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FORMULATION OF EXPERIMENTAL FEEDS FOR AQUACULTURE OF THE SEA URCHIN *Paracentrotus lividus* (LAMARCK, 1816), IN A CIRCULAR ECONOMIC PERSPECTIVE

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ABSTRACT

The classical linear economy model based on extraction, transformation, production and waste seems no longer functional in a world where natural resources are beginning to be depleted. Through the circular economy model, the concept of a green economy based on a more eco-sustainable vision can be supported. For these reasons, the aim of this study is to assert the concept of circular economy by providing a concrete example of reusing waste of the sea urchin *Paracentrotus lividus* from the catering service industry, thus valorising waste by-products to create new, eco-sustainable feeds for sea urchins with benefits for the urchin aquaculture and the reduction of wates.

This work is a contribution to the BRITEs (Byproduct Recycling: Innovative TEchnology from the Sea) project. The aim of BRITEs project is to reuse sea urchin waste from the catering and fishing industry to generate valuable by-products, such as biocarbonates, used in the production/integration of fish and invertebrate feed, and collagen, a structural protein that can be used in tissue engineering/regenerative medicine applications.

The present study evaluated the performance of two pelletised feeds, formulated in the laboratory: one using as source of carbonates the grinded test of the urchins derived from catering service industry, while the other with inorganic carbonate. The two pelletised diets were compared between each other and with a control diet based on marcoalgae (i.e., *Ulva* spp. and *Ellisolandia elongata*) in terms of somatic and gonadal development. In addition, the total protein and lipid contents of the gonads of the sea urchin *Paracentrotus lividus* were determined.

The study was conducted for 2 months and in two different seasonal periods, June-August and October-December, in a specially developed indoor marine system located in the Laboratorio Marino of Camogli (CNR-IBF, Italy).

The use of artificial diets per se can be advantageous because they have the potential to provide predictable growth performance. Artificial diets based on environmentally sustainable feed, using ingredients derived from fish waste or parts thereof and/or from the catering and fishing industry, provide the additional advantage of generating a sustainable full-cycle production of *P. lividus*. Furthermore, sustainable full-cycle sea urchin production will potentially benefit from a reduction in the time taken during the growth phase by increasing growth rates of juvenile sea urchins.

The results of the present study show an efficacy of the two formulated diets, even greater in the one containing sea urchin flour (FSU) which produces larger somatic growth and gonadosomatic index already in the short term. In addition, in summer, sea urchins fed formulated feeds, in particular FSU, have a higher lipid content in the gonads, an aspect certainly appreciated by consumers, as it provides more flavour and taste to these edible parts. Considering that the FSU feed, in addition to containing biocarbonates is also rich in the main macronutrients, such as proteins, lipids, carbohydrates and fibers, the use of an artificial diet can be a consistent and economically convenient tool in the implementation of closed echinoculture systems.

This improvement in sea urchin aquaculture can represent an important step forward in the protection of *Paracentrotus lividus*, affected by the depletion of wild stocks, to meet the growing demand of the gonad market. In addition, the reuse of sea urchin waste, besides improving waste management and valorisation, is a valid tool to reduce the environmental impact of fishing.

For all these reasons, this study is part of the circular economy model, with particular regard to the protection of the seas, oceans, biodiversity and the exploitation of marine environmental resources, which through technological innovation (blue biotechnologies) favor the reuse of resources and the creation of a sustainable economic system in waste management.

INTRODUCTION

Nowadays, with the increase in fish consumption, most of the wild fish stocks are classified as fully exploited, with several species threatened with extinction (Delgado, CL et al., 2003). In addition, a considerable share of waste is represented by discarded fish, which includes many species and by-catch (Caruso, G., 2015). It is estimated that fishery waste exceeds 20 million tonnes per year, about 25% of the annual global harvest (Kim, SK & Mendis, E., 2006; Caruso, G., 2015) It is therefore an increasingly emerging problem, not least because fish industries are suspected to be responsible for significant environmental risks (Arvanitoyannis, SI et al., 2014). Fish wastage occurs for several reasons: products caught but not sold because of low commercial value, damaged goods, inadequate supply chain management, improper storage of the product, and finally waste from domestic consumption. For example, the consumption of species such as sea urchins, generates abundant waste due to the high content of inedible parts such as tests, spines and viscera (Raman, M. & Gopakumar, K., 2018). Failure to manage fish waste and overfishing have several negative implications on ecosystems and marine species protection (*e.g.* habitat damage, illegal fishing, endangered fish species).

The circular economy (CE) is not yet a common economic model. This is due to application limitations such as, the impossibility of recycling all waste, as the recycling and recovery process of waste materials requires additional energy (Georgescu-Roegen, N., 1995). Moreover, the recovery of waste and related materials will never be complete, but will produce other waste or by-products. A solution to this problem, at least partly theoretical, could be to always use renewable energy from the sun, for the entire recycling process. This solution, however, would require large investments and much more work to complete the entire cycle.
In an idealised situation, the physical material and energy flows recovered in the CE model would reduce the use of new raw materials, energy inputs, waste management costs and waste generation (Korhonen, J. et all., 2018). As a result, there would be a number of cascading benefits, from reduced raw material and energy costs, to diversification of reuse along the value chain. This would trigger new job opportunities and growth in the sense of community, with the birth and/or conversion of companies projected towards a sharing economy and an eco-friendly vision.
The circular economy, therefore, remains a widely debated topic among economists and academics, however, among the most optimistic visions it seems to have win-win potential for three dimensions of sustainable development: economic, environmental and social.

Thus, the reuse of sea urchin waste is a clear example of how a circular economy model can be adopted and is a valuable tool to reduce the environmental impact of fishing (Caruso, G., 2015). In addition improving the management of waste and its reuse to obtain biomaterials or energy sources, it is also possible to maximise the value of food production and reduce waste; it also promotes new forms of sustainable enterprise through a more virtuous economy.

The harvesting of different species of sea urchins as a food resource has been carried out since the beginning of the 17th century, causing a dramatic impoverishment of populations, with cascading ecological consequences worldwide. The world market demand for gonads has increased significantly since the early 1970s (especially in Japan), both with the natural growth of the world population and the growing interest in this delicacy (Williams, 2002; FAO, 2011). Sea urchin roe (gonad), which is the edible part of the urchin, is a prized delicacy and is valued both for its size and quality criteria (taste, texture and colour). Interest in sea urchin cultivation has increased in the last two decades due to the depletion of wild stocks due to over-exploitation (Sartori & Gaion, 2016). Despite good improvements in echinoculture, market demand is still mainly met through wild harvesting, as its

production on an industrial scale remains marginal due to cultivation methods that are not yet economically viable for producers (Ciriminna et al., 2020). Along the Mediterranean coasts, the most intensively exploited species is *Paracentrotus lividus*, whose orange-red gonads owe their quality to their high nutritional content, especially due to the considerable presence of carotenoids (Sartori et al., 2015). In Italy, although the harvesting of this echinoid is regulated by the D.M. of 12 July 1995, *P. lividus* exploitation is widespread in the southern regions and is often practised throughout the year by illegal gatherers (Tortonese, 1965; Guidetti et al., 2004; Pais et al., 2012). In addition to uncontrolled harvesting and overexploitation of these species, climate change, such as ocean acidification, has caused the reduction of sea urchin populations from previously abundant areas, especially in the shallow subtidal rocky reefs of the Mediterranean (Cohen-Rengifo M. et al., 2013; Dupont S. & Thorndyke M., 2013).
Consequently, there is an urgent need to develop good breeding and feeding strategies to respond to these problems.
According to the latest data released by the FAO (2013), the production of *P. lividus* through aquaculture systems is 10 t/year in Europe, and must support a landing activity of 108 t/year (Sartori et al. 2015). The main objective of echinoculture is to bridge the gap between supply and demand for eggs (Pearce C. M., 2002), providing the market with edible sea urchins with excellent gonads throughout the year. One of the limitations to its development is that the gonads of specimens reared on artificial feed are usually not of acceptable quality (Shpigel et al., 2005). Aquaculture also requires a long time with critical steps to obtain market-size sea urchins (5 cm) and about 2-3 years to rear new spawners (Castilla-Gavilán et al, 2018; Aminur Rahman M. et al., 2014). In fact, *P. lividus* is a slow-growing species (Frantzis et al., 1988; Bouderesque and Verlaque, 2013) with a maximum gonadal yield (measured as gonado-somatic index, GSI) of adult sea urchins ranging between 15% (Machado et al., 2019) and 18% (Rocha et al., 2019). In the wild, it can take up to 5 years to reach a test diameter (TD) > 45 mm, with a maximum growth rate between 5.8 and 7.7 mm year $^{-1}$ (Turon et al., 1995). To address these two bottlenecks research has followed two parallel paths: improving the gonad quality of wild caught sea urchins; and developing economically viable full-cycle production. In both cases, it is essential to develop cost-effective artificial feeds that meet the nutritional requirements of the species for achieving: i) successful offspring production; ii) high somatic growth rates during the growth phase; and iii) high commercial quality of sea urchin roe (Pearce et al., 2002; McBride, 2005; Schlosser et al., 2005; Bouderesque and Verlaque, 2013). The optimal diet should promote good somatic and gonadal growth with a long shelf-life (Pearce et al., 2002), but also produce gonads with colour, flavour and texture suitable for the market (McBride et. al., 2004; Shpigel et al., 2005).

Wild seaweed is currently harvested to feed farmed sea urchins, however, this practice is not environmentally sustainable and should therefore be avoided and replaced by artificial seaweed farms or feeds (Carboni et al., 2013). The development of artificial diets represents the main challenge for viable sea urchin aquaculture (Pearce et al., 2002).

Trials conducted on different sea urchin species have shown faster gonad growth when using formulated feeds compared to natural algal feeding (Lawrence et al., 1992; Hiratsuka and Uehara, 2007; Siikavuopio et al., 2012). In particular, for *P. lividus*, Prato et al. (2018) showed that a diet consisting of 50% Ulva sp. and 50% pelletised diet can lead to a significant increase in gonado-somatic index.
On the other hand, several studies have tested new artificial and sustainable food types, which resulted in an increase in edible tissue weight and preserved 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 G. et al., 2016).
The nutritional value and feed type of diets offered to sea urchins also have a high impact on feed intake and feed conversion ratio (Fernandez and Boudouresque, 2000; Spirlet et al., 2001; Prato et al., 2017). Like other organisms, *P. lividus* regulates feed intake to meet physiological needs regardless of the protein or energy level of the diet (Heflin et al., 2016; Lourenço et al., 2020).
All these studies provide quite promising insights into the implementation of aquaculture and improved feeding strategies of *Paracentrotus lividus* to meet the growing dietary demands of the human population.

The current work is a contribution to the BRITEs Project (Byproduct Recycling: Innovative TEchnology from the Sea), funded by the PRIN programme (Projects of Significant National Interest, MIUR), and involving the Universities of Padua, Milan and Genoa. BRITEs project is investigating how to re-use sea urchin waste from the catering/fishing industry to generate valuable by-products, such as biocarbonates, to be used in the production/integration of fish and invertebrate feed, and collagen, to produce two-layer skin substitutes (epidermis and dermis), useful to accelerate the healing of wounds and burns (Ferrario C. et al, 2020).
Marine collagen is very similar to that found in human tissues such as bones, blood vessels, ligaments and skin. With regard to the mammalian source currently used (bovine, equine, porcine), most collagen is obtained after hydrolysis, a process that partially 'destroys' the molecular structure, thus reducing its mechanical strength. The positive aspect of sea urchin collagen is that it is obtained without the need for destructive methods, which allows its structural integrity and thus its mechanical performance to be preserved, a key element in the design of biomaterials. Due to its characteristics, marine collagen is a valuable support for the production of biomaterials, biomedical devices, dermal implants, cosmetic and pharmaceutical products.
In recent years, the production of films from natural polymers has increased significantly in the food industry as an alternative to petroleum-based synthetic films. According to recent scientific studies, (Somia Hamil et al., 2020), sea urchin powder has been incorporated into chitosan films, as it provides hydrophobicity properties and results in improved antioxidant and thermal stability of the films.

Considering that the edible portion of *P. lividus* is limited to the reproductive organs (the gonads), the waste constitutes the majority fraction (up to about about 90% of the organism), producing a considerable amount of waste.
In particular, the sea urchin test, made up of calcium carbonate, represent the highest waste fraction. Using the biocarbonate derived from grounding this fraction, a pelletised biocarbonate-based feed was prepared in the laboratory.
The objective of this study was to evaluate the performance of this bio-pellet/feed, supplemented by organic carbonate (FSU), compared to another pelletised diet also prepared in the laboratory, having the same composition but supplemented by inorganic carbonate (FSC). The two pelletised diets were then compared to a control diet made up of two fresh macoralgae (50% *Ulva* sp. and 50% *Ellisolandia elongata*).
The performance of the provided diets was evaluated by means of morpho-functional parameters of the organisms and biochemical analysis of the total protein and lipid content of the gonads.

2. MATERIAL AND METHODS

2.1. Specimens collection and laboratory setup

A total of 144 *Paracentrotus lividus* specimens were collected manually (carefully detached from the rocky substrate to avoid damage and injury) in shallow rocky cliffs in front of Vernazzola (Genova, Italy, north-western Mediterranean Sea). Sea urchins were chosen to be relatively uniform in size (mean diameter 2.77mm ± 0.11 SD) and presumably in age, to minimise variation in growth potential, potential feed consumption and initial gonad weight. The decision to use small-size sea urchins is also due to the expected faster growth rate, possibly detectable in a short term experiment.
Two sets of 72 specimens were collected, respectively on 25 May 2022 and 10 October 2022.
These specimens were then rapidly transported in seawater soaked tissues to the Camogli Marine Laboratory (CNR-IBF), around 20 km form the sampling location, and left to acclimatise for a few days in aquaria.
After the acclimatisation period, the urchins were not fed to standardise the animals' appetite levels as reported in the study by *Pearce et al. 2002*.

Sea urchins were randomly placed in 12 tanks (3.7 litres each; 6 individuals per tank), so that there were 4 replicates for each diet type (3 diet types, see below). The tanks were equipped with a water recirculation system and an aeration system to maintain a high oxygen level and continuous water movement; in addition, each tank was individually fed by a constant flow with a turnover of 1 L/minute (approximately 3 minutes for a total turnover), so as to have independent replicates. The seawater was taken directly from the sea. Each tank was covered by a net to prevent urchins escape.

The experiment has been repeated in two different periods: June-August 2022 and October-December 2022.

On 6 June and 18 October 2022, corresponding to T_0 of each experimental rearing period, body weight and size (diameter without spines, in triplicate) were first measured for each specimen using a slide caliper (± 0.1 mm). Then the different diet was administered for the first time.

During the experimental periods (June-August and October-December), temperature was measured continuously with a HOBO datalogger (Onset Computer Corporation, Pocasset, Massachusetts, USA), while salinity and oxygen concentration were monitored twice a week with a probe, as well as the health and welfare of the specimens were continuously monitored.

2.2. Experimental diets

Sea urchins were fed two laboratory pelletised diets, FSU and FSC, and an algal-based control diet (a 50% mix of two species).
The FSU pelletised diet contains carbonate of organic origin (biocarbonate) obtained from urchin tests; the other diet, FSC, also contains carbonate but of mineral origin.
The rectangular pellets were approximately 1-2cm long and 0.5-1cm thick.

Table 1 shows the biochemical composition (in %) of the pelletised feed, used in the feeding trials, differing only in the form of carbonate, precisely consisting of:

Ingredients	FSC	FSU
Fish gelatin	25	25
Corn gluten	15	15
Sodium alginate	5	5
Linseed oil	2,5	2,5
Soy lecithin powder	2	2
Macroalgale (*Ulva* sp.)	25	25
Antioxidant powder (Oxivia)	0,5	0,5
Ca carbonate	25	0
Urchin powder	0	25
Total %	100	100

Table 1: Composition of the sea urchin feed formulation: FSU: (Urchins powder) organic carbonate; FSC: inorganic Carbonate.

For the algae-based comparison diet, two species of macroalgae were selected from an extensive literature review on the basis of their nutritional characteristics and complementary organoleptic properties: *Ulva* spp, a green alga rich in minerals and trace elements, in particular magnesium, calcium and iron, with a rather high protein content (approx. 15%) and all essential amino acids; *Ellisolandia elongata*, a red coralline alga, whose articulated thallus consists mainly of calcium carbonate. They are also a source of vitamin C and B vitamins (especially B1, B2, B6 and B12), minerals (iron, calcium and magnesium) and other trace substances (chromium, zinc, selenium, iodine and bitter tonic substances).

The 2 species of macroalgae selected for the diet (Ulva spp. and Elissolandia elongata) were collected on a weekly basis in the coastal area of Camogli and stored in tanks filled with constantly oxygenated seawater.

Sea urchins were fed twice a week at a feeding rate of 2.5% for pellets and 5% fresh seaweed, of body weight per day-1 (Prato et al, 2018; Boudouresque and Verlaque, 2020; Grosjean et al., 1998), i.e. 4gr ±0.10 pellets with organic carbonate (FSU), 4gr ±0.10 pellets with inorganic carbonate (FSC) and 6gr ±0.10 algae (50% Ulva sp and 50% E. elongata). The quantity of food provided was calculated for 3.5 days. Before feeding, each tank was cleaned to remove faeces and uneaten food and new fresh feeds were provided.

2.3. Measurement of morpho-functional parameters, index calculation and biochemical analysis of the gonads

At the beginning of the experiment (T_0), the size (without spines) and weight of each specimen was measured.

At the end of each rearing period, sea urchins were transferred to the University of Genoa Laboratory and dissected for morpho-functional measurements. For each specimen, in addition to the total weight and diameter, the following parameters were measured:

- Weight of the theca (with spines, g)
- Gonad weight (g)
- Gut weight (g)
- Weight of the Aristotle's lantern (g)

The data collected made it possible to calculate three functional indices:

1. Gonado-somatic index (GSI): ratio of gonad weight to total body weight, expressed in %.
2. Repletion Index (RI): ratio of gut weight to total body weight, expressed in %.
3. Lantern Index (LI): ratio of lantern weight to total body weight, expressed in %.

During the first experimental phase (June-August), especially during the period at the end of July/beginning of August, the recorded seawater temperature was about 2°C higher than the seasonal average, causing the death of several individuals. The work, therefore, was conducted and completed with 31 out of 72 sea urchins. In addition, when dissected at the end of the two experimental periods, the gonads of several individuals were not quantifiable, while others were treated differently for other analyses.
Therefore, the gonads from a total of 94 individuals (31 for the summer period and 63 for the winter period) after being weighed were stored in a freezer at -18°C and then freeze-dried for testing to determine the content of:

Total proteins according to the colorimetric method "*Hartree 1972*" a modification of the "*Lowry method*", which provides a linear photometric response.
The extraction and quantification are performed as follows:

- Add 1ml of milliQ water to the samples, about 1mg of dry gonad, then shake with vortex.
- Added 0.9ml of "solution A", consisting of 2g of NaK tartrate and 1g of Na_2CO dissolved in 500ml of NaOH 1N all brought to 1l with milliQ water, then placed for 10 minutes in a water bath at 50 ° C.
- Subsequently, 0.1ml of "solution B" is added to the mixture, consisting of 2g of Nak Tartrate and 1g of $CuSO_4$ penta hydrate dissolved in 90ml of milliQ+10ml of NaOH 1N water and left to rest at room temperature for 10 minutes.
- After 10 minutes, the Folin-Ciocalteu reagent and milliQ water (1:16 vol/vol) are added and placed in a water bath at 50°C for 10 minutes: this procedure produces a stable blue color and proportional to the protein concentration of the reaction mixture.
- After centrifuging the samples at 800g for 10 minutes, they were analyzed by spectrophotometer at a wavelength of 650 nm.

For the calculation of the total protein content, values were reported at a calibration curve obtained from a standard solution of bovine albumin, then expressed in μg of total protein per mg of dry gonad compared to the standard solution of albuminbovine (BSA).

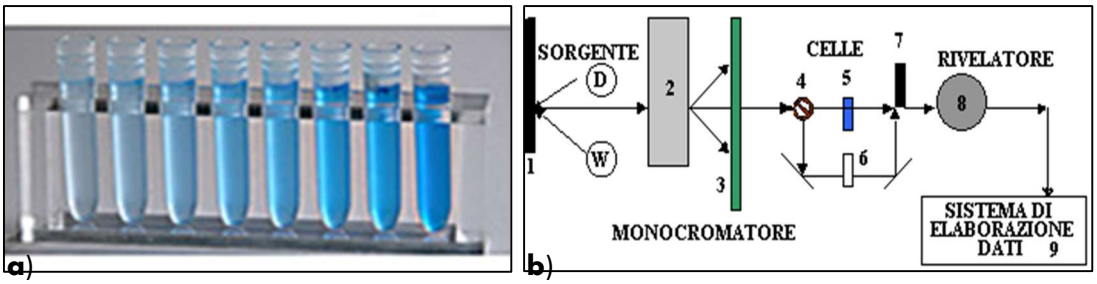

*Figure 1.: **a**) samples with different protein concentrations; **b**) spectrophotometer operation diagram.*

Total lipids extracted according to the method of *Bligh & Dyer (1956), Marsh & Weinstein, (1966)*. The extraction and quantification are performed as follows:

- Add 1ml of milliQ water to the samples, about 1mg of dry gonad, and shake for 1 minute with vortex Add methanol and chloroform (2:1 vol/vol), before leaving them to rest in the fridge for 10 minutes at 4°C.
- After being centrifuged at 800g for 10 minutes two phases are obtained: an upper lipid and a lower solid; the upper lipid phase is transferred into Pyrex tubes to which milliQ water and chloroform (1:1 vol/vol) are added, shaken with vortex for 1 minute and then centrifuged at 800g for 10 minutes, which determines the appearance of two phases: a lower lipid and an upper aqueous one that is eliminated with a Pasteur;
- the test tubes containing the lipid phase are placed in a dry thermostated bath at 80-100°C for 20 minutes in order to evaporate the chloroform.
- After that, concentrated hydrogen sulphide (H_2SO_4) is added and placed in a dry thermostated bath at 200 ° C for 15 minutes: this process determines the carbonization of lipids.
- The samples are left cooled in ice for 15 minutes, then 3ml of milliQ water are added, then analyzed by spectrophotometry.

The reading was made with spectrophotometer at a wavelength of 375 nm. The calculation of the lipid concentration was carried out on the basis of calibration curves obtained by reacting standard solutions (in chloroform) of tripalmitin according to the same method. Concentrations were expressed in µg tripalmitin equivalents per mg dry gonad.

*Figure 2.: **a**) samples with different lipid concentrations; **b**) dry thermostat bath.*

2.4 Statistical analyses

All experimental values are reported as mean and standard error (SE). In order to evaluate the effect of the tested diets (FSC, FSU, algal diet), periods (summer, autumn) and their interaction, on all the response variables considered, i.e. GSI, lipid content and protein content of sea urchin gonads, a crossed two-way ANOVA design was applied, using "diet" and "period" as fixed cross-factors. Additionally, differences in protein and lipid content of the provided diets (FSC, FSU, ALGA XX,

ALGA XX) were tested through a one-way ANOVA using the factor "diet" as fixed. For all the ANOVA analyses, the normality and homogeneity of the variances were verified for all response variables considered using the Kolmogorov-Smirnov test and the Bartlett tests, respectively. All statistical analyses were performed using the R software (R Core Team (2021).

3. RESULTS

3.1. Somatic growth: diameter (cm), weight (g) and gonado-somatic index

The total body weight (8.70 ± 2.39 g) and the diameter of the test (2.9 ± 0.28 cm) of the sea urchins displayed a different seasonal trend: in the experimental summer period no change in the total weight and diameter of the urchins was observed; in autumn, on the other hand, a constant growth was observed in terms of total weight and diameter of all individuals (Fig.3 and Fig. 4). This different trend is most likely due to the seasonality of the life cycle and therefore of the reproductive cycle of organisms: a larger effort on the reproductive output in summer implies less energy invested.

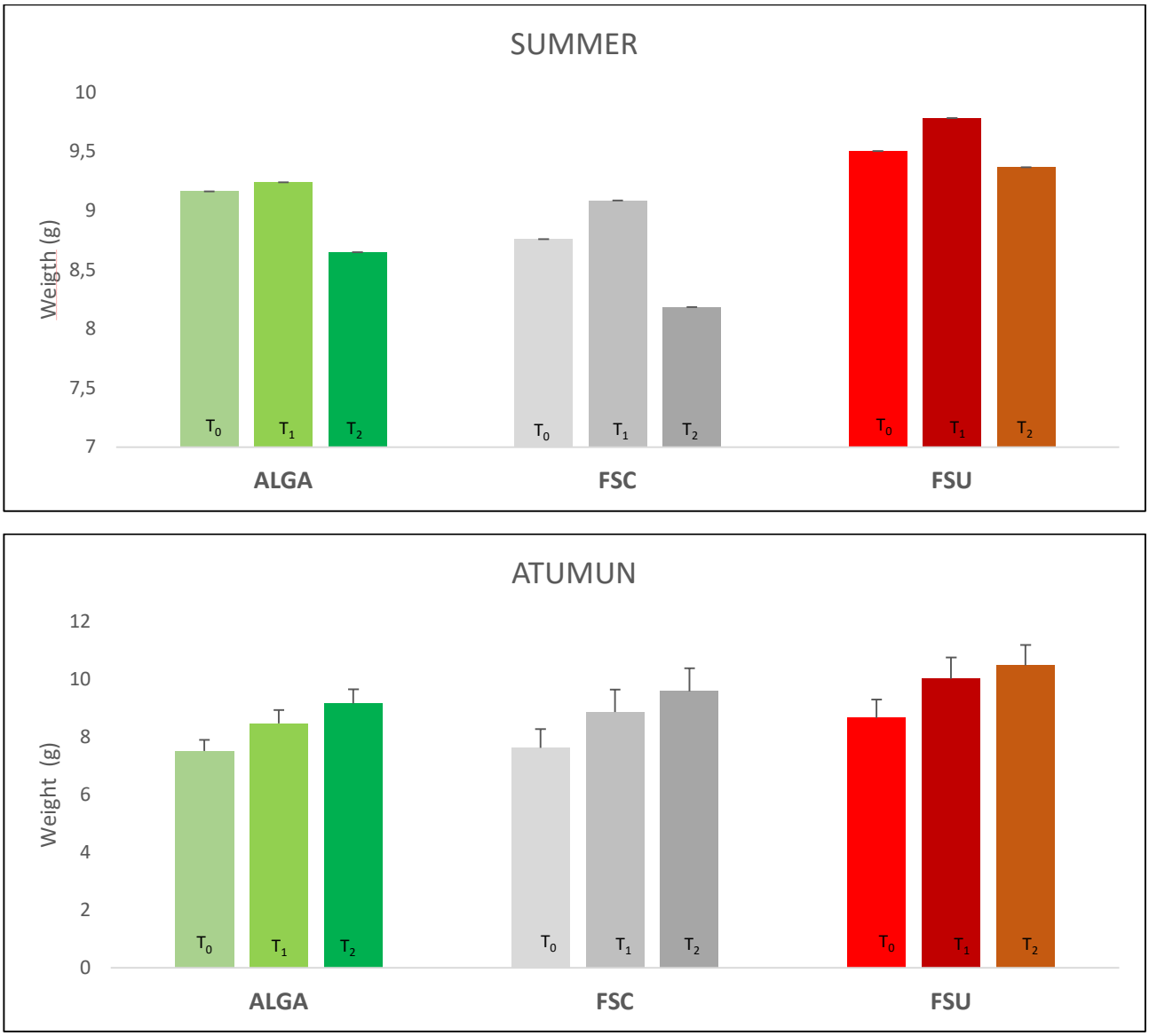

Figure 3: Somatic growth of the weight (in g) in the two breeding periods and in the three times: T0: start of experiment; T1: during experiment; T2: term experiment (sectioning), compared to the 3 experimental diets. The values shown represent the average values with standard error bar.

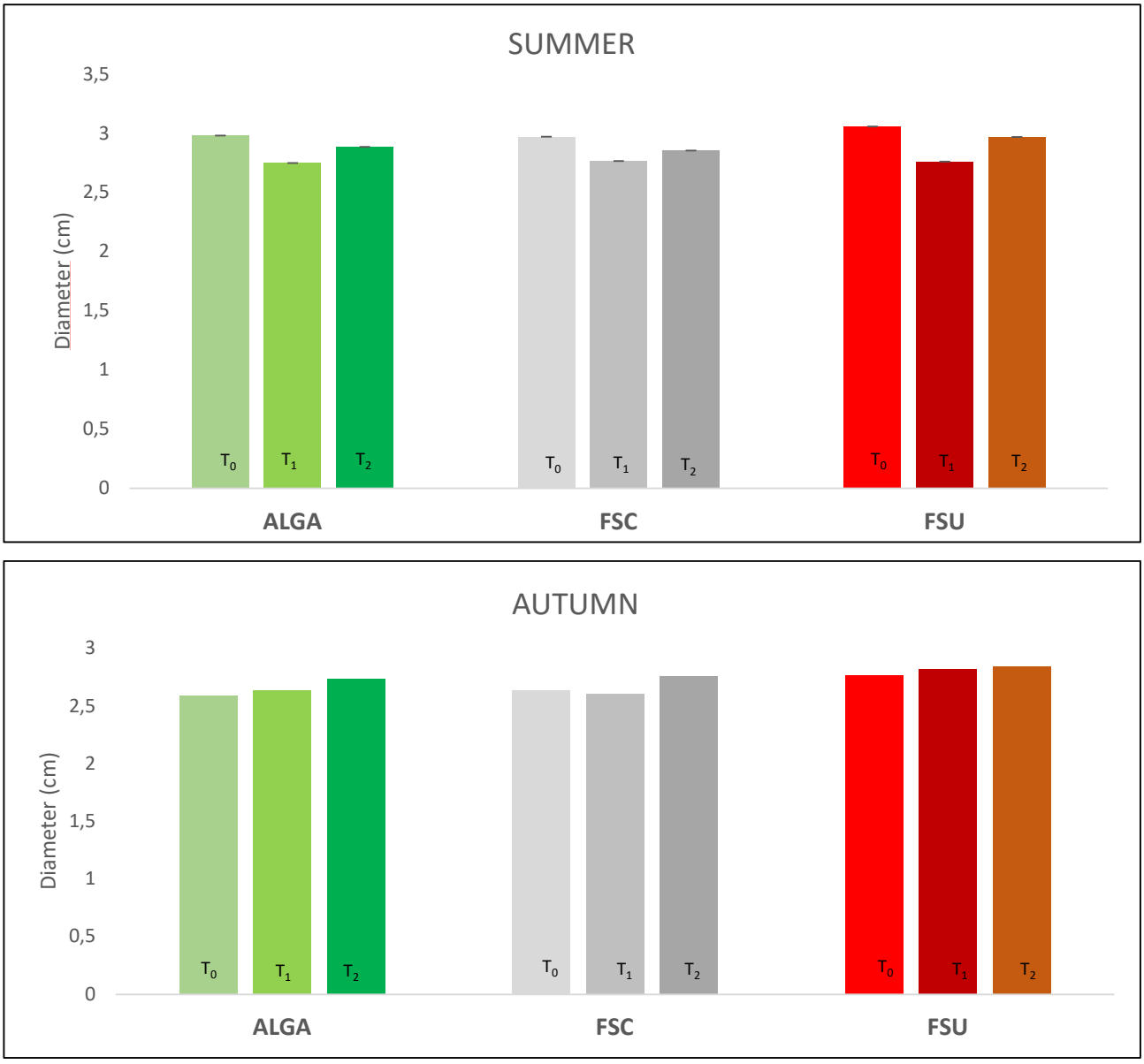

***Figure 4**: Somatic growth of the diameter (in mm) compared for the 3 experimental diets and in the three times: T0 : start of experiment; T1: during experiment; T3: end of experiment (sectioning), in the two experimental periods. The values shown represent the average values with standard error bar.*

GSI values (Fig. 5) were similar in the two pelletized diets, while significant differences were observed between the algal diet and the two experimental feeds ($p < 0.001$): the two feeds FSC and FSU provided a much higher GSI than the algal diet, which indicates adequate somatic and gonadal growth.

The feed with organic carbonate (FSU) provided a slightly higher GSI. As shown in Fig. 5, the gonado-somatic index (GSI) was higher in the summer: this aspect indicates that it is the best time to take advantage of this resource.

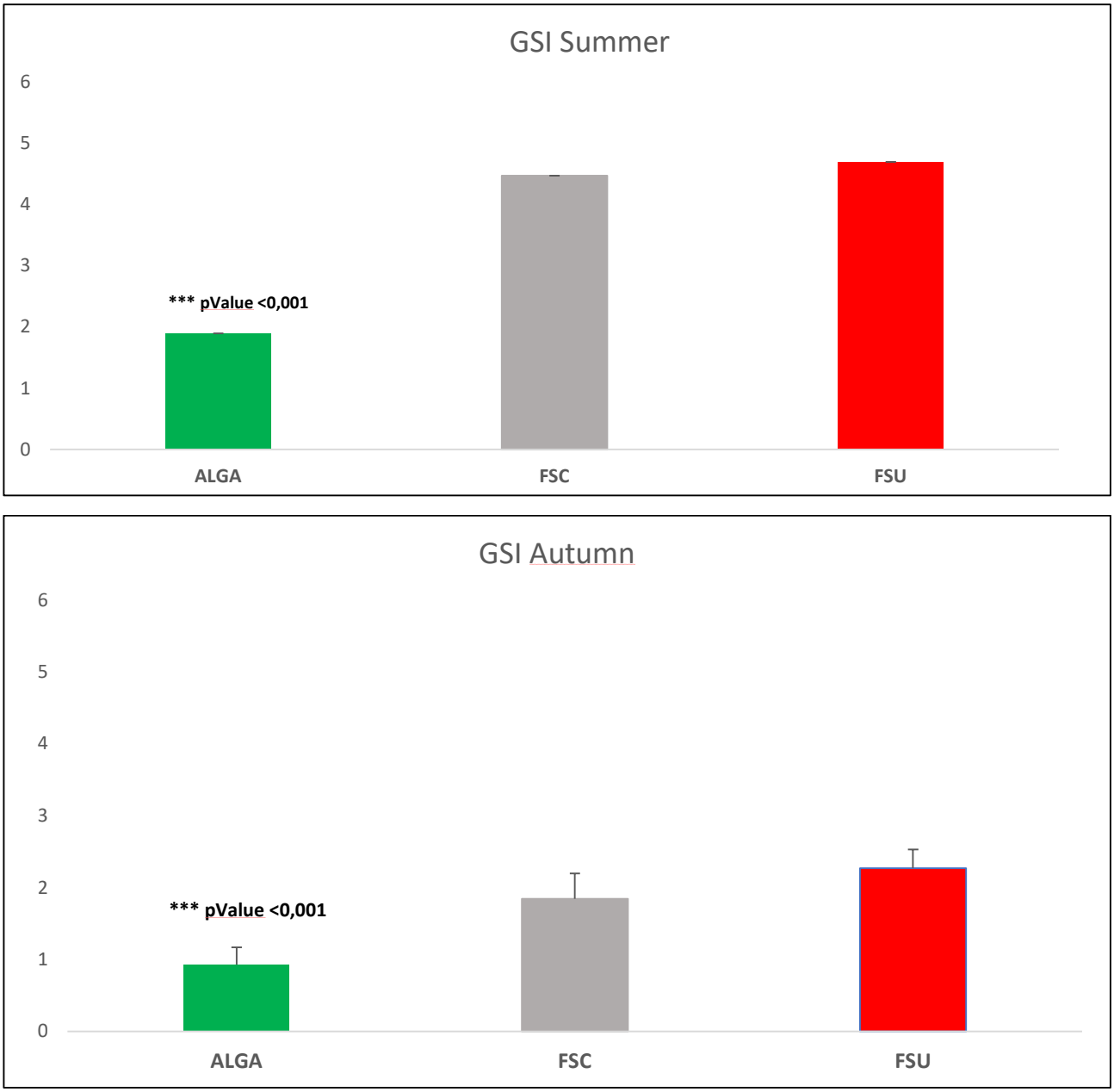

***Figure 5**: GONADO-SOMATIC INDEX (GSI) compared for the 3 experimental diets in the two breeding periods. The values shown represent the average values with standard error bar.*

3.2 Total lipid content in diets

According the analysis of variance (ANOVA) (Table 2), the FSC and the FSU diet were not significantly different. In addition, FSC and FSU provided a significantly higher lipid content than ALGA ($p < 0.001$) (Fig. 6). Seaweed data are reported twice, since analyses were performed on two different batches to cope with the possibly variable content of the natural diet along time due to seasonal intrinsic patterns (that resulted not significant).

	Sum Sq	Df	F value	p-value	
ALGA9_06 - ALGA3_23 == 0	1,53	5,525	0,277	0,992	
FSC - ALGA3_23 == 0	37,928	5,525	6,865	<0.001	***
FSU - ALGA3_23 == 0	62,106	5,525	11,241	<0.001	***
FSC - ALGA9_06 == 0	36,398	5,525	6,588	<0.001	***
FSU - ALGA9_06 == 0	60,577	5,525	10,964	<0.001	***

FSU - FSC == 0	24,178	5,525	4,376	0,0102	*

***Table 2**: ANOVA among the different types of diets: ALGA, FSU AND FSC. Seaweed reported twice: it is the same algal species but from different sampling times.*

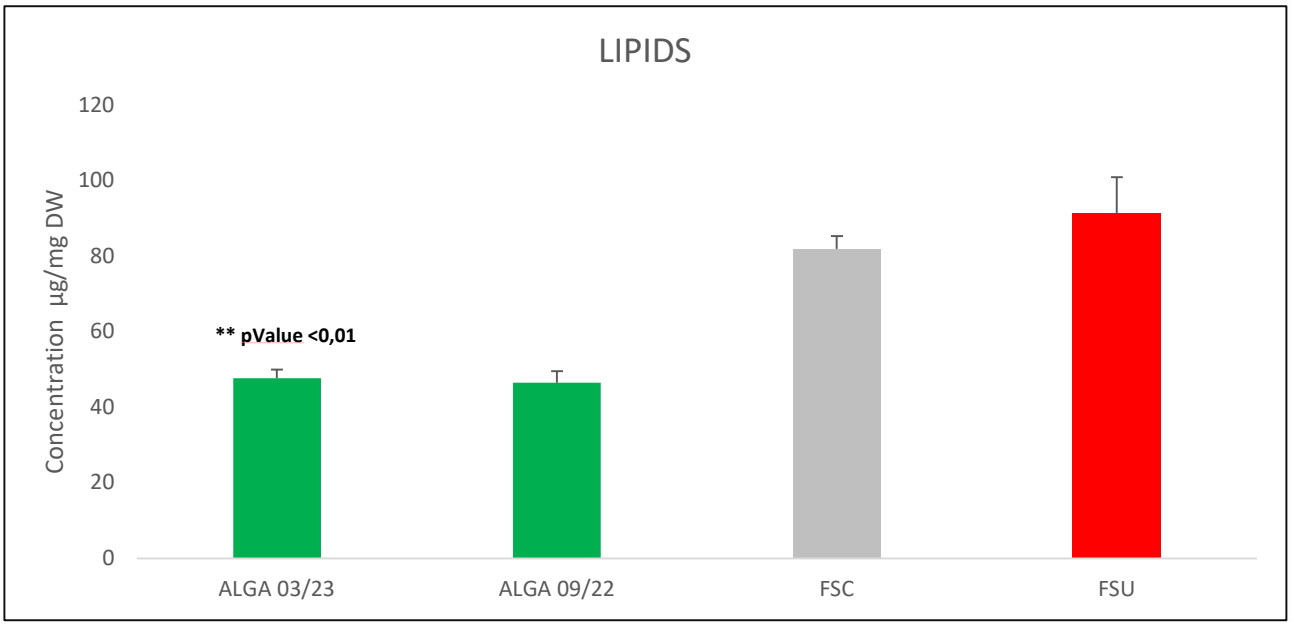

***Figure 6**: Total lipid content in the three different diets. The values shown represent the average values with standard error bar.*

3.3 Total protein content in diets

From the analysis of variance (ANOVA) (Table 3) the FSC diet and the FSU diet had a significantly higher protein content than the ALGA one ($p < 0.001$). In addition, urchin powder enriched (FSU) diet had a significantly higher protein content than FSC, in relation to the fact that it does not contain only calcium carbonate (Figure 7).

	Sum Sq	Df	F value	p-value	
ALGA9_06 - ALGA3_23 == 0	1,53	5,525	0,277	0,992	
FSC - ALGA3_23 == 0	37,928	5,525	6,865	<0.001	***
FSU - ALGA3_23 == 0	62,106	5,525	11,241	<0.001	***
FSC - ALGA9_06 == 0	36,398	5,525	6,588	<0.001	***
FSU - ALGA9_06 == 0	60,577	5,525	10,964	<0.001	***
FSU - FSC == 0	24,178	5,525	4,376	0,0102	*

***Table 3**: ANOVA among the different types of diets: ALGA, FSU AND FSC. Seaweed reported twice: it is the same algal species but from different sampling times.*

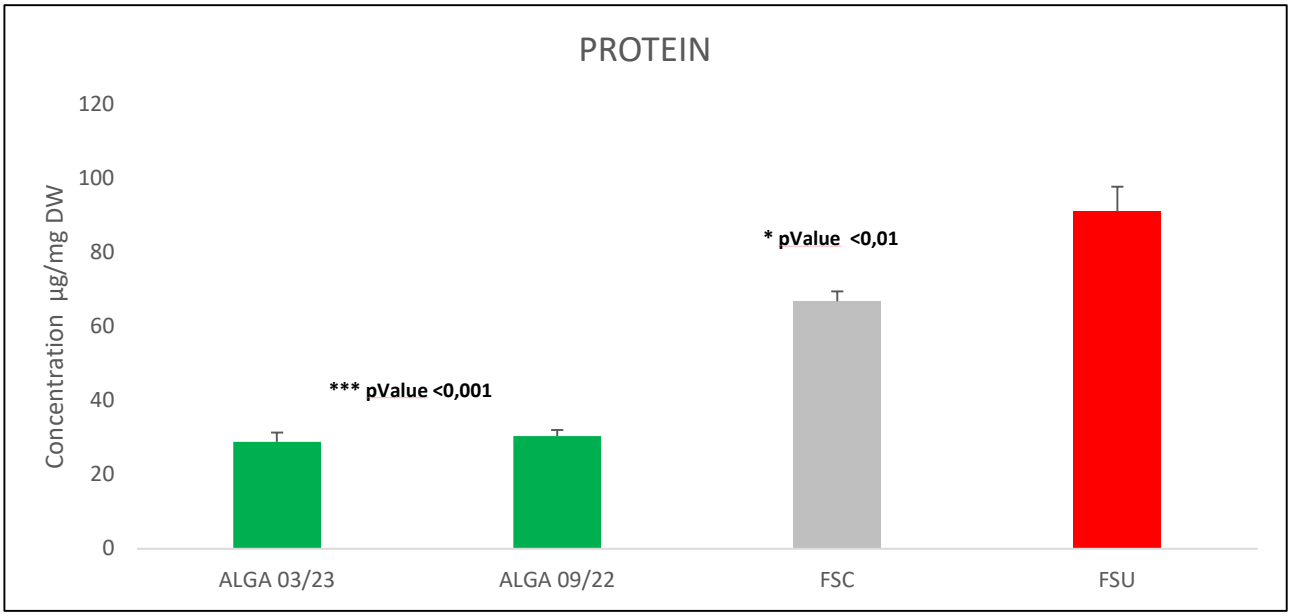

***Figure 7**: Total protein content in three different diets. The values shown represent the average values with standard error bar.*

3.4 Total lipid content in gonads

Analyses of the total lipid content of *P. lividus* gonads showed that the macroalgae diet (50% *Ulva* sp and 50% *E. elongata*) showed significant differences in the two experimental periods, in particular with higher values in autumn (p=0.03). In addition, the algal diet and the FSU pelletized diet were significantly different in summer (p=0.005), with even higher values in the FSU diet compared to the FSC one (Fig.8) In addition, in the FSU diet the total lipid content showed significant differences between the summer and autumn period (p=0.01) (table 4).

In the summer experimental period, urchins fed with formulated feed, in particular with FSU, had a higher lipid content in the gonads: an aspect certainly more appreciated by consumers as it gives more flavor and taste (Fig. 8).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Feed	2	82631	41316	3,668	0,02965	*
Period	1	4728	4728	0,4198	0,518792	
Feed:Period	2	155320	77660	6,8947	**0,001679**	**
Residuals	85	957411	11264			

***Table 4**: ANOVA of the total lipid content of the gonads of the urchins fed with the different types of diets: ALGA, FSU and FSC in the two experimental periods.*

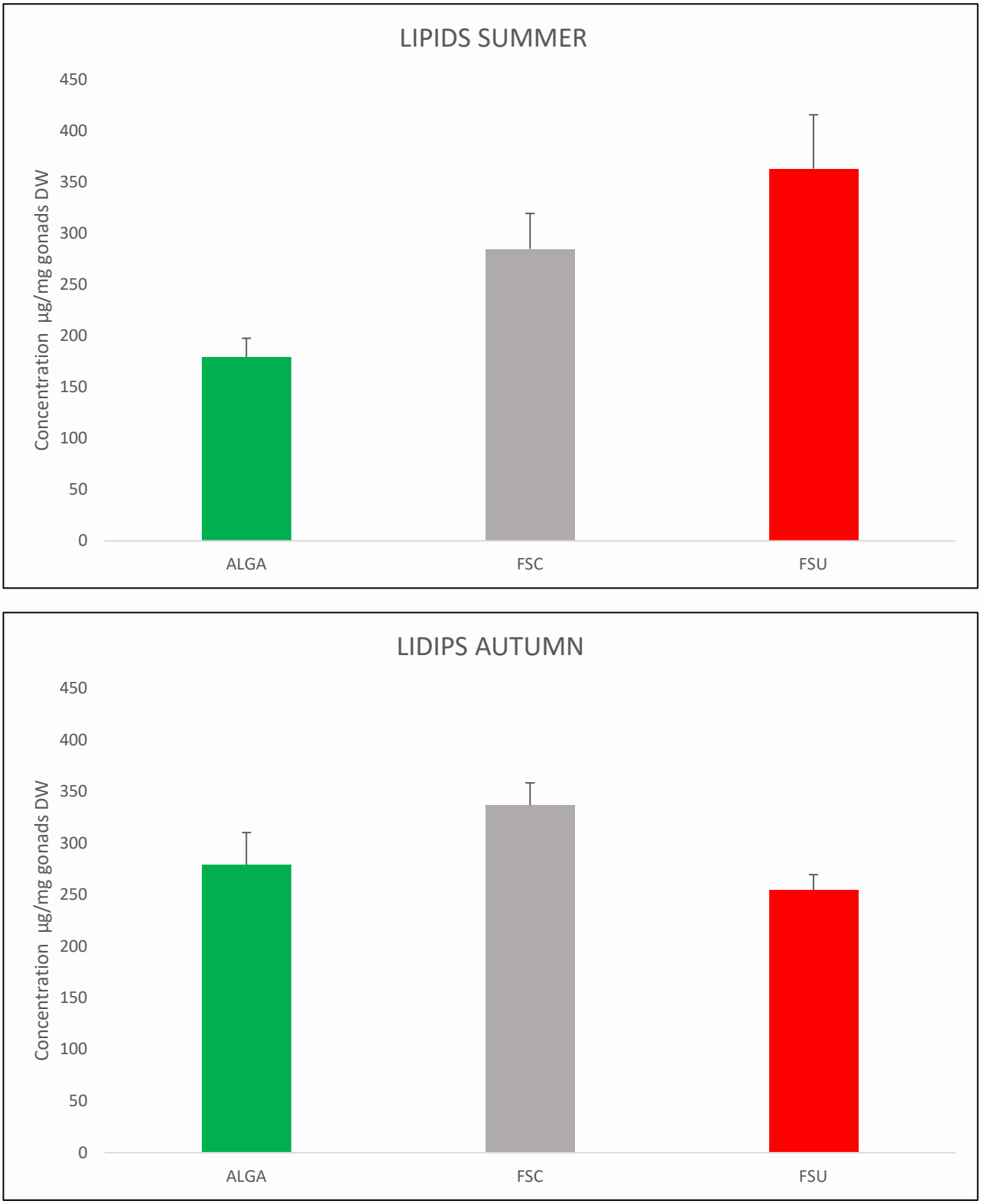

Figure 8: *Total lipid content of the P. lividus gonads according to the different diet in the two experimental periods. The values shown represent the average values with standard error bar.*

3.4 Total protein content in gonads

Analyses of the total protein content of *P. lividus* gonads showed that the algal diet (50% *Ulva* sp and 50% *E. elongata*) and the FSU pelletizing diet were significantly different in summer (p=0.005). In particular, the FSU diet differed significantly between the summer and autumn period (p=0.01). the

same applies to the algal diet, as it differed significantly between the summer and autumn period (p=0.03) (Table 5).

The protein content of the gonads did not show significant differences between the 3 diets, this means that the formulated feeds do not show deficiency of this component (Fig.9).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Feed	2	22254	11127	2,788	0,06711	.
Period	1	44375	44375	11,119	**0,00126**	**
Feed:Period	2	7093	3547	0,889	0,41494	
Residuals	86	343221	3991			

Table 5: *ANOVA of the total proteins content of the gonads of the urchins fed with the different types of diets: ALGA, FSU and FSC in the two experimental periods.*

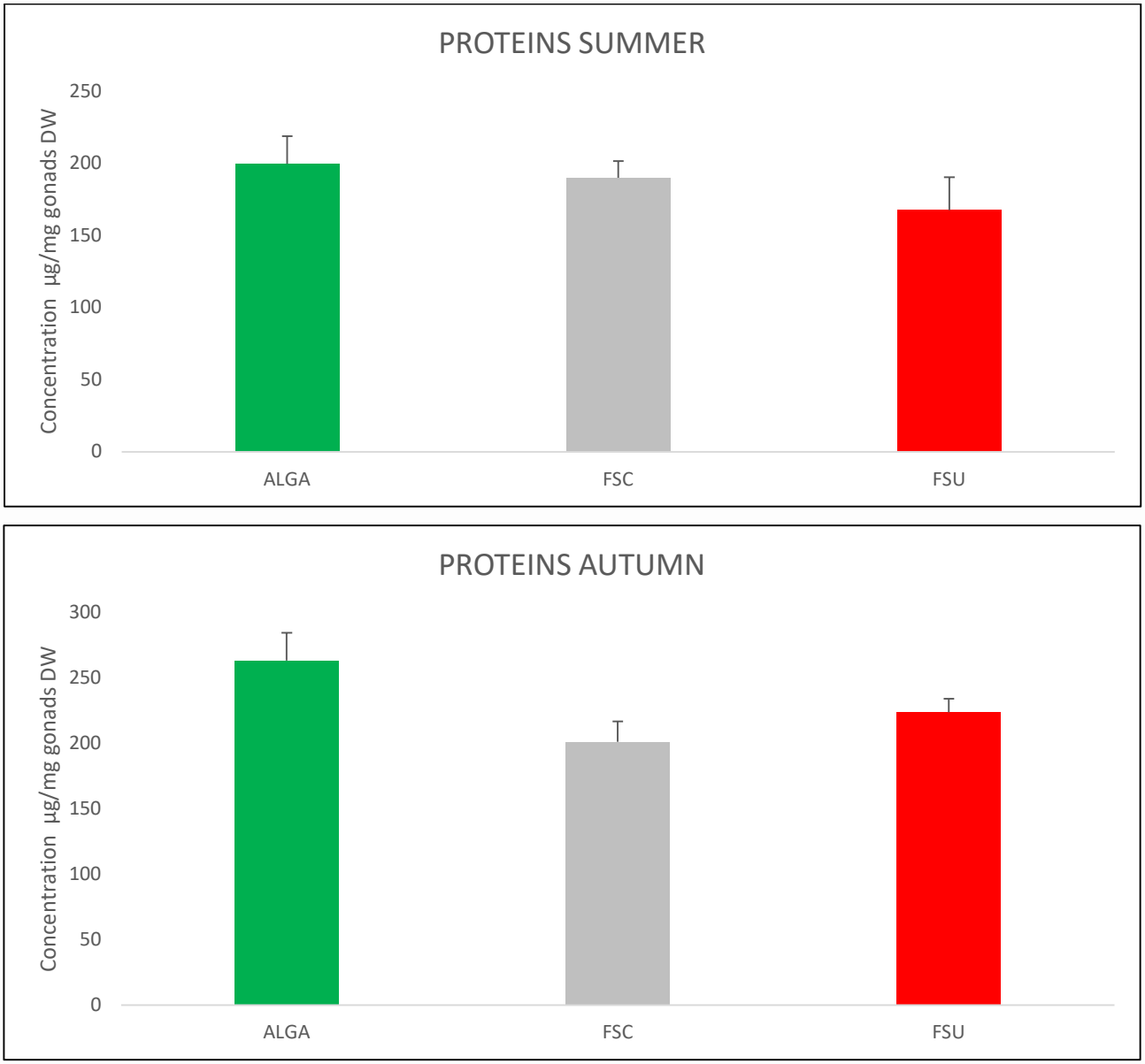

Figure 9: *Total protein content of the P. lividus gonads according to the different diet in the two experimental periods. The values shown represent the average values with standard error bar.*

4. DISCUSSION

The quality of the gonads is mainly determined by its size, taste, color and texture, which are influenced by the content of proteins, lipids, carbohydrates and carotenoids of diets (Cuesta-Gomez and Sánchez-Saavedra, 2017; Taylor et al., 2017). Several factors can influence both the yield and the quality of the gonads, such as: i) abiotic factors (e.g. temperature, salinity, photoperiod), ii) nutritional factors (e.g. feed ingredients and chemical composition), iii) and farming conditions (e.g. livestock density, water circulation) (Lourenço et al., 2018b; Rocha et al., 2018; Rocha et al., 2019).

To move towards a sustainable echinoculture, also in order to reduce harvesting pressure on wild populations, its economic viability and market acceptance depend heavily on the development of suitable, cost-effective and nutritionally balanced diets capable of producing high-quality gonads for the growing global demand for this delicacy (Eddy et al., 2012; Pearce et al., 2002; Woods et al., 2008). It is now clear that the success and sustainability of commercial sea urchin aquaculture, its economic viability and market acceptance depend heavily on the development of suitable, cost-effective and nutritionally balanced diets capable of producing high-quality gonads for the growing global demand for this delicacy (Eddy et al., 2012; Pearce et al., 2002; Woods et al., 2008).

The present study evaluates and proposes a sustainable feed formulation through the reuse of waste from the food industry and the fishing supply chain. The use of the flour obtained from the wastes of urchin food industry (Table 6) provides an ingredient that not only contains a large amount of biocarbonates (88.11%), but it also contains the main macronutrients such as lipids and proteins that contribute respectively for 1.71% and 5.52%, in addition to carbohydrates (3.78%) and fibers (0.89%).

FARINA DA SCARTO LIOFILIZZATO								
CAMPIONE		umidità	ostanza secca medi	ceneri	proteina (Nx6,25)	lipidi grezzi	fibra grezza	i inazotati(carboidrati)
		%	%	% sostanza secca	% sostanza secca	ó sostanza secc	% sostanza secca	sostanza secca
RICCIO		0,95	99,05	88,11	5,52	1,71	0,89	3,78

Table 6: Biochemical composition of sea urchin flour obtained by crushing of sea urchin test, expressed in %

In addition to diet, gametogenesis and water temperature can also have a marked influence, promoting the indispensable mechanisms of synthesis, selective storage and mobilization of certain fatty acids to meet nutritional needs (Martinez-Pita et al., 2010a, Martinez-Pita et al., 2010b). Depending on the stage of maturation, the gonads contain a different ratio between germ cells (eggs and sperm) and somatic cells (nutrient phagocytes), and are generally characterized by a different gonado-somatic index (GSI), not closely related to each other (Byrne, 1990, Lozano et al., 1995, Soualili and Guillou, 2009).

Given that *P. lividus* takes at least 2-3 days to consume the feed offered in confined conditions (Fabbrocini et al., 2015), the prepared diets were easily consumed by sea urchins in both trials, therefore they represent a suitable choice in the production of sustainable feed for indoor echinoculture. The evaluation of the gonado-somatic index carried out in this study shows that significant differences are observed in both experimental periods between the two experimental diets compared to the algal diet: overall, significant differences in GSI were observed regardless of diet in both feeding tests. Organic carbonate (FSU) feed provided a slightly higher GSI, but for more accurate evaluations and/or further evaluations longer experiments are required, as in a whole production cycle (e.g. 1 year) and the benefits may be much more evident.
This different trend is most likely due to the seasonality of the life cycle and therefore of the reproductive cycle of organisms: a larger effort on the reproductive output in summer implies less energy invested.

Proper nutritional composition of feed is crucial in echinoculture. With particular regard to the lipid content of the gonads of sea urchin fed with FSU and in the summer, higher values are observed and consequently significant differences compared to the algal diet. Food lipids play a key role as structural components, energy sources and precursors of bioactive molecules (Carboni et al., 2013), and also influence the FA composition and organoleptic attributes of eggs (Martínez-Pita, García, & Pita, 2010; Siliani et al., 2016; Vizzini et al., 2019). Consequently, a high lipid content of the diet can promote the development of the gonads and contribute to the restoration of energy intake following hunger, during which sea urchins tend to consume the nutrients present in their tissues (Guillou & Lumingas, 1998).

Also, the source of proteins is important, as revealed by Fernandez and Boudouresque (2000). As far as the total protein content, among the three diets ALGA, FSC and FSU in the same breeding period no significant differences are observed; therefore, this important component in the FSU diet is not limiting. This is in relation to the fact that sea urchin flour, as can be seen from chemical analysis (Table 6), is not composed exclusively of biocarbonate, which represents the largest component (88.11%), but also of organic components such as proteins. On the other hand, there is a significant difference of the the protein content of the gonads between the summer and autumn period with higher values in autumn.

5. CONCLUSIONS

The analytical data obtained in the 2 experimental feeding periods allowed to provide new evidence on the use of feed prepared for the culture of *Paracentrotus lividus* intended for human consumption. Although the duration of the experiments was relatively short (2 months for each breeding period) the results show that the diet containing sea urchin test powder (FSU) was able to increase both somatic and gonadosomatic index, as well as lipid and protein content of the gonads.
The use of an artificial diet can be a consistent and inexpensive tool in the installation of indoor echinoculture systems. This strategy can represent an important improvement in the protection of *Paracentrotus lividus*, affected by the depletion of wild stocks, to meet the growing demand of the gonad market.

For all these reasons, this study is part of the circular economy model, with particular regard to the protection of the seas, oceans, biodiversity and exploitation of marine environmental resources, which through technological innovation (blue biotechnologies) favor the reuse of resources and the creation of a sustainable economic system in waste management.

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