface and is more calcified than the opposite side, the “lower” surface (Iluz et al., 2017). Calcification of the frond amounts to approximately 11% content by dry weight (Benita et al., 2018). The calcium carbonate gives little support to the plants (Miyata, Okazaki & Furuya, 1977).

The content of antioxidants in seaweed is widely studied because of its therapeutic properties. The family of Dictyotaceae has been extensively studied for its wide variety of bioactive diterpenes with marked biological activities (Zubia et al., 2009).

D. dichotoma extract showed high total flavonoid and total alkaloid contents. Flavonoids have been reported to be antioxidants, scavengers of a wide range of ROS and inhibitors of lipid peroxidation, and also to be potential therapeutic agents against a wide variety of diseases (Rania et al.,2019). Antioxidants play a vital role against various diseases like cancer, cardiovascular diseases, inflammation, ageing process, rheumatoid arthritis, diabetes, as well as disease associated with cartilage and Alzheimer’s disease (Tariq et al., 2015).

Also the profiles of *P. pavonica*, evaluated using HPLC, reported to contain five phenolic compounds, suggesting using *Padina pavonica* as alternative for various diseases including diabetic, cardiovascular etc (Sudha & Balasundaram, 2018).

In light of the above, those macroalgae have been used to test the effect of different content of calcium carbonate and antioxidant in sea urchin diet, measuring different morpho-functional parameters indicative of animal health and development: weight, test and lantern size, robustness, jaw/test ratio, gonadosomatic index, repletion index, lantern index.

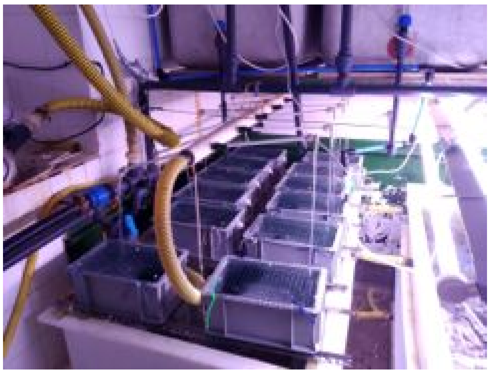
2. Materials and methods

2,1. Specimens collection and laboratory setup

We collected specimens of *A. lixula* and *P. lividus* in shallow rocky reefs, along the portion of the coast located between Genoa and the Portofino Promontory (GE). Sea urchins were carefully detached from the rocky substrate to avoid damaging spines and injury.

They were then quickly transported to the Marine Laboratory of Camogli (CNR-IBF), located in NW Mediterranean Sea, Italy, and allowed to acclimate for some days in aquaria to limit the shock due to sampling.

We randomly choose 24 specimens of *P. lividus* and 24 of *A. lixula*. The sea urchins were divided into 12 tanks of 3,7 liters each (4 individuals per tank), in order to have two replicates for each species, for each diet. The tanks were fed individually by a constant flow of sea water, in order to have independent replicates. The sea water was taken directly from the sea. The turnover of the water is 1 L /minute (around 3 minutes for a total turnover). Each tank was covered with a net in order to prevent urchin escape from it.



Prior to the start of the experiment, sea urchins were starved in the experimental setup for 2 weeks.

During the experimental period, temperature was measured continuously with a data logger, while salinity and oxygen concentration were monitored twice a week with a probe.

We collected the 3 species of the selected macroalgae (*Elissolandia elongata, Padina pavonica* and *Dictyota dichotoma*) with a weekly frequency, in the same coastal area where the sea urchin specimens were collected. The algae were stored in specific tanks filled with sea water and constantly oxygenated, in order to supply them to the sea urchin in conditions as similar as possible to natural ones.

The 9th of July (T0), we first measured for each specimen the body weight and the size (test diameter without spines, in triplicates) using a vernier calliper (±0.1 mm). Then we fed the sea urchin for the first time. After collecting numerous data from previous breeding experiments of these echinoderms (Prato et al.; 2018, Boudouresque and Verlaque, 2020; Grosjean et al., 1998), it was decided to feed each specimen with a daily amount of algae equal to 2% of their body weight.

We fed the sea urchins twice a week. Before feeding we cleaned each tank in order to remove the feces, and we weighed the quantity of the macroalgae not eaten. The food they discarded was thrown away in order to feed them with fresh algae.

The breeding of the specimens lasted 3 months, during which the health and well-being of the specimens was continuously monitored. We measured the test size (without spines) and the weight of each sea urchin 4 times during the experiment: at the beginning of the experiment (T0) and three further dates distant approximately one month each other (T1, T2 and T3). The T3 (6th of October) coincides with the end of the experiment.

Unfortunately, in the middle of the experiment an unexpected event occurred: the sewer of Camogli had troubles and the sea water pumped into the tanks was organically contaminated. The laboratory takes sea water directly from an area of sea that is not far from the sewer outlet. Some specimens died in a few days and others got sick and died one or two weeks later. *Arbacia lixula*, compared to *Paracentrotus lividus,* is the species most sensitive to organic pollution. At the end of the experiment we lost 75 % of *Arbacia lixula* (18 specimens) and 8 % of *Paracentrotus lividus* (2 specimens).

At the end of the three months breeding experiment, the sea urchins were moved to the Laboratory of the University of Genova to perform morpho-functional measurements.

Besides the total weight and the test diameter, we measured for each specimens the following parameters:

- Test robustness (g/mm)
- Aristotle's lantern weight (g)
- Aristotle's lantern size (mm)
- Test weight (with spines, g)
- Gonads weight (g)
- Gut weight (g)

Test robustness was measured in alive sea urchin specimens using a custom-made device designed to measure the static force required to crush sea urchin tests (used by Asnaghi et al., 2013, 2019). Sea urchins were positioned upside down (in order to mimic fish predator attack) in a glass column. Then, a hollow piston, built to fit and run within the column, was inserted inside the column and progressively filled with lead pellets in order to increase the pressure, until the crushing of the urchin test. The static force required to crush sea urchin tests was measured as the weight (g) of piston and lead added. Test robustness has been normalized dividing the fracture force/surface on which the force is applied.

To measure the length of the demi-pyramids of Aristotle's lantern, it was necessary to immerse the Aristotle's lantern in "Amuchina" Solution, which contains 2% of sodium hypochlorite, for 30 min, in order to remove organic matter and facilitate the separation of structural elements (according to Asnaghi et al., 2013).

The Aristotle’s lantern and the gonads were stored in a freezer at around -18°C, to preserve them for future studies.

The data collected allowed us to calculate four important indexes:

1. Jaw/Test ratio (JTR): the ratio between the length of the jaw pyramids of the Aristotle’s lantern and the diameter of the test, expressed in %.
2. Gonado-somatic index (GSI): the ratio between the gonad weight and the total body weight, expressed in %.
3. Repletion index (RI): the ratio between the gut weight and the total body weight, expressed in %.
4. Lantern index (LI): the ratio between the lantern weight and the total body weight, expressed in %.

2,2. Statistical analyses

The measured morpho-functional features (*i.e.* gonadosomatic index, repletion index, test robustness and jaw-test ratio), have been used to perform multivariate analyses. We calculated the triangular matrix of dissimilarity using the Euclidean distance.

Potential differences between the two sea urchin species due to algal diet were tested through PERMANOVA, using the factor Species (2 levels), the factor Algal diet (3 levels) and their interaction.

Results are visually displayed through the MDS (Multidimensional scaling) plot, a multivariate technique that enables visualizing the level of similarity of individual cases of a dataset.

All statistical analyses were performed using R software.

3. Results

The multivariate analyses highlighted differences between the two sea urchin species and an effect of Algal diets.

3.1. MDS ordination

The plot in Figure 1 shows the results of MDS analysis. It is clear that the 2 species *Arbacia lixula* and *Paracentrotus lividus* are well separated in two groups.

Regarding the Algal diets, the plot shows a net division between *Ellisolandia elongata* and *Padina pavonica* , whereas *Dictyota dichotoma* is more dispersed in the plot.

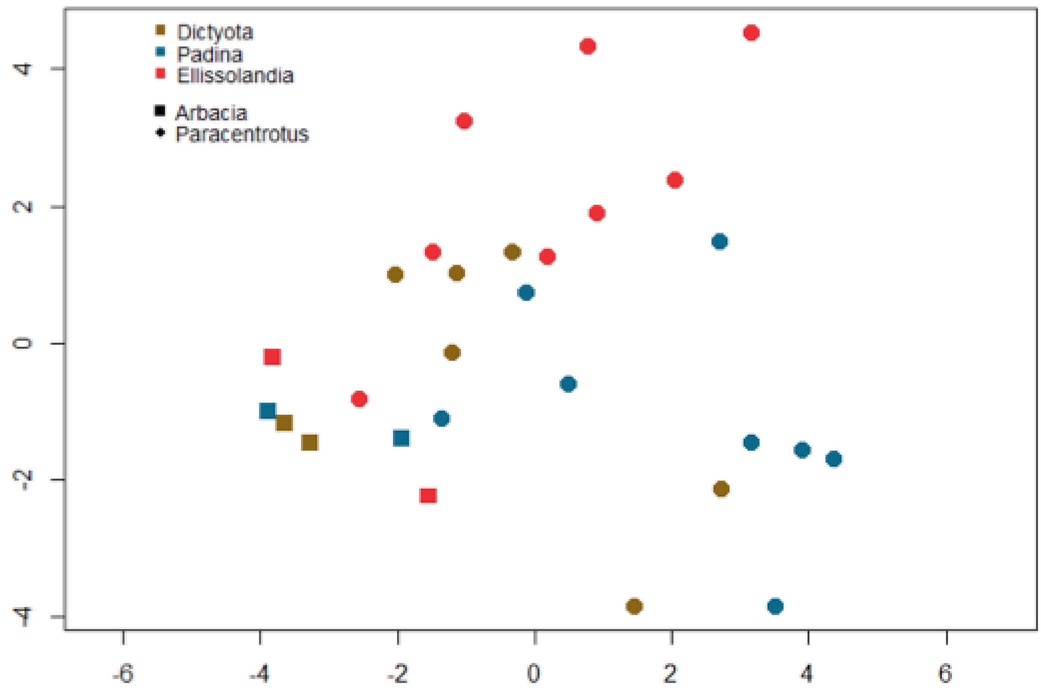
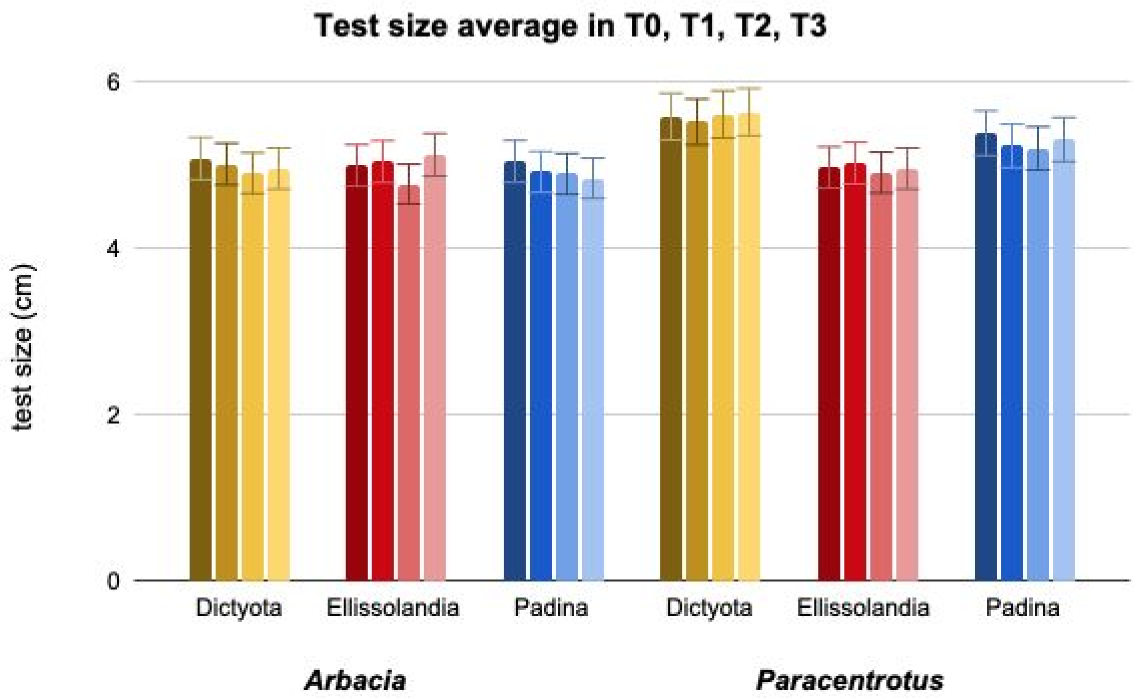


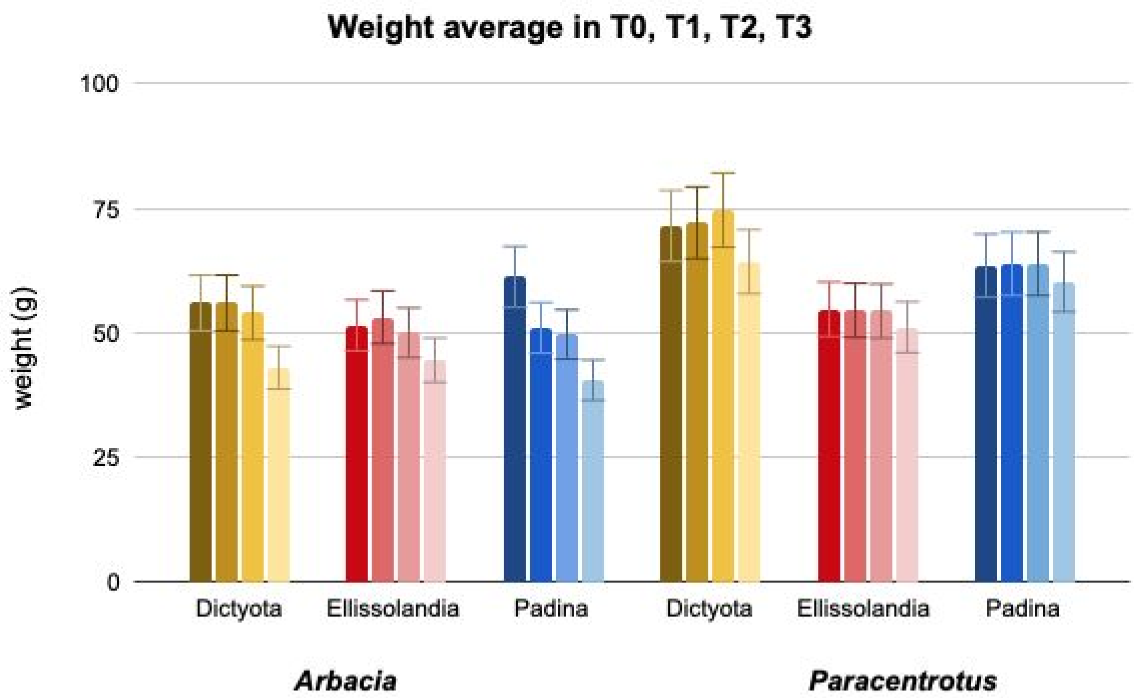
Figure 1

3,2. Test size and weight

We measured the test diameter and the weight of each specimen monthly during the experiment (T0= start of experiments, T1, T2 and T3 with a month of distance).

The specimens did not vary significantly their size during the experiment, as it is shown in the following graphs. This can be expected since we used adult specimens and we performed the experiment during the summer season, when organisms consume more energy for feeding and grow less.





3,3. Algal consumption

The feeding day we also weighed the quantity of macroalgae not eaten from the sea urchin in each tank. Figure 2 shows the box-plot of the algal consumption, that means the percentage of algaes consumed from the specimens during the experiment.

Arbacia lixula left much more food compared to *Paracentrotus lividus.* The algal species preferred by *A. lixula* is *Elissolandia elongata*, followed by *Padina pavonica*. *Dictyota dichotoma* is the most rejected species.

P. lividus results instead to consume all the food supplied, except occasional events.

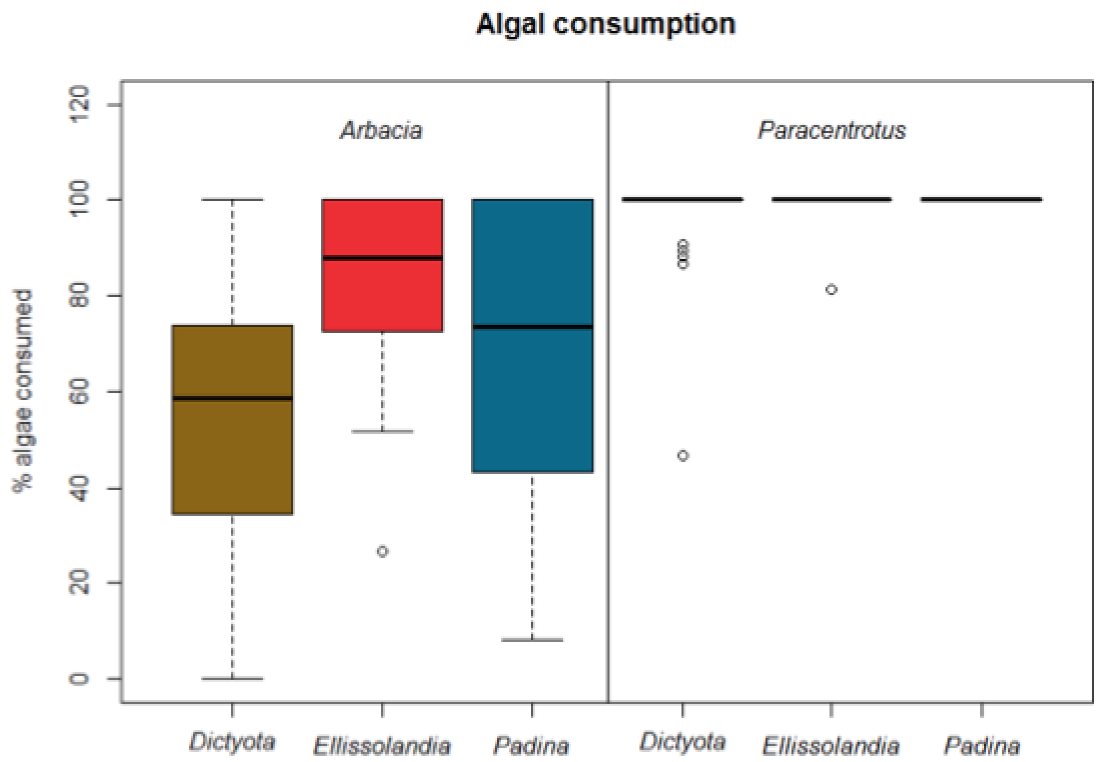


Figure 2

3,4. Gonado-Somatic Index

The box-plot in Figure 3 shows the Gonado-Somatic Index calculated for each specimen of the two species after 3 month of breeding.

There is a clear difference between the two species of sea urchin: *P. lividus* has gonads much more developed compared to *A. lixula.*

Furthermore, for both species, *the Elissolandia elongata* diet confers more development of gonads.

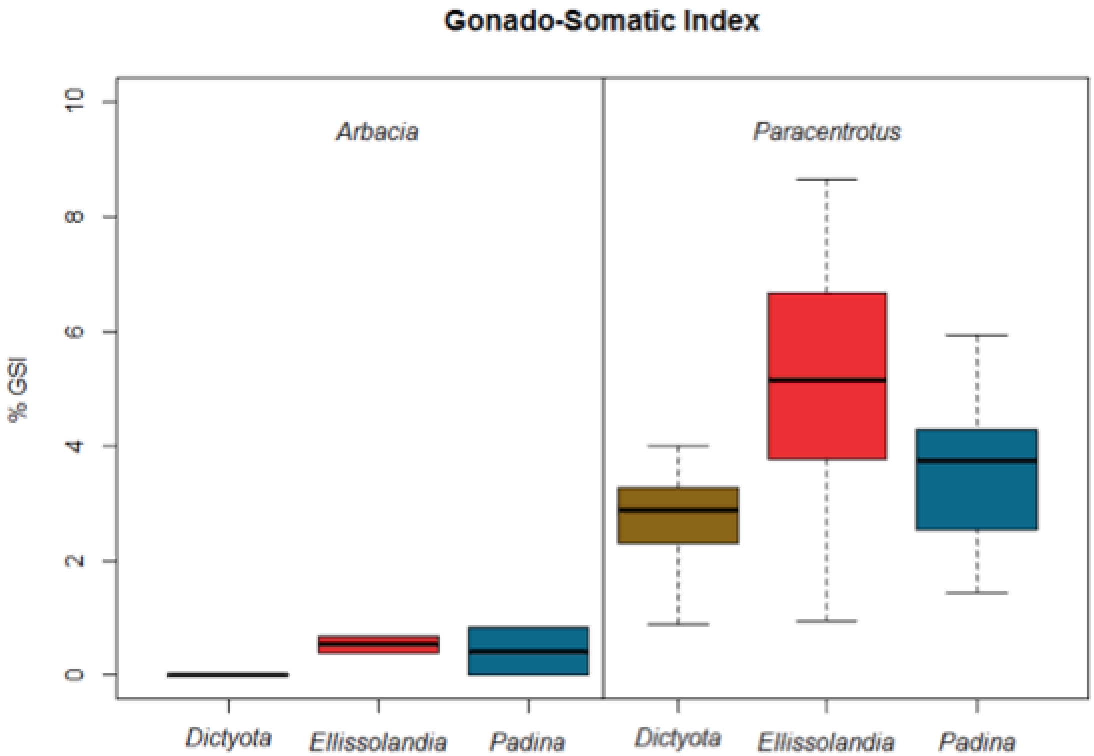


Figure 3

3,5. Repletion Index

The Repletion Index (RI) is the ratio between the gut weight and the total body weight. The box-plot in Figure 4 shows that *A. lixula* has similar values for the three diets, instead *P. lividus* presents higher values of RI for *P. pavonica* diet.

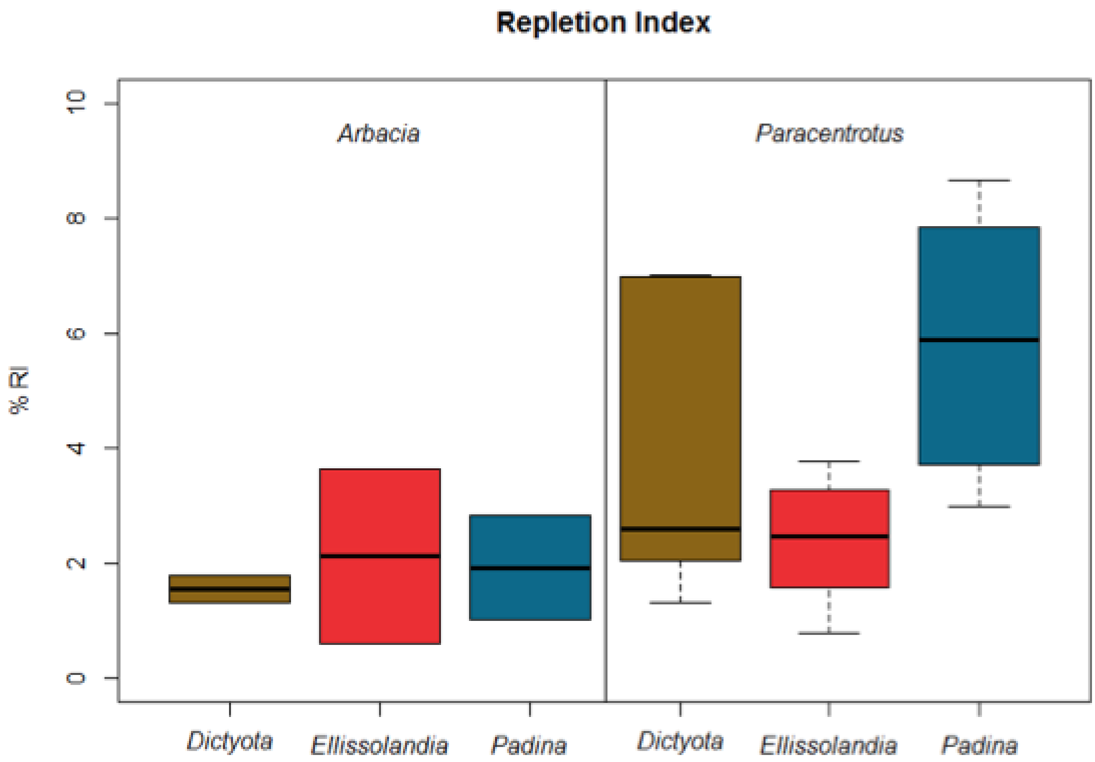


Figure 4

3,6. Test robustness

The box-plot in figure 5 shows the test robustness of sea urchin. Contrary to what expected, *E. elongata* diet (the diet with more calcium carbonate content) does not lead to higher test robustness.

A. lixula shows similar value with D*. dichotoma* and *E. elongata* diets, higher than the one of urchins fed *P. pavonica*. *P. lividus* has higher values with D*. dichotoma* diet.

For both species *P. pavonica* diet leads to lower values of test robustness.

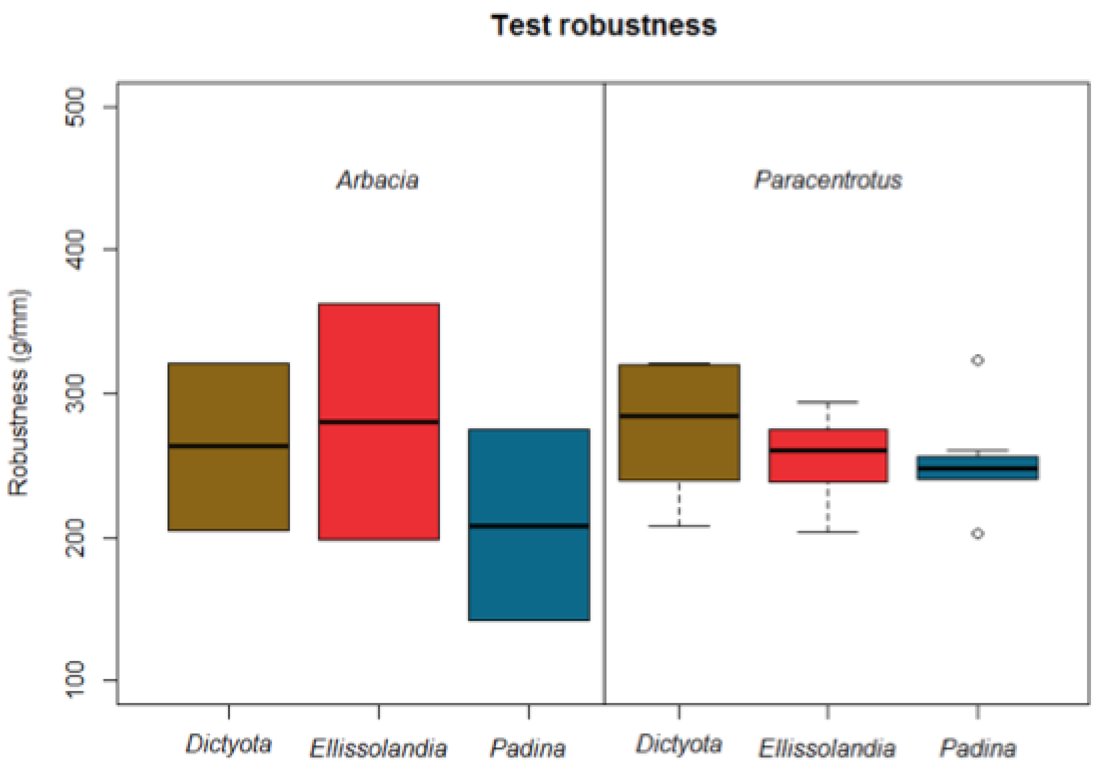


Figure 5

3,7. Jaw/Test ratio

The Jaw/Test ratio (JTR) indicates the ratio between the length of the jaw pyramids of the Aristotle's lantern and the diameter of the test. It indicates the growth of the "chewing apparatus" in respect to body dimension during the 3 months breeding experiment.

Figure 6 shows the value of JTR for the two species, at the end of the experiment. It exhibits higher values for *A. lixula,* especially for the specimens fed *D. dichotoma* diet.

Regarding *P. lividus,* there are very small differences among the three diets.

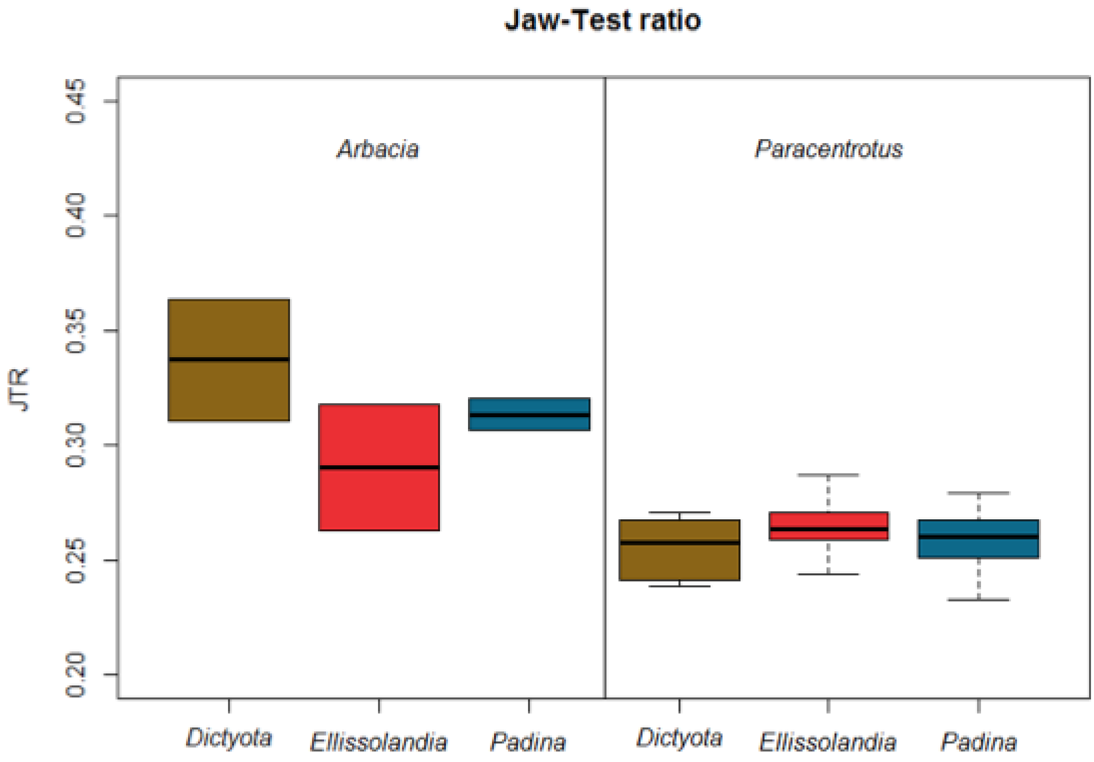


Figure 6

4. Discussion

Sea urchins' breeding have been widely investigated in the last year (Carboni et al., 2014, Marta Castilla-Gavilán et al., 2019, DeVries Maya S et al., 2019, Grosjean et al., 1998, Prato et al., 2018, Privitera et al., 2008, Vielmini at al., 2005), mainly because the demand as a food resource has increased and at the same time the natural stocks have dramatically dropped (Williams, 2002; FAO, 2011).

Supplying the human demand of sea urchin is not easy since the echinoculture presents some limits compared to fish breeding: the gonads of the specimens reared with artificial feed usually do not have an acceptable quality (Shpigel et al., 2005), and also it requires 2-3 years to obtain marketable sized sea urchins (5 cm). The fish breeding allows to obtain the marketable size with only few months, in an easier way since the development of a fish does not require a larval settlement.

The natural stocks of sea urchins are threatened both for the human demand, both for climate changes, as acidification of the oceans. Sea urchins have carbonate structures (skeleton and grazing apparatus) made up of the very soluble high-magnesium calcite, particularly sensitive to a decrease in pH. The biomechanical properties of their skeletal structures are of great importance for their individual fitness, because the skeleton provides the means for locomotion, grazing and protection from predators (Asnaghi et al., 2019).

In a future scenario of ocean acidification a decrease of sea urchins' density is expected, due to lower defense from predation, as a direct consequence of pH decrease, and to a reduced availability of calcifying macroalgae, important component of urchins' diet (Asnaghi et al., 2013).

In the present study, we confirmed the importance of calcifying macroalgae in sea urchin diet, since they are the species which lead to the highest value of Gonado-Somatic Index and the most appreciated from both species of sea urchin studied.

Most of the breeding studies focus on finding an artificial diet able to confer tasty, big and well-coloured gonads and analysing the growth stages of sea urchins.

Recent studies proved that gonads of *Paracentrotus lividus*, fed new sustainable feed produced with cheap and recycled ingredients obtained from farming discards (egg white was added as protein source), achieved a good colour and high quality, hence representing a commercially valuable product (Vizzini et al., 2019). Another study tested two experimental formulations, obtained using discarded endive (*Cichorium endivia*) leaves and anchovy (*Engraulis encrasicolus*) industry discards in different proportions, and agar as a binder. At the end of the experiment, an increase in gonado-somatic index was recorded.

We chose to use fresh macroalgae as diet in order to reproduce their natural food, providing different amount of calcium carbonate and antioxidant according to algal characteristics.

Another study confirmed the suitability of *P. palmata* and fresh diets for *P. lividus* nutrition, but without denying the great growth performances of sea urchins when these diets are replaced with dried macroalgae. These feeds could therefore be used by farmers without detriment to production, especially during short periods when, for example, the algal biomass is less abundant. Further investigations could be done on the effect of these diets on the sea urchin gonad enhancement and quality. However, for a fattening phase of the production cycle, the lower cost of feeding with natural dried algae compared to the cost of formulated diets could allow small producers to diversify their activities (Marta Castilla-Gavilán et al., 2019).

During our three months breeding experiment *A. lixula* seems to be the species more sensitive to organic pollution, since we lost much more specimens of this species as a consequence of a sewer contamination of the laboratory seawater pump. Studies have already shown that these two species can have different degrees of pollutant bioaccumulation while they develop in polluted sediment, with *A. lixula* showing slightly higher sensitivity to heavy metals than *P. lividus* (e.g. Carballeira et al., 2011, 2012; Portocali et al., 1997).

4,1. Gonado-Somatic Index

The analysis of the Gonado-Somatic Index is really important since the main purpose of echinoculture is to find the best way to generate sea urchin with big and tasty gonads. In our study *Ellisolandia elongata* diet is the one performing better to reach this goal, compared to the other diets tested. For both species we calculated higher values of GSI with a calcified macroalgae diet: the GSI is higher for *E. elongata*, lower for *P. pavonica* and much lower for *D. dichotoma* diet.

The values obtained with our study show that the Gonado-Somatic Index is very different between the two species. For both species the higher values are obtained with *E. elongata* diet, but *A. lixula* presents much lower values for all diets compared to *P. lividus*.

The explanation of this difference is the period of gonads maturation of the two species.For P. lividus, Guettaf et al. (2000) showed that the maximum values of GSI were found in August. The gonadosomatic index of *A. lixula* follows a seasonal cycle which peaks in May-July and attains its lowest values in October-November every year (Wangensteen et al., 2013). Another study confirmed that the GSI for *Arbacia lixula* peaked during May-June-July in the French Riviera (Fenaux, 1968).

We measured the GSI in the first week of October, therefore for *P. lividus* this period coincides with the maximum gonadal maturation, whereas for *A. lixula* is the lowest period. This explains and confirms the big differences of GSI values between the two species.

4,2. Repletion Index and algal consumption

The dietary preferences of the two species of sea urchins are usually studied analysing the gut contents (Privitera et al.,2008, Vielmini at al., 2005). *A. lixula* guts have been found to be dominated by encrusting corallines, while *P. lividus* by non-encrusting macrophytes (Privitera et al.,2008).

Assessing the values of the Repletion Index (ratio between the gut weight and the total body weight) of our experiment, we can observe similar results: *A. lixula* has similar values for the three diets, slightly higher for *E. elongata* diet and lower for *D. dichotoma* diet, instead *P. lividus* presents much higher values of RI for *P. pavonica* diet.

The RI values for all diets are much lower for *A. lixula* compared to *P. lividus:* this data reflect the algal consumption during the rearing experiment, since the RI is the weight of the gut and therefore represents the food inside the gut.

A. lixula is the species that rejected much more food, especially *D. dichotoma* diet and the lower values of RI is exactly for this macroalgae. *E. elongata* is the most consumed diet, and the RI of *A. lixula* is higher for this diet.

Regarding *P. lividus* it was expected no difference of the RI for the different diets, since he consumed almost all the food supplied during the experiment. On the contrary the values of RI for *P. lividus* are much higher for *P. pavonica* diet, therefore we can affirm that the *P. pavonica* is the algal that remains for more time in the gut. Hence it should mean it is a macroalgae difficult to digest. We can hypothesize that a reason for this feature is the composition of this algae: *P. pavonica* is a brown algae with also a little calcification which could complicate its digestion. It is necessary to do further analysis of the gut contents in order to have more information about this index.

4,3. Test robustness

Assessing test robustness is really important, since this parameter shows how a specimen of sea urchin is able to defend from predator attacks. Asnaghi et al., 2013 showed that sea urchins fed the more calcified macroalga sustain higher forces before breakage than the one fed the non-calcified algae.

Our analysis of the test robustness shows values different to what expected. For both species *P. pavonica* diet leads to lower values of test robustness. *A. lixula* shows similar value with D. *dichotoma* and *E. elongata* diets, while *P. lividus* has higher values of robustness with D*. dichotoma* diet.

Regarding *P. lividus,* the test robustness does not seem to be influenced by the different macroalgal diets, maybe because this species, being in the maturation phase of the gonads, has invested more energy in that sector, rather than in growth.

Moreover, these results of test robustness are probably due to the size of the sea urchins tested. Since we did the experiment with adult specimens, it is not easy to observe differences in test robustness with short term exposure to experimental treatments. Differences in this parameter could have been visible if the specimens were younger, and so they significantly grew their tests during the experimental period. With adult specimens, in only three months of experiment, the differences in this feature is unfortunately not evident.

4,4. Jaw/Test ratio

The Jaw/Test ratio defines how much the grazing apparatus is developed relative to the size of the specimen. The jaw of the sea urchin is composed of 5 demi-pyramids and is called Aristotle's Lantern.

The lengths of the jaws (demi-pyramids) change relative to test diameter in response to variation in food abundance, whereby jaw length becomes longer when food is limited (DeVries Maya S. et al., 2019).

A. lixula is a more selective species, usually grazing on calcareous algae, and has commonly bigger jaws compared to *P. lividus*. In our study *A. lixula* shows, indeed, higher values of Jaw/Test ratio compared to *P. lividus*, especially for *D. dichotoma* diet. This reflects the algal consumption during the experiment: *A. lixula* rejected a big part of the food supplied, in particular *D. dichotoma* diet. Hence the algae supplied do not provide enough nourishment to *A. lixula*, which develops a higher JTR, especially if fed with brown algae.

Instead, regarding *P. lividus,* the results of JTR do not show differences between the three diets. Conversely, other studies showed that the JTR of *P. lividus* changes according to the diet: it is hypothesized that *P. lividus* juveniles need a larger grazing apparatus (relative to the body size) when fed more calcified algae (Asnaghi et al., 2013). They develop a bigger grazing apparatus in such a way that they have more strength to chew the calcified thalli of macroalgae as *E. elongata.*

Our experiment does not show these differences in the jaws of urchins fed the calcified macroalgae diet. The reason could be the same as the test robustness results, since the Jaw/Test ratio is another parameter that needs time to be appreciated in terms of variability in response to different diets.. Therefore, a three month breeding experiment with adult specimens is not enough to study the effect of different diets on jaw lengths.

5. Conclusions and future outlook

The increasing demand for sea urchin gonads has paved the way for further research and exploration of echinoculture as an alternative to natural stocks harvesting, since this species is in serious danger.

In the past decade significant advances have been made: prepared diets for echinoculture to reach high gonads quality have been found, studies on larvae stages defined how to obtain an adult sea urchin in land-based system and at the same time some projects also looked for a way to produce new feeds recycling industry wastes, including the big quantity of sea urchin wastes discarded from restaurants, in a circular economy perspective.

A circular economy is an economic system aimed at eliminating waste and the continual use of resources. The circular, or ecological, economy employs reuse, sharing, repair, remanufacturing and recycling to create a closed-loop system. All "waste" should become "food" for another process. This regenerative approach is in contrast to the traditional linear economy, which has a "take, make, dispose" model of production.

The scientific research aims to find innovative ways to create a circular economy (Vizzini et al., 2019, Ciriminna et al., 2020), hence with our study we would contribute to improve the efficiency of echinoculture and, at the same time, provide to another project (BRITEs Project) useful information to exploit sea urchin waste.

Results obtained with the present experiment provide useful information about echinoculture: we confirm the good performance of the *E. elongata* diet which, despite the short experimental period, leads to high value of GSI, and therefore this calcified algae rich in calcium carbonate promotes gonadal development. This supports the usefulness of adding biocarbonates to the feeds used in aquaculture and could be considered in the identification of diets for sea urchin breeding.

This study also highlights the importance of considering the period of maximum gonadal development: for *P. lividus* we confirm an elevated development of gonads in August/ October, therefore this could represent a good period to exploit sea urchin for the gonads requests.

Moreover the present work, born in the context of the BRITEs Project, is a first experiment that allows to understand some features of the sea urchin breeding that will be used in the future steps of this project.

The two species are different both for the food preference and for the breeding resistance: *A.lixula* rejected a big part of the food supplied, mostly *D.dichotoma* diet, and is more sensitive to organic pollution. The algae supplied do not provide enough nourishment to *A. lixula*, which has a higher JTR, especially if fed with brown algae. Hence we confirm the suitability of *P. lividus* for breeding, which resulted the species being more tolerant and less selective.

Test robustness of adult organisms does not seem to be influenced by the different macroalgal diets, maybe because *P.lividus*, being in the maturation phase of the

gonads, has invested more energy in that sector, rather than in growth. Therefore using juvenile organisms is preferable to appreciate differences in the morpho-functional parameters in the short period. Alternatively, it is necessary to plan longer periods of exposure.
For a future perspective further experiments with juvenile specimens are necessary to assess the effect of different diets on the variation of Test robustness.

We are confident that the echinoculture, through scientific research, will reach an adequate improvement able to satisfy, almost in part, the demand of sea urchin gonads and to slow down the decline in natural stocks of sea urchins.

Also the profiles of *P. pavonica*, evaluated using HPLC, reported to contain five phenolic compounds, suggesting using *Padina pavonica* as alternative for various diseases including diabetic, cardiovascular etc (Sudha & Balasundaram, 2018).

In light of the above, at the end of the experiment we measured different morpho-functional parameters and index for each specimen: weight, test and lantern size, robustness, jaw/test ratio, gonadosomatic index, repletion index, lantern index. Analysing the results we assessed the difference between the two species fed different macroalgal diets, keeping in mind the species and macroalgaes features.

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